# Dynamic Modelling of Microalgae Growth Under Micro-Aeration Conditions

Ting Zhang[a],* ,Aiguo Feng[b,c] , Chunjiang Liu[b]

[a] School of Civil Engineering, Liaoning Technical University, Fuxin 123000, China
[b] School of Chemical Engineering and Technology, State Key Laboratory of Chemical Engineering, Tianjin University, Tianjin 300072, China
[c] College of Food Science and Engineering, Hainan University, Haikou 570228, China
 memchem@163.com

Microalgae cultivation is a critical prerequisite of biomass and biofuels production, which is conductive to the reduction of fossil energy consumptions, but there are the hydrodynamic challenges. This study proposed a dynamic modelling of microalgae growth using experimental and computational fluid dynamics (CFD) methods. Photosynthesis performances of unicellular microalgae in photobioreactors (PBRs) were investigated. In a regions-dividing airlift PBR-B, flow field and cell-cycle progression of *Chlorella vulgaris* were studied. It demonstrated adverse effects of the Karman Vortex Street on microalgae exponential growth. High-levels biomass productivity in flow stagnate region emerges. In a PBR-O, cell density and fluorescence intensity of *Chlorella vulgaris* were elevated by 2.62 % and 46.69 % in the 9th day of cultivation. Therefore, *Chlorella vulgaris* growth profiles were predicted using this mathematical modelling successfully. It benefits for the retrofits of commercial shear-sensitivity microalgae culture that require aeration in bio-energy industry.

## 1. Introduction

Microalgae, as prominent sunlight-driven cell factories, can be adopted for producing biofuels (Yin et al., 2020). Some microalgae, capable of producing up to 70-75% w/w lipids under suitable cultivation conditions, have emerged as popular bio-resources (Menegazzo & Fonseca, 2019). There is a published way by sparging anthropogenic greenhouse gases into microalgae fluids for high-efficiency microalgae cultivation (Faried et al., 2017). However, the aeration-based culture progress is still limited by poor understanding of microbial growth behaviours (Visca et al., 2017). Moreover, in some aspect of the Photobioreactors (PBRs), environmental constrains act on microalgae photosynthesis under some critical culture conditions attract less attentions, such as the effects of light and nutrients on the growth of cells. Adoption of aeration technique into PBRs is a practical way to promote cell yields (Huang et al., 2017). In the aeration culture system, microbial communities move very fast (Raina et al., 2019). Yet now controls of the microbial growth rates in the air sparger-based PBRs have some mistakes under complex culture conditions in the published literatures (Egli, 2015). Design of large-scale microalgae PBRs become one of the most crucial embranchment for high-density algal cultures, but often resulting in deleterious effects, such as algae sedimentation, membrane destruction, nutrient loss, and others (Rojas et al., 2020). Experimental and numerical studies can demonstrate flow mixing to improved light distribution and carbon fixation adapting well-designed PBRs for microalgae cultivation (Li et al., 2020). For example, computational fluid dynamics (CFD) models have been adopted as ways to investigate hydrodynamics effects on bubble column PBRs for accurate prediction of microalgae productivity (Bitog et al., 2014). Recently, hydrodynamic effects have been recognized as one of a significant subject of microalgae culture (Garcia-Ochoa et al., 2020). There are still few researchers prefer to focus on fluid dynamic impacts of bubbles, liquids and microalgae growth in the industrial PBRs due to complexity. Modelling allows an integration of multiple abiotic stimuli that modulate algae growth and generation of biofuels (Xu & Boeing, 2014). However, earlier published model of algal finds favor by choosing a description of the theoretical hypotheses based on black-box methods and does not identify how environmental parameters regulate bioprocesses. The empirical models provide limited knowledge between the algae biomass growth and neutral

lipid production (Brennan & Collins, 2015). Non-uniform distribution of bubbles in a PBR had adverse effects on microalgae growth due to the adverse micro-eddies (Li et al., 2019), and ignored the inductions of hydrodynamic effects on microalgae lipids production, but there is the requirement for more comprehensive models (Concas et al., 2019). The fluctuated micro-bubbles carrying roles affect the disturbance intensity in algae liquids, and then it has a theoretical guidance for design of the PBRs. Therefore, a simple representation of dynamic algae growth without understanding fluid behaviors may not capture the detailed regularities of microalgae cultivation. Therefore, this study proposed a regions-dividing PBR model and an atomizing PBR model with micro-aeration. These models were studied by CFD and practical culture experiments, aiming to predicting microalgae growth profiles of *Chlorella vulgaris*. Moreover, this works defined four regions in the PBR-B and identified biomass production in response to critical environmental stimuli. The newly-developed modelling frame facilitates understanding of the cellular lipids production patterns of microalgae under a wide range of hydrodynamics, such as flow separation. It broadens insights of algae growth in PBRs, thus benefiting achievement of goals of Carbon neutrality and Sustainability.

