Proceedings of the Canadian Society for Mechanical Engineering International Congress 2022
CSME Congress 2022
June 5-8, 2022, Edmonton, AB, Canada

EFFECTS OF TURBULENT MIXING VERSUS ORBITALLY SHAKING ON CELL GROWTH IN ACTIVE FLUIDS

Zahra Samadi[1*], Malihe Mehdizadeh-Allaf[1], Reza Saifi[1], Christopher T. De Groot[2], Hassan Peerhossaini[1,2,3]
[1]Department of Civil and Environmental Engineering, The University of Western Ontario, London, ON, Canada
[2]Department of Mechanical & Materials Engineering, The University of Western Ontario, London, ON, Canada
[3]Astroparticles and Cosmology Lab,Université de Paris, Paris, France
*zsamadi@uwo.ca

Abstract— Mixing displays a major role in the growth of photosynthesis microorganisms in active suspensions: it gives access to nutrients, improves the microorganisms' exposure to light, and homogenizes temperature distribution in photobioreactors (PBRs). Therefore, mixing is an essential factor in the design and optimization of PBRs for biomass culture. However, different mixing modes can affect cell growth differently. In this study, three different modes of mixing, ranging from turbulent stirring, as the upper bound of mixing, orbitally shaken flow, as the lower bound of mixing, and simple molecular diffusion, were applied for cultivations of the cyanobacterium *Synechocystis* sp. CPCC 534. Results showed that imposing mixing on the culture can improve the growth rate as well as biomass yield production compared to simple molecular diffusion. The diffusion model shows the lowest growth rate and the lowest yield production in this study.

Keywords: Microorganisms; Bacteria; Algae; Cyanobacteria; Synechocystis; Yield production; Photobioreactor (PBR); Incubator; Incubator shaker; Magnetic stirrer; Molecular diffusion.

I. INTRODUCTION

Photosynthetic microorganisms such as unicellular microalgae, bacteria, and plant cells are promising resources in various applications: biofuel production, carbon capture and storage [1], wastewater treatment, animal feed, pigments production, cosmetics, pharmaceuticals, and bioplastics production [2].

Suspensions of self-propelled particles such as photosynthetic microorganisms are generally referred to as "living" or "active" fluids [3]. The properties of active fluids are basically different from those of conventional or "passive" fluids. In passive fluid, pressure, velocity, or temperature gradients are driving forces for the flow, while in active fluid, cells as the microstructural elements of the fluid, use nutrients or the light to activate molecular motors and carry out other metabolic functions and navigate in the media, which these directed movements are called chemotaxis or phototaxis, respectively [4,5] In this study, *Synechocystis* sp., which is a strain of unicellular freshwater cyanobacteria is suspended in a medium fluid to constitute the working active fluid [6].

In industrial applications, photosynthetic microorganisms are cultivated in photobioreactors (PBR) [7]. Several key parameters affect the growth and biomass production of microorganisms, among which light, nutrient availability, pH, and temperature play an important role[8]. Mixing is the underlying mechanism in making accessible the above conditions. It breaks the gradients of temperature and pH, improves the accessibility of nutrients, and light to the microorganism cells, enhances mass transfer, avoids cell sedimentation and cell attachment to the photobioreactor walls [9,10], and improves the overall performance of the photobioreactor[11]. In industrial photobioreactors mixing can be attained by various methods including mechanical agitation, bubbling, aeration, and pumping or the combinations of these modes [12].

Cell cultures in the laboratory are generally performed with two main mixing methods (modes); turbulent stirring (TS) and orbitally shaking (OS). The main objective in mixing is to make direct contact between the cell and nutrient or light at a local level. However, different mixing modes achieve this aim differently. In this study, we compare the efficiency of TS and OS mixing modes in this relation, and compare it to a non-mixing (simple molecular diffusion) mode, using cell growth as a proxy.

