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# Different types of bubbly flows in a confined channel with the aim of limiting microalgae biofilm development – Part II: study of the development and removal of microalgae biofilm

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## Abstract

This study aims at investigating the role of bubbles passage to prevent biofouling in thin-gap bubble column photobioreactors (PBRs) where the biomass concentration is important and the reactor thickness is low. Different types of gas sparging are tested in a square channel with a cross-section of 4x4 mm<sup>2</sup> mimicking a thin-gap bubble column PBR.

Firstly, it is shown that it is very difficult to detach the biofilm once it has adhered to the wall: only the bubbling conditions with high wall shear rate fluctuations are able to detach part of the superficial layers of the biofilm, but no effect is observed on the detachment of the conditioning layer.

Secondly, bubbling generating either high shear rate fluctuations, or high mean shear stress are tested to assess their ability to delay biofilm development: both accentuate biofilm adhesion, showing that the biofilm may be compacted by highly confined bubbles.

Thus, small bubbles whose diameter (1.4 mm) is smaller than the side of the square channel are investigated, allowing bubbles oscillations and a 3D hydrodynamics around the bubble. This bubbling, which creates the most important shear rate fluctuations, is the most adapted to limit the development of biofilm in thin-gap PBR.

**Keywords:** microalgae biofilm, cleaning, bubbling, wall shear stress, thin-gap bubble column

## 1. Introduction

In nature, microorganisms can adopt two radically different lifestyles, either the planktonic mode in which free-living microorganisms float in a liquid environment, or the biofilm mode in which they form microbial aggregates embedded within a matrix of extracellular polymeric substances (proteins, lipids, exopolysaccharides, nucleic acids) and are attached to a surface. This last mode is the most common in aquatic ecosystems since it guarantees a better resistance against environmental aggressions (acids, bases, antibiotics, viruses, grazers, UV exposure, etc...). Some biofilms, composed of non-pathogenic strains and called “positive biofilms”, are used for biotechnological purposes since fixed microorganisms can have better performance, such as in fixed bed bioreactors for wastewater treatment. On another side, biofilm development may become a real problem in many fields like the medical sector or the food industry. When it is undesirable, the biofilm may be responsible for infectious diseases and may pose significant health risks. In industry, biofilms can result in losses of process performance and therefore significant economic losses, such as biofilm contamination in food processing equipments. In photobioreactors (PBRs) used for photosynthetic microorganisms’ cultivation (microalgae and cyanobacteria), biofilms can grow at the walls and be responsible for the decrease of light transmission. Thereafter, a decrease in microorganisms’ productivities is observed [1] [2]. When the biofilm development is too significant, this requires the culture interruption for cleaning, which will result in significant maintenance cost. Moreover, culture intensification by increasing biomass concentration in PBRs of reduced thickness (such as thin-gap bubble columns) will tend to intensify the development of biofilm at walls [3] [4]. For these reasons, many studies have aimed at eliminating biofilms, or at least limiting biofilms development at the equipment walls. It must be pointed out that this issue

has been mainly addressed for bacterial biofilms and that studies dealing with photosynthetic biofilms are missing.

Generally, biofilm establishment occurs in three successive steps: (i) cell adhesion, (ii) cell growth and polymer excretion and (iii) dispersal. Cell adhesion begins with the conditioning film establishment due to the contact between the support and the culture medium. This film is the result of macromolecules deposition (glycoproteins and polysaccharides) and favors the initial attachment of biomass [5]. At this stage, microorganisms start attaching to the surface with some possible influence of factors such as physicochemical characteristics of the wall material [6]. In dynamic conditions, microorganisms adhesion at walls results from the transport phenomenon to the support which is mainly governed by convection, diffusion and Brownian movements [7]. This step is called reversible adhesion. During this phase, low bond strengths are involved like Van der Waals forces and electrostatic bonds, so the occurrence of biofilm detachment from the surface is high. Then, irreversible adhesion occurs, it is characterized by the presence of multiple weak bonds and the presence of acid-base interactions. During this step, microorganisms will also secrete extracellular polymeric substances (EPS) which will favor the fixation of other cells [8]. During this second step, the biofilm development continues with the growth of already adhered cells, and the continuous supply of other microorganisms that settle at the superficial layers. Finally, partial detachment of the biofilm may occur, caused by the shear stress applied at superficial layers.

To limit the biofilm development at the wall, several authors have focused on the first stage of microorganisms adhesion, either by modifying the surface properties of the materials by surface treatment techniques or by using coatings containing bactericidal agents [7]. Other solutions consist in modifying hydrodynamics to limit biofilm development. Hydrodynamics

may be modified to reduce the convective transport of bacteria toward the surface [5]. Indeed, El Khatib [5] limited convective transport in a coaxial-cylindrical Couette-Poiseuille reactor by canceling the near-wall velocity gradient by forming a stagnant zone in the vicinity of the reactor surface. Under these hydrodynamic conditions, the bacterial transfer is only due to Brownian diffusion and sedimentation. This allowed inhibiting the biofilm growth on the outer cylinder for 48 hours compared to a configuration where the shear rate across the gap is constant. Some other authors noticed that shear stress at walls can entail biofilm detachment. Pechaud et al. [9] showed that below a shear stress value of 2.5 Pa, the detachment of the biofilm is predominant. However, by increasing this shear stress, compression and consequently consolidation of the biofilm takes place. Other authors have shown that extreme conditions of very high shear stress can cause biofilms deformation and detachment from surfaces [10]. Thus, there is some contradiction about the shear stress effect on biofilms. For some authors, wall shear stress does not prevent the formation of biofilm, but makes it thinner and denser with increasing shear strength [11] [12] [7]. In fact, hydrodynamics is a complex parameter to optimize because it can either promote the access of the microorganisms to the support, and thus promote the biofilm development or it can exert sufficient shear stress on the biofilm, resulting in detachment phenomena.

Another interesting approach of hydrodynamics modification to prevent biofilm issue is the use of non-stationary flows, such as pulsed flows. These flows can contribute to the increase of the mean and the fluctuating components of the wall shear rate [13] [14]. In bubble columns, non-stationary flows are observed, they are likely to have the same interest as pulsed flows [15] [16] [17] [18]. In conventional – unconfined – bubble columns used for photosynthetic microorganisms cultivation and with a thickness of a few centimeters, bubbles injection allows mixing and ensures gas-liquid transfers necessary for the growth of

microalgae. However, bubbling in this configuration, *i.e.* bubble columns of characteristic dimensions much larger than the diameter of bubbles, has a limited impact on walls, particularly in terms of shear stresses. In 2D confined bubble reactor [4], with a gap between the walls of the same order of magnitude as the capillary length, the effect of the bubbles is important at the walls. In a companion article, Thobie *et al.* [19], characterized the hydrodynamics generated by the injection of different bubble sizes at different flow rates in confined conditions. In airlift or bubble columns PBRs, bubbling allows avoiding the sedimentation of cells, in addition to CO<sub>2</sub> supply and O<sub>2</sub> stripping. Thus, limiting the development of biofilm by adapting bubbling conditions would be ideal. However, the application of excessive shear forces may have adverse effects on microorganisms. Indeed, the physiological state of photosynthetic microorganisms cells can be disturbed or modified [20] by factors such as flow turbulence or bubbling conditions [21] and high shear rates. This may result in the excretion of exopolysaccharides (EPS) [22] [23], physical alteration of the cells or cell destruction [24].

Despite the advantage of intensifying the volumetric productivity by increasing microalgae concentration, PBRs of low thickness present issues of biofilm development that need to be solved [3]. In the present paper, the role of bubbles to limit biofilm formation or lead to its detachment in the context of microalgae culture in thin-gap PBR is investigated. In particular, the impact of different types of gas sparging conditions (bubbles size and shape, flow rate, etc.) is tested in culture conditions to assess its effect on photosynthetic biofilm. The final goal is to propose strategies to limit biofilm development at walls of thin-gap PBRs. For a better control of bubble passage, the characterized geometry is not the 2D thin-gap bubble column described in [4] but a square confined channel with a cross-section of 4x4 mm<sup>2</sup> and a height of 20 cm. Hydrodynamics of bubbly flow in such configuration is described in detail in another

study of the same authors [19]: in particular, the mean and fluctuating components of the wall shear stress are characterized by electrochemical measurements, bubbles size, shape and velocity are described via shadowgraphy visualizations and liquid velocity field is determined via particle image velocimetry (PIV) measurements.

