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Active Fluids: Effects of Hydrodynamic Stress on Growth of Self-Propelled Fluid Particles

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ABSTRACT

Under the current global energy crisis the interests in developing a third generation of biofuels produced from non-food feedstock such as microalgae and cyanobacteria have clearly increased. Hydrodynamic stress, always present in cultivation process of these microorganisms, is an essential factor to ensure mixing inside bioreactors; however the importance of its intensity is usually ignored by applying a random agitation (energy consumption) which is unnecessarily overestimated. In this work, two types of agitation, stirring in agitated photobioreactors (APBR) and bubbling air in draft tube airlift photobioreactors (DPBR), are applied to study the effects of hydrodynamic stress on the growth and pigment content evolution of the cyanobacteria *Synechocystis sp. PCC 6803*, a self-propelled microorganism. The range of applied shear stress was between 0 and 400 mPa. Similar results are obtained for both agitation mechanisms, indicating that the effects of shear stress are limited to the breakdown of the cell colonies; once they are broken down any further increase in shear stress has no significant effect on their growth rate. Moreover, the variation in pigments concentration appeared to be linear with the cellular concentration and independent from shear intensity.

Keywords: Hydrodynamic stress; Photobioreactor; Cyanobacteria; Biofuel.

NOMENCLATURE

A	PBR cross sectional area
APBR	Agitated photobioreactor
b	width of magnetic agitation bar
Ch_a	chlorophyll a concentration
d	length of magnetic agitation bar
D	PBR diameter
DPBR	draft tube airlift photobioreactor
g	gravity
K_v	kolmogorov length scale
n	cellular concentration
N	rotational speed
N_P	Power number
OD	optical density
P	power input
PBR	Photobioreactor
Q	airflow rate
U	fluid mean velocity
U_g	superficial gas velocity
V_{PBR}	PBR volume (l)
α	exponential phase growth rate
β	coefficient in Eq. (4)
γ	average shear rate
μ	dynamic viscosity
ρ	density
τ	average shear stress
ω	agitator rotational speed

1. INTRODUCTION

The demand of energy production relying on renewable sources has become a global need. Biofuels offer a transition towards a world of renewable energy supply and production. The

interests in developing a third generation of biofuels produced from microalgae and cyanobacteria, which are generally efficient in converting solar energy into biomass have clearly increased (Chisti *et al.*, 2007; Schenk *et al.*, 2008; Converti *et al.*, 2009; Jacob-Lopes *et al.*, 2009). This new technology has far less impact on the environment and on the world's food supply than conventional methods of biofuel production from crops. Microalgae and cyanobacteria have greater caloric values and lower viscosity and density compared with crops used in 1st and 2nd generation biofuels, which make them more suitable for biofuel production than lignocellulosic materials (Miao and Wu, 2004). They also have a higher lipid content and semi-steady state production; they can grow in various climates (Subhadra, 2010). Since the cost-effective production of biofuels at large scale remains a major challenge, constructing easily implementable culturing systems with high production efficiency is essential for controlling the growth of photosynthetic microorganisms. However, all technological processes dealing with biofuel production from photosynthesis microorganisms involve aqueous suspensions of these creatures, known as active fluids.

Active fluids face the science of fluid mechanics to a historical challenge. Active fluid refers to the fluids of self-propelled particles such as bacteria or algae. They display properties that differ fundamentally from the passive fluids. Contrary to the conventional fluid flows in which one needs gradients of pressure, velocity and temperature to break equilibrium and drive the flow, in active fluids, cells as the microstructural elements of the fluid convert the chemical energy of nutrients into mechanical energy for driving the flow. Therefore, by using their molecular motors to activate their flagella (or pilis), microorganisms in active fluids can develop complex spontaneous fluid motions in the absence of external gradient.

Needs for knowledge build-up on active fluids goes far beyond the technological bioprocesses. Large numbers of microorganisms are suspended in temperate aqueous environments. A great collection of widely varied microorganisms is hosted in oceans, rivers, ponds, droplets, and the internal fluid of mammals. Their presence is not easily obvious, but they constitute the major part of the world's biomass (Pedley and Kessler, 1992). Microorganisms are present everywhere in nature and originally appeared on the Earth 3.8 billion years ago compared to human existence of only a few million years. It is estimated that approximately 5×10^{30} bacterial cells exist on the Earth.

One of the interesting, but also intriguing character of active fluids is the fact that the flow interacts with its constituent microstructural elements, the microorganisms, which undergo biomechanical transformations and then in turn change the nature of the active fluid. In this sense, active fluids resemble the rheologically complex fluids, with the difference that the rheological mechanism in active fluids has the capacity of analysis and action, though rudimentary. Among the important flow-microorganism interactions in active fluids is the effect of hydrodynamic stresses on microorganisms, which is the focus of this work.