Turbulent stirring (TS) is achieved, in laboratory experiments, by a magnetic stirrer. It is a laboratory tool, which utilizes an external rotating magnetic field to make a cylindrical stirring rod, put in a liquid, rotates very fast, so mixing it (Figure 1). This mode of mixing is used in chemistry and microbiology. By gradually increasing the rotation speed of the magnetic field, one can generate a laminar vortex or an almost uniform turbulent micromixing field. In the latter case, the velocity and shear stress distribution becomes rapidly complex and an efficient mixing regime is established. For further details, the reader is referred to Figure 1. Turbulent stirrers are normally placed in incubators to maintain optimal conditions for incubating cells.

Orbital shaking is another mode of mixing that is achieved by an orbital shaker; a piece of laboratory device, which is utilized to mix the materials in a flask by shaking mechanism. An orbital shaker has a circular shaking motion with a slow speed (25-500 rpm) (Figure 2). It is suitable for the cultivation of microorganism cells and general mixing in a low shear stress environment to avoid damaging the cells in the suspension. Orbital shakers are commonly integrated into an incubator to generate an incubator shaker. Incubator shakers contain a horizontally shaking board that flasks or breakers are placed inside it. An incubator shakes and maintains optimal conditions for incubating microorganism cells simultaneously. The velocity field and shear stress distribution in an orbital shaker are less uniform than in a turbulent stirrer and it can be expected that this difference affects the growth cell.

In this study, we investigated, the impacts of mixing modes on the growth of the cyanobacterium *Synechocystis* sp. CPCC 534. *Synechocystis* was cultivated in lab photobioreactors by applying three different mixing modes; turbulent stirring, TS (using a magnetic stirrer), orbitally shaking, OS (using an incubator shaker), and molecular diffusion, MD (no mixing or shaking).

II. MATERIALS AND METHODS

A. Strain and Culture Conditions

Synechocystis sp. CPCC 534 wild-type was purchased from the Canadian Phycological Culture Center (Waterloo, ON, Canada), and was cultured in chemically defined liquid BG11 medium at 20±1 °C. The initial cell concentration was 5×10^5 cell.ml^{-1} and the culture were carried out under the light cycle of 12/12 h and photon flux of 50±5 µmole.m^{-2}.s^{-1}.

B. Mixing Modes

Cells were cultivated under controlled conditions in three different mixing modes. In turbulent stirring (TS) mode, digitally controlled magnetic stirrers (VWR Canada) were used to create turbulent mixing inside the lab photobioreactors placed in a fully controlled PHCbi incubator (light intensity and temperature were 50±5 µmole.m^{-2}.s^{-1} and 20±1 °C, respectively). Cylindrical magnetic stirring bars with a diameter of 7.9 mm and a length of 19.8 mm were used to mix the sample. In the orbitally shaken (OS) mode, lab photobioreactors were placed in a fully controlled Eppendorf Innova S44i incubator shaker (light intensity and temperature, were 50±5 µmole.m^{-2}.s^{-1}, 20±1 °C, respectively). In the un-mixed or molecular diffusion (MD) mode, lab photobioreactors were placed standstill in controlled conditions (light intensity and temperature were 50±5 µmole.m^{-2}.s^{-1} and 20±1 °C, respectively). The turbulent stirring and orbitally shaking mixing devices are schematically shown in Figures (Figures 1 and 2)

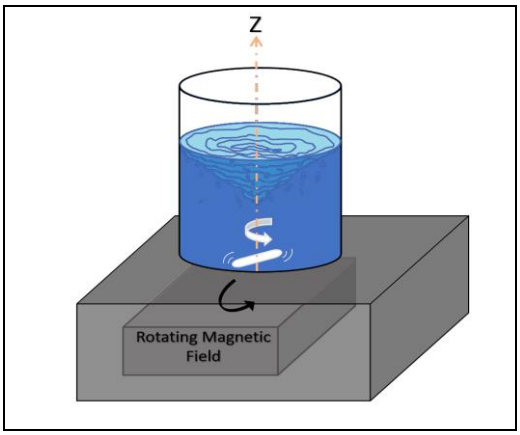

Figure 1. Schematic illustration of a turbulent stirring system.