## **2. Materials and methods**

### **2.1. Experimental set-up**

#### **a. Lab-scale bubble column PBR used in the preliminary study**

A preliminary study is performed to assess the impact of bubbles on biofouling in a classical – unconfined – bubble column PBR. The lab-scale column used in this first step is a parallelepipedic tank, with a 2 cm thickness and a volume of 0.23 L. Gas is sparged locally at the bottom center, bubbles of around 3 mm are generated at the sparger. Culture of *Chlorella vulgaris* is carried out for 30 days under the incident light flux of  $700 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , which causes stress conditions favorable for biofilm development. Biofilm establishment and detachment are followed by measuring light flux attenuation through the culture volume. This is possible since the PBR is made of transparent poly(methyl methacrylate) – PMMA - to let the light through. Four PMMA plates are inserted flush with the wall so as not to modify the hydrodynamics. At the end of the culture phase, these plates are disassembled and analyzed using a digital microscope, which allows estimation of the biofilm thickness at different wall zones.

#### **b. Confined square channel**

The confined bubble contactor used in this study is a vertical channel with a  $4\times 4 \text{ mm}^2$  cross-section and a height of 200 mm above the gas injection. This experimental device **allows**

generating different sizes of bubbles at different frequencies and to control their trajectory (Figure 1). A mass flowmeter (Bronkhorst) is used to control the gas flow rate at the inlet of the channel. A pump is used to inject the liquid phase at the bottom of the channel with a low velocity to avoid emptying the channel with the bubble passage: a constant liquid flow rate of  $1.3 \times 10^{-7} \text{ m}^3 \cdot \text{s}^{-1}$  is applied in the channel. This flow rate corresponds to a liquid superficial velocity of  $0.007 \text{ m} \cdot \text{s}^{-1}$  and generates a wall shear stress of approximately  $0.002 \text{ Pa}$ , therefore largely below the wall shear stress found in the presence of bubbles [19]. To maintain a constant liquid flow rate, a constant pressure chamber is placed after the pump.

Air is injected 2 cm above the liquid inlet. Two types of air injections can be performed: an injection through a capillary with an internal diameter of 2 mm, to generate slightly oscillating bubbles in the channel, and a T-injection with air and liquid injected through channels of  $4 \times 4 \text{ mm}^2$  cross-section to obtain bubbles confined in the channel (Taylor bubbles) with an average Sauter diameter greater than the channel size and a higher average wall shear stress than created via the previous injection (Figure 1). The outflow of both phases occurs at the top of the channel. Hydrodynamics characterization for the different conditions of bubbling in this channel is presented in [19] for the liquid phase (velocity and vorticity fields) and the bubbles (size and shape, frequency, velocity); mean and fluctuating wall shear stresses at the wall are also characterized.

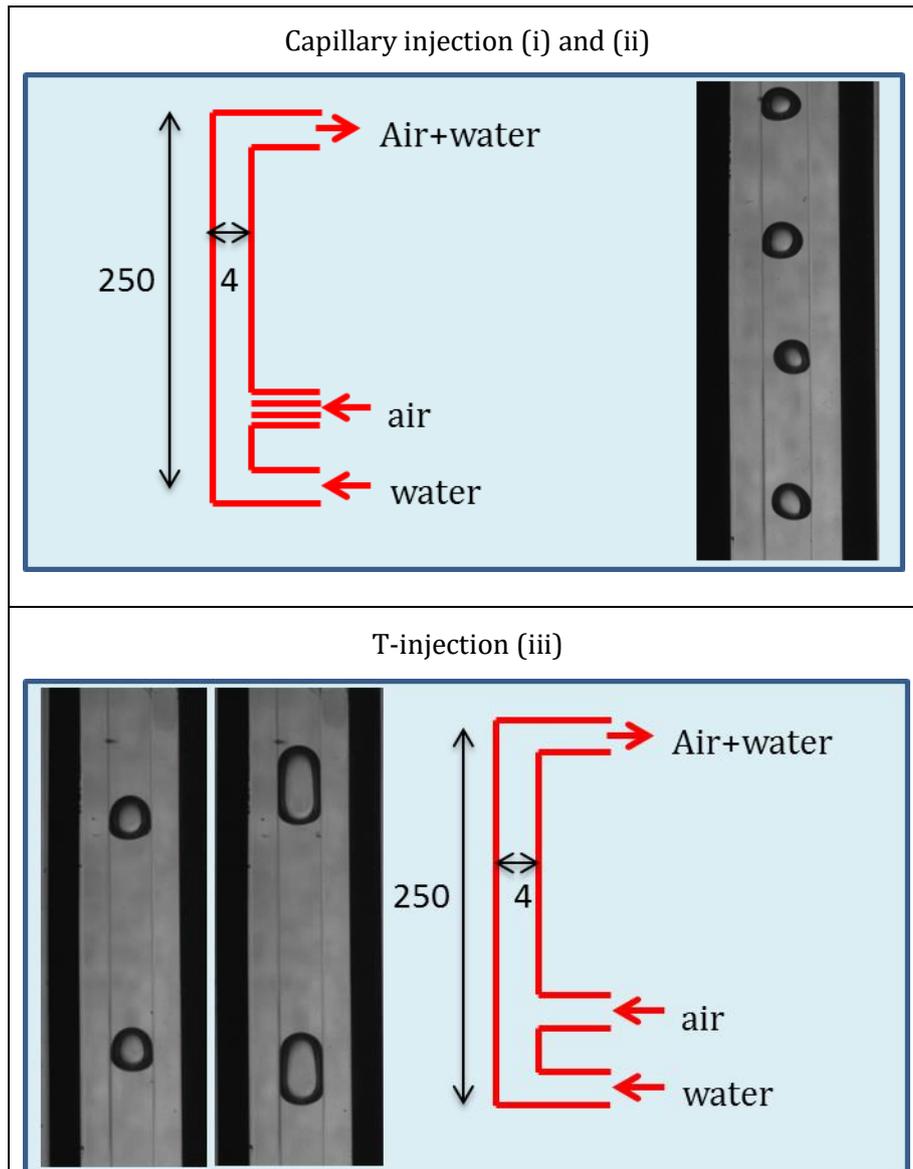


Figure 1: Square channels of 4 mm x 4 mm x 250 mm used for biofilms experiments and their gas injection system (dimensions in mm)

This channel contactor is placed in derivation of an airlift photobioreactor (1 L) to study the effect of bubbles on the biofilm in a real culture of *Chlorella vulgaris* microalgae (Figure 2). The airlift photobioreactor (5) is operated in continuous mode with a constant microalgae concentration of 3-4 g.L<sup>-1</sup>. The culture is injected into a buffer tank (1) using a volumetric pump. This buffer tank (1) is placed high and is intended to avoid fluctuations of velocity and pressure generated by the pump, which would impact the hydrodynamics in the channel. A valve is used to adjust the liquid flowrate injected into the channel (4). The microalgae culture

is returned into the airlift PBR from the outlet of the channel contactor and the buffer tank. Concerning bubble injection into the channel, the gas flow rate is adjusted via a pressure reducer followed by a mass flowmeter. Between the two, an air filter is inserted in order to work in axenic conditions for the culture. The mass flowmeter (EL-FLOW®-BRONKHORST) makes it possible to precisely adjust the air flowrate injected into this channel contactor (4).

