Scale-up of photo-bioreactors for microalgae cultivation by π -theorem

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Abstract

Robust microalgae cultivation at industrial scale needs complex scale-up of photobioreactors since the same yields obtained at lab-scale are hardly reached during pilot or industrial production. In this paper we propose a procedure based on Buckingham π -theorem to perform the scale-up of photobioreactors used to cultivate *Chlorella Vulgaris* fed by CO₂ and wastewater rich in glycerol. This theoretical approach is usually overlooked in favor of the synergy of modeling tailored on the process and piloting, but it grants excellent generalizability. An experimental campaign at three levels was designed and carried out to evaluate the characteristic dimensionless numbers individuated by the theoretical formulation. Since scale-up regards both geometrical dimensions and type of reactor, passing from lab-scale stirred tanks (STRs) to pilot scale tubular and airlift, particular attention was devoted to defining characteristic lengths inside the dimensionless numbers. Moreover, since scale-up also regards the operating mode, scaling from discontinuous to semi-continuous to continuous, some interesting dimensionless numbers arise other than Re, Sh, Da_{II}. They are mainly related to the type of biological process and its operating mode and are the ratios O₂/CO₂ and T/T_{opt}, the ratio between the incident light intensity and the saturation constant, the absorbance, the

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ratio between the final and the initial concentration c/c_0 , the ratio between the maximum increase in cell population and its initial concentration, the ratio between the estimated specific kinetic constant and a variable representing the characteristic time of mixing inside the chosen reactor.

Preliminary outdoor tests confirmed the operability of the scaled-up airlift reactors reaching $c/c_0 = 5.3 - 7.5$, with $c = 1.15 \text{ g L}^{-1}$ at extraction intervals of 5 days. They were operated under optimal light conditions of incident light greater than *Chlorella v.* saturation constant and absorbance =40 and at incipient churn flow ($Re=10000 - 11000$) with dimension of fluegas bubbles around 5 mm, apt to guarantee $Sh= 1500-2500$; and with a calculated Da_{II} around 25.

Keywords: π -theorem, scale-up, dimensionless numbers, airlift photobioreactor, *Chlorella vulgaris*, kinetics.

Declarations of interest: none

1. Introduction

Biofuels synthesized from microalgae (third generation biofuels) are an interesting option in the context of a circular economy that is increasingly mentioned and sought after by both policymakers and corporations. They align effectively with the new holistic approach aimed at exploiting the interdependence of different areas, having the potential to use waste products as raw materials and treat wastewater, thus bio-fixating waste streams from other processes. Not only microalgae can feed on nitrogen (N), phosphorus (P) and heavy metals compounds, but also, through photosynthesis, they capture carbon dioxide (CO_2). Accordingly, they can provide noteworthy quantities of carbon compounds and bioproducts (lipids, vitamins, pigments, ...), thus being used also for high-value chemicals, pharmaceuticals (antioxidants, anticancer drugs, antimicrobial

drugs), cosmetics and nutrient supplements production on top of being sold directly as food [1–3], all processes viable at large-scale from an economic point of view. In a nutshell, microalgae reduce the pressure on resource allocation, overcome the problems of food crop competitiveness and offer the aforementioned advantages of a multipurpose environmental tool, on top of showing higher lipid productivity compared with plants [4]. Nevertheless, this biofuel production route is not yet widely followed for commercialization as its current economic balance is still not always advantageous [5]. Some solutions at production scale consist of using simple design reactors as open ponds [6] or tubular bubble reactors made by inexpensive material [7]. However, to achieve high yield raceway ponds are not considered the optimal solution, as they are characterized by low productivity. Robust industrial scale production typically entails some downstream processing that accounts for about 60% of the overall biodiesel production cost [8]. To reduce it, one can intervene by advancing the technology behind the engineering of production processes and optimizing both operating conditions and cost of the devices, or by improving biological efficiency, increasing the growth rate and lipid content inasmuch as possible [9]. To the former approach refer the studies on the optimal design of biorefineries, in which biomass to bioenergy production processes are coupled with microalgae cultivation [10]. To the latter path belongs the efforts to further improve the characteristics of microalgae with bioengineering techniques. This would lead to fourth generation biofuels, for which metabolic engineering and genome editing methods [11], like Clustered Regularly Interspaced Palindromic Sequences (CRISPR)/CRISPR-associated protein 9 (Cas9), are still being investigated and scarcely employed outside laboratories, albeit they seem promising [12]. In this work, we focused on the technological route, and we devised an integrated biorefinery exploiting waste frying oils,

solid organic wastes and algal biomass to produce biodiesel and energy while simultaneously minimizing waste fluxes and possibly bio-fixating CO₂ and treating flue gases and wastewater [13]. It comprises three main sub-processes. The first two are biodiesel synthesis by Trans-Esterification (TE) and syngas production by gasification. The third, algal biomass production in airlift photobioreactors fed by recycled process wastewater and flue gases, is also the most critical one.

AirLift Reactors (ALRs) are flexible and markedly suitable to be incorporated into biorefineries and integrated power generation systems, due to the multitude of process parameters that can be used to optimize their operation. For the growth of microorganisms, they are considered superior to both tubular bubble reactors and open ponds, with shear distribution homogeneous throughout the ALR translating in a relatively constant environment, with minimization of sharp changes in the mechanical forces acting on suspended particles [14]. Moreover, in ALRs it is possible to obtain very fast photoperiods and good mixing without high energy demand. In fact, the interest in ALRs for microalgae growth is especially spiked by high yields net of energy consumption for medium/low mixing.

However, complexity is due primarily to the interrelation between key physical aspects, transport phenomena and fluid dynamics *in primis*. A good reactor design should concurrently cover all the aspects of multiphase kinetics of the algal biomass, where the cells are in solid phase, some nutrients like CO₂ are in gas phase and some others are dissolved in liquid phase, moreover oxygen (O₂) is both a byproduct or a reactant depending on the different night/day growing conditions and finally also light can be considered as a reactant. A complete detailed simulation of both biochemical reactions and mass transport processes inside the reactor is challenging and models frequently

consider these aspects separately, focusing either on the mass transfer (e.g. [15]) or on the hydrodynamics, usually with the aid of Computational Fluid Dynamics (CFD) (e.g. [16]) or only on kinetics. During operation, residence times into the different zones of the reactor must contemporary guarantee good mass transfer, the best CO₂ distribution and O₂ release possible on top of optimal light/dark cycles that can be guaranteed with part of the airlift reactor made of not-transparent material.

For these reasons, the pilot-scale experimental test planning is mandatory in order to correctly set up and tune process variables before the final plant installation. Moreover, even if the design was optimized, in some cases the final operating conditions differ from the designed ones due to the biological nature of the reactants and the complexity of the system. Even if theoretical scale-up procedures from batch mixed photobioreactors at lab-scale to ALRs at pilot-scale are not usually carried out, the approach adopted in this paper proposes to use the π -theorem to define the main dimensionless groups at lab-scale and to keep their values as desired at pilot-scale. Thus, since due to their hydrodynamics ALRs offer more degrees of freedom than STRs, some of these values can be tuned inside the identified ranges of the operating variables, then assuring a good continuous operation of the ALRs as components of integrated pilot-scale biorefineries. In this perspective, we choose *Chlorella vulgaris* as the nurtured species, by virtue of its remarkable versatility. It is a fast-growing freshwater unicellular eukaryotic green algae belonging to the *Chlorophyta* phylum that can efficiently adapt to a wide range of surrounding environments. Rich in lipid content, these microalgae can proliferate under conditions of high nutrient concentrations, effectively feeding also on wastewaters that can even enhance their growth [17,18]. Studies highlight notable removal capabilities of N (up to about 90%), P (even complete elimination) and the ability to bind heavy metals [19,20],

with an average deletion efficiency of 72% for N and 28% for P [21]. *Chlorella vulgaris* can tolerate high CO₂ concentrations, showing good sequestration rates [22] with reasonable growth; for this reason it is often suggested for the treatment of exhaust gases in biofuel production processes [23]. *Chlorella v.* can withstand temperatures between 5 and 35 °C, with an optimal range between 25 and 30 °C [24], and can absorb light energy at different wavelengths (420 ÷ 475 nm, 630 ÷ 700 nm [25,26]).

2. Materials and Methods

2.1. Photobioreactors

The scale-up presented in this work is intended as discussed in [27,28] and regards dimensional, operating, and automation scale-up, following the scheme reported in Figure 1.

Figure 1: Schematic representation of the intended scale-up.