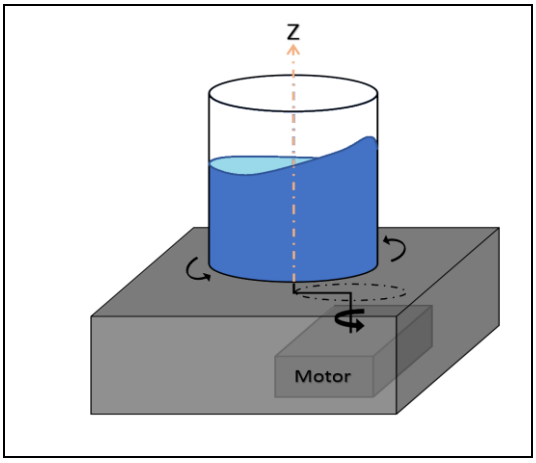

Figure 2. Schematic illustration of an orbital shaker.

C. Experimental Protocol

Each mixing mode experiment was performed in triplicates and was carried out in 125 ml Pyrex flask lab photobioreactors. The optical density (OD) of *Synechocystis sp.* CPCC 534, as a proxy of growth, was measured daily at 750 nm wavelength using a Spectronic 200E spectrophotometer. A calibration curve was prepared to estimate the number of cyanobacteria cells per 1 milliliter. For this purpose, a dilution series of *Synechocystis* sp. CPCC 534, all grown at the same temperature and light intensity, were prepared and the optical density and the number of cells.ml^{-1} were measured using the Spectronic 200E spectrophotometer and a Hausser Scientific hemocytometer, respectively. The calibration curve (also known as standard curves) was generated to calculate the number of cyanobacteria cells in the sample solution from the OD measurements.

D. Calculation of Cynobactrium Growth Rate (r) and Doubling Time (k)

The growth rate (r) of the population was calculated during the exponential growth phase to estimate the rate of growth in cells number per unit time by using equation (1) [13].

$$r = \frac{ln\ N_t - ln\ N_0}{\Delta t} \quad (1)$$

where N_0 and N_t are the size of the population on the first day and at the end of the cultivation time interval, and Δt is the duration of the growth time interval (t_t - t_0).

The growth rate (r) is used to estimate the number of cell divisions per day known as doubling per day (k), by equation (2) [13].

$$k = \frac{r}{ln2} = \frac{r}{0.6931} \quad (2)$$

The cell yield of *Synechocystis* sp. CPCC 534 was computed by integrating the area under the growth curve surface from the beginning of the exponential phase until the end of the stationary phase.

E. Statistical analysis

The significant differences in growth, doubling per day, yield production, and Chl_a production was investigated by a one-way ANOVA followed by the Tukey multiple comparison test. statistical analyses were estimated by OriginPro 2017 (OriginLab Corporation, Northampton, MA, USA), and significance was considered when $p < 0.05$.

III. RESULTS AND DISCUSSION

A. Standard Curve

In the first step, a serial dilution of *Synechocystis* sp. CPCC 534 was prepared. Then, the OD of each sample was measured by the spectrophotometer and the number of *Synechocystis* sp. cells of each sample was counted by hemocytometer under Nikon microscope (Nikon ECLIPSE Ti2 inverted microscope). Then the calibration graph was plotted, as is shown in Figure 3. The obtained equation from the calibration curve was used to calculate the number of *Synechocystis* sp. cells (Figure 3).

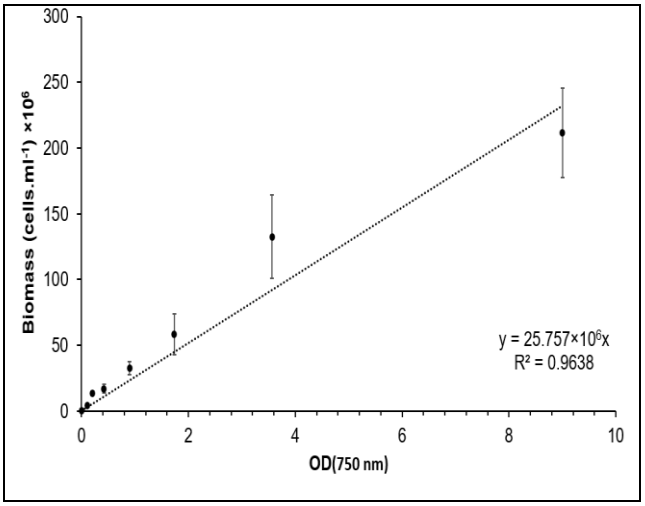

Figure 3. Calibration curve of Synechocystis sp. CPCC 534 solutions.