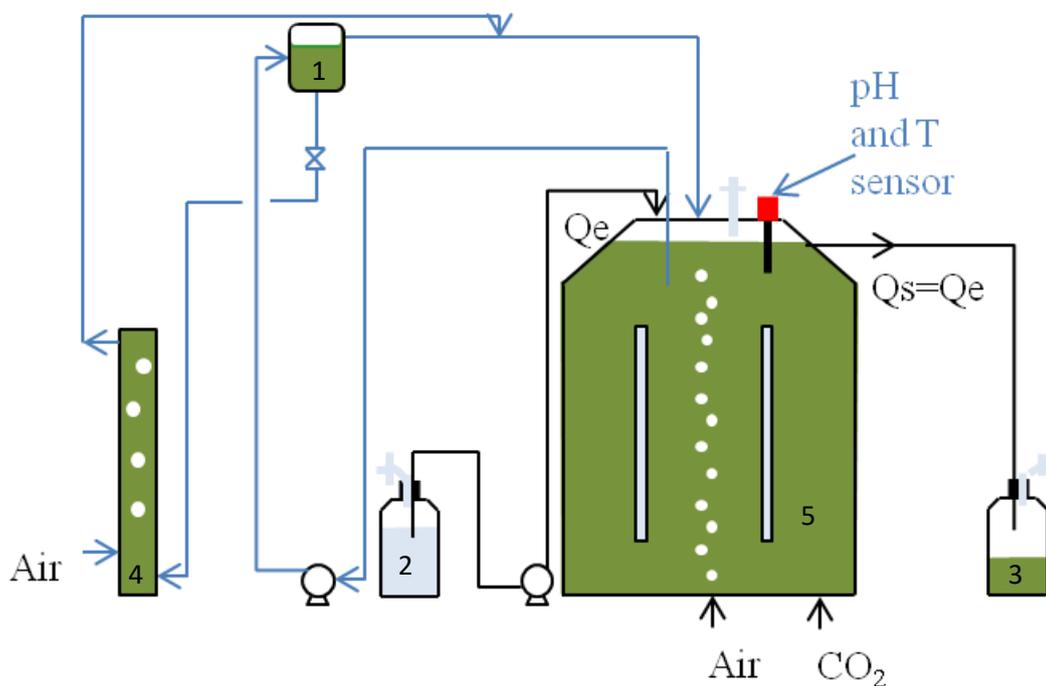


Figure 2: Square channel placed in derivation of the airlift culture of *Chlorella vulgaris*. 1: Buffer Tank; 2: BBM medium; 3: Harvest tank; 4: Channel placed in derivation; 5: Airlift closed PBR.

## 2.2. Biofilm establishment and characterization

### a. Microalgae and culture medium

The microalgae strain used in this study is *Chlorella vulgaris* (CCAP 211/19). This strain is cultivated in continuous mode in Bolt Basalt Medium (BBM). The nutrient composition to

obtain dry biomass of 1 g.L<sup>-1</sup> is given in Table 1. The composition of Table 1 is adapted to allow reaching 4 g.L<sup>-1</sup> of cell concentration after cultivation. Inorganic carbon is provided *via* pH regulation at 7.5 by the addition of air enriched with carbon dioxide. This microalgae strain is cultivated in 1 L airlift PBR (cf. 2.1.b) made in PMMA and enlightened using a LED panel. The incident luminous flux is 700 μmol.m<sup>-2</sup>.s<sup>-1</sup> and the dilution rate is 0.01 h<sup>-1</sup>. The same conditions are used in the preliminary study.

Table 1: Composition of the modified Bolt Basalt Medium (1 L) to produce a biomass concentration of 1 g.L<sup>-1</sup>

Compound	Molecular weight (g.mol <sup>-1</sup> )	Concentration in the culture medium (g.L <sup>-1</sup> )	Concentration in the culture medium (mmol.L <sup>-1</sup> )
NaNO <sub>3</sub>	85.0	0.750	8.824
MgSO <sub>4</sub> , 7H <sub>2</sub> O	246.5	0.225	0.913
CaCl <sub>2</sub> , 2H <sub>2</sub> O	147.0	0.025	0.170
Additive A		1 mL	
Additive B		1 mL	
EDTA Na <sub>2</sub> , 2H <sub>2</sub> O	372.2	0.050	0.134
FeSO <sub>4</sub> , 7H <sub>2</sub> O	278.0	0.014	0.050
K <sub>2</sub> HPO <sub>4</sub>	174.2	0.150	0.861
KH <sub>2</sub> PO <sub>4</sub>	136.1	0.123	0.900
NaHCO <sub>3</sub>	84.0	1.26	15.000

Additive	Compound	Molecular weight (g.mol <sup>-1</sup> )	Concentration in the culture medium (g.L <sup>-1</sup> )	Concentration in the culture medium (mmol.L <sup>-1</sup> )
<b>A</b>	ZnSO <sub>4</sub> , 7H <sub>2</sub> O	287.5	0.222	0.772
	Co(NO <sub>3</sub> ) <sub>2</sub> , 6H <sub>2</sub> O	291.0	0.044	0.151
	CuSO <sub>4</sub>	159.6	0.079	0.000
	H <sub>3</sub> BO <sub>3</sub>	61.8	2.86	0.046
	MnCl <sub>2</sub> , 4H <sub>2</sub> O	197.9	1.81	0.009
<b>B</b>	Na <sub>2</sub> MoO <sub>4</sub>	205.9	0.22	1.063

*b. Daily culture monitoring*

To control the state of the microorganisms, microscopic analysis is made regularly during the *Chlorella vulgaris* culture. The dry biomass concentration is also followed daily. The method consists, first of all, in weighing a filter (Whatman GF / F brand fiberglass filter, 0.7  $\mu\text{m}$ ) which has been previously oven-dried at 110 °C for 24 h. Then, a constant volume of culture is filtered through this filter. A rinsing step is performed with demineralized water to remove the mineral salts from the filtered biomass. Finally, this filter is placed in an oven at 110 °C for 24 hours, then cooled in a desiccator for several minutes and weighed. The biomass concentration ( $C_x$ ), given in  $\text{g}\cdot\text{L}^{-1}$ , corresponds to the difference between the two weights of the filter divided by the filtered volume of the culture. The dry biomass concentration measurement is performed in triplicate.

*c. Biofilm establishment monitoring in the channel*

Once the concentration is stabilized in the airlift PBR, part of the culture is sent into the channel to study the bubbling effect on biofouling. Biofilm establishment at the transparent walls is responsible for the light intensity decline. Therefore, the daily monitoring of this criterion is a good way to follow the biofilm development. Thus, every day, the channel is emptied of the microalgae solution to make measurements of light intensity in the same conditions, *i.e.* without culture solution. Measurements are performed with light meter LI-250A (LI-COR®, USA) placed at 6 positions behind the PMMA channel (Figure 3).

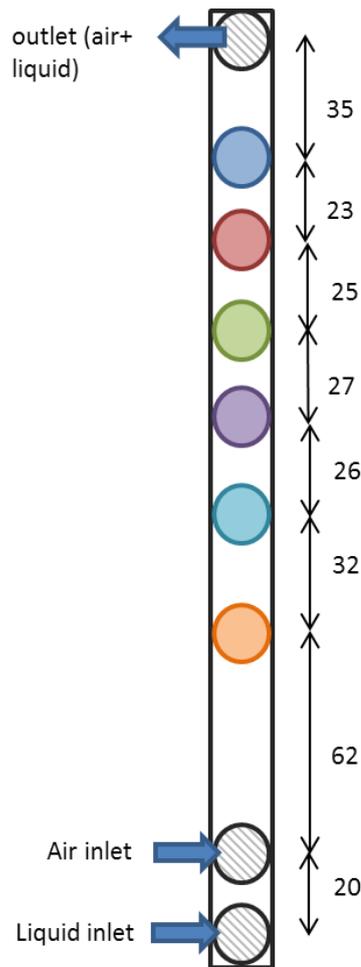


Figure 3: Positions of light measurements at different heights in the channel using a Quantum meter - Positions in mm.

*d. Preliminary characterization: biofilm observation with a numerical microscope*

For this preliminary step, *Chlorella vulgaris* culture is carried out continuously for 35 days in the 0.23 L PBR described in paragraph 2.1a. Biofilm establishment is analyzed on the plates numbered from 1 to 4 which are mounted flush with the wall. At the end of the culture phase, the PBR is emptied and the plates are removed and analyzed by microscopy. The thickness of the biofilm is determined using a digital microscope (Keyence - VHX-5000, VH-Z100R / Z100T lens) and transmission LED lighting. This microscope allows precise measurements ( $\pm 5 \mu\text{m}$ ) and the acquisition of 3D data via the analysis of the degree of blur of a 2D image.