2.1.1. Discontinuous cultivation at lab-scale

Open top cylindrical glass containers (beckers) were used for the discontinuous cultivation of *Chlorella vulgaris*, with a useful volume of 500 mL and an internal diameter of 100 mm. Test batteries consisting of six stirred tanks are mixed using a Velp Scientifica FC 6S flocculator with individually adjustable mixing speed ranging from 10 to 250 rpm. It is equipped with different types of impellers (self-built radial and axial) with variable diameters ranging from 40 to 80 mm and lighted by six FLUORA Osram FD-T26-L-30-77 (30W, 1000 lm at 25 °C) lamps under 12 light/ 12 dark photoperiods. Seed cultures are prepared by adding 50 mL of the stock culture to 450 mL of BBM medium. The primary feed, consisting of CO₂ mixed with air, simulates flue gas. This gaseous mixture is dispersed from the bottom of the reactor by means of a ring-shaped ceramic diffuser with an outer diameter equal to 100 mm and internal diameter equal to 90 mm. The secondary feed, consisting of synthetic wastewater containing glycerol, is manually inserted at pre-established time intervals.

2.1.2. Semi continuous cultivation at lab-scale

At semi-continuous lab-scale microalgae cultivation was carried out in two glass-lined clamped top stirred tank reactors, having a useful volume of 2500 mL and an internal diameter of 120 mm. The diameter/height ratio of the two reactors is 1:2, not in the range of classical stirred tanks, but specially designed in order to approximately keep at this scale the same average light intensity obtained inside the smaller reactors at discontinuous lab-scale. At this intermediate scale, microalgae cultivation was carried out under two different light conditions: the former with reactors lighted by eight FLUORA Osram FD-T26-L-30-77 lamps under 12 light/ 12 dark photoperiods; the latter in outdoor during the

spring season. The glass top of each reactor is designed with three nozzles (diameter equal to $\frac{3}{4}$ ") for probes introduction and one central nozzle (diameter equal to 1") for the stirrer axis. Mixing is performed by two Velp Scientifica PW overhead stirrers, with speed ranging from 20 to 1200 rpm. No baffles are provided inside the reactors. Both radial and axial impellers are available with diameters ranging from 40 mm to 100 mm. Air enriched by CO₂ simulating flue gas is continuously dispersed by a ring-shaped ceramic diffuser installed on the bottom of each reactor; its external diameter is equal to 120 mm while its internal diameter is 110 mm. CO₂ concentration can be regulated. Synthetic wastewater is inserted at chosen time intervals by peristaltic pumps and cyclic injection can be managed. Microalgae extraction can be cyclically operated. Seed cultures are prepared using directly the stock culture into BBM medium. One of the two reactors is shown in Figure 2.

Figure 2: Semi-continuous STR at lab-scale

2.1.3. Continuous cultivation at pilot-scale: ALRs

The experimental campaign at continuous pilot-scale was carried out in four external loop airlift reactors (EL-ALRs) properly designed and assembled to conduct the tests.

Figure 3: Two of the EL-ALRs.

Both the riser and the downcomer are made by transparent polymethyl methacrylate (PMMA), while the material adopted for the horizontal collectors is not-transparent PVC.

This choice allows a good dark/light cycle alternation at relatively short residence times, a fact that plays a decisive role in improving algae growth, avoiding photo-inhibition for the culture. EL-ALRs are shown in Figure 3.

The ratio between the riser and the downcomer diameter is 2.2, falling within the range $1.0 \div 3.0$ suggested as optimum for airlift bioreactors design [29] and the total volume of each ALR is about 10.5 L. The main dimensions of each pilot ALR are reported in Table 1.

Air simulating flue gas is insufflated by a sparger installed into the base of the riser and made by a perforated stainless steel plate coupled with a porous sponge diffuser.

pH, conductivity and dissolved oxygen probes are installed in the midsection of the lower collector (see Figure 5). pH measurements are carried out during the hydrodynamic experimental tests and used for an indirect estimation of carbon dioxide dissolved in circulating water. Additional conductivity measurements, executed at the top of both the riser and the downcomer, can be used to estimate residence times by NaCl tracing.

Table 1: ALR geometric data.

Geometric data	Value
Length of the riser	0.780 m
Diameter of the riser	0.110 m
Length of the downcomer	0.780 m
Diameter of the downcomer	0.050 m
Length of the horizontal collectors	0.385 m
Diameter of the horizontal collectors	0.050 m

Two EL-ALRs were operated for algae cultivation in outdoor conditions during the spring season.

2.2. Monitoring and automation

2.2.1. Monitoring output variables

Microalgae concentration was daily measured at discontinuous lab-scale through both direct dry cell weight measurements and spectrophotometric analysis. The former procedure was carried out by filtering the extracted samples (Buchner and 45 μm filters), then by drying the filters for 24 h at 70 °C into a Mechanical Convection Oven (Binder Microprocessor-Controlled WZ05012) and finally by weighing them using an analytical balance (Mettler Toledo) to obtain the dry cell mass (g L^{-1}). Spectrophotometric analysis was performed on daily collected algae samples by UV-VIS Hach DR-6000 spectrophotometer. Calibration curves constructed at different wavelengths (430, 434, 480, 550, 650, 663, 666, 680) were used to correlate the measured absorbance with concentration measures. Microalgae growth was estimated by using concentration values obtained by both dry cell mass measures and absorbance measures. In the latter case, kinetic parameters were estimated at the different wavelengths, in order to choose the best ones, to test the robustness of the estimation procedure using spectrophotometric data with respect to dry cell mass measurements and to obtain statistical information about measurement errors.

At semi-continuous lab-scale, microalgae concentration was only indirectly measured by daily spectrophotometric analysis at different wavelengths (430, 434, 480, 550, 650, 663, 666, 680). Kinetics parameters were estimated at all the considered wavelengths also to obtain statistical information about the robustness of the kinetic model.

At continuous pilot-scale, samples are collected at the top of the downcomer and only two wavelengths were used to construct the calibration curves adopted to derive the

concentration measures. Kinetics were estimated using data at the considered wavelengths.

2.2.2. *Monitoring internal variables*

At discontinuous lab-scale internal variables as pH, conductivity (C), dissolved oxygen (DO_x) and temperature (T) were off-line acquired by WTW multi-parameter portable system 340i and related probes SenTix 41, TetraCon 325, CellOx 325. At semi-continuous lab-scale and at pilot-scale the internal variables pH, C , DO_x and T were on-line acquired at 10 Hz frequency by B&C Electronics series 7685 Microcontrollers and related probes. The probes were respectively inserted into the dedicated nozzles in the glass top of each reactor and in the midsection of the lower collector of the EL-ALRs. At both semi-continuous lab-scale and pilot-scale the B&C systems were adopted to acquire and convert the measures, show their values in the lab and to send the acquired values ($4 \div 20$ mA signal) to both a PXI National Instruments system and MEMO monitoring system, this one being a real-time, remotely accessible operating database that continuously collects, filters and stores the measures (ECPLab – University of Genoa, Italy). At pilot-scale, the B&C conductivity controller and the pH controller were also used to respectively regulate the NaCl used as a tracer and pH into the ALRs.

2.2.3. *Monitoring input variables*

The incident light intensity (I_0) to the PBRs was measured by a luxmeter (CA 1110 Chauvin Arnoux; $0.1 \div 200000$ lux). The average light intensity to each cell inside the reactors was calculated by the Lambert-Beer law, with an absorption coefficient of daylight source for *Chlorella v.* assumed equal to $0.45 \text{ m}^2 \text{ g}^{-1}$. At both semi-continuous and pilot-scale in outdoor light conditions, the photon flux density (PFD) for the

photosynthetically active radiation (PAR) range was directly measured by two Apogee SQ-500 PAR light sensors. Incident photon flux density (PFD_0) was measured on the reactor surface, while local measures of PFD were taken by inserting the probes respectively into one dedicated nozzle of the glass top of each reactor and into the riser/downcomer. The signal in output from the sensors was sent to the MEMO system after conversion by MCP3008 A/D converter.

2.2.4. Operating mode and automation

The CO_2 concentration in the simulated flue gas (air enriched by CO_2) dispersed into the stirred tank reactors was regulated by Brooks Instrument 5850S smart mass flow controllers. At discontinuous lab-scale, wastewater introduction and microalgae extraction were manually operated.