B. Growth Curve and Comparison of Three Mixing Modes

A growth curve, shown in Figure 4, was used to monitor the number of *Synechocystis* sp. CPCC 534 cells over time. In cyanobacteria, cultures move from the lag phase into a logarithmic growth phase, which continues until reaching a linear growth phase period. After the linear growth phase, the non-growing stationary phase starts. The self-shading of cells often obstacle the accessibility of microorganism cells to the light source to get a certain cell density during the linear growth period. Figure 4 shows that the *Synechocystis* cells that were grown under TS and OS mixing modes enter the logarithmic growth phase around day 4, while the motionless culture enters the logarithmic phase around day 7. The faster start of the exponential phase in mixed cultures can be attributed to the abundance of nutrients and light available to cells due to their spatial displacement.

In the same manner, the cultures grown under TS and OS mixing modes reached the linear growth phase later than the cultures grown in motionless PBRs; day 15 for the formers and around days 12 or 13 for later. Here again, efficient movement of cells inside the batches, caused by mixing, allows them to uptake nutrients from unexplored regions and reach light more efficiently even in the presence of self-shading caused by the more populated environment compared to the motionless culture.

Regarding growth rate, Figure 4 shows a better growth rate for cultures grown under TS and OS mixing modes in comparison with cultures that were grown under motionless conditions (see Figure 5). Nevertheless, the growth rate for TS is slightly higher than that of OS, suggesting that turbulent stirring is more efficient in mixing the active fluid than orbitally shaking. A closer analysis of the fluid mechanics of TS and OS modes (not shown here) confirms the superiority of TS mixing above and over that of OS mixing. However, the OS growth curve overtakes the TS growth curve at the beginning of the linear phase and overlaps with it for the whole period of linear growth. The later start of the logarithmic phase, smaller growth rate, and earlier start of the linear phase in motionless culture resulted in a significant lag in biomass yield, as is shown in Figure7.

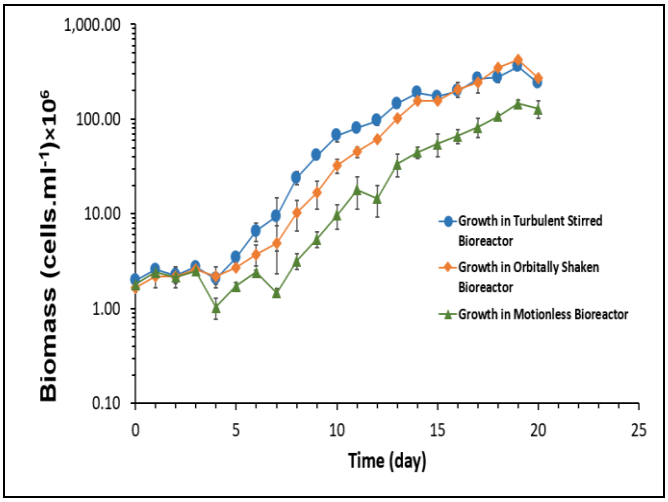

Figure 4. Growth curve of Synechosystis for three different mixing modes.

The growth rate and doubling per day for all modes were estimated during the exponential phase, and the results are presented in Figures 5 and 6. The results showed that under mixing conditions, there is a significant change in a growth rate and doubling per day of *Synechsystis* in comparison to non-mixing (motionless) conditions. Mixing increases the accessibility of microorganisms to nutrients and light; therefore, it improves the growth rate as well as doubling per day [14].