### 3. Results and analysis

#### 3.1. Preliminary study to assess the effect of bubbling in a classical PBR

Figure 4 presents a photograph of the back wall of the PBR with the 4 plates after 35 days of cultivation. The bubble passage zone, which includes the whole width covered by the bubbles during **their** oscillations, is indicated by dashed lines. In this area, no biofilm development is observed: plates 1 and 3 are the most impacted by this effect, as shown by visual observation. A few centimeters above the injection, the bubble plume expands with bubbles rising preferentially on the left side of the PBR, which explains that biofilm is less present in that half of the back wall. In the vicinity of the air sparger (Plates 2 and 4), biofilm implantation is more important, which corresponds to a zone where bubbles plume is concentrated on a limited width above the sparger and does not reach Plates 2 and 4.

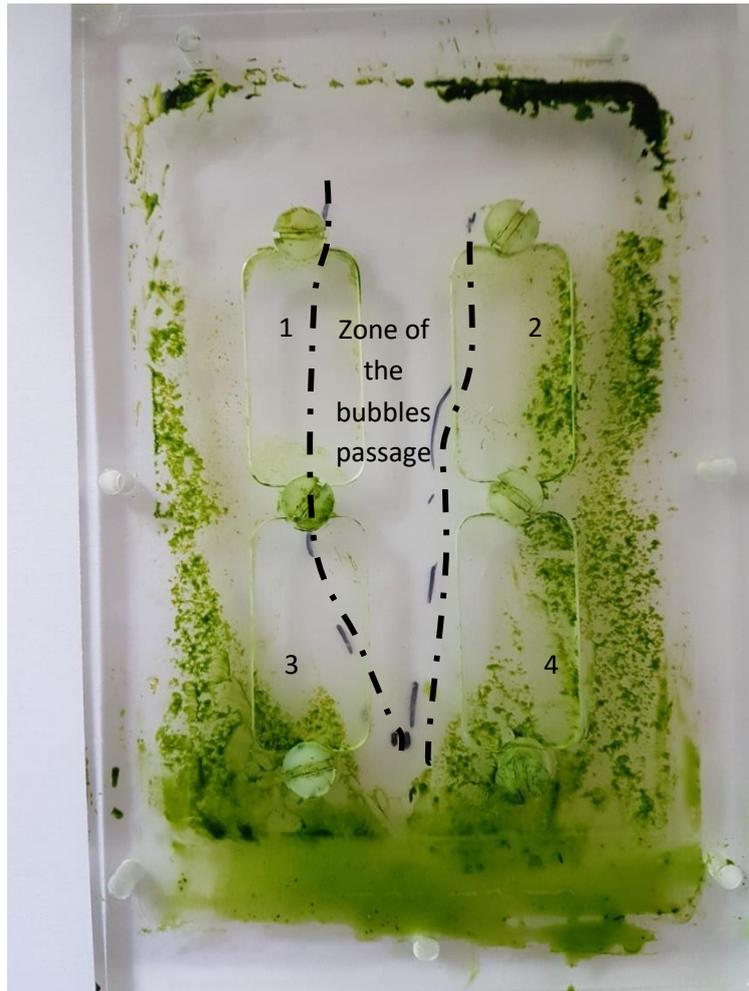


Figure 4: Photo of the back wall of the photobioreactor with the 4 plates and the zone of passage of the bubbles

To have a better understanding of the biofilm aspect and the influence of bubbling, digital microscopy observations are realized; an example of biofilm 3D topography is given in Figure 5.

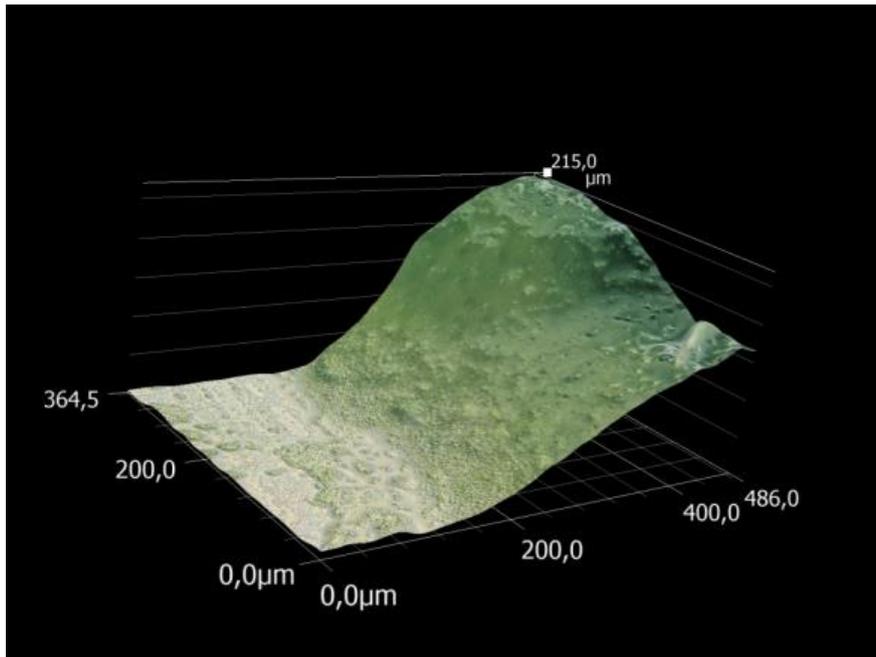


Figure 5: 3D topography of the biofilm taken from the Keyence<sup>®</sup> digital microscope

Photos of the 4 plates, with the areas where biofilm thickness is determined, are shown in Figure 6. Some local values or local thickness ranges are also indicated. These areas of measurements have a height of 1 mm and a length (shown in Figure 6) which varies according to what is interesting to measure. The choice of these lines allows linking biofilm thickness distribution with bubble passage. Figure 6.e. presents a typical image of the biofilm topography in area A6.

The repartition of the biofilm layer is non-homogeneous. The thickness distribution depends mainly on the trajectory of the bubbles injected in the PBR. In Figure 6.a., visual observation shows that the biofilm does not seem to be developed on plate 1, and yet, microscopic analysis reveals a deposit whose thickness is not negligible ( $\approx 6 \mu\text{m}$ ). To give an order of magnitude, the diameter of the microalgae *Chlorella vulgaris* is about  $5 \mu\text{m}$ , which suggests that this layer can be identified as a conditioning one, formed mainly by the compounds of the culture

medium and excretions of microalgae cells like exopolymeric substances, and does not result from the adhesion of living or dead cells.

On plate 2, the thickness of the biofilm is greater on area A3 than on area A2, which can be explained by the enlargement of the bubble plume which widens with the height of the PBR. The top of plate 3 which is impacted by bubble passage does not seem to have a biofilm, however, a thickness between 5 and 10  $\mu\text{m}$  was determined on area A4. Finally, plate 4, which is not impacted by bubble passage, has a biofilm deposited on most of its surface with very important thicknesses compared to other plates.

