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Hydrodynamics as a Tool to Remove Biofilm in Tubular Photobioreactor

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Biofilm formation is one of the crucial limiting factors which influences the microalgae production because sufficient light conditions for microalgae cultivation are required. One of the possibilities, how to prevent biofilm formation, is to properly set-up hydrodynamic conditions. Generally, when a minimum value of wall shear stress at the internal surface of the illuminated area is reached, then biofilm can be removed. Studies of the relationship between hydrodynamics and biofilm removal for tubular photobioreactor system were performed in the laboratory stand for biofilm removal under flowrate in the range 1 – 40 L min^{-1} in DN20 glass/PMMA tube. The biofilm removal was observed for the wall shear stress values ranging between units and low multiples of tens of Pascal. These novel results are recommended to be respected during photobioreactor´s operation to avoid biofilm formation.

1. Introduction

The problematics of biofilm removal has an essential issue in microalgae cultivation systems – photobioreactors for biofuels. The biofilm is usually visible as a slimy layer of sediments; its thickness ranges from units up to hundreds of micrometres. Generally known, biofilm formation can reduce the efficiency of light illumination into the culture medium. Huang et al. (2016) reported that it is not possible for light to penetrate through the microalgal biofilm formed on the illuminated surface of the photobioreactor when the biofilm density exceeds 40 g m^{-2} of photobioreactor irradiated area. In other study presented by Schnurr et al. (2014), algal cells in culture medium receive only 20 µmol m^{-2} s^{-1} at the light intensity of 100 µmol m^{-2} s^{-1} and biofilm thickness of 150 µm. From this information follows that biofilm causes a limitation of light penetration into the system, decreases the photosynthetic efficiency of the cultivation system and biomass productivity. As highlighted by Zeriouh et al. (2017), biofouling also leads to a series of further undesirable phenomenon including degradation of the culture's cell pigmentation, contamination by microbes and bacterium, and incrustation on the photobioreactor surface. Finally, Cicci et al. (2014) stated that sufficient available light intensity is the essential process parameter for the growth of microalgae.
Therefore, biofilm removal on the inner walls of closed photobioreactors is one of the most necessary steps for the improvement of the performance of the microalgae bioprocess in photobioreactors.

1.1 The process of biofilm formation

The formation of biofilm is influenced by material, adhesion, algae strains and their physicochemical properties, the chemical composition of the medium, by the geometry of the photobioreactor and hydrodynamics (Barros et al., 2019). The force effects on the microalgae cells is a crucial factor for biofilm formation. It means a force balance between gravitational forces, buoyancy forces, adhesive forces, and drag forces. Generally known, the adhesive forces are dependent on the surface properties of the material and parameters of the fluid medium. Drag forces are dynamic forces, which depend on hydrodynamic conditions. So, the disbalance among these forces can be reached by changes in hydrodynamic conditions that reflect variations in wall shear stress. Detailed information about the process of the biofilm formation can be found in studies presented by Boelee (2013).

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1.2 The examples of biofilm removal techniques

The elimination of biofilm on the illuminated surface of photobioreactor can be realised by optimising factors, that also support biofilm formation. There are plenty of methods how to remove biofilm, for example by the choice of the appropriate material (Burns, 2014), by geometry (Schott, 2017), by suitable hydrodynamics (Grobbelaar, 2010), mechanically cleaning (Silva et al., 2015), ultrasonic technology (Hielscher, 2019) and others (Nawar et al., 2017). The based on the data synthesis from previous information, it can be stated, that biofilm removal techniques can be generally divided into three groups, i.e. to mechanical method, mutual optimisation in geometry and hydrodynamics, and innovative physical and chemical techniques, see Table 1.