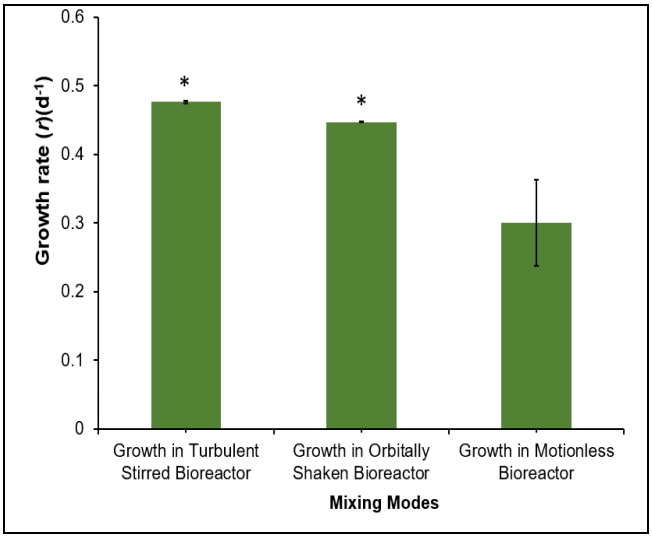

Figure 5. Growth rate (*r*) of *Synechocystis* sp. CPCC 534 under various mixing conditions. * indicates the significant effect at the level of 0.05 among different mixing modes.

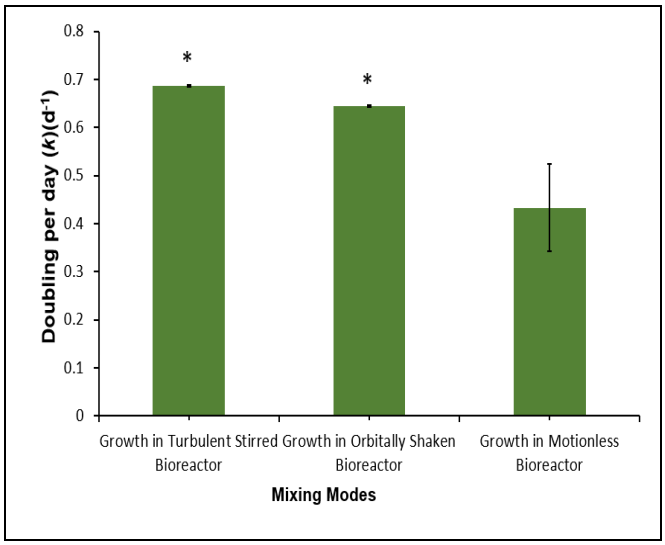

Figure 6. Doubling per day (*k*) of *Synechocystis* sp. CPCC 534 for different mixing modes. * indicates the significant effect at the level of 0.05 among different mixing modes.

The *Synechocystis* sp. CPCC 534 yield under different mixing conditions was plotted in Figure 7. Based on the presented data, the biomass yield production was significantly higher when cells were grown under mixing conditions. The biomass yield production showed an increase of over 213% for TS mode and 197% for OS mode compared to motionless cultures. Mixing enhances the ability of cells to visit different places in the culture, so microorganisms can easily get access to CO_2, nutrients, and light, from this point of view, both mixing modes play a curial role in biomass production[14].

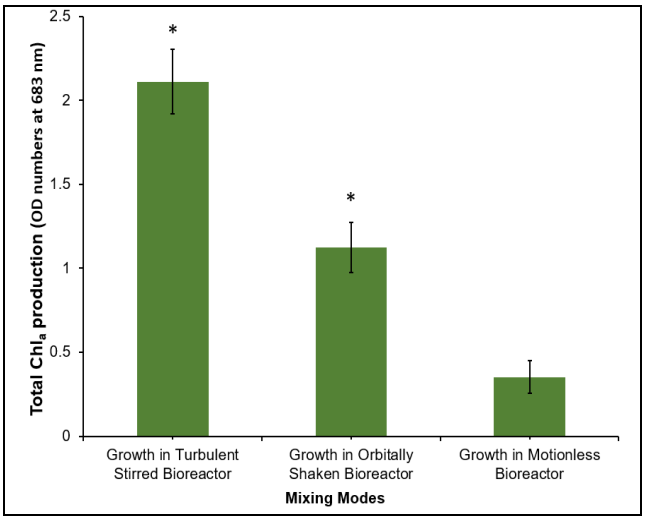

Figure 7. Profile of yield for *Synechocystis* sp. CPCC 534 under different mixing modes. * indicates the significant effect at the level of 0.05 among different mixing modes.