At semi-continuous lab-scale, cyclic injection of secondary feed, i.e. simulated wastewater rich in glycerol, was regulated by the PXI National Instrument system plus Labview. The operator was able to set feed mass and injection intervals and the system could accordingly act on two inlet Gilson Minipuls 3 peristaltic pumps by manipulating the on-off switch and their flowrate. The same strategy was adopted to regulate microalgae extraction. A scheme is reported in Figure 4.

At pilot-scale during night, CO_2 concentration could be set to zero and gas flowrate could be accordingly regulated by manipulating (through the PXI National Instrument system plus Labview) the pressure on the compressor valve in order to keep a constant gas velocity into the riser. Wastewater inlet and microalgae extraction were controlled with the same strategy adopted at the semi-continuous lab-scale. In growing mode, the B&C Microcontrollers were expected to work both as acquiring units for the MEMO monitoring system and as controllers. Moreover, at pilot-scale, the MEMO system

provided in addition to the online filtering also the gross errors elimination and alarms managing. A scheme is reported in Figure 5.

Figure 4: Schematic representation of semi-continuous STR and relative automation system.

Figure 5: Schematic representation of EL-ALR and relative automation system.

2.2.5. Hydrodynamics and bubble dynamics measurements

Bubbles image acquisition was performed by a Canon EOS-10D camera and a self-developed software was adopted to process images (b/w transformation) and to evaluate bubble diameter and global G-L ratio. pH measurements carried out during bubbles acquisition (WTW multiparameter portable system 340i and B&C Electronics series 7685 pH Microcontroller respectively set at the top of the riser and the top of the downcomer) allowed to indirectly estimate dissolved carbon dioxide concentration in circulating water.

NaCl was used as a tracer to evaluate liquid residence times in the different sections of the ALRs; its concentration was evaluated by indirect conductivity measurements at the top of both the riser and the downcomer and in the midsection of the lower collector (WTW multiparameter portable system 340i and B&C Electronics series 7685 Conductivity Microcontroller).

Different solid tracers were employed to simulate microalgae, whose movement was recorded by a Hamamatsu C5405 CCD camera, with a resolution of 752(H) · 582(V), a depth of 10 bits and a minimum object illumination of 0.3 lux. Tracers were flat reflective aluminum particles, polymers and TiO₂ particles (mean diameter range 0.5 ÷ 4 mm).

2.3. Microalgae culture

In the present work, we employed the *Chlorella vulgaris* CCAP 211 (Culture Collection of Algae and Protozoa, Argyll, UK) strain. It was cultivated in Bold's Basal Medium (BBM), the composition of which, in distilled water, is as follows [30]: 250 mg L⁻¹ NaNO₃, 25 mg L⁻¹ CaCl₂ · 2H₂O, 75 mg L⁻¹ MgSO₄ · 7H₂O, 75 mg L⁻¹ K₂HPO₄, 175 mg L⁻¹ KH₂PO₄, 25 mg L⁻¹ NaCl, 31 mg L⁻¹ KOH, 50 mg L⁻¹ Na₂ EDTA · 2H₂O, 4.98 mg L⁻¹ FeSO₄ · 7H₂O, 1.44 mg L⁻¹ MnCl₂ · 4H₂O, 1.57 mg L⁻¹ CuSO₄ · 5H₂O, 0.71 mg L⁻¹ MoO₃, 11.42 mg L⁻¹ H₃BO₃, 8.82 mg L⁻¹ ZnSO₄ · 7H₂O, 1.84 mg H₂SO₄, 0.49 mg L⁻¹ Co(NO₃)₂ · 6H₂O.

The strain was stored at 25 ± 1 °C in 250 mL Erlenmeyer flasks, kept under a photon flux of 80 μmol m⁻² s⁻¹.

3. Theoretical development

3.1. Scale-up by π -theorem

Scientific scale-up has been carried out both for discontinuous to semi-continuous process in STRs and for pilot ALRs. This means employing the so-called Buckingham's π -theorem.

In essence, π -theorem states that it is possible to reformulate a physical law interconnecting some physical quantities as a function of dimensionless numbers, traditionally indicated as π . Operatively, it can be performed by identifying the main influencing variables of the process under scope, summarized in a relevance list, building a dimensional matrix and obtain dimensionless numbers from its completely reduced form [31].

The relevance list has many common elements both for airlift photobioreactors and the lab-scale stirred ones, as the main aspects to cope with are fluid dynamics, mass transfer and light conditions. Moreover, geometric factors should be taken into account as well.

Generally speaking, from the point of view of dimensional analysis, chemical engineering problems refer to a 5-parametric dimensional system: [M, L, T, Θ , N] where [M] is mass, [L] is length, [T] is time, [N] is the amount of substance and [Θ] is temperature. If also light influences the process a 6-parametric dimensional system must be considered by adding luminous intensity [J].

While mass, heat transfer and light transmission /absorption are scale dependent, the same cannot be said of thermodynamics laws and stoichiometry. A simplification is hereinafter employed, as the process of algae nurturing is assumed quasi-isothermal, with culture temperature depending only on the external temperature. This entails that the process relationships obtained through dimensional analysis are valid for any constant process temperature to which the numerical values of the physical properties are related.

In the first place, let us consider the problem of scaling up the lab-scale cultivation of microalgae in a batch stirred reactor. Flow field characterization is inferable, in first approximation, on the base of two material properties, namely fluid density ρ and dynamic viscosity μ , two geometric parameters, i.e. the diameters of the reactor d and of the stirrer d_s , and a process one, the stirrer speed n . The list can be reduced by lumping together ρ and μ and considering in their place the kinematic viscosity $\nu = \mu \rho^{-1}$.

To identify the variables controlling the biochemical aspect of the process is more delicate. In order to try and develop a convincing mathematical description of microalgae growth, numerous studies have been carried out over the years, producing a wide cosmos of models, ranging from the well-known Monod equation to far more complex ones. A thorough review of such models is found in Lee et al. [32]. However, it is known that microalgae growth depends on multiple factors, mainly temperature, pH, dissolved oxygen and CO₂ concentrations, availability of nutrients (in particular nitrogen and phosphorus) and light. It follows that the substrate concentration S can be added to the relevance list.

Since pH is mathematically defined as the common logarithm of the reciprocal of the proton activity in an aqueous solution and activity is deemed a dimensionless number, pH is a dimensionless number as well. Thus, it can be considered as a π -number and be excluded from the list.

The concentration of dissolved oxygen (DO_x) in water is influenced by a number of factors, including water temperature, salinity and atmospheric pressure. As alkalinity of the aqueous solution is measured by pH and regulates chemical equilibrium, the aforementioned concentration can be thought of as functions precisely of pH other than the overall mass-transfer coefficient K_{OG} and oxygen concentrations. Ditto for dissolved

CO₂ concentration. Carbon dioxide diffusion coefficient D is taken into account as well (diffusion coefficient in water, neglecting the effects of solutes dissolved in the culture medium). Hereinafter, CO₂ is assumed to be the only substrate. In addition, two more biotic factors should be accounted for: microalgae initial concentration and actual microalgae concentration, as the latter can indicate self-shading and thus autoinhibition. To model algae growth, kinetic models oftentimes resort to a formulation comprising a pseudo-first-order constant k_1 , of dimension [T⁻¹], and a second kinetic constant related to the asymptotic behavior of the concentration, i.e. the maximum concentration obtainable [M L⁻³]. Since we will use the Beruto kinetic model [33] for estimating the global kinetics of *Chlorella v.* growth, we hereinafter will denote this second constant as k_a .

Finally, light plays a vital role, as luminous energy cannot be stored by microalgae and thus needs to be provided adequately. According to the Tamiya model, which is probably the most widely applied algae growth model among those taking light conditions into consideration, light-related parameters are a saturation constant K_I and the incident light intensity I . When $I < K_I$ microalgae growth is limited by light according to first-order kinetics while, whenever $I \gg K_I$, it is a light-independent phenomenon and $k_1 \rightarrow \mu_{\max}$, where $\mu_{\max}$ is the corresponding of k_a for the Beruto model but has the same dimension as k_1 . Incident light intensity on microalgae cells can be described by means of the Lambert-Beer law, which states that $I = I_0 \exp(-\varepsilon cl)$, where I_0 is the light intensity entering the material composing the reactor excluding surface reflection, ε is the absorption per concentration coefficient, c is the concentration of the absorbing material and l is the path length. In this case, one can assume that $l = d$ and that light absorption by perspex/glass are negligible.

An additional operative hypothesis is that all parameters are uniform, i.e. perfect mixing is assumed.

Overall, the relevance list for the scaling of microalgae farming in a stirred, transparent batch reactor is the following one.