## 2. Materials and methods

### 2.1 Novel flat-plate PBR design

There are realized in two types of systems available for algae culture, open raceways or PBRs. Artificial ponds have several problems, such as contamination from other invading species, excessive space requirements, which causes a limitation in microbial production sustainability. The closed system is expensive to construct since they need high quality materials and intensive energy to scale-up (Assunção & Malcata, 2020). PBRs, one of the most used systems for algae cultivation, are designed to overcome the limitation of the algae ponds. Airlift and mixing are guaranteed with the design of baffles, which are likely to be the promising techniques for extensive cultivation. In this study, the flowchart is presented in Figure 1.
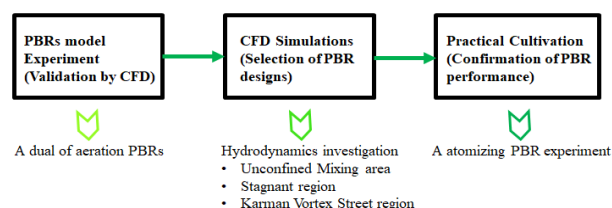


*Figure 1: Flowchart of this study*

The steps of this study are PBRs model, CFD simulation and practical cultivation. In this study, a PBR-B with four newly-dividing regions was proposed. Geometrical parameters are presented in Table 1. The PBR-B model in this work was an plat-plate PBR designing an air spager, square baffles and hexagonal columns.

*Table 1: PBR-B characteristics.*

| Items | Details |
|---|---|
| PBRs volume/working volume | 1950 mL/1650 mL |
| Dimensions of PBR-B (L × W × H) | 100 mm × 60 mm × 325 mm |
| Height and width of vertical baffles | 100 mm/65 mm |
| Height and degree of sloping baffles (1#—2#) | 100 mm, 50 mm/15 °, 20 ° |
| Height of hexagonal columns (1#—6#) | 65 mm, 50 mm, 45 mm, 40 mm, 35 mm, 30 mm |
| Height and diameter of air sparger | 30 mm/10 mm |
| Working liquid level height | 275 mm |

The hydrodynamic effects on the performance of *Chlorella vulgaris* growth in the PBRs were identified. A contrast PBR (PBR-A) and the PBR-B were equipped with a submerged air sparger. After concerning the stagnant flow fields of microalgae cultivation, CFD simulations were conducted. Then, the practical cultivation periods were investigated and validated the simulation results. Finally, a delicate-designed atomizing PBR (PBR-O) was analyzed. The working volume of the PBRs is 1.75 L (1-2.5 L is the most commonly used value). There are several horizontal baffles placed in the PBRs to prevent sedimentation of microalgae, giving the water a speed between 5 and 22 cm/s for microalgae cultivation (Ye et al., 2018). In the PBR-B, the photosynthesis process was influenced by four considerations regarding four regions. There were airlift stream, looped downstream, Karmen vortices flow and stagnant stream. The airlift stream was definitely

affected by six hexagonal round flow columns where the microalgae liquid underwent a first concentration by suspension. The separated stream was mixed while the large vortices stay above the looped downstream. In this micro-aeration condition, the operating time required by the microalgae cell division can be different from the time in the looped downstream. To sum up, there are Karman vortex street region, unconfined mixing area, looped area and flow stagnant region in the PBR-B (Figure 2(a)). This work predicts microalgae lipids productivity.