The intensity of hydrodynamic stress in the flow can strongly contribute to the growth of microorganisms (Bronnenmeier and Märkl, 1982; Grima *et al.*, 1997; Camacho *et al.*, 2000; Chisti and Jauregui-Haza, 2002; Sobczuk *et al.*, 2006; Dragone *et al.*, 2010; Kaiwan-Arporn *et al.*, 2012; Leupold *et al.*, 2013). In photobioreactors (PBR) used for production of biofuels this stress is usually applied by stirring in agitated photobioreactors (APBR) or by bubbling air in draft tube airlift photobioreactors (DPBR). It helps to prevent cells settling, avoids thermal stratification, and distributes nutrients. It also breaks down diffusion gradients at the cell surface to remove photosynthetically generated oxygen and to ensure that the cells experience alternating light/dark periods. Some hypotheses suggested that shear stress induced in different PBR types, like mechanical agitation in stirred tanks and aeration or bubbling in airlift and bubble column PBRs would increase the growth rate of some types of microalgae and cyanobacteria, probably due to the improved access of the cultured cells to nutriments, CO_2 and light. However, at critical values of shear stress, the yield starts to decrease as a function of agitation intensity. Although mixing can improve the biomass production, excessive mechanical agitation in the presence of aeration can cause severe damage to the cells since it decreases the size of gas bubbles rupturing at the cellular surface that is more damaging.

The complexity of active fluids comes also from the fact that the effects of hydrodynamic stress are not limited to the growth of microorganisms. Stimulating effects on the physiological processes within the cells, mainly on the photosynthetic activity have been also reported. The photosynthetic activity has been studied by measuring the variation of pigment content; and accumulation of the pigments in the biomass has been presented as an indicator of physiological state of the culture (Wellburn, 1994; Lichtenthaler and Buschmann, 2001; Mirón *et al.*, 2002; Pápista *et al.*, 2002; Macias Sanchez *et al.*, 2005; Ritchie, 2006; Henriques *et al.*, 2007; Touloupakis *et al.*, 2015; Zavřel *et al.*, 2015).

Most of the existing work highlighted the effects of shear stress at a certain phase of the growth cycle, mostly during the stationary phase and not during the whole growth cycle. However, from the point of view of process analysis, the role of hydrodynamic stress should be determined during all stages of the microorganism growth. Moreover, only a few studies have addressed the effects of shear stress on pigment content evolution; effects of shear stress on chlorophylls and carotenoids concentrations during the growth cycle are yet to be investigated. This work aims to investigate, for the first time to the best of our knowledge, the effects of shear stress generated by both stirring and bubbling in APBRs and DPBRs respectively, on cultivation process of the cyanobacterium *Synechocystis*. The results of this kind of studies contribute to the construction of constitutive laws to be integrated in the governing

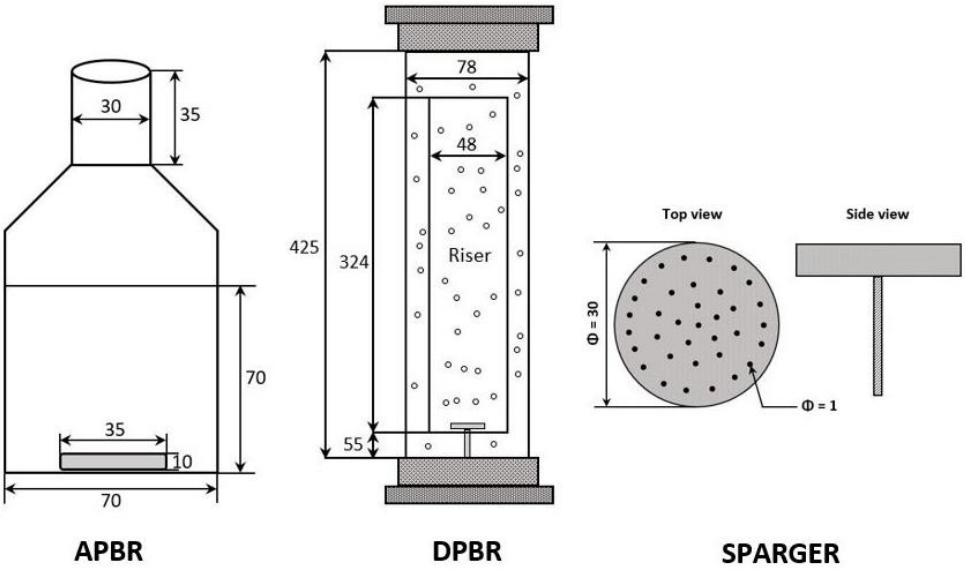


Fig. 1. Schematic representation showing from left to right geometries and dimensions of the APBR, DPBR and an airjet sparger (mm).

equations of active fluids. The paper is organized as follows: section 2 describes the microorganism, the geometry of photobioreactors and their operating conditions, the methods applied for growth and pigment measurements, and finally the models and correlations used for calculation of hydrodynamics shear stress inside each bioreactor. The results are presented and discussed in section 3; then the concluding remarks are summarized in section 4.