Table 2: Relevance list for scale-up of batch microalgae growth in stirred tank reactors.

Geometric parameters		Diameter of the reactor d	[L]
		Diameter of the stirrer d_s	[L]
Material properties	► Fluid dynamics	Kinematic viscosity ν	[L ² T ⁻¹]
	► Mass transfer	Microalgae concentration c	[M ¹ L ⁻³]
		Microalgae initial concentration c_0	[M ¹ L ⁻³]
		Diffusion coefficient D	[L ² T ⁻¹]
		Substrate concentration S	[L ⁻³ N ¹]
		Dissolved oxygen concentration DO_x	[L ⁻³ N ¹]
		Overall mass-transport coefficient K_{OL} or K_{OG}	[L ¹ T ⁻¹]
		Maximum specific growth rate k_a	[M L ⁻³]
		Growth rate k_1	[T ⁻¹]
	► Microalgae	Saturation constant K_l	[J L ⁻²]
		Absorption coefficient ε	[L ² M ⁻¹]
		Optimal temperature for algae growth	[Θ]
Process parameters		Stirrer speed n	[T ⁻¹]
		Temperature T	[Θ]
		Light intensity I_0	[J L ⁻²]

The relevance list consists of 17 parameters hinging on 6 SI base quantities: [L], [M], [T], [N], [Θ], [I]. It follows that $17 - 6 = 11$ non-dimensional numbers are to be expected. Since T cannot be controlled (e.g. by manipulating a proper heat exchange system) and it is pseudo-constant inside the PBRs, this parameter can be excluded from the relevance

list as it does not directly alter the others. The ratio between the temperature inside the reactor and the optimal temperature required for microalgae growth T/T_{opt}^{-1} can be directly considered *a priori* as a π -number. Similarly, at least 5 other trivial π -numbers can be pinpointed beforehand: the purely geometrical ratio $d_s d^{-1}$, the ratios $DO_x S^{-1}$, c/c_0^{-1} , plus the kinetic ratios $k_a c_0^{-1}$ and $I_0 K_I^{-1}$.

Hence, 5 π -numbers remain to be ascertained, related to parameters measured through 4 fundamental units: [M], [L], [T] and [N].

A final note regards the choice of the characteristic length inserted in the dimensional matrix. For an STR the usually adopted representative scale is the stirrer diameter, so it would make sense to use precisely that. However, when dealing with variations in the type of employed devices, the need of using different characteristic lengths may arise (e.g. for an ALR, where the impeller is absent, one could focus on the sparger diameter or on bubble diameter). To keep the discussion more general, we employ here the reactor diameter d . This choice is not relevant from a theoretical perspective since it is possible to switch *a posteriori* the characteristic lengths by conveniently combining dimensionless groups.

	c	d	n	C	ν	D	K_{OL}	k_1	ε	
M	1	0	0	0	0	0	0	0	-1	
L	-3	1	0	-3	2	2	1	0	2	(1)
T	0	0	-1	0	-1	-1	-1	-1	0	
N	0	0	0	1	0	0	0	0	0	

By the means of the Gaussian reduction algorithm, the matrix is found to be equivalent to:

	c	d	n	C	ν	D	K_{OL}	k_1	ε	
M	1	0	0	0	0	0	0	0	-1	(2)

L	0	1	0	0	2	2	1	0	-1
T	0	0	1	0	1	1	1	1	0
N	0	0	0	1	0	0	0	0	0

397 The following π -numbers ensue:

$$\Pi_1 = \frac{\mu}{\rho d^2 n} = \text{Re}^{-1} \quad (3)$$

$$\Pi_2 = \frac{D}{d^2 n} \quad (4)$$

$$\Pi_3 = \frac{K_{OL}}{dn} \quad (5)$$

$$\Pi_4 = \frac{k_1}{n} \quad (6)$$

$$\Pi_5 = \varepsilon dc = A \quad (7)$$

398 Π_1 is the reciprocal of the impeller Reynolds number whilst Π_5 is the absorbance.

399 The number Π_2 can be rewritten in a different way resorting to Π_4 , gaining a different

400 interpretation in the process; the inverse of the Damköhler number Da_{II} appears:

$$\Pi_2 \Pi_4^{-1} = \frac{D}{d^2 k_1} = \text{Da}_{\text{II}}^{-1} \quad (8)$$

401 Analogously, by combining Π_2 and Π_3 , Sherwood number is found:

$$\Pi_3 \Pi_2^{-1} = \frac{K_{OL} d}{D} = \text{Sh} \quad (9)$$

402 Concluding, the complete set of the 12 π -numbers (recalling the presence of pH)

403 describing the problem at hand is:

$$\left\{ \text{Re}, \text{Da}_{\text{II}}, \text{Sh}, \frac{k_1}{n}, \text{pH}, \frac{T}{T_{opt}}, \frac{I_0}{K_l}, A, \frac{d_s}{d}, \frac{c}{c_0}, \frac{\text{DO}_x}{S}, \frac{k_a}{c_0} \right\} \quad (10)$$

404 **3.2. Correlations for π -numbers**

405 By analyzing the list (10), we can single out three main categories of dimensionless

406 numbers and group them in subcategories accordingly:

1) Known dimensionless numbers. To this group belong the Reynolds number Re , the Sherwood number Sh and the second Damköhler number Da_{II} . Absorbance is considered in this group as well.

Reynolds number, defined as the ratio between inertial and viscous forces, can be used to determine if the flow in which algae are dispersed is turbulent (strong prevalence of inertial forces) or laminar. Sherwood number compares convective mass transfer to the rate of diffusive mass transfer and for the considered application it can refer both to the dissolved CO_2 and O_2 . Da_{II} is useful for determining whether diffusion or reaction is the controlling phenomenon. As regards microalgae growth, it compares the diffusion of the key reactant (CO_2) and the growth rate (k_1).

2) Operational π -numbers. Six of them can be individuated in set (9): $c\ c_0^{-1}$, $k_a\ c_0^{-1}$, $DO_x\ S^{-1}$, pH , $T\ T_{opt}^{-1}$ and $I_0 K_l^{-1}$. The first three are related to characteristic process quantities as yield, maximum yield, the ratio between key byproduct and key reactant, while pH is a process tuning parameter. They can be manipulated during continuous operation. $T\ T_{opt}^{-1}$ and $I_0 K_l^{-1}$ are related to process parameters that cannot be usually manipulated in outdoor conditions.

3) Reactor-related π -numbers. They encompass crucial data of the reactor, either geometric ($d_s\ d^{-1}$) and functional ($k_1\ n^{-1}$). They cannot be used if the shape or the structure of the reactor is changed.

According to what was said, dimensionless numbers Re , Sh , Da_{II} and A here listed contain the generally defined characteristic length d (diameter of the reactor). By combining the original dimensionless numbers with the reactor-related π -numbers they can be reformulated in order to encompass appropriate characteristic lengths.

As it is to be expected, by employing Buckingham's π -theorem for the up-scaling of ALRs, more dimensionless numbers emerge (e.g. Weber and Eötvös numbers) as more phases are involved. Yet, these additional π -numbers only concern fluid dynamics and material properties of the gas and liquid phases and should be used to further scale-up ALRs. Moreover, in accordance with what has been said on reactor-related π -numbers, the two dimensionless groups of (10) related to the presence of the impeller disappear. Concluding, at least two degrees of freedom remain and are exploitable. The procedures and calculations used to evaluate the π -numbers are presented in the following sections.

3.2.1. Fluid dynamics in STRs and hydro-bubble dynamics in ALRs

In order to choose the optimal impeller diameter and geometry at lab-scale, we carried out some computer fluid dynamics simulations by SimScale Multiphase Flow. Only biphasic liquid systems as glycerol-water and dissolved carbon dioxide-water are simulated. Microalgae fluid dynamics has not been taken into consideration due to the required high computation time, even if SimScale allows it by using particle-flow approaches. From this study, the optimal $d d_s^{-1}$ ratio and stirrer speed n are estimated in order to obtain in the STRs impeller related Reynolds numbers suitable for algae growth. They are calculated by Eq. (11) as:

$$Re = \frac{\rho d^2 n}{\mu} \left(\frac{d_s}{d} \right)^2 \quad (11)$$

Strictly speaking, to perform the scale-up in STRs, this Reynolds number should be kept unaltered.