The production of Chl_a was evaluated under mixing conditions. Chl_a is one of the major pigments in photosynthesis with the ability to capture light and convert it into chemical energy [15]. The Chl_a was measured at the wavelength of 683 nm, and the average Chl_a production during the exponential phase is reported in Figure 8. Experiments under three different mixing modes show turbulent stirring produces more Chl_a in comparison to other modes and there is a significant increase in production of Chla under mixing conditions.

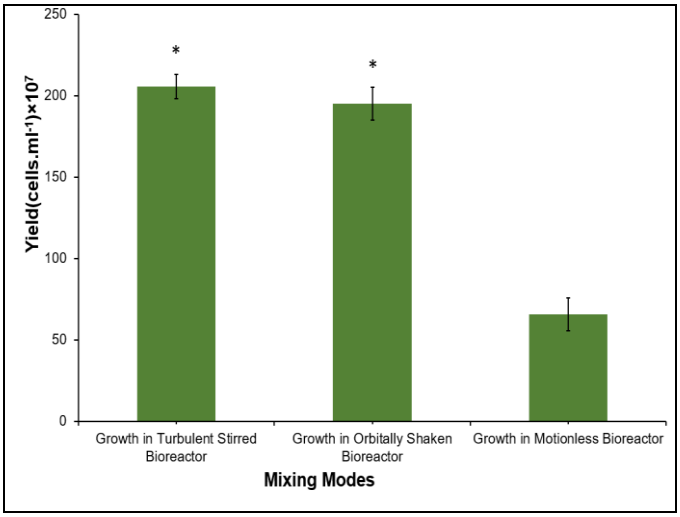

Figure 8. Profile of Chl_a production for *Synechocystis* sp. CPCC 534 under different mixing conditions. * indicates the significant effect at the level of 0.05 among different mixing modes.

Mixing by turbulent stirring seems to be more efficient, as appears in most cell growth metrics, than mixing by orbitally shaking. This difference can be attributed to the dissimilarity in the fluid motion and wall shear stress distribution between

the two mixing modes. In orbitally shaking, the fluid undergoes a solid body rotation about the center of the cylindrical vessel, and most of the fluid and cell particle movement is concentrated in the peripheral zone of the reactor. In solid rotation, the fluid body is not sheared thus, the relative distance between fluid particles remains constant. Fluid particles trapped in the solid rotation zone have a very limited chance (except by molecular diffusion) to visit other places in the medium for nutrient uptake, and especially getting close to the free surface and wall vicinity where they can be exposed to light. While this difference can affect the logarithmic growth period (shorter in TS than in OS) however, the OS growth curve catches up to this delay and reaches the TS growth curve in the linear growth period.

IV. CONCLUSIONS

In this study, the impact of two types of mixing (turbulent stirring and orbitally shaking), on various growth parameters of *Synechocystis* sp. was investigated and compared with stationary (motionless) culture. The results revealed that mixing *Synechocystis* cultures can improve growth rate, doubling per day, yield, and Chl_a production in comparison with the cultures where no mixing was imposed. Mixing by turbulent stirring seems to be more efficient, as appears in most of the cell growth metrics, than mixing by orbitally shaking. This difference can be attributed to the dissimilarity in the fluid motion and wall shear stress distribution between the two mixing modes. Mixing enhances the nutrient uptake as well as homogenizes the distribution of light and temperature.

ACKNOWLEDGEMENTS

The researchers appreciate Dr. Charles G. Trick for providing *Synechocystis* strain for this work. HP acknowledges the financial support of NSERC (RGPIN-2019-05156) which partially funded MMA, ZS, and RS. CD acknowledges the financial support of NSERC (RGPIN-2017-04078) which partially funded ZS.