2. MATERIALS AND METHODS

2.1 Microorganism and Culture Medium

A strain of *Synechocystis sp.* called PCC 6803 (Pasteur Culture Collection of Paris) was used in these experiments. *Synechocystis* is a prokaryotic unicellular freshwater cyanobacterium of 3-7 µm cellular diameter (Vourc'h *et al.*, 2018). It is capable of both phototrophic growth by oxygenic photosynthesis during light periods and heterotrophic growth by glycolysis and oxidative phosphorylation during dark periods. It can grow under photoautotrophic conditions, mixotrophic conditions with glucose as a carbon source and heterotrophic conditions with a daily short pulse of light (Lopo *et al.*, 2012). Quantitative hydrocarbon yields of the unicellular *Synechocystis*, as percent dry weight of biomass, ranges from 0.03 % to 0.09 % (Coates *et al.*, 2014).

The *Synechocystis* suspension was maintained in BG11 culture medium containing $NaNO_3$, 15 g; K_2HPO_4, 0.3 g; $MgSO_4$ $7H_2O$, 0.75 g; $CaCl_2$, $2H_2O$, 0.36 g; citric acid, $1H_2O$ 0.063 g; EDTA, 2Na, $2H_2O$ 0.009 g; Na_2CO_3, 0.4 g; $(NH_4)_2$ Fe $(SO_4)_2$, $6H_2O$ 0.12 g; 1 ml of metal traces solution and 1 l of Milli-Q water.

2.2 Photobioreactors

Two kinds of photobioreactors were used: an agitated photobioreactor (APBR) and a draft tube airlift bubble column photobioreactor (DPBR). The configurations of photobioreactors and their dimensions are presented in Fig. 1. The APBR is a cylindrical flat-bottomed vessel fabricated from Pyrex glass. Four identical vessels of 70 mm inner diameter were fabricated and filled with microorganism suspension in such a way that the aspect ratio of the liquid free surface height over vessel's diameter was 1. Magnetic agitation bars of diameter d = 35 mm and width b = 10 mm were used for stirring the suspension in the vessels that are placed on digitally controlled magnetic agitators. Three different rotation speeds "ω" (360, 540, 900 rpm) were used for the APBR with one non-agitated vessel that serves as a reference unit.

Regarding the DPBR, it consists of two concentric transparent Polymethylpentene (PMP) cylinders of 3 mm wall thickness and 2 l working volume. The external cylinder diameter is D = 78 mm while the internal cylinder, called draft tube, is of 48 mm diameter and 324 mm height. The height of liquid free surface was 425 mm. The working fluid is mixed with air sparging through perforated cylindrical stainless steel jet-spargers (40 holes of 1mm diameter) as shown in Fig. 1. Seven different airflow rates "Q" (0.05, 0.2, 0.5, 1, 2, 3.5 and 5 l/min) were used.

For all the experiments, *Synechocystis sp.* was cultured over a period of 3-4 weeks starting with an initial dilution factor of 1/300 ($ml_{suspension}/ml_{total\ volume}$) under a homogenous light distribution of 6 µmole/m^2/sec (450 lux) intensity and 20 ºC surrounding temperature.

2.3 Growth Measurements

The cellular concentration was determined spectrophotometrically (1 cm light path) by measuring the culture absorbance at a wavelength of

580 nm using a lambda 25 UV/VIS PerkinElmer spectrophotometer. Then using a microscopic cell counter, the cellular concentration was measured for different concentrations, and correlated with corresponding optical densities.

A calibration curve established the following dependence between the optical density (OD_{580}) and the cellular concentration n (cell/ml):

$$n = 25\times10^{6}\times OD_{580} \tag{1}$$

2.4 Pigment Measurements

The spectrophotometric method of *Lichtenthaler* was used for estimating the pigment content in the cells (Lichtenthaler and Buschmann, 2001). A culture volume of 1 ml was centrifuged at 13000 rpm for 10 minutes to recover the cells. The biomass left in the tube after extracting the culture medium was re-suspended in an ethanol-water mixture (1 ml, 95% v/v ethanol) to extract the pigments. Then, the tube was ultrasonicated and kept in dark at 45ºC for 45 minutes before being centrifuged again in order to finally recover the supernatant and measure its optical density in a 1 cm light path quartz cuvettes at 470, 648.6, and 664.1 nm wavelengths. If the absorbance value exceeded 0.8, the sample was diluted with the solvent to bring the measurement within the range. It is reported that *Synechocystis* is a strain that does not contain chlorophylls *b* like most of the cyanobacterium species (Ritchie, 2006; Zavřel *et al.*, 2015); therefore, the concentration of chlorophylls *b* is assumed to be zero in this work. Thus, the concentrations of chlorophylls *a* and carotenoids can be determined using the following correlations corresponding to 95% ethanol-water solvent (Lichtenthaler and Buschmann, 2001):

$$Ch_a = 13.36OD_{664.1} - 5.19OD_{648.6} \tag{2}$$

$$Carotenoids = \left(10^{3}OD_{470} - 2.13Ch_a\right)/209 \tag{3}$$

where Ch_a and *Carotenoids*, are the chlorophyll *a* and carotenoids concentrations (mg/l) respectively, and OD_λ is the optical density measured at the specified wavelength λ.