Table 1: Overview of biofilm removal techniques

Technique	Principle	Advantages	Disadvantages
Mechanical	abrasive or erosive wear of biofilm due to its shearing by inert solid materials, (see Figure 1)	- effective - widely used	- addition of particles - interruption of operation - the scratch of the irradiated surface
Optimisation in geometry and hydrodynamics	achievement of suitable hydrodynamic conditions to reach cohesiveness of biofilm	- without interruption of the cultivation process	- energy demand to reach intensive turbulence
Innovative physical and chemical	principles like ultrasonic cleaning, (see Figure 2), using of ozone, use chemicals	- intensive	- high cost - high energy demand - complicated design

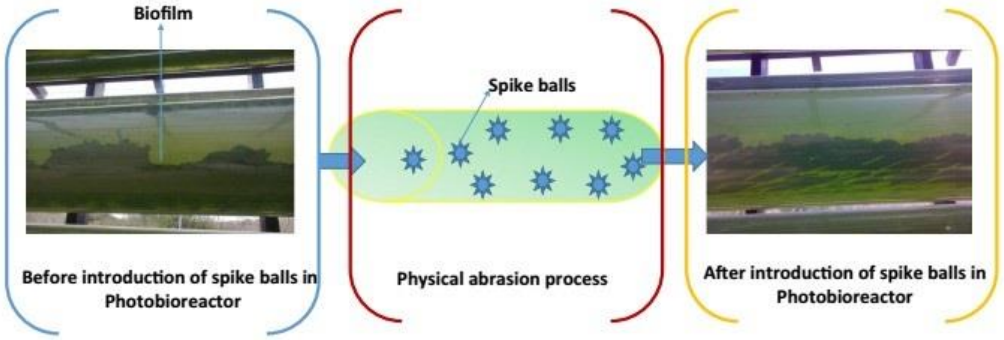

Figure 1: Effect of spike balls (Nawar et al., 2017)

Figure 2: Ultrasonic cleaner (Hielscher, 2019)

As Table 1 shows, there is a definite option for geometry and hydrodynamic methods for photobioreactor cleaning and biofilm removal. Biofilm removal by hydrodynamics is a simple method, and if it is well designed by suitable geometry, the effectiveness and functionality are ensured. Except this, the most important parameter of this method is to find an appropriate value of wall shear stress. However, there are two limitations. If the value of shear stress is over 80-100 Pa, microalgal cells are negatively affected or damaged (Wang et al., 2016).

On the other hand, when the value is too low, the biofilm still adheres to the surface. Therefore, a suitable amount of shear stress is a necessary process parameter. Thus, this work aimed to verify the possibility of hydrodynamics application to biofilm removal and to quantify the values of hydrodynamic parameters.

2. Methods

This experimental study was conducted in the form of two stages of experiments, where the biofilm formation and the biofilm elimination were monitored. The critical parameter was the influence of the liquid flowrate on the biofilm stability and the corresponding wall shear stress. The first experiments were focused on the cultivation of the biofilm and the second one was focused on the biofilm removal in different ranges of flowrate.

2.1 The experimental setup

2.1.1 Biofilm formation

This approach was realised in the model system of cultivation – tubular laboratory system in which microalgae *Chlorella vulgaris* was cultivated. The apparatus consisted of transparent tubes with a diameter 20 mm and a length of 0.5 m and a volume of 80 ml. The material of the tubes was glass and PMMA. The tubes were aerated by air from compressor using a stainless-steel needle with a hole of 3 mm diameter because microalgae cells receive CO_2 and nutrients for their growth. Moreover, the oxygen was also removed from the system and accumulation was avoided. The tubes were exposed to artificial lighting – the LED panel was used for artificial illumination. The photosynthetic photon flux density (PPFD) of LED panel was 100 µmol m^{-2} s^{-1}, the luminance: 5,400 lm and the colour temperature of a light source was 4,000 K, what represents "cool colours". This value corresponding to the wavelength about 500 nm and only solar irradiation within the range of 400 and 700 nm is the absorption spectrum for the chlorophylls, and the algal photosynthesis runs in this range with the high efficiency (Suh and Lee, 2003).
An inoculum of *Chlorella vulgaris* was used with a concentration of 3 g L^{-1}. These samples were cultivated in the tubes. The volume of the nutrient medium in the tube was 80 mL. The nutrients medium, which were used, had the following composition (per liter): 550 mg $(NH_2)_2CO$, 118.6 mg KH_2PO_4, 102 mg $MgSO_4 \cdot 7H_2O$, 19.8 mg $C_{10}H_{12}O8N_2NaFe$, 44 mg $CaCl_2$¸ 0.416 H_3BO_3, 0.472 $CuSO_4.5\ H_2O$, 1.647 mg $MnCl_2 \cdot 4H_2O$, 0.308 $CoSO_4 \cdot 7H_2O$, 1.339 $ZnSO_4 \cdot 7H_2O$, 0.086 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.007 $(NH_4)\ VO_3$. The microalgae inoculum of 2 mL was added into the cultivation tube with a nutrient medium of 80 mL. The biofilm cultivation was carried out under ambient room temperature and lasted from 7 to 10 days. The experiment was stopped as soon as fully developed biofilm on the internal tube wall was visually recognised, see Figure 3.