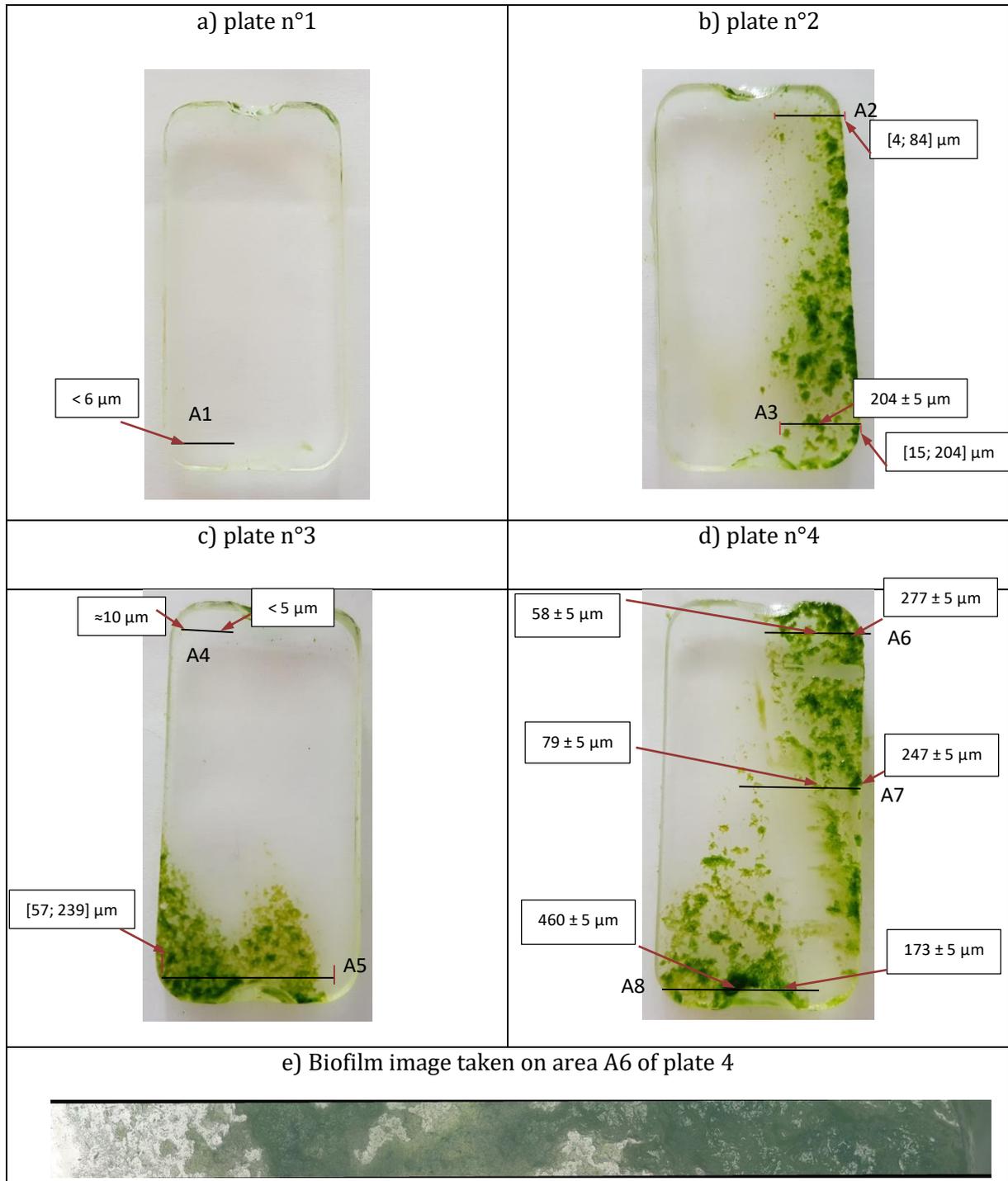


Figure 6: Photos of the plates (2.5x5 cm<sup>2</sup>) with the positions of the different areas (from A1 to A8) analyzed under Keyence<sup>®</sup> microscope (6a to 6d) and example of biofilm image (6e)

It can therefore be concluded that although bubbling has a significant effect on biofilm development, it cannot prevent the establishment of the first layer of biofilm deposition (conditioning layer), even if the biofilm is not visible from a macroscopic point of view.

Another analysis was carried out to identify the effect of biofilm development on the decrease of the light flux transmitted through the photobioreactor. For this, before starting the culture, a measurement of the light flux transmitted through the photobioreactor is made at the back façade of the 4 plates flush-mounted to the wall. After 35 days, the photobioreactor is emptied and a measurement of the light flux transmitted is carried out at the same locations as the initial measurement to assess the effect of the biofilm development on the decrease of the light intensity. These results are summarized in Table 2.

Table 2: Light flux ( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ ) before and after the biofilms on the 4 plates inserted in the wall of the 0.23 L photobioreactor

Areas		Light flux before biofilm development at $t_0$ ( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ )		Light flux after biofilm development during 35 days ( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ )	
plate 1	plate 2	356.4	320.1	316.2 (-11%)	242.4 (-24%)
plate 3	plate 4	399.2	346.8	253.9 (-36%)	256.3 (-26%)

Despite the absence of a visual observation of biofilm on plate 1 and also the very small thickness determined by the digital microscope ( $< 6 \mu\text{m}$ ) on area A1, an attenuation of the transmitted light flux of 11% is obtained on this zone after 35 days of culture. Plates 3 and 4 located at the bottom of the PBR (Figure 6) show a greater drop in light flux with respectively 36% and 26% reduction compared to the initial flux. Overall, at the top of the photobioreactor the transmitted light flux decreases less than at the bottom. This is explained by the trajectory of bubbles that gives rise to a plume that expands and oscillates, thus avoiding the presence of a very thick biofilm on a surface that widens with increasing height. This result allows putting forward the effect of bubbling on the biofilm since, in the zone where there is a

passage of bubbles, the biofilm is thinner and covers less surface. However, the bubbling does not prevent the implantation of the first layer of biofilm (wall conditioning layer) which is responsible for a drop in transmitted light flux of more than 10% after 35 days of culture.

### **3.2. Effect of different bubbling conditions on microalgae biofilm establishment**

#### *a. Bubbling effect on biofilm already adhered to the wall*

To study the effect of bubbling on the development of a microalgae biofilm in intensified conditions, the confined gas-liquid contactor described in paragraph 2.1.b is used (Figure 1).

The first experiment aims to develop a biofilm at the walls of the channel, and then to test different bubbling configurations and their impact on biofilm detachment. For 33 days, the channel is only supplied with a culture of *Chlorella vulgaris* at 4 g.L<sup>-1</sup> and with a constant flow rate of 1.3x10<sup>-7</sup> m<sup>3</sup>.s<sup>-1</sup>. Thus, no bubble injection is performed in the first step to avoid the effect of shear stresses due to bubbles passage on biofouling. Daily measurement of the transmitted light through the channel is made at different heights of the channel (Figure 3) with a quantum meter. To avoid the effect of the cell concentration variations on light transmission, the channel is emptied before each measurement. For each height, measurements are taken 10 times and the luminous flux is then averaged over all heights to have only one curve. Figure 7 shows the evolution of the average light flux versus time. 14% decrease in light transmitted is observed after 33 days, therefore around 0.42%/day.