2.5 Average Shear Stress in APBRs

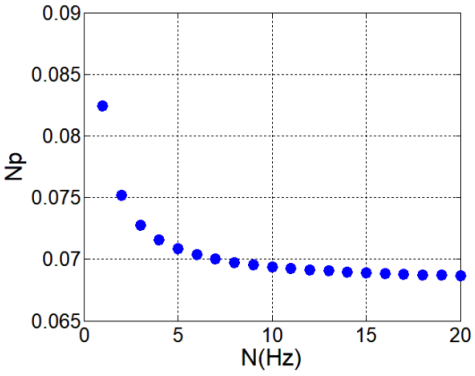
The magnitude of the average shear rate induced by agitation in APBRs is estimated using Eq. (4) that gives the shear rate γ in turbulent flows inside stirred tank as a function of rotation speed N (Hz) of the agitator, the geometrical parameters of the stirred tank and Newtonian fluid properties (Rushton *et al.*, 1950a,b; Sánchez Pérez *et al.*, 2006).

$$\gamma = N^{\frac{3}{2}}.\left(\frac{4N_p\rho d^2}{27\pi\mu}\right)^{\frac{1}{2}} = \beta.N^{\frac{3}{2}} \tag{4}$$

In this correlation γ is the average shear rate, N represents the rotation speed in Hz, d stands for the agitating bar diameter, μ and ρ are respectively the dynamic viscosity and density of the fluid. In this study, the density and the dynamic viscosity are assumed equal to those of water at 20 ºC, i.e. 1.002 x 10^{-3} Pa.s and 998.2 Kg/m^3 respectively. N_p is called "power number" and defined according to the following equation where P is the input power :

$$N_p = \frac{P}{\rho N^3 d^5} \tag{5}$$

To determine the average shear rate γ in Eq. (4), the power number is needed. It is determined using empirical correlations developed for propeller and pfaudler-type impellers in agitated reactors (Furukawa *et al.*, 2012; Kato *et al.*, 2009). The variation of N_p as a function of rotation speed N used in this experiment is showed in Fig. 2(a). Then, the coefficient β in Eq. (4) is calculated for different values of N_p corresponding to each rotation speed N, and eventually the average shear rate γ is determined. The corresponding average shear stress ($\tau = \mu\gamma$) is calculated and plotted as a function of the rotation speed N in Fig. 2(b).



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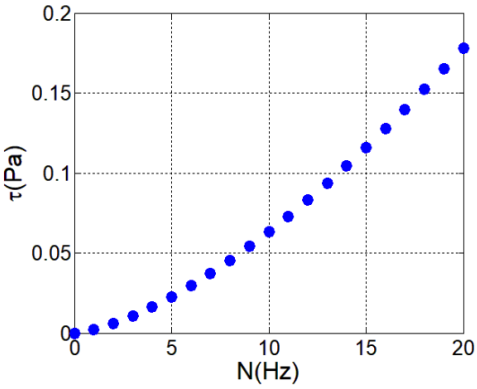


(b)

Fig. 2. Power number as a function of rotation speed (a). Average shear stress τ induced in the agitated reactors as a function of rotation speed (b).

2.6 Average Shear Stress in DPBRs

In airlift PBRs the pneumatic power input provided for isothermal expansion of the sparged gas is the source of agitation. Thus, the average shear rate depends on the superficial gas velocity U_g and the

rheological properties of the fluid (Metz *et al.*, 1979; Sánchez Pérez *et al.*, 2006). The specific energy dissipation rate depends on the average shear rate γ and the average shear stress τ inside the PBR:

$$\frac{P}{V_{\mathrm{PBR}}} = \tau\gamma \tag{6}$$

where P is the power input and V_{PBR} is the working volume of the fluid in the PBR. Knowing that $\tau = \mu\gamma$ for Newtonian fluids we obtain:

$$\frac{P}{V_{\mathrm{PBR}}} = \mu\gamma^2 \tag{7}$$

Thus, the shear rate γ can be determined by:

$$\gamma = \left(\frac{1}{\mu}\frac{P}{V_{\mathrm{PBR}}}\right)^{\frac{1}{2}} \tag{8}$$

The power input per unit volume of the working fluid is related to the superficial gas velocity as follows:

$$\frac{P}{V_{\mathrm{PBR}}} = gU_{\mathrm{g}}\rho \tag{9}$$

where U_{g} is the superficial gas velocity. Substituting Eq. (9) in Eq. (8) gives:

$$\gamma = \left(\frac{1}{\mu} g\,\rho U_{\mathrm{g}}\right)^{\frac{1}{2}} \tag{10}$$

Thus, the average shear stress τ is determined from Eq. (11).

$$\tau = \left(\mu g\,\rho U_{\mathrm{g}}\right)^{\frac{1}{2}} \tag{11}$$

The fluid properties are assumed to be those of water. Thus, the average shear stress τ in the airlift PBR as a function of the superficial gas velocity U_{g} can be expressed as:

$$\tau = 3.13 U_{\mathrm{g}}^{\frac{1}{2}} \tag{12}$$

The relation between U_{g} and the airflow rate Q is based on the definition of the superficial gas velocity (Kantarci *et al.*, 2005; Saravanan *et al.*, 2009):

$$Q = U_{\mathrm{g}}A \tag{13}$$

where $A = \pi D^2/4$ represents the cross-sectional area of the PBR. Using Eq. (12) and Eq. (13), where D is equal to 78 mm, the average shear stress as a function of airflow rate can be estimated by Eq. (14).

$$\tau = 0.185 Q^{0.5} \tag{14}$$

where Q and τ are respectively in l/min and Pa. This correlation is plotted in Fig. 3.