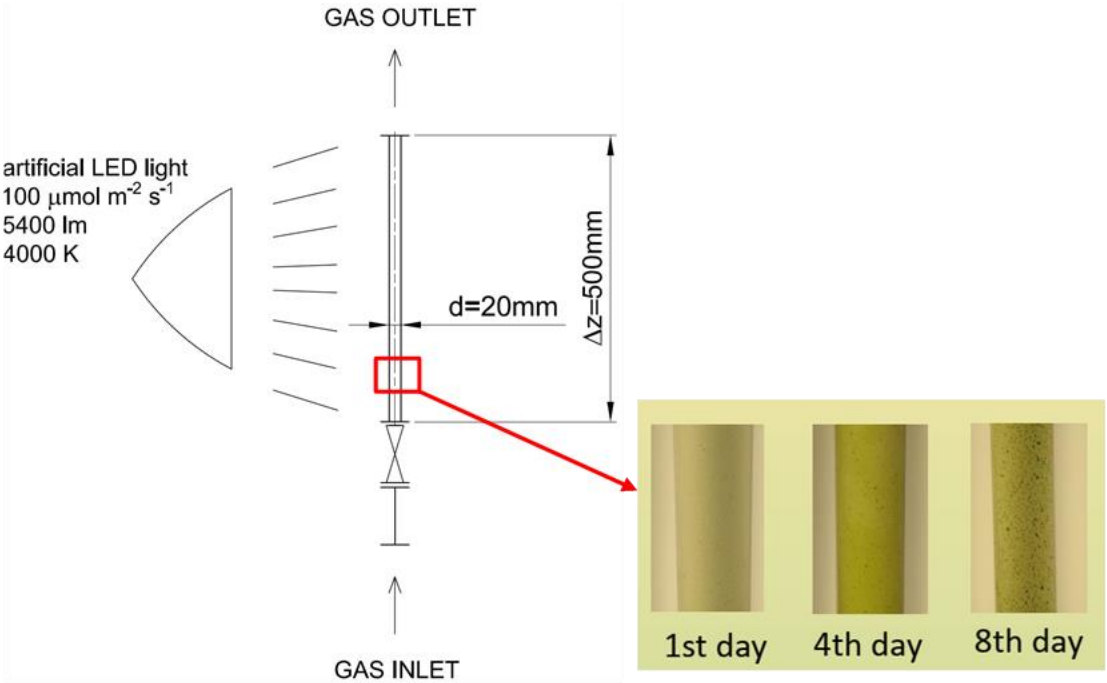

Figure 3: Removable glass or PMMA tube assembly for biofilm formation

2.1.2 Biofilm removal

The second step of the experiment was realised in laboratory equipment with the closed hydrodynamic circuit, see Figure 4. It consists of a storage vessel with the volume of 100 L filled by water, a centrifugal pump with a maximal flowrate 250 L min^{-1} and a removable tube assembly, in which biofilm was cultivated. The diameter of the whole circuit was DN20 PN6.