## 2.2 Model descriptions

In this study, the *Chlorella vulgaris* cultivation system was constructed by a mathematical model. Then, the grids were refined and the number of mesh was set as 838,430. An unsteady state analysis was used because the microalgae growth rate is time dependent. The validation of time step independence was conducted in an earlier paper (Li et al., 2020), then the time-step for the PBR was smaller than 0.007, and the numerical calculation time was chosen as 0.0001 s. Overall, the model is a differential algebraic equations system. VOF multi-phase model was reported to predict the gas injection in the PBRs (Yeo et al., 2018), and then the governing equations are composed of a set of momentum and continuity equations. This study did not list the equations and they can be referred to by previous studies (Liu et al., 2019). The standard k-ε model, as a widely used model, was chosen to depict the turbulent fluid behaviors in the PBRs considering previous successful studies (Bitog et al., 2011). The microalgae growth behaviors were identified through data of the culture cell number and fluorescence intensity. Then, the overall dynamic growth rates were used to express the microalgae growth process based on the growth curve of microalgae from practical cultures.

## 2.3 Practical cultures

Measurement of flow velocity field and concentration aim to characterization turbulent mixing behavior (Aslanbay Guler et al., 2019). In the experimental part, microalgae species of *Chlorella vulgaris* is cultivated in the PBRs. These apparatus were operated under micro-aeration, micro-diffusion conditions. The cultivation temperature is 25℃—28℃. Within the 0—9,18 cultivation days, the absorbance degree of the microalgae liquids was measured by a UV-visible spectrophotometer (Tianjin Gangdong Co., Ltd F380) every 24 h. Figure 2(a) pointed four sampling sites of algae liquid (S1-S4), which were located in the center of the PBR-B and PBR-A. There were two sampling sites (S0 and S00) in the center and corner of the compared PBR-A, it was noted by a red arrow. In Figure 2(b), the PBR-A apparatus was manufactured and placed at the right of the PBR-B. The injected air volume of PBRs was adjusted by a volume flow meter, and the air flow rate is 1 L/min. Figure 2(c) presented an in-situ photo of PBR-O installations. In PBR-O, the dimension of a Venturi tube was 12 mm, 3 mm and 14 mm (Throat tube, Shrink tube and diffusion tube), the degree were 11.7° and 10.1° (Shrink angle and Divergence angle).
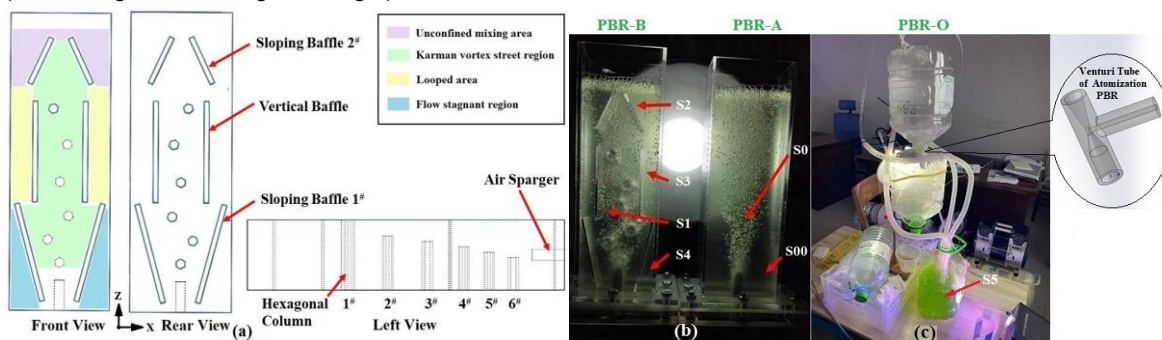


*Figure 2: Schematic of the regional-divided PBR-B(a), PBR-A/PBR-B(b) and PBR-O algae culture system(c)*

The correlation parameters between the absorption coefficient at a wavelength of 681 nm need be determined (Li et al., 2020). At different cultivation days, the correlation between the absorption coefficient of the microalgae of *Chlorella sp.* at wavelength of 681 nm and the microalgae biomass concentration was determined in the earlier disclosed researches. And then, the biomass concentration in these newly-proposed PBRs (c, g/ml) was calculated by an equation which was presented as follows.