Comparing the average shear stress range applied in APBRs (see $N \leq 15$ Hz in Fig. 2(b)) and that applied in DPBRs (see $Q \leq 5$ l/min in Fig. 3), shows that the values of shear stress obtained in APBRs correspond to those obtained for flow rates lower than 0.5 l/min in DPBRs. In other words, using the airflow rates greater than 0.5 l/min in the DPBRs we can investigate higher shear stresses compared to the APBRs.

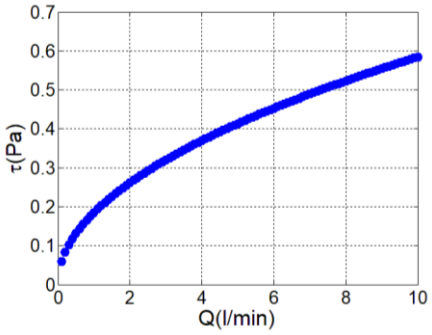


Fig. 3. Average shear stress induced in air lift reactors as a function of the airflow rate.

3. RESULTS AND DISCUSSIONS

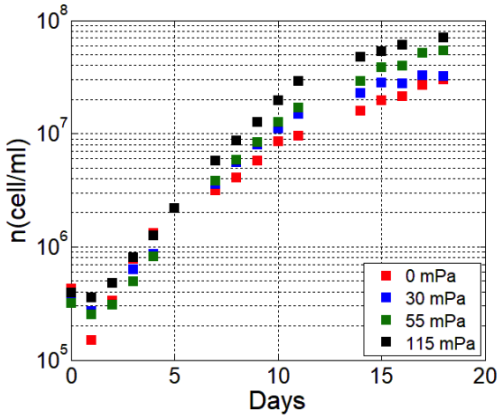
For different shear stress intensities applied in the form of stirring and bubbling air, the growth of *Synechocystis sp. PCC 6803* is monitored with time; different growth phases are distinguished and the variations in the growth behavior are identified. Moreover, the evolution of pigment content was determined by monitoring the concentrations of chlorophyll *a* and carotenoids during the growth under different shear stress conditions. The results are presented and discussed in this section.

3.1 Shear Stress Effects on Growth Rate of Synechocystis

Optical densities at 580 nm wavelength were measured daily, then by using the Eq. (1) the cellular concentrations, n in cell/ml, were estimated for each corresponding value of optical density. The results are presented for both APBR and DPBR in Fig. 4. In the first week of growth, a linear relation between n and time has been observed for all samples when the concentration is presented in semi logarithmic scale versus time. This corresponds to the exponential growth phase, followed by a quasi-stationary phase where the growth slows down and the concentration tends to a constant value after two weeks approximately.

The exponential growth phase corresponds to the highest cellular division rate of the microorganism. The exponent of the curve, fitted on the experimental data of this phase, is called exponential growth rate and is represented by "α". The exponential growth rate of *Synechocystis* is determined from Fig. 4 and plotted in Fig. 5 as a function of shear stress corresponding to different rotation speeds and airflow rates applied in APBRs and DPPRs respectively. The variation of α with shear stress intensity, plotted in Fig. 5(a), shows that values of τ lower than 30 mPa in APBR appear to be enough to increase the growth rate by ~ 50% compared to non-agitated case. While there is a significant difference

between growth rate in the presence and absence of agitation; the intensity of agitation seems to have no major effect on α (varies between 0.45 and 0.48 day^{-1}, thus 6.5%) when the applied stress increases from 30 to 115 mPa. The low value of α in the non-agitated vessel observed in Fig. 5(a) can be attributed to the fact that the motility of *Synechocystis* is very low thus; it has a small diffusion coefficient.