On the other hand, since the ALRs have a different morphology with respect to the lower scale reactors, some distinctions are necessary. In the first place, ALRs employed in this

research are designed to contain the bubble phase only in the riser, not in the downcomer. Consequently, considering the difference in their cross sections as well, it is evident that Re is nonequivalent in these two parts of the photobioreactor. Clearly, even the formulation of Re appears different: by making use of the π -theorem to derive dimensionless groups for the ALR, Reynolds number is obtained again but liquid velocity naturally substitutes the velocity given by the product of stirrer diameter and impeller speed. Besides, in order to optimize its operation, the multiphase flow regime needs to be investigated. The preferential situation should be on the border between the heterogeneous (churn) and the homogeneous (bubble) regimes. The former corresponds to an environment in which, due to the manifestation of clustering and coalescence, both small and large bubbles are present concomitantly. Large bubbles can assume spherical cap shapes and zigzagging trajectories, providing better area-to-volume ratios and longer permanence times, subsequently entailing better G-L mass transfer. On the other hand, fluid velocities in the riser are higher, and turbulence phenomena can affect the integrity of microalgae cells. This critical issue is solved if the established regime is the homogeneous one. In this scenario, where fluid velocities are more modest, there are no substantial interactions between the bubbles to be reported, their ascending path is mostly straight and the bubble size distribution is narrow, with small diameters and prevalence of spherical shapes [34].

Hence, even if Reynolds numbers should be kept unaltered, for the aforementioned reasons it might not be possible and the optimal choice could be related to the desired contact time between microalgae and bubbles and to the right recirculation conditions that also assure convenient photoperiods.

The experimental campaign apt to examine the hydrodynamic picture inside ALRs has been carried out on the EL-ALR fed with injected air and filled with water. Liquid velocity was experimentally measured in the downcomer. The same was not feasible in the riser, as the formation of eddies at different scales, in conjunction with the fact that the hypothesis of unidirectional flow does not hold due to the presence of the bubbles. Instead, we availed ourselves of an empirical formula [35] to calculate the average liquid velocity in the riser $u_{L,\text{riser}}$ (Eq. (12)).

$$u_{L,\text{riser}} = \sqrt{\frac{2gh_{\text{down}}(\varepsilon_{G,\text{riser}} - \varepsilon_{G,\text{down}})}{\xi \left(\frac{1}{(1 - \varepsilon_{G,\text{riser}})^2} + \left(\frac{A_{\text{riser}}}{A_{\text{down}}} \right)^2 + \frac{1}{(1 - \varepsilon_{G,\text{down}})^2} \right)}} \quad (12)$$

Here, g represents the gravitational acceleration (m s^{-2}), h_{down} the downcomer height (m), ξ the (dimensionless) friction loss coefficient, related to the geometrical parameters of the reactor, and A the cross-sectional area (m^2). Subscripts down and riser are used to refer the variables to the downcomer and riser respectively. Finally, ε_G denotes the gas hold-up (dimensionless), computed for the riser with Eq. (13) and for the downcomer with Eq. (14).

$$\varepsilon_{G,\text{riser}} = \frac{V_{G,\text{riser}}}{V_{G,\text{riser}} + V_{L,\text{riser}}} \quad (13)$$

$$\varepsilon_{G,\text{down}} = 0.79 \varepsilon_{G,\text{riser}} - 0.057 \quad (14)$$

where $V_{G,\text{riser}}$ is the volume of the gas phase whilst $V_{L,\text{riser}}$ that of the liquid phase, both in the riser (m^3).

PIV analysis was used to gauge the gas velocity in the riser $u_{G,\text{riser}}$ as well as microalgae motion, the latter with the aid of different tracers.

The results of the aforementioned investigations allow calculating Re for the riser and the downcomer.

3.2.2. Mass transport

Carbon dioxide is the considered substrate in the relevance list, and oxygen is the product of photosynthesis, hence continuous operating in steady-state requests fixed ratio between them: this is the meaning of the operational π -number $DO_x S^{-1}$ to be conserved for scale-up in daylight conditions (during night only air is supplied to the reactors). Obviously, mass transport characteristics of the dissolved CO_2 to the suspended algae (and dissolved O_2 produced by them) in the liquid phase must be also kept into account during scale-up. With reference to the performance of the discontinuous and semi-continuous lab-scale stirred reactors it is necessary to evaluate the optimal average mass flux of the substrate to the algae. It depends on the bulk concentration of carbon dioxide, its diffusion coefficient in water, the dimension of microalgae, fluid properties as density and viscosity and algae velocity relative to the fluid.

The following assumptions are here adopted to scale-up from lab-scale to pilot-scale: the resistance to mass transport due to the cellular membranes do not change in scale-up and the algae clusters moving into the reactors (STRs and ALRs) have the same dimension. Since we decide to keep not very high Re in order to not damage algal cells and oxygen is rather soluble, liquid mass transport is the controlling phenomenon, and $K_{OL} = k_L$. Dynamic experimental tests as discussed in [36] are then performed in the Discontinuous Tank Reactors (DSTRs) and the volumetric mass transfer coefficient $k_L a$ is obtained. By considering the oxygen mass balance in the STR:

$$\frac{dC_{A,bulk}^L}{dt} = k_L a (C_A^{L*} - C_{A,bulk}^L) \quad (15)$$

where C_A^{L*} is the saturation concentration in the liquid phase and $C_{A,bulk}^L$ is the measured concentration in the liquid phase; $k_L a$ can be estimated by Eq. (15) from the collected

measures of $C_{A,\text{bulk}}^L$ during the dynamic physical test, being sure that the sensor response time is very small. A $k_L a$ two-points estimate is easily calculated as:

$$k_L a = \frac{1}{t} \ln \left(\frac{C_A^{L*} - C_{A1,\text{bulk}}^L}{C_A^{L*} - C_{A2,\text{bulk}}^L} \right) \quad (16)$$

As regards ALRs, the volumetric mass transport coefficient $k_L a$ could be experimentally determined in a similar manner or it can be computed by using correlations, e.g. as that reported for EL-ALRs in Bello et al. [37]:

$$k_L a = 0.79 \left(1 + \frac{A_{\text{down}}}{A_{\text{riser}}} \right)^{-2} u_{G,\text{riser}}^{0.8} \quad (17)$$

Since many correlations are proposed for computing k_L and $k_L a$ and sometimes it is difficult to distinguish between them, we verified the obtained experimental values also by directly calculating Sherwood number with the following empirical correlation (Eq. (18)) [38].

$$\text{Sh} = 0.322 \text{Re}^{0.7} \text{Sc}^{0.33} \quad (18)$$

where Sc is the Schmidt number, in this case equal to $\text{Sc} = \mu \rho^{-1} D^{-1}$.

As regards ALRs, the specific surface of the bubbles a ($\text{m}^2 \text{m}^{-3}$), i.e. the ratio between the sum of the surface of all the bubbles inside the ALR and the reactor volume is experimentally estimated by image acquisition.

3.2.3. Light conditions for algae growth

The desired light intensity in the PAR range for most microalgae is $10 \div 250 \mu\text{mol m}^{-2} \text{s}^{-1}$. If microalgae are cultivated in phototrophic conditions, light is the most important parameter for their growth; on the contrary in outdoor at Mediterranean latitudes ($40^\circ \div 45^\circ \text{N}$) sunlight yields a PFD_0 up to $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$, causing light to be a possible cause of photoinhibition instead of a limiting factor for their growth, especially

during summer season: at PFD greater than $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, photoinhibition is usually present in most microalgae [39].

As reported in [40], the photosynthetic activity of *Chlorella v.* reaches a light-independent state at very low light intensity for diluted microalgae concentration (0.066 g L^{-1}) whereas photosynthetic activity still depends on the incident light intensity even for values around $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ in concentrated suspension (1.064 g L^{-1}). Since *Chlorella v.* concentration in our PBRs exceeded this value, a saturation constant K_I equal to $1100 \mu\text{mol m}^{-2} \text{s}^{-1}$ is assumed, and incident light intensities equal to or greater than $1100 \mu\text{mol m}^{-2} \text{s}^{-1}$ are the optimal desired operating condition. Light-related dimensionless numbers at these operating conditions can be used as limiting factors; i.e. geometrical scale-up must be carried out in order to have similar average light intensity inside all the PBRs. As regards scale-up to EL-ALRs, the presence of the bubbles in the riser could reduce light absorption. Moreover, illumination into the downcomer should be better than into the STRs due to its smaller diameter.

Local light intensity (or local photosynthetic PFD) to the single cell is computed by the Lambert-Beer law, while the average light intensity I_{av} inside the PBRs can be calculated for cylindrical vessels as in [41].

3.2.4. Global kinetics of algae growth

Many kinetic models for microalgae growth are available in the literature [29].