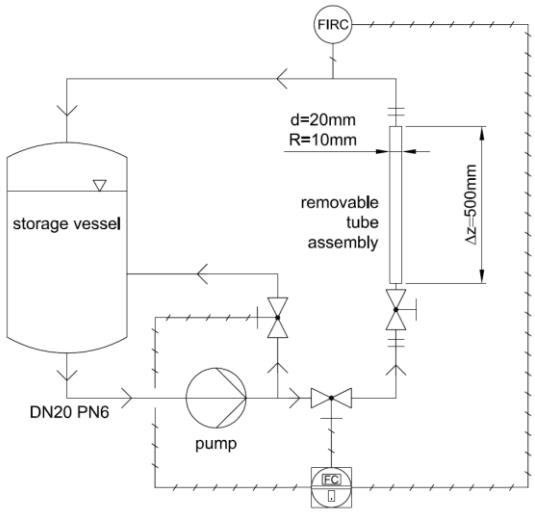

Figure 4: Laboratory stand for biofilm removal

Its working principle is based on such a procedure. Removable glass or PMMA tube assembly with the cultivated biofilm were placed to the equipment, a proper volumetric flowrate Q (L min^{-1}) of water was set up. A flowrate was set up using a set of manual control valves, 1.4–33.6 L min^{-1} for glass tubes and 18.6 to 63.0 L min^{-1} for PMMA tubes, respectively. Water flowrate was measured merely as a given volume in time. Finally, biofilm removal was visually studied to find its value at which it was entirely removed from the internal tube surface. Based on its knowledge and theoretical background, minimum wall shear stress value was determined.

2.2 Hydrodynamic conditions

The experimental data were used to determine wall shear stress value for given flowrate according to this methodology. Using the measured flowrate values, the mean volumetric liquid velocities were calculated. To establish whether the flow was laminar or turbulent, the Reynolds number was determined by:

$$Re = \frac{d\,u\,\rho}{\mu} \tag{1}$$

where d (m) is the internal tube diameter, u (m s^{-1}) is the mean volumetric velocity, ρ (kg m^{-3}) is the density of the culture medium, and μ (Pa s^{-1}) is the dynamic viscosity.
Both, glass tubes and PMMA tubes were considered to be a hydraulically smooth. The Blasius correlation was used for calculation of dimensionless friction factor

$$\lambda = 0.316\,Re^{-0.25} \tag{2}$$

The dependence between pressure loss Δp_Z (Pa) and wall shear stress τ_W (Pa) was derived based on the general momentum balance equation for flow in the tube. Resulting derived equations for pressure loss and wall shear stress are following

$$\Delta p_Z = \lambda \frac{\Delta z}{d} \frac{u^2}{2} + \rho\, g\, \Delta z \tag{3}$$

$$\tau_W = \frac{\Delta p_Z}{\Delta z} \frac{R}{2} - \rho\, g \frac{R}{2} \tag{4}$$

where Δz (m) is the height of the media level in the tube, g (m s^{-2}) is gravitational acceleration, and R (m) is the internal tube radius.

3. Results and discussion

Both experimental results of biofilm removal from glass and PMMA tubes are listed in Table 2. Biofilm removal for glass tube (Figure 5a) was reached for mean volumetric velocity 0.07 m $s^{-1,}$ and corresponded wall shear stress 0.03 Pa since the biofilm layer was too weak. This state was not representative, and it was therefore neglected in overall results. The full biofilm removal was reached for water mean volumetric velocity of 1.77 m s^{-1} and wall shear stress of 9 Pa, see Figure 5c.

Table 2: Hydrodynamic parameters for glass and PMMA

Material	Experiment	Fig.	Q (L min^{-1})	u (m s^{-1})	Re (-)	Δp_z (Pa)	τ_W (Pa)
Glass 1	1.	5b	1.4	0.07	1,440	4,900	0.03
Glass 2	1.	5d	18.6	0.99	19,850	5,220	3.3
	2.	5e	33.6	1.77	35,300	5,800	9.0
PMMA 1	1.	6b	18.6	0.99	19,850	5,220	3.3
	2.	6c	33.6	1.77	35,300	5,800	9.0
	3.	6d	42.6	2.27	45,400	6,300	14.0
PMMA 2	1.	6f	12.6	0.66	13,200	5,060	1.6
	2.	6g	18.6	0.99	19,850	5,220	3.3
	3.	6h	20.2	1.32	26,470	5,440	5.4
	4.	6j	42.6	2.27	45,380	6,300	14.0