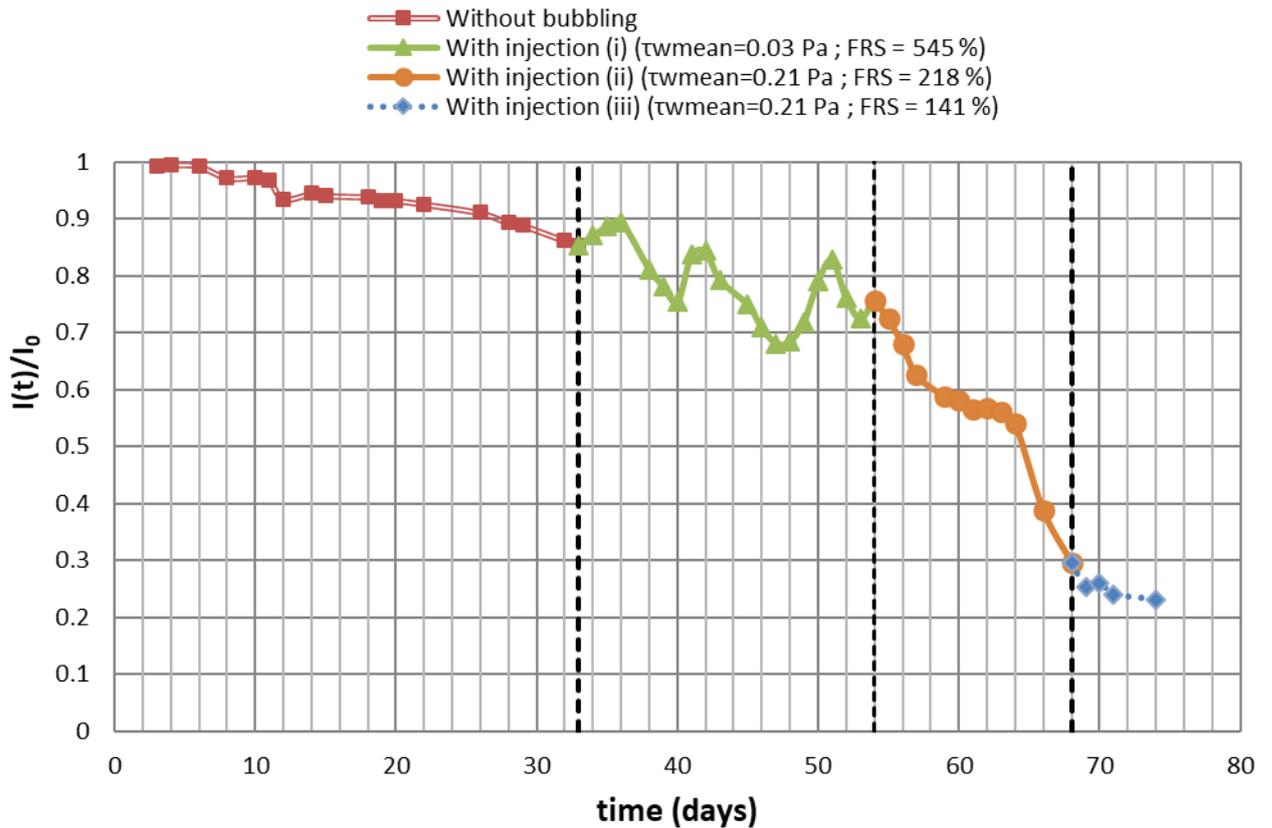


Figure 7: Evolution of the normalized light flux as a function of time - Biofilm detachment tests

Also, only slight cloudiness is observed on the channel walls with a very slight green deposit in the last days of this experiment. This slight cloudiness corresponds to the surface conditioning layer which is generally composed of nutrients, cell fragments and other products secreted under stress conditions, such as exopolymeric substances. This result confirms the observations made on the PMMA plates installed in the classical PBR of 0.23 L (Figure 6).

After this first step of biofilm deposition (from day 33), different bubbling strategies are tested to assess their impact on biofilm removal. Three types of bubbling are compared. The properties of these bubbles (size, shape and velocity) have already been identified by the shadowgraphy method in a previous study [19] and are summarized in Table 3. The mean wall

shear stress and the wall shear rate fluctuation intensity (FRS) generated by bubbling have been characterized by a polarographic method and are also summarized in Table 3. The chosen bubbling strategies are the following:

- (i) Bubbling inducing high wall shear rate fluctuations: this can be obtained via capillary injection to generate bubbles with a Sauter diameter a little smaller than the channel size and thus, oscillating slightly in the channel. It is characterized by a mean wall shear stress and wall shear rate fluctuation intensity (FRS) respectively equal to 0.03 Pa and 545 %.
- (ii) Bubbling inducing high average wall shear stress and intermediate fluctuations: these wall shear stress characteristics can be obtained via capillary injection (2 mm) with a higher gas flow rate than the first type of bubbling. Average wall shear stress is 0.21 Pa and 218% wall shear rate fluctuations are obtained.
- (iii) Bubbling inducing high average wall shear stress and low fluctuations: T-injection, with air and liquid injected through channels of 4x4 mm<sup>2</sup> cross-sections, allows generating bubbles confined in the channel (Taylor bubbles) with an average equivalent Sauter diameter greater than the channel size and a high average shear stress (0.21 Pa) but lower fluctuations' intensity (141%).

Table 3: Characteristics of the different bubbling sequences tested to eliminate the formed biofilm

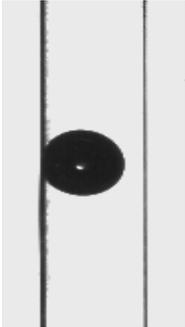
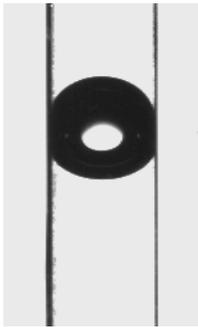
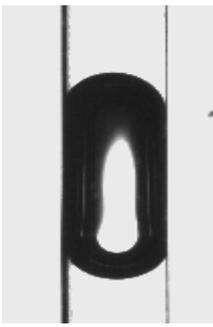
Type of bubble injection	(i)	(ii)	(iii)
Bubble inlet	Capillary - 2 mm	Capillary - 2 mm	T injection - 4 mm
$d_{32}$ (mm)	$3.7 \pm 0.14$	$4.3 \pm 0.17$	$7.1 \pm 0.54$
$V_{\text{bubbles}}$ ( $\text{m.s}^{-1}$ )	$0.10 \pm 0.001$	$0.12 \pm 0.002$	$0.13 \pm 0.005$
$F_{\text{bubbles}}$ (Hz)	0.65	4.15	1.59
$\tau_w$ (Pa)	0.03	0.21	0.21
FRS (%)	545	218	141
Shadowgraphy images			

Figure 7 shows that with (i) bubbling (day 33 – day 54), the light transmitted through the channel begins to oscillate with time. This result can be explained by the periodical adhesion and detachment of the superficial layers of the biofilm, considered as the least adhered. But the very first layers of the biofilm do not detach, and provide adhesion support for new cells. The passage of 3.7 mm bubbles generates high wall shear rate fluctuations and thus allows detaching the superficial layers of the biofilm. This is in agreement with the work of [25] who showed the same phenomenon of formation and detachment of the superficial layers of a bacterial biofilm subjected to hydrodynamic shear stress.

Then, (ii) bubbling is applied (day 54 – day 68), it generates slightly confined bubbles with a Sauter diameter of 4.3 mm and thus creates more friction at the wall. Bubbling applied from the 54th day seems less effective to detach the superficial layers of the biofilm, since there is no longer fluctuation of the light flux (Figure 7). In addition, confined bubbles even tend to

promote the development and the consolidation of the biofilm. Indeed, the decrease in light flux is more pronounced for this bubbling than with the previous injection (Figure 7): the slope of the curve is more important (i.e. about 3.2%/day) than with the previous injection (about 0.5%/day).

Finally, another bubbling sequence has been studied with the (iii) T injection (day 68 – day 76). As shown in Figure 7, this bubbling does not detach the biofilm, and the light intensity continues to drop by about 1.2%/day.

These two last bubbling conditions, therefore, seem to be less effective in detaching the superficial layers of the biofilm. They tend even to favor its development. This is in agreement with the work of several authors [26] [27] [11] [12] [7], who have shown that, once the conditioning layer is formed, wall shear forces do not prevent the biofilm formation anymore, but make it thinner, denser and more resistant to tearing forces. [9] also showed that below a certain value of shear stress, the detachment of the biofilm predominates; but, by increasing this constraint, compression of the biofilm can take place. Moreover, as shown by [13] [28] during the cleaning studies of agri-food equipment, it is better to have high wall shear rate fluctuations than high average shear stresses. Results obtained in this work are therefore in agreement with these authors, since the smallest bubbles, which oscillate in the channel, create more disturbances at the wall. This causes detachment of the superficial layers of the biofilm, unlike flattened bubbles that tend to compact the biofilm and make it denser.

However, none of the bubbling conditions tested is effective to detach the first layers of the biofilm, also known as wall conditioning layers. Some bubbling conditions even tend to accelerate the development of the biofilm. This could be explained firstly by the generation of wall friction allowing its consolidation and, secondly, by the supply of nutrients necessary for

its development, due to the convective exchanges between the culture medium and the layers that adhere to the wall.

