Characterization of shear-stresses in an oscillating grid bioreactor using bioluminescent micro-organisms

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Introduction and context

Microalgae and photobioreactors

The promotion of transfers within a reactor is often the keystone of the enhancement of a chemical or biochemical process. In the case of microorganism cultures within a bioreactor, this **transfers intensification** should not be accompanied by a **shear stress** increase inducing mechanical stress on the micro-organisms that can lead to their lysis.

For the culture of **microalgae**, heterogeneous group of photosynthetic micro-organisms, several configurations and geometries of **photobioreactors (PBR)** are available, each one with its own advantages and disadvantages[1,2,3] :

Open culture systems

Culture medium circulation provided by paddle wheels. Systems sensitive to external conditions

Open raceway pond

Closed culture systems

Mixing provided by bubbling and shear stress depends on bubble sizes

Bubble column

Flat plate airlift

Culture medium circulation provided by centrifugal pump generating shear stress

Tubular PBR

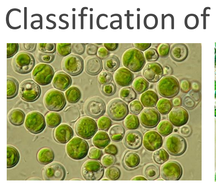
Culture stirred by rotating mobiles generating shear stresses in their vicinity

Stirred tank PBR

Poor mixing properties
Low induced shear-stress

Good mixing properties
High induced shear-stress

Classification of microalgae according to their **shear stress sensitivity**:[2.]

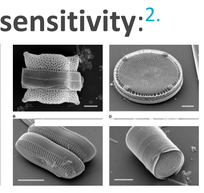


Green microalgae

Cyanobacteria

Haptophyta

Red microalgae



Diatoms

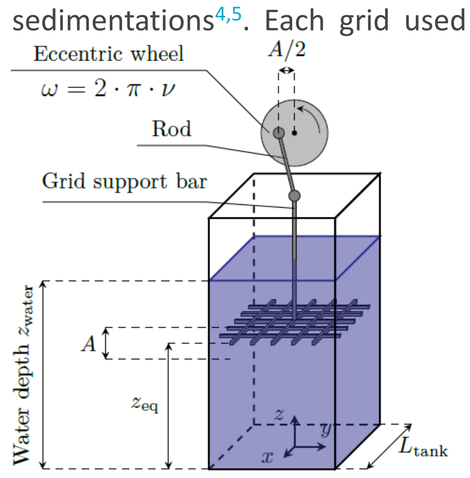
Dinoflagelates