$$c = 0.394\,OD_{681} + 0.055 \tag{1}$$

According to disclosed references (Yang, 2011), the specific growth rate of microalgae (u, day-1 or s-1) is calculated through Eq. (2—4). The volumetric microalgae growth rate (f (t), day-1 or s-1) can be calculated as following Eq(3):

$$u = f(t)/c(t) \tag{2}$$

$$dc/dt = f(t) \tag{3}$$

## 3. Results

In this study, CFD measurements of PBR-B demonstrated that the unconfined mixing area reached a relative low flow velocity, a circular flow and mixing occurred, with high values of TKEs between four adjacent baffles and hexagonal columns were calculated, the un-uniform flow fields were created for microalgae growth. The small TKE intensity gradually decreased with increasing of H (Z values), which contributed to the large velocity differences and indicated the different mixing performance over the Karman Vortex Street region and flow looped area in the PBR-B. In the unconfined mixing area, both of the TKE value and velocity were low (Figure 3(a)). Here, the uniform liquid flow in stagnant region was suitable for creating less difference in liquid velocity among the zones for algal growth. Herein, there was a major balance of turbulent intensity and carbon dioxide ($CO_2$) concentration during the nutrient mass transfer. The performance of aerobic bioprocess depends not only on bubble-to-cell collisions frequency, but also due to the influences of operation stabilities, such as time-dependent weak velocity fluctuations, controlling to the microbial cell divisions. In Figure 3(b), the red and black bars represent $OD_{681}$ value of microalgae liquids, the growth rates of microalgae in the PBR-A (red bar) was larger than that in the PBR-B (black bar). Overall, the four regions in the PBR-B obtained different growth rates, while high density of microalgae was measured in the flow stagnant region. Moreover, it was found that the Karman vortex street region attained low-level microalgae cell density, and the cell number in the bottom (sampling point S00) of the PBR-A were relative higher than that area of the center of the PBR-A.

*Table 2: PBR-A and PBR-B experimental data.*

| Day | Cell number (cells*10^5/ml) | | | | | OD681 (A.U.) | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | PBR-A | | PBR-B | | | PBR-A | PBR-B |
| | S00/S0 | S1 | S2 | S3 | S4 | S0 | S1 |
| 0 | 7.93 / 7.93 | 7.93 | 7.93 | 7.93 | 7.93 | 0 | 0.035 |
| 2 | 25.1 / 18.4 | 13.4 | 17.5 | 17.2 | 20 | 0.221 | 0.184 |
| 4 | 59.2 / 49.6 | 44.3 | 48.5 | 46.1 | 50 | 0.609 | 0.527 |
| 5 | 81 / 76.5 | 44.4 | 41.5 | 50.7 | 68.1 | 1.142 | 0.812 |
| 6 | 74.5 / 72.1 | 58.9 | 56 | 58.7 | 70.6 | 1.29 | 0.994 |
| 7 | 87.8 / 80.7 | 78.7 | 78.3 | 78.4 | 78.9 | 1.32 | 1.178 |
| 9 | 129 / 125 | 89.1 | 85.6 | 80.4 | 92 | 2.181 | 1.54 |
| 11 | 154 / 153 | 116 | 102 | 112 | 120 | 2.484 | 1.696 |
| 13 | 164 / 158 | 130 | 124 | 126 | 139 | 2.78 | 2.036 |
| 15 | 179 / 170 | 132 | 129 | 128 | 157 | 2.996 | 2.268 |
| 17 | 184 / 175 | 120 | 128 | 125 | 145 | 3.04 | 2.516 |
| 18 | 190 / 156 | 115 | 119 | 118 | 138 | 2.876 | 1.98 |

In Table 2 and Figure 3(c), the cell growth rate of PBR-A was higher than that in PBR-B. Seriously, in the PBR-B, the microbial growth performance in the flow stagnant region was relatively higher than that in the other three regions. Moreover, the fitting equations of $OD_{681}$ were obtained.

$$u = 2.22345\,e^{-4} + 7.72637\,e^{-4}t + 0.06638t^2 - 0.00698t^3 + 3.24733\,e^{-4}t^4 - 6.36627\,e^{-6}t^5 \tag{4}$$

$$u = 0.05373 - 0.18507t + 0.14855t^2 - 0.02262t^3 + 0.00144t^4 - 3.31146\,e^{-5}t^5 \tag{5}$$

where u represents the growth rate of microalgae, the unit of u is also corresponding to microalgae growth (represented by $OD_{681}$). The fitting equations reached higher R-Square and Adj. R-Square ($\approx 1$), and these equations meet the standards for primary microalgae growth prediction in pilot-scale PBRs in bio-resource industry. Herein, above microalgae growth equations were presented by polynomial fitting based on the actually measured microalgae growth curves in the 18 days cultivation periods (Table 2). Moreover, we could deduce that large vortices have adverse impacts on microalgae exponential growth phase.