*b. Bubbling conditions effect on development of the first biofilm layer*

The study of the biofilm behavior vs wall shear stress detailed in the previous paragraph emphasizes the difficulty of detaching it once it is formed at the wall. A complementary study is therefore carried out to verify the effect of bubbling on the development of the first layer of biofilm. This part aims to identify the right choice of bubbling conditions, from the beginning of the cultivation, which could delay as far as possible the inevitable development of the conditioning layer. This bubbling strategy could allow extending the duration of PBR operation before the biofilm establishment at walls.

First, before studying the effect of different types of bubbling on the biofilm establishment, we must be sure of the reproducibility of the results, since in culture tests several parameters are involved (light, dilution rate, culture medium, pH, temperature, possible bacterial contamination, etc.). A repetition of the experiment of culture circulation without bubbling (day 1 to day 32 of Figure 7) is thus carried out to confirm the effect of the conditioning layer on light attenuation. This result is presented in Figure 8. The same tendency is observed since the two experiments show a light attenuation of approximately 8 to 10 % at the end of the 30th day, and have globally the same aspect ( $\pm 3\%$  difference between the experiments during the first 30 days) which proves the reproducibility of this test.

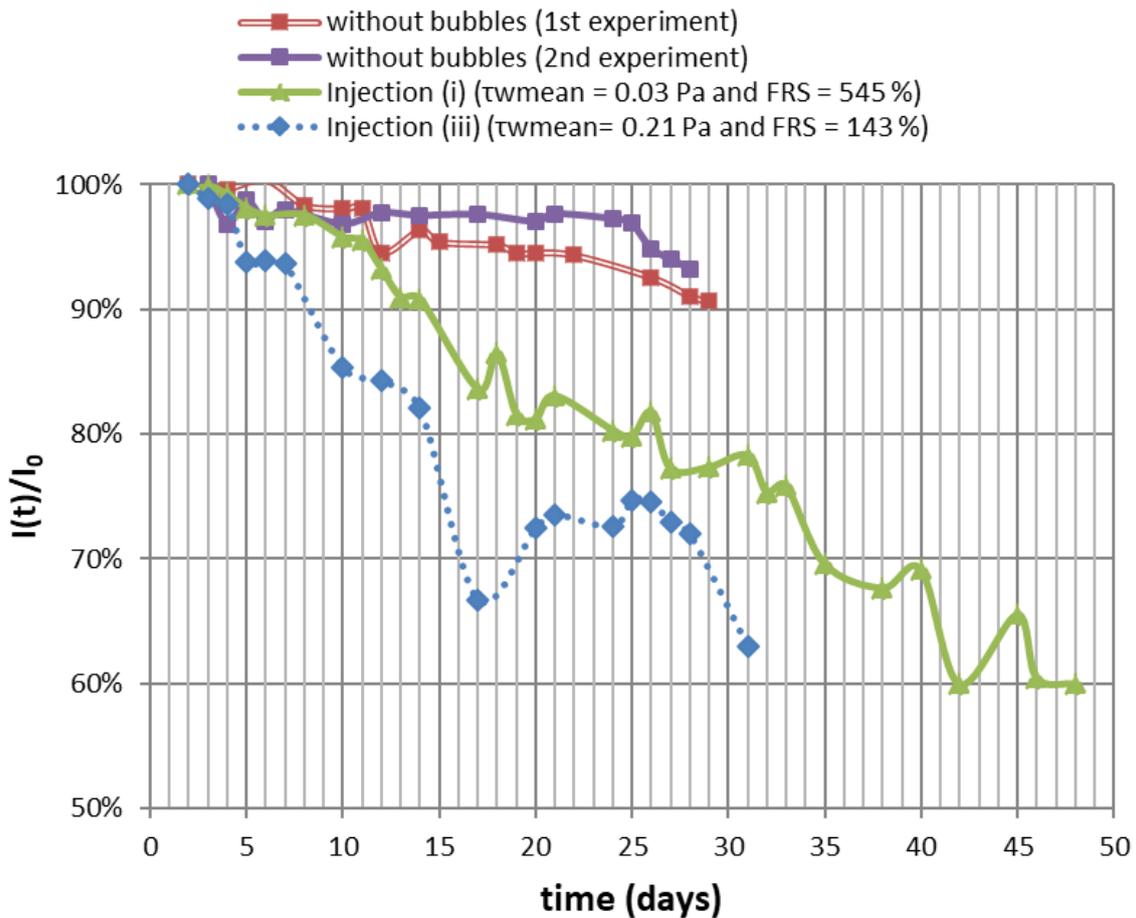


Figure 8: Evolution of the normalized light flux as a function of time - Tests with or without bubbles

In a second step, bubbles injection is performed from the beginning of the culture circulation. Indeed, the preliminary experiment carried out in a classical photobioreactor, has shown that in the zone where the bubbles are present, the thickness of the biofilm is thinner and the luminous flux which passes through the PBR is higher than in the zones without bubbling.

Given the results of the previous paragraph where a capillary injection of type (i) allows eliminating some of the superficial layers of the biofilm, the choice is therefore first turned towards this type of injection to limit the appearance of biofilm on the walls of the channel. An experiment is thus carried out with this first injection from the beginning of the circulation of the culture in the channel (i.e. no biofilm at the beginning of the bubbling experiment).

Figure 8 shows that during the first 10 days, the evolution of the biofilm development is the same as in the case without bubbling. This information is deduced indirectly by following the light transmission through the channel with and without bubbling, which presents the same tendency. After this duration, bubbles action on the walls of the channel promotes the biofilm establishment, even if slight fluctuations in transmitted light with time are observed. A decrease of 23 % in the light flux transmitted through the PBR is recorded after 30 days with this first injection, while it was only 10 % in the case without bubbling. Therefore, this bubbling is not adapted to avoid biofilm.

It is also necessary to see if the injection of more confined bubbles, creating more friction at walls, and resulting in higher shear stresses, would be more suitable to avoid biofilms establishment. For this reason, the T injection is used (injected of type (iii)). This type of bubbling favors the development of biofilm since, from the 5th day, a more important decrease of the light transmission is observed than without bubbling (Figure 8). Moreover, from the 2nd day, a slight green deposit is observed at the channel walls and at the end of the 7th day, a green biofilm is already well installed. After 30 days, 35% decrease in light flux occurs with this bubbling. This type of bubbling is therefore to be avoided, since it does not prevent the formation of biofilm, but makes it denser by compacting it. In this type of confined configuration, bubbling does not prevent the establishment of the biofilm, but tends to favor it.

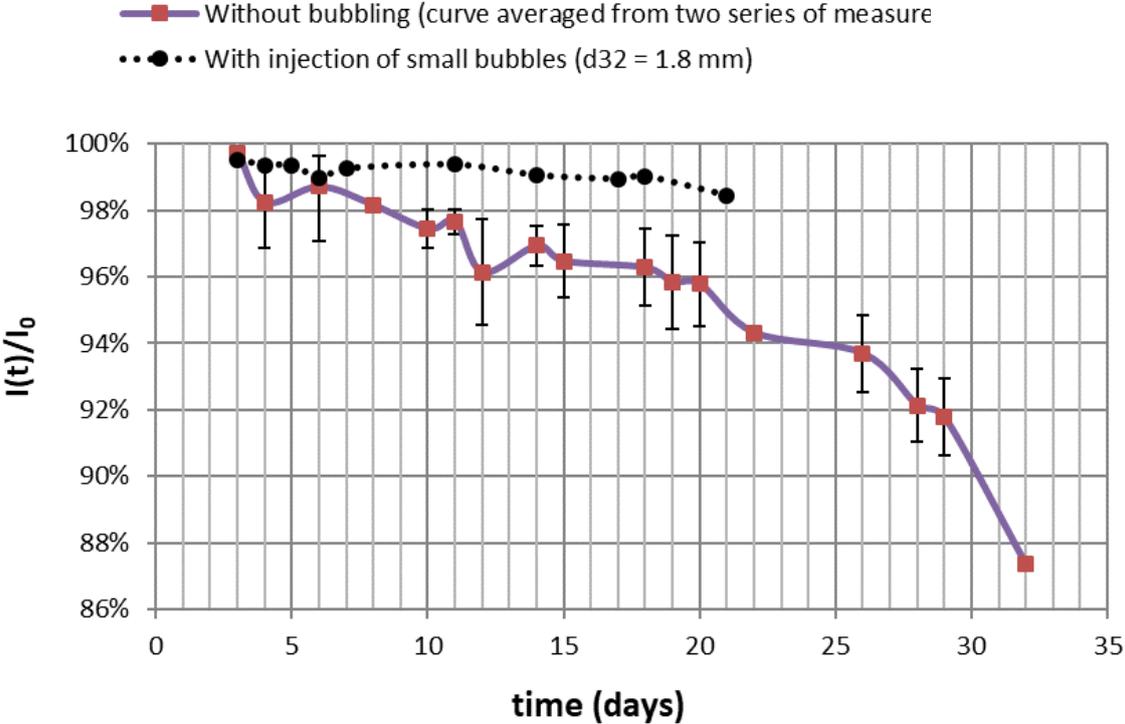
These results about the impact of bubbling in the confined channel seem to be contradictory with the observations in conventional – unconfined - airlift or bubble column PBRs. Indeed, in unconfined bubbly flow, if bubbling does not prevent the first layer of biofilm deposit, it avoids too great a decrease in the light transmitted through the PBR compared to areas without