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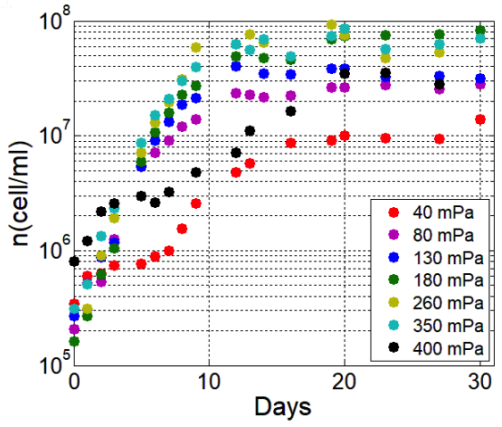
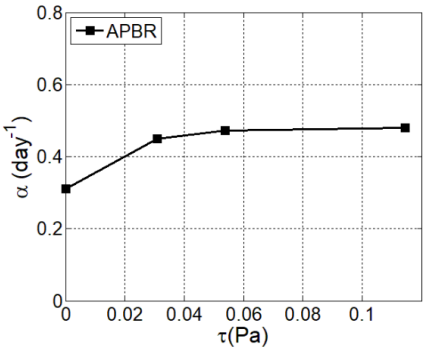


(b)

Fig. 4. Population growth n as a function of time for different values of τ induced by agitation in APBR (a) and bubbling in DPBR (b).

Therefore, the cells are accumulated and grow in colonies, which lead to the formation of large micro-scale flocculation and long macro-scale filaments. The flocculation breaks down under the effect of shear stress once agitation starts, then the cells have more access to light and nutriment and thus have a higher growth rate. However, only a slight agitation seems to be enough for this break down and any further increase in the agitation intensity does not change the growth rate. A similar behavior has been observed in DPBRs as is described in the following paragraph.

In DPBRs, Fig. 5(b) shows that increasing the shear stress intensity from 80 mPa to 180 mPa increases the growth rate by ~ 30%. Beyond this range, increasing τ up to 350 mPa had no significant effect on the variation of α; it increased from 0.6 and 0.63 day^{-1} (5 %). The increase noticed in the exponential growth rate between 40 and 80 mPa is attributed to the fact that mixing is extremely low at 40 mPa. As the airflow rate slightly increases, mixing is improved and thus the culture is mixed more evenly even though α is still considered low at these flow rates. This observation implies that the agitation generated by bubbling at low airflow rates is not enough to completely homogenize the culture in the reactor. In fact, sedimentation and flocculation of cells occur at low flow rates, similar to non-agitated APBRs where cells sediment and filaments start to form thus, α is very small. For the highest stress value, 400 mPa, we notice a drop in α. This can be attributed to the fact that extremely high intensities of shear stress may damage cells and consequently the cellular structure. It can also be argued that the cells do not have enough access time to light and nutriments in the case of high bubbling intensity.



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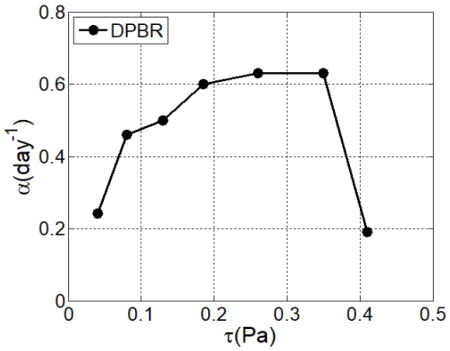

(b)

Fig. 5. Variation in exponential phase growth rate α as a function of average shear stress τ induced by agitation in APBR (a) and bubbling in DPBR (b).

These results obtained for both systems (APBR and DPBR) suggest that *Synechocystis* is highly resistant to shear, and the effects of shear stress are limited to the breakdown of the cell colonies; once they are broken down any further increase in shear stress has no significant effect on their growth rate. This behavior can be explained by the fact that *Synechocystis* has a small cellular size (3-7 µm), which is smaller than the Kolmogorov length scale "K_v" of the flows tested here (K_v between 18 and 40

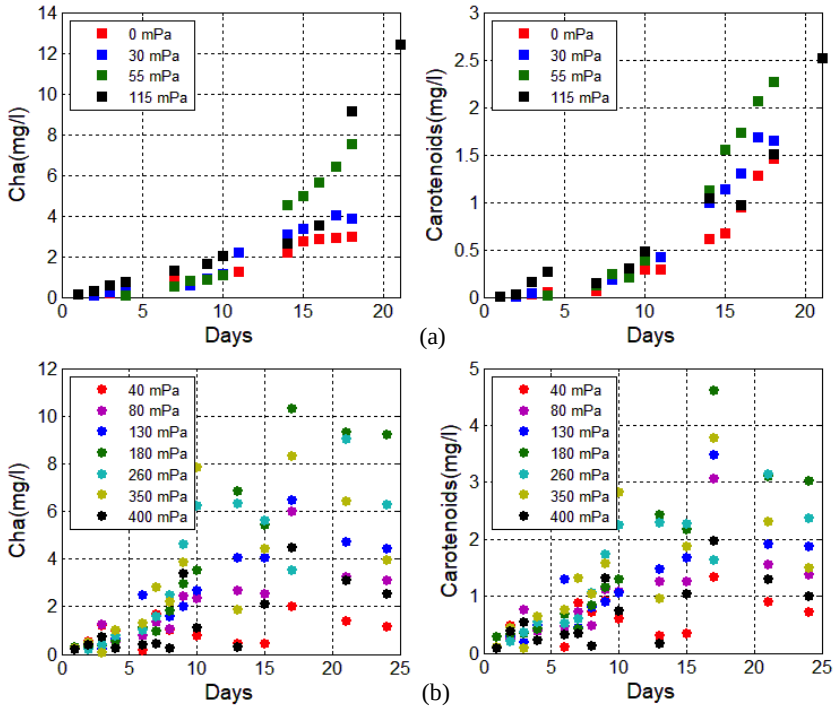

Fig. 6. Variation in concentration of chlorophyll a (left) and carotenoids (right) with time for different shear stress values in APBR (a) and DPBR (b).