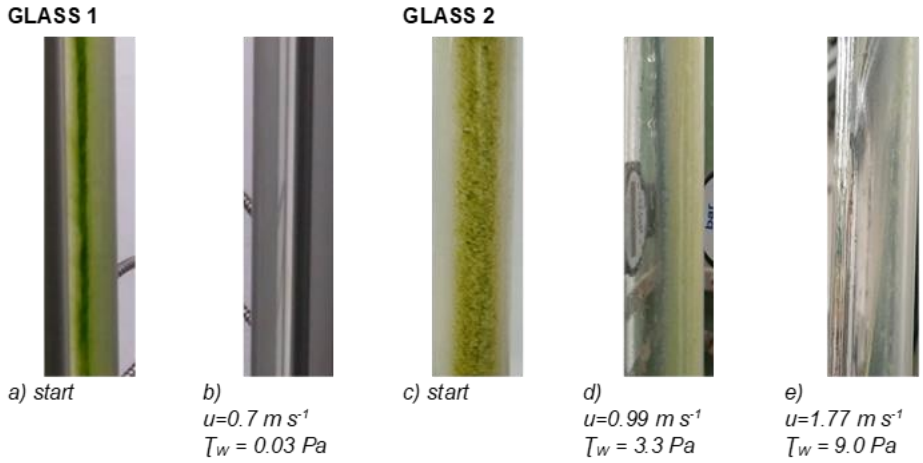

Figure 5: Process of experiments for glass tubes

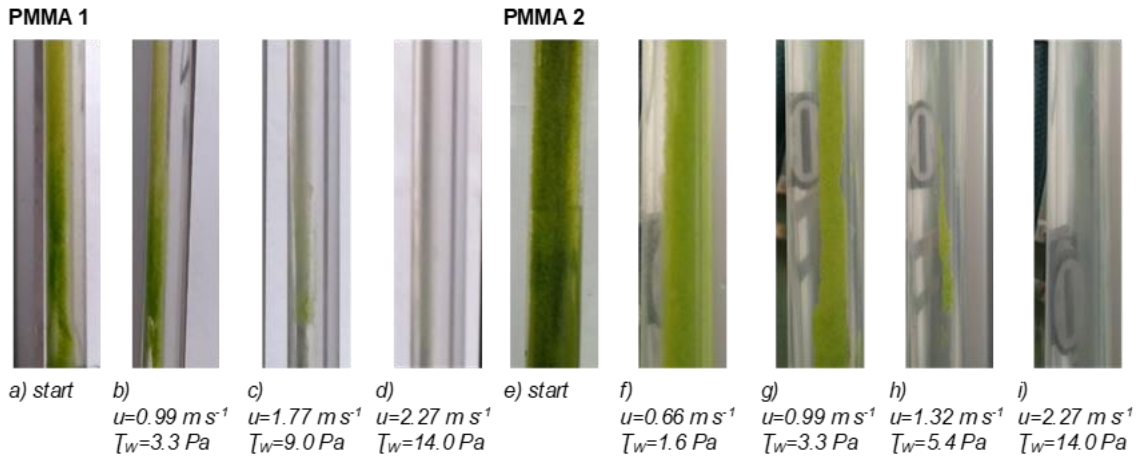

Figure 6: Process of experiments for PMMA tubes

The same hydrodynamic conditions in which biofilm was removed in a glass tube, were tested as the initial process parameters for PMMA tubes. As presented in Figure 6, no biofilm removal was recognised at these conditions. Therefore, there was a need to increase water flowrate gradually. The full biofilm removal was reached for water mean volumetric velocity of 2.27 m s^{-1} and wall shear stress of 14 Pa, see Figure 6d and Figure 6i. As Table 2 shows, measured data confirmed definitely that the hydrodynamic parameters in the case of glass are different than for PMMA - the biofilm is more accessible to remove on the glass tube, and a smaller value of shear stress on the wall is needed.

4. Conclusion

The biofilm removal is ensured by the effect of wall shear stress when units or low multiples of tens of Pascal are reached. To make the photobioreactor operation more efficient, it is possible to achieve these values only at short intervals. Thus, the mean volumetric velocity of culture medium in photobioreactor can be varied. Results of this work can be used for pilot scale and industrial systems as well. The values of the measured wall shear stresses can be adapted for further scale-up due to the transport phenomena methodology.

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