In this work, since scale-up is intended for operating in outdoor conditions, we decided to consider separately the effect of light by means of related dimensionless numbers and to employ the kinetic model suggested by Beruto et al. as it allows to implicitly take into account different factors by following the perspective of cell mitosis. The advantage is

that the concentration curve, of sigmoid shape, is obtained employing a rather streamlined equation (Eq. 19).

$$c(t) = c_0 + \frac{k_a}{1 + \frac{k_a - c_0}{c_0} \exp(-k_1 t)} \quad (19)$$

c_0 denotes the inoculum concentration (g L^{-1}) at the initial time whilst k_a is a specific rate constant representing the total average probability that cells have to form clusters and, considering its limit for $t \rightarrow +\infty$, it can be viewed as the maximum increase in the cell population (g L^{-1}) in the reactor. k_1 is a specific kinetic rate constant (s^{-1}) that can be computed through an Arrhenius-like equation as it is related to temperature, and accounts for the frequency of mitotic events and for the enthalpy barrier hindering cell duplication. Since the reactors are operated at pseudo-constant temperature, oscillating around 25 °C, the dependence of k_1 on T is neglected.

The Maximum Likelihood (ML) method is adopted to carry out parameter estimation. Under the assumption of explicit exact models, i.e. neglecting model uncertainty, the objective function with ML represents the estimated experimental error. This method reduces to un-weighted Non-Linear Least-Squares (NLLS) or Weighted Non-Linear Least Squares (WNLLS) if normally distributed and independent errors [42] are considered. The use of weighted or unweighted least-squares reflects a different choice about the experimental error due to the analytic instrumentation and the experimental conditions. If different accuracy is devoted to measuring low concentration and high concentration values, the residual is proportional to the measured concentration and weighted least squares are adopted with a multiplicative error. On the contrary, if the same random error is supposed for all the measures of concentration, unweighted least-

squares with additive errors are adopted. Results obtained with both the approaches are here reported.

From the estimated values of the kinetic parameters both the Da_{II} number and the $k_a c_0^{-1}$ number can be computed. The same can be said of $k_1 n^{-1}$ at lab-scale. Up-scaling to airlift, this number, as said previously, cannot be kept as it is and gas velocity, multiplied by a , could substitute n in its evaluation.

4. Results and Discussion

4.1. Fluid dynamics in STRs and hydro-bubble dynamics in ALRs

4.1.1. Lab-scale STRs

According to Eq. (11), the impeller Reynolds number assumes values in the range $1.798 \cdot 10^3 \div 2.809 \cdot 10^4$. These extrema correspond to the STR operation at 60 rpm with a stirrer diameter of 40 mm and at 150 rpm with a stirrer impeller of 100 mm respectively.

It follows that the hydrodynamic environment in the reactors can be on average a turbulent one, and at least the regime is of transition to turbulence ($Re > 10^3$).

Using CAD design of the different impellers available at the lab-scale, mixing in STRs at both 0.5 L and 2.5 L scale was simulated using SimScale. By comparing the different simulations, and remembering that high speeds can damage microalgae, the chosen solution was to use a radial impeller at 20 mm from the bottom of the reactor, with diameters of 80 mm and 100 mm respectively, at 90 rpm and 60 rpm for the STRs at the different scales. Thus, the chosen Re is about 10800 for the lab-scale DSTRs and 11000 for the lab-scale semi-continuous reactors and the whole cultivation campaign was carried out in these fixed conditions.

4.1.2. Pilot-scale ALRs

The experimental runs were devised to test both the bubble flow (Table 3) and churn regimes (Table 4). As regards the former, liquid velocity and related residence times in the downcomer were measured simultaneously using conductivity measurements of injected NaCl (tests 1, 2, 3) and solid particles as seeds (tests 4, 5, 6). Tests investigating churn regime were carried out only with NaCl.

Table 3: ALR in bubble flow; main variables experimental determined.

Test number	$u_{L,\text{down}}$ (m s ⁻¹)	Downcomer residence time (s)	Average time cycle (s)	$u_{G,\text{riser}}$ (m s ⁻¹)	Mean d_b (mm)	Bubbles per cross section	Bubbles in the riser
1	0.390	2.0	21.2	0.131	3	51	4505
2	0.357	2.2	24.7	0.111	4	45	3975
3	0.301	2.6	21.9	0.090	4	39	3445
4	0.367	2.1	20.8	0.131	3	51	4505
5	0.346	2.3	19.9	0.111	4	45	3975
6	0.325	2.4	18.0	0.090	4	39	3445

Table 4: ALR in churn flow; main variables experimental determined.

Test number	$u_{L,\text{down}}$ (m s ⁻¹)	Downcomer residence time (s)	Average time cycle (s)	$u_{G,\text{riser}}$ (m s ⁻¹)	Mean d_b (mm)
7	0.168	4.8	27.6	0.573	5
8	0.200	4.0	26.2	0.586	5
9	0.235	3.5	20.4	0.642	7

In Table 4 the evaluation of the number of bubbles, both per cross section and riser volume, is not reported since, due to the complexity of the heterogeneous flow, it was difficult to assess it without professional PIV instrumentation.

$u_{L,\text{riser}}$ and Reynolds number for both the riser and the downcomer were respectively computed according to Eq. (12) and the ALR version of Eq. (11) and are presented in Table 5.

Table 5: Main hydrodynamic variables in the ALR computed with empirical correlations.

Test number	$u_{L,\text{riser}}$ (m s ⁻¹)	Re in the riser	Re in the downcomer
1 & 4	0.056	5244	18843 – 17731
2 & 5	0.057	5337	16717 – 17248
3 & 6	0.056	5244	14543 – 15219
7	0.084	7865	9438
8	0.110	10300	11236
9	0.117	10955	13202

Fluid dynamics conditions in churn regime (Re number) thus evidence a good correspondence with those at lab-scale.

4.2. Mass transport

4.2.1. Lab-scale STRs

The experimental tests to estimate the overall mass transport coefficient were carried out in lab-scale STRs at different Re numbers (obtained by varying the stirrer speed), at different temperatures and in both pure water and BBM (concentration monitored by conductivity measurements). Results for O₂ are considered also valid for CO₂, since among the various variables influencing the value of the overall mass transport coefficient K_{OL} , the effect of fluid velocity is the greater one.

The $k_L a$ values are slightly influenced by the medium. Values quantified by applying Eq. (16) at 25 °C are in the range $4.3 \cdot 10^{-4} \div 7.5 \cdot 10^{-4}$ for both the 0.5 L and 2.5 L STRs at their chosen stirrer speeds, respectively of 90 and 60 rpm. It is to be noted that the estimated probe lag (polarographic DO_x measures) is 4 s and no correction was applied to the acquired data.

The impeller Sherwood number is computed using these experimental data by applying the formula $Sh = k_L a d_s^2 D^{-1}$, instead of $Sh = K_{OL} d_s D^{-1}$, as it is often the case when experimental measures of $k_L a$ are available [43].

Sh ranges between 1450 (smaller $k_L a$ in DSTR) and 3950 (bigger $k_L a$ in semi-continuous STR) while, by using Eq. (18), $Sh \cong 1600$. An intermediate value can be kept as reference in ALRs.

4.2.2. Pilot-scale ALRs

From the experimental data reported in Tables 4 and 5, it is possible to compute the volume of the gas phase $V_{G,riser}$ and the specific surface of the bubbles a . By using Eq. (17) with the data relative to bubble flow tests, we obtained values of $k_L a$ in the range $0.08 \div 0.11$ whilst for churn regime $k_L a$ was in the range $0.35 \div 0.38$. In order to compute Sherwood number for the ALRs, we can use again the formula $Sh = k_L a d_b^2 D^{-1}$ where d_b in this case is the characteristic length for mass transport in the ALRs and reasonably equal to the diameter of the bubbles. The resulting Sh ranges between 360 and 880 in bubble flow and between 4575 and 9800 in churn regime. Since a can be also calculated as the product of the number of bubbles in the riser and the mean surface of the bubble divided by the volume of the riser, it results in a range of $17 \div 27$ in bubble flow (the number of bubbles is available only for this regime). The relative k_L is comprised between 0.003 and 0.006 and the resulting Sh takes values in the interval $450 \div 1200$.

On the other hand, resorting to Eq. (18), the Sherwood number (in the riser) for the bubble flow is $\cong 1010$ whilst for churn flow stands in the range $1360 \div 1700$.