Based on above results, a newly-proposed PBR-O was then designed. It would receive more sufficient light and $CO_2$, probably meet the nutritional needs of algae growth and bio-production, thus probably promoting the mature algae divisions, enriching nutrients transfer and then accelerating the new born algae growth, mature, division, while the algae grow slowly in traditional PBRs. In this practical cultivation, the cell density of the

Venturi aeration PBR-O was 2.62 % higher than the air-spargering PBR, the growth trend of cell density is elevated during the 9 culture days (Table 3).

*Table 3: PBR-A and PBR-O experimental data.*

| Day | Cell density (r, g/ml) | | |
|-----|------|------|------|
| | PBR-A | PBR-O | Difference ratio |
| | S0 | S5 | $r_{PBR-A}/r_{PBR-O}$ |
| 0 | 0.98621 | 0.99211 | 0.9940 |
| 3 | 0.99484 | 1.00645 | 0.9885 |
| 5 | 0.99986 | 1.01351 | 0.9865 |
| 7 | 1.00381 | 1.02379 | 0.9805 |
| 9 | 1.01031 | 1.03683 | 0.9744 |

Microalgae lipids productivity was further demonstrated by high-level fluorescence intensity. In Figure 3(d), algae lipid productivity produced by atomizing photobiological reactor was represented by fluorescence intensity as 46.69 % higher than that in PBR-A. Obviously, the increased fluorescence intensity within culture days in the PBR-O indicated significant improvement on lipids productivity in microalgae cells. Then, the PBRs with appropriate considerations of hydrodynamics is suitable for microalgae cultivation under Venturi tube based micro-aeration conditions, which is conducive to accumulation of neutral lipids, may get better economic benefits in industrial production. In sum, the newly-proposed atomization PBR-O supports elimination of adverse vortex effects and attains satisfied culture performance. These results benefit the emergences as the bases of microbial growth and lipids productivity predictions.
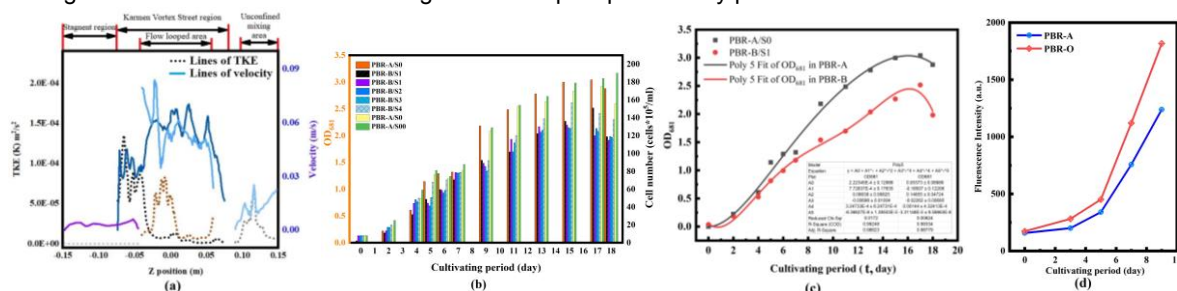


*Figure 3: Effect of hydrodynamics on TKE(a), microalgae fluid velocity(b) and growth profiles of Chlorella vulgaris in the PBR-A/PBR-B (c) and PBR-O(d)*

## 4. Conclusions

Microbial growth performance was investigated in the novel PBRs. The microbial growth rates in flow stagnant region of the PBR-B were significantly higher than that in the regions with large vortex flows. Gas and liquid velocities in Karman vortex street region were enhanced, accompanying low biomass concentration, while the relatively increased microalgae growth rate with weak mixing phenomenon occurs in the unconfined vortex area. Then, adopting micro-aeration conditions to PBRs operations by introducing a Venturi tube-based hydrodynamic internals into the atomizing PBR-O, the PBR-O reached 46.69 % higher microalgae concentration, lipids productivity and accumulation than conventional microalgae cultivation. Therefore, this modelling works benefit understanding hydrodynamic effects, such as $CO_2$ concentration gradients, microalgae liquid turbulent flow characteristics on microalgae growth, thus providing supports for high-efficiency PBR designs, operation strategy development, and scale-up strategies of bio-based industry.

**Nomenclature**

c – Biomass concentration of photobioreactor, g/ml

f(t) – Volumetric microalgae growth rate, day-1 or s-1        u – Specific growth rate of microalgae, day-1 or s-1

**Acknowledgements**

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