REFERENCES

[1] Huang, Q., Jiang, F., Wang, L., & Yang, C. (2017). Design of photobioreactors for mass cultivation of photosynthetic organisms. Engineering, 3(3), 318-329.

[2] Samadi, Z., Johlin, E., DeGroot, C., & Peerhossaini, H. (2021, August). Modelling Optical Properties of Algae Using the Finite-Difference Time Domain Method. In Fluids Engineering Division Summer Meeting (Vol. 85307, p. V003T05A016). American Society of Mechanical Engineers.

[3] Mehdizadeh Allaf, M., Habibi, Z., de Bruyn, J. R., DeGroot, C. T., & Peerhossaini, H. (2022). Rheological and Biophysical Properties of Living Fluids Under Shear: Active Suspensions of Synechocystis sp. CPCC 534. Journal of Fluids Engineering, 144(2).

[4] Fadlallah, H., Jarrahi, M., Herbert, É., Ferrari, R., Mejean, A., & Peerhossaini, H. (2019). Active Fluids: Effects of Hydrodynamic Stress on Growth of Self-Propelled Fluid Particles. Journal of Applied Fluid Mechanics, 13(2), 561-570.

[5] Vourc'h, T., Leopoldes, J., & Peerhossaini, H. (2020). Light control of diffusion coefficient of active fluids. J. Fluids Eng., 142, 031109.

[6] Heidorn, T., Camsund, D., Huang, H. H., Lindberg, P., Oliveira, P., Stensjö, K., & Lindblad, P. (2011). Synthetic biology in cyanobacteria: engineering and analyzing novel functions. Methods in enzymology, 497, 539-579.

[7] Voloshin, R. A., Rodionova, M. V., Zharmukhamedov, S. K., Veziroglu, T. N., & Allakhverdiev, S. I. (2019). Biofuel Production from Plant and Algal Biomass. Alternative Energy and Ecology (ISJAEE), (7-9), 12-31.

[8] Bitog, J. P., Lee, I. B., Lee, C. G., Kim, K. S., Hwang, H. S., Hong, S. W., ... & Mostafa, E. (2011). Application of computational fluid dynamics for modeling and designing photobioreactors for microalgae production: A review. Computers and Electronics in Agriculture, 76(2), 131-147.

[9] Anjos, M., Fernandes, B. D., Vicente, A. A., Teixeira, J. A., & Dragone, G. (2013). Optimization of CO2 bio-mitigation by Chlorella vulgaris. Bioresource technology, 139, 149-154.

[10] Carvalho, A. P., Meireles, L. A., & Malcata, F. X. (2006). Microalgal reactors: a review of enclosed system designs and performances. Biotechnology Progress, 22(6), 1490-1506.

[11] Al Taweel, A. M., Shah, Q., & Aufderheide, B. (2012). Effect of mixing on microorganism growth in loop bioreactors. International Journal of Chemical Engineering, 2012.

[12] Wang, B., Lan, C. Q., & Horsman, M. (2012). Closed photobioreactors for production of microalgal biomasses. Biotechnology advances, 30(4), 904-912.

[13] Wood, A. M., Everroad, R. C., & Wingard, L. M. (2005). Measuring growth rates in microalgal cultures. Algal culturing techniques, 18, 269-288.

[14] Mehdizadeh Allaf, M., Habibi, Z., Samadi, Z., DeGroot, C. T., Rehmann, L., de Bruyn, J. R., & Peerhossaini, H. (2020, July). Physical and Rheological Properties of Active Fluids Under Shear Stress: Suspensions of Synechocystis. In Fluids Engineering Division Summer Meeting (Vol. 83723, p. V002T03A026). American Society of Mechanical Engineers.

[15] Vinyard, D. J., Ananyev, G. M., Dismukes, G. C. (2013). Photosystem II: the reaction center of oxygenic photosynthesis. Annual review of biochemistry, 82, 577-606.