A very good agreement is found between the Sh evaluated by the three different correlations in bubble flow. On the contrary, some discrepancy is observed between the

Sh evaluated from experimental data by employing Eq. (17) and that evaluated by Eq. (18) in churn regime. This is to be expected and strongly depends on the experimental errors other than on the adopted correlation, that depends only on Re and Sc numbers. Anyway, the ALR in our tested bubble regime seems to guarantee slightly lower mass transport characteristics than those obtained for the STRs; conversely, in churn regime mass transport is better than in the STRs. This could justify the adoption of the bubble regime, on the edge of the transition to churn, for EL-ALRs devoted to biochemical processes as adopted in other works [44].

4.3. Global kinetics of algae growth

4.3.1. Lab-scale STRs

The following variables are measured at discontinuous lab-scale:

- 3 input variables: concentration of CO₂, concentration of glycerol, incident light intensity;
- 3 operating input variables: stirrer velocity, feeding time interval, extraction time interval;
- 4 internal variables: temperature, pH, conductivity, dissolved oxygen;
- 2 output variables: absorbance at different wavelengths; dry cell mass weight.

Daily measurements of all the internal and output variables are carried out in six DSTRs. The experimental conditions of the first three DSTRs are defined in order to test the combined effects of the two “chemical” input variables, i.e. concentration of CO₂ and concentration of glycerol, and the two operating input variables (feeding time interval and extraction time interval) on the microalgae growth.

The values chosen for these input variables are in the range of the possible process conditions. The third input variable, incident light intensity, is kept fixed and equal to $1100 \mu\text{mol m}^{-2} \text{s}^{-1}$, so as the third operating input variable, stirrer speed, that is kept fixed at a previously optimized value (see next paragraph) of 90 rpm for all the tests. The experimental operating conditions of DSTRs 4, 5, 6 are the same of DSTRs 1, 2, 3 as regards the values of the input operating variables and incident light intensity, while glycerol and CO_2 concentration are set to zero. In this way reactors 4, 5 and 6 are useful to evaluate kinetics when microalgae are fed only with nutrients (BBM medium), at different operating conditions.

The experimental campaign lasted 90 days and its scheme is reported in Table 6.

Table 6: Experimental tests at discontinuous lab-scale.

Test number	Reactor number	Glycerol (g L^{-1})	CO_2 (%)	Feeding interval (days)	Extraction interval (days)	Cultivation time (days)
1	1	5	5	3	3	10
2	1	15	5	3	4	10
3	1	20	5	3	3	10
4	1	0	5	4	7	15
5	1	0	15	4	7	15
6	1	10	10	4	7	15
7	1	0	20	5	5	15
8	2	0	30	5	5	20
9	2	30	0	5	5	20
10	2	30	30	6	3	20
11	2	30	15	6	5	30
12	3	15	30	6	5	30
13	3	15	18	5	6	30
14	3	20	18	6	6	30

The kinetic parameters inside the growth model expressed by Eq. (19) have been estimated using concentration values derived by both dry-mass weight data and

spectrophotometric data at different wavelengths, by the construction of proper calibration curves.

Table 7 and Table 8 show some results obtained using respectively NLLS and WLNNS for test 5 and test 6 described in Table 6. The fitted growth curves, with reference to test 6, and computed by both NLLS and WNLLS, are finally shown for two wavelengths in Figure 6.

Table 7: Kinetic parameters estimated through NLLS fitting.

	NLLS	c_0 (g L ⁻¹)	k_1 (day ⁻¹)	k_a (L g ⁻¹)	ε	R^2
Reactor number 1, test 5	$\lambda = 430$ nm	0.0605	0.2790	0.4296	0.0517	0.9323
	$\lambda = 650$ nm	0.0683	0.2558	0.4375	0.0373	0.9338
	Dry weight	0.0817	0.2000	0.6797	0.0517	0.9182
Reactor number 1, test 6	$\lambda = 430$ nm	0.0514	0.2967	0.4261	0.0279	0.9831
	$\lambda = 650$ nm	0.0574	0.2880	0.4184	0.0200	0.9871
	Dry weight	0.0573	0.2346	0.6123	0.0279	0.9180

Table 8: Kinetic parameters estimated through WNLLS fitting.

	WNLLS	c_0 (g L ⁻¹)	k_1 (day ⁻¹)	k_a (L g ⁻¹)	ε	R^2
Reactor number 1, test 5	$\lambda = 430$ nm	0.0634	0.2483	0.4530	0.0483	0.7149
	$\lambda = 650$ nm	0.0713	0.2308	0.4576	0.0351	0.7926
	Dry weight	0.0784	0.2338	0.6276	0.0537	0.7326
Reactor number 1, test 6	$\lambda = 430$ nm	0.0540	0.2773	0.4360	0.0256	0.9261
	$\lambda = 650$ nm	0.0603	0.2701	0.4255	0.0176	0.9539
	Dry weight	0.0383	0.3163	0.5387	0.0765	0.6399

The obtained results at discontinuous lab-scale allow to both restrict the range of the possible input conditions and to reduce the costs for the measurements of the output variables. Good yields are obtained for tests 4, 5, 7 and 13, this suggests to investigate the input conditions of these tests at semi-continuous lab-scale. The kinetic parameters estimated with concentration data coming from different measurement methods are in

good accordance: at semi-continuous lab-scale only spectrophotometric data can be used, also expecting to design an automated procedure for sample collection and related analysis. The expected error could be considered additive since R^2 computed by using NLLS is always higher than R^2 computed by WNLLS, even if the estimated parameters are very similar. The kinetic model is suitable and the estimation procedure is robust: at semi-continuous lab-scale, only one or two wavelengths can be used to compute microalgae concentration.

a)

b)

Figure 6: Growth curves for Reactor 1, Test 6 for (a) $\lambda = 430$ nm and (b) $\lambda = 650$ nm, evaluated with NLLS and WNLLS.

The possible optimal operating conditions, resulting from the best kinetics among the experimental tests described in Table 6, are summarized in Table 9. The corresponding ranges of internal variables, measured during the experimental tests, are also reported in Table 10.

Table 9: Optimal operating ranges of the input variables at DSTR lab-scale.

Glycerol (g L ⁻¹)	CO ₂ (%)	I ₀ (μmol m ⁻² s ⁻¹)	Feeding interval (days)	Extraction interval (days)	Cultivation time (days)
15 ÷ 20	15 ÷ 20	>1100	3 ÷ 5	5 ÷ 7	10 ÷ 15

Table 10: Measured and optimal operating ranges of the internal variables at DSTR lab-scale.

	Temperature (°C)	pH	Conductivity (μS)	DO _x (mg L ⁻¹)
Measured	16 ÷ 28	7.5 ÷ 11	600 ÷ 2500	10 ÷ 21
Optimal	25 ÷ 28	7.5 ÷ 9	800 ÷ 1500	12 ÷ 20

From the analysis of the collected results, we decided to investigate the scale-up of test 6.

For this case the six operational π -numbers can be directly inferred ($\text{DO}_x S^{-1}$, pH, $T T_{opt}^{-1}$, $I_0 K_l^{-1}$) or calculated. The ratio $c c_0^{-1}$ ranges approximately in $6.3 \div 8.8$ whilst $k_a c_0^{-1}$ in $6.4 \div 8.1$. Maximum absorbance, calculated in the center of the PBR at final microalgae concentration, is equal to 10.1. The reactor-related geometric π -number $d_s d^{-1}$ is 0.80 and functional π -number $k_1 n^{-1}$ is $2.0 \cdot 10^{-6} \div 2.3 \cdot 10^{-6}$. Finally, the calculated Damköhler number Da_{II} is in the range $10 \div 12$.

At semi continuous lab-scale we consider the same variables studied at discontinuous lab-scale with the only difference that only one output variable is measured (cell concentration by spectrophotometric analysis) on a daily basis and the four internal variables are on-line measured.

Tests are carried out in two glass clamped top reactors, at incident light intensity equal to $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$, the former being in the tested conditions (test 6 of the previous scale) and within the interval of optimal operating ranges reported in Tables 9 and 10 and the latter is operated as control reactor with the two input variables related to feed set to zero. The cultivation test lasted 90 days, and the automatic management of all the semi-continuous operations as feeding, extraction, brought to volume was performed by the self-built control tool developed on Labview 12 for the PXI National Instruments PXI system. Alarms for the internal variables were set on the MEMO system.

Growth curves are obtained for different wavelength and the estimated parameters obtained with both NLLS and WNLLS for two wavelengths are summarized in Table 11. Figure 7 shows the fitted growth curves.