μm). It should be reminded that according to the energy cascade model in turbulence the Kolmogorov length scale, $K_v \sim (\frac{\nu^3 d}{U^3})^{0.25}$, is the order of smallest eddies in a turbulent flow at which viscosity dominates and the turbulent kinetic energy is dissipated into heat. Another argument that may explain the high resistance of *Synechocystis* against shear stress is the absence of flagella, which is usually the most fragile and shear sensitive organ of the motile microorganisms.

3.2 Shear Stress Effects on Pigment Evolution during Synechocystis Growth

In the experiments on the evolution of chlorophylls and carotenoids as a function of shear stress during the growth cycle of *Synechocystis*, we followed the protocol of pigments measurement described in 2.4.

Using Eq. (2) and Eq. (3) the concentrations of chlorophyll *a* and carotenoids are determined as functions of time for different shear stress intensities in both APBRs and DPBRs. Results are plotted in Fig. 6.

For APBR, Fig. 6(a) shows that the increase in the pigment content does not vary linearly with time. For shear stresses of 0.30 and 55 mPa, the concentrations of both chlorophyll *a* and carotenoids increase slightly as time increases between day 2 and day 10 (which corresponds to the exponential growth phase); the concentration ranges from 0.1 to 1 mg/l for chlorophyll *a* and from 0.1 to 0.5 mg/l for carotenoids. Beyond that the concentration starts to increase sharply with time until reaching its peak at day 18 where the concentration increases from 1 to 6 mg/l for chlorophyll *a* and from 0.5 to 2.5 mg/l for carotenoids. For shear stress of 115 mPa, the same pattern is seen but with different time ranges (probably because the shear stress intensity of this unit is very high compared to the others): a smooth increase was noticed from day 2 up to day 14, beyond which the slope increases largely between day 14 and 21. It is also noticed from Fig. 6(a) that the chlorophyll *a* and carotenoids concentrations increase with τ. Pigments concentrations for the 115 mPa unit surpass the concentrations obtained for the other units and reach the highest concentrations after 21 days. Similarly, the concentrations for the

55 mPa unit are higher than those of the 30 mPa and non-agitated units, with higher concentrations between day 10 and day 18. This behavior is explained by the fact that light attenuation inside the PBR is directly related to the biomass concentration increase, and follows a non-linear pattern with time similar to that of the cell concentration evolution.

For the DPBR, it can be noticed from Fig. 6(b) that the increase in the pigments content starts smoothly during the first week then, the concentrations of both chlorophyll *a* and carotenoids increase slowly during the exponential growth phase. At this stage, the concentration ranges between 0.1 and 1.5 mg/l and 0.1 and 0.5 mg/l for chlorophyll *a* and carotenoids respectively; then the concentration starts to increase rapidly with time. As the growth reaches the stationary phase, the concentration reaches values between 5 and 8 mg/l for chlorophyll *a* and from 0.5 to about 2.5 mg/l for carotenoids. It is difficult to

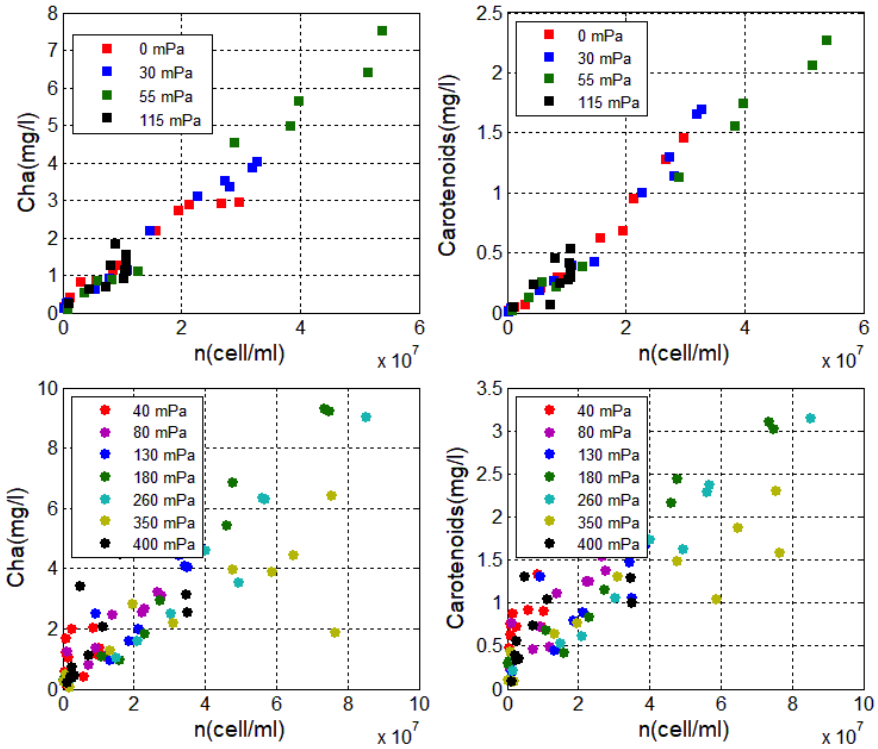

Fig. 7. Variation in the concentration of chlorophyll a (left) and carotenoids (right) as a function of cellular concentration for different shear stress values in APBR (a) and DPBR (b).