bubbling, as was observed in the preliminary study in the airlift PBR of 0.23 L (Figure 4). The difference in bubbling effect on biofilm development for both configurations can be explained by the ratio of equivalent bubble diameter to reactor thickness. In the case of the confined channel, the ratio is of the order of 1 in the case of the capillary injection with an injection diameter of 2 mm and greater than 1 in the case of the injection in T. For the reactor of 0.23 L, this factor is around 0.15. Moreover, in this type of confined bubble contactor at the 4 sides, the hydrodynamics of the liquid phase is very constrained. Liquid flow is mainly directed upward between the bubbles and has to go downward in the film between the bubbles and the wall [19]. In the case of conventional unconfined PBRs, the liquid can flow freely between the bubbles in 3 dimensions.

The last bubbling test is thus performed in the confined channel with bubbles of a diameter smaller than the channel thickness to test the hypothesis that smaller diameter bubbles would be more effective in preventing biofilm development. This type of bubble injection should allow generating important fluctuations of wall shear stress despite a lower average value, whereas the liquid can flow more freely around the bubble than in a film. This type of bubble injection is achieved using a 0.254 mm diameter capillary. With this type of injection, shadowgraphy measurements were carried out and showed that the average diameter of the bubbles is 1.4 mm for an average bubble velocity of  $0.20 \text{ m}\cdot\text{s}^{-1}$ . It would be interesting to carry out electrochemical measurements to know more precisely the average and the fluctuating values of the wall shear stress for this type of injection.

In Figure 9, the two curves obtained without bubbling are averaged and compared with the curve obtained with small bubbles of 1.4 mm average diameter. The transmitted light obtained with the injection of small bubbles remains between 98% and 100% of its initial value

over 21 days of culture (Figure 9): therefore a 2% decrease in light transmitted is obtained whereas, without bubbling, the decrease is of around 5% in the first 21 days. The comparison with confined bubbles shows that after 21 days, the transmitted light flux decrease across the channel was about 16% with the capillary injection of type (i) (Figure 8). The experiment could not be prolonged more than 21 days (Figure 9). However, it is clear from Figures 8 and 9 that bubbling with small bubbles of equivalent diameter significantly smaller than the channel size limits biofouling compared to confined bubbles. This can be explained by the fact that with this type of bubbles the disturbances created at the wall are important (due to the high shear rate fluctuations), which therefore limits the development of biofilm, but also to the fact that the liquid can come down on all sides of the bubble which is not the case for flattened bubbles in the channel where the liquid is forced to pass through a film on all four sides.



. Figure 9: Evolution of the normalized light flux as a function of time - Comparison between the curves obtained with injection of small bubbles and without bubbling

#### 4. Conclusions

The development of biofilm is undesirable during microalgae cultivation since it is responsible for a decrease in the light flux transmitted through the bioreactor and therefore causes a decrease in biomass productivity. This problem has been investigated in this study for bubbly flow in the context of bubble columns or airlift PBRs operating in intensified conditions (high biomass concentration, low reactor thickness of a few millimeters). The objective is to propose bubbling strategies allowing to limit the biofilm formation, and thus to avoid the extra costs caused by frequent PBR cleaning or culture stop.

Preliminary experiments in a PBR of 2 cm thickness have put into evidence the effect of bubbling on biofilm development in a conventional PBR geometry. In areas without bubbling, a thick biofilm is created with a significant drop in the light transmission. In areas impacted by bubbling and without visible biofilm, microscopic analysis showed the presence of a thin transparent layer with a thickness less than 10  $\mu\text{m}$ . This layer is responsible for a drop in light transmission of about 11 % after 35 days. Bubbling, therefore, makes it possible to limit the development of biofilm but does not prevent it completely. The first so-called conditioning layer still adheres to the wall.

Then, experiments have been carried out in a confined 4x4 mm<sup>2</sup> channel. This study has shown that it is very difficult to detach the biofilm once it has adhered to the wall. Only the bubbling conditions with high fluctuations rate of the wall shear stress have been able to detach the superficial layers of the biofilm. No effect was observed on the detachment of the first layer of the biofilm (or conditioning layer).

Since it is difficult to detach a biofilm once formed, attempts were made to avoid or delay its development. For this, the transmitted light curves obtained without bubbling were compared to bubbling conditions with capillary injection and T injection. These two air injections allow

obtaining bubbles with mean diameters equal to 3.7 mm and 4.5 mm respectively. Contrary to the observations of the preliminary study on a classical photobioreactor (2 cm thick), bubbling accentuates biofilm adhesion at the surface of the channel. It was also noticed that the phenomenon was worse with the injection of bubbles highly confined in the channel, which can be explained by the fact that the biofilm is compacted by highly confined bubbles. The last experiment was thus carried out with small bubbles whose diameter (1.4 mm) is smaller than the dimensions of the thin-gap channel. This makes it possible to approach the hydrodynamics obtained in a conventional PBR, in which the ratio of bubble diameter to reactor thickness is commonly less than 0.15. Indeed, in the channel, if the bubbles are confined, a 1D effect appears, forcing the liquid to go downwards as a film around the bubbles. If the ratio of the bubble diameter to the thickness of the column is much less than 1, a 3D hydrodynamic takes place allowing the liquid to flow around the bubble. With a ratio of bubble diameter to channel thickness of 0.35 corresponding to 1.4 mm bubble diameter, the experiment has demonstrated that this type of bubbling is the most adapted to limit the development of biofilm compared to other bubbling conditions. Also, it is the only bubbling tested that seems to lead to good results in confined flow, limiting the development of the conditioning layer compared to the case without bubbling.

The strategy to be tested in the case of future applications in high volumetric productivity PBRs with a low culture thickness (< 1 cm) is therefore to have the smallest bubbles as possible that will create intense fluctuations of shear rate at walls. It is also important to extend the study to the case of viscous and non-Newtonian cultures such as those of high cell concentration and to verify that mixing and gas-liquid mass transfer are not altered, especially with the coalescence phenomena that are likely to be more present in such conditions.

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## Nomenclature

$C_x$	dry biomass concentration ( $\text{g.L}^{-1}$ )
$d_{32}$	bubble Sauter diameter obtained by shadowgraphy method (mm)
$F_{\text{bubbles}}$	frequency of passage of bubbles determined by shadowgraphy and electrochemical methods (Hz)
FRS	shear rate fluctuations intensity (%)
$I(t)$	light flux passing through the channel at time $t$ ( $\mu\text{mol.m}^2.\text{s}^{-1}$ )
$I_0$	light flux passing through the channel at time $t_0$ (before the culture) ( $\mu\text{mol.m}^2.\text{s}^{-1}$ )
$V_{\text{bubbles}}$	velocity of bubbles obtained by shadowgraphy method ( $\text{m.s}^{-1}$ )

### Greek letters

$\tau_w$	mean wall shear stress (Pa)
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