743 Table 11: Kinetic parameters estimated with both NLLS and WNNLS fitting for semi-
744 continuous operation.

	λ (nm)	c_0 (g L ⁻¹)	k_1 (day ⁻¹)	k_a (L g ⁻¹)	ϵ	R^2
NNLS	430	0.4980	0.5087	0.9857	0.0131	0.7890
	650	0.5024	0.4516	1.0494	0.0104	0.8884
WNNLS	430	0.4978	0.4999	0.9853	0.0130	0.8462
	650	0.5018	0.4482	1.0498	0.0104	0.9199

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Figure 7: Growth curves for the tested semi continuous lab-scale reactor for (a) $\lambda = 430$ nm and (b) $\lambda = 650$ nm, evaluated with NLLS and WNLLS.

Also for this case, the six operational π -numbers can be directly inferred or calculated.

$DO_x S^{-1}$ is kept similar to that of test 6 at discontinuous lab-scale, since CO_2 concentration is regulated and a mean value of 16 mg L^{-1} was measured for O_2 (see Figure 8).

Figure 8: Measured DO_x trend and relative mean value.

The ratio $c c_0^{-1}$ is $2.9 \div 3.0$ and $k_a c_0^{-1}$ is around $2.0 \div 2.1$. It is important to notice that the experimental growth conditions at semi-continuous lab-scale are different from those tested in the DSTRs. By comparing Figures 6 and 7 it can be observed that in the DSTRs the estimated kinetic curves, starting from the inoculum, well describe the lag and the exponential phases of microalgae growth, while the kinetic curves at the larger lab-scale represent only the rapid growth phase until the stationary one, since a great part of the transferred cells were in the exponential growing phase (concentrated inoculum from past cultivation). Then, attention must be devoted to comparing kinetics related π -numbers as $c c_0^{-1}$, $k_a c_0^{-1}$ and $k_1 n^{-1}$ if the initial conditions are not the same at the different scales. In order to understand if at a larger scale the STRs work equally or better than the lab-scale DSTRs, the values here obtained must be correctly compared with those calculated at the discontinuous lab-scale but in the same growth phase. By observing Figure 6 it happens at the inflection point of the kinetic curves, about after 8 days of cultivation and with a corresponding initial concentration of about 0.3 g L^{-1} . By recalculating the dimensionless numbers with this c_0 value, both $c c_0^{-1}$ and $k_a c_0^{-1}$ in semi-continuous STR

are equal or better than the corresponding values in DSTRs. Maximum absorbance, calculated in the center of the PBR at the final microalgae concentration is 39.1 while $I_0K_l^{-1}$ is 1.18. Despite the higher $I_0K_l^{-1}$, I_{av} inside the semi-continuous STRs is less than I_{av} in the discontinuous ones, due to the higher cell concentration.

The reactor-related geometric π -number $d_s d^{-1}$ and functional π -number $k_1 n^{-1}$ are respectively 0.83 and $5.2 \cdot 10^{-6} \div 5.9 \cdot 10^{-6}$.

Damköhler number Da_{II} is in the ranges from about 27 to 31 depending on the considered k_1 . Finally, the product $Da_{II} Sh^{-1}$, which compares kinetics and overall mass transfer is in the range $8 \cdot 10^{-3} \div 2 \cdot 10^{-2}$ thus highlighting how the algae growth in the mixed conditions remains the controlling phenomenon.

A 15-days cultivation was repeated in outdoor conditions in order to evaluate $I_0K_l^{-1}$ and absorbance. Measured incident light intensity at 12.00 a.m. was in the range $1300 \div 1680 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($70000 \div 91000 \text{ lx}$), and an average value of $1100 \div 1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ can be considered as supplied to the microalgae during daylight, so $I_0K_l^{-1}$ is around $1 \div 1.1$. Microalgae concentration reached a maximum of 1.2 g L^{-1} with $c c_0^{-1}$ and $k_a c_0^{-1}$ slightly worse than the corresponding ones in indoor conditions. This is mainly due, in our opinion, to $T T_{opt}^{-1}$, whose mean value is 0.69 (smaller than the corresponding value equal to 0.85, obtained in indoor tests) rather than to light, absorbance maximum value being 32.4.

Photosynthetic PFD measures inside the STRs were taken in the liquid phase at a distance from the reactor wall equal to 0.03 m and a depth of 0.1 m. Measurements resulted to be sensitively influenced by mixing. Values ranged between $60 \text{ and } 180 \mu\text{mol m}^{-2} \text{s}^{-1}$.

4.3.2. Pilot-scale ALRs

In order to verify the scale-up procedure, two EL-ALRs were simultaneously operated with semi-continuous STRs in the 15-days outdoor test, trying to keep similar the previously calculated hydrodynamic π -numbers. Synthetic flue gas was used and no glycerol was added in both the reactor types for this comparison test. Estimated parameters k_1 , k_a and c_0 contribute to the determination of π -numbers $k_a c_0^{-1}$, $\text{DO}_x S^{-1}$ and $k_1 n^{-1}$, with the latter that for the EL-ALRs becomes $k_1 u_{G,\text{riser}}^{-1} a^{-1}$ as no impeller is present. To keep such dimensionless groups unaltered in EL-ALRs, one can exploit the available degrees of freedom. In practice, this task can be accomplished by choosing the appropriate initial concentration of microalgae, by diluting the exhaust gases with air and managing bubble sizes, eventually by varying the design of the diffuser. Microalgae concentration during this test reached a maximum of 1.15 g L^{-1} (at extraction time = 5 days), the obtained ratio $c c_0^{-1}$ is between 5.3 and 7.5 and $k_a c_0^{-1}$ is around $5.8 \div 8.1$, that means a similar yield as obtained in the semi-continuous STRs at the same outdoor and feeding conditions. EL-ALRs were operated under optimal light conditions with $I_0 K_l^{-1}$ around $1 \div 1.1$ and at incipient churn flow ($\text{Re} = 10000 \div 11000$) with dimension of fluegas bubbles around 5 mm, apt to guarantee $\text{Sh} = 1500 \div 2500$; and with a calculated Da_{II} of around 25. Photosynthetic PFD measures taken in the center of the downcomer at a depth of 0.1 m seems not to be influenced by mixing, with values ranging between 120 and $170 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Since $c c_0^{-1}$ is the dimensionless number representing the bioprocess yield, it can be designated as the target variable, so an adequate choice of time intervals for feeding with glycerol and for concurrent extraction and brought to volume can contribute to the optimal set-up of the production in order to further increase this value.

A battery of external loop recirculated reactors has also been tested (Figure 9). In these PBRs the tubular units have the same diameter of the downcomer in order to reduce absorbance, and Re and Sh are kept in the optimal range by using the recirculating pump as a manipulating variable.

Figure 9: Battery of tubular recirculated PBRs.

5. Conclusions

In this study, the scaling up and setting up of *Chlorella vulgaris* cultivation in photobioreactors are examined. Through rigorous scale-up, carried out by resorting to π -theorem, 12 dimensionless numbers were identified. This entails that by maintaining the same values of said numbers at different scales, the same conditions should be realized.

Albeit other approaches, mainly based on dynamic simulation, are typically chosen by industries to perform the scale-up of their processes, we studied the application of Buckingham's π -theorem to the general practice of microalgae growth in PBRs due to its generalization qualities that may very well end up determining time and economic savings.

Experimental tests were carried out in lab-scale STRs to investigate different operating conditions. The correspondent optimal output values obtained at such scale were then maintained as desired for the pilot-scale ALRs. Mass transport, global kinetics and dimensionless numbers adopted to perform scale-up are obtained from 0.5 L DSTRs to semi-continuous 2.5 L STRs by experimental campaigns. A mixed approach coupled with fluid dynamics experimentation is proposed to scale-up from semi-continuous 2.5 L STRs to semi-continuous 10 L ALRs. Finally, scale-up verification at pilot-scale ALRs was performed by computing from the experimental campaign in outdoor conditions the remaining dimensionless numbers related to the kinetics of algae growth and process yield. The pinpointed target variable is the ratio between the final and initial concentrations c/c_0 . At DSTR lab-scale, its value was estimated to range between 6.3 and 8.8. The data gathered from such preliminary outdoor tests confirmed the operability of the scaled-up airlift reactors reaching c/c_0 of $5.3 \div 7.5$, with $c = 1.15 \text{ g L}^{-1}$ at extraction intervals of 5 days.

Broadly translated, the findings reported in this paper provide a set of dimensionless numbers that can be exploited to successfully scale microalgae cultivation in PBRs.

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