quantify the effect of shear stress generated by bubbling on the evolution of pigments concentration. However, it can be seen that the amplitudes of chlorophyll *a* and carotenoids curves increase as τ increases from 40 to 180 mPa at which the highest concentration is measured. Beyond 180 mPa, the concentrations slightly decrease for 260 and 350 mPa and suddenly drop for 400 mPa. If these results are compared to the growth curves obtained in Figs. 4(b) and 5(b) it can be noticed that the highest growth rate was obtained between 180 and 350 mPa which is also the case for chlorophyll *a* and carotenoids concentrations. Moreover, at 40 and 400 mPa the growth rate was minimal due to sedimentation resulted from poor mixing and due to high bubbling intensity respectively. Thus, it can be concluded that the shear stress has a similar effect on both pigments content and growth rate.

For some DPBR units, especially the ones with low shear stress intensity, the measured values look scattered. This is attributed to the fact that at low airflow rates mixing is not efficient thus, the culture is rather inhomogeneous due to sedimentation. This leads to sampling problems; the samples taken are not often representative of the whole reactor. For these reasons samples were taken from different points in the reactor (upper, middle and bottom part) and the average values were used.

Concentration variations of chlorophyll *a* and carotenoids with respect to the culture's cellular concentration are plotted in Fig. 7. The results show that for all experiments conducted in both types of PBRs, the concentrations of chlorophyll *a* and carotenoids increase almost linearly with the cellular concentration (except for two airflow rates because of the reason mentioned in the previous paragraph). Increasing the cellular concentration leads to a decrease in the light intensity inside the PBR (photo-limitation) and thus the cells respond by increasing the pigments concentrations (carotenoids and chlorophyll *a*) trying to harvest most of the available light especially for those who are in the reactor bulk (Grima *et al.* 1997). This linear correlation between the pigment concentration and the cellular concentration seems to be almost independent from the agitation intensity. This implies that agitation affects the pigments and the cellular concentration of *Synechocystis* in the same way.

4. CONCLUSIONS

Among the important flow-microorganism interactions in active fluids is the effect of hydrodynamic stresses on microorganisms. Effects of shear stress on the cultivation process of *Synechocystis* were studied when two types of agitation, stirring in agitated photobioreactors (APBR) and bubbling air in draft tube airlift photobioreactors (DPBR), are respectively applied. The results obtained from both types of bioreactors confirmed similar effects of shear stress in the range of 0 and 400 mPa.

Experiments carried out in APBRs, where stirring is

the main mechanism of mixing, revealed that *Synechocystis* was highly resistant to shear stress. The effects of shear stress on *Synechocystis* were limited to the breakdown of the cell colonies. A gentle agitation generating a shear stress intensity less than 30 mPa was enough to increase the exponential growth rate, α, by ~ 50% compared to non-agitated cases; increasing the agitation intensity (up to $\tau \sim 120$ mPa in shear stress) had no significant effect on α. This behavior is attributed to the low motility of *Synechocystis*, absence of flagella, and also its small size (3-7 μm) which is smaller than the Kolmogorov length scale of the tested flows; the cells were not significantly affected by the generated turbulence.

Effects of shear stress induced by bubbling inside DPBR confirmed the high resistance of *Synechocystis*. This time, the maximum applied shear stress was about 400 mPa; i.e. more than three times higher than that applied in APBR experiments. Initially, the exponential growth rate α increased by ~ 30% as τ increased between 80 mPa and 180 mPa. Beyond these values no significant change in α takes place as long as the airflow rate is sufficient (Q = 1 l/min or $\tau \sim 180$ mPa) to provide appropriate mixing conditions. Only at extreme shear stress intensities ($\tau \sim 400$ mPa) a sharp drop in α was observed, probably due to cells damage at such high sheer stresses. These results show that once the shear stress intensity is high enough to homogenize the suspension (break down colonies), no significant variation in α is noticed, suggesting that *Synechocystis* is highly shear resistant specie.

In addition to these observations, the variations in the pigments concentrations as a function of time and agitation intensity were also measured for *Synechocystis*. Chlorophyll *a* and carotenoids concentrations increase almost linearly with the cellular concentration. The independence of this linear correlation from the agitation intensity implies that the shear stress affects both the pigments and the cellular concentration of *Synechocystis* in a similar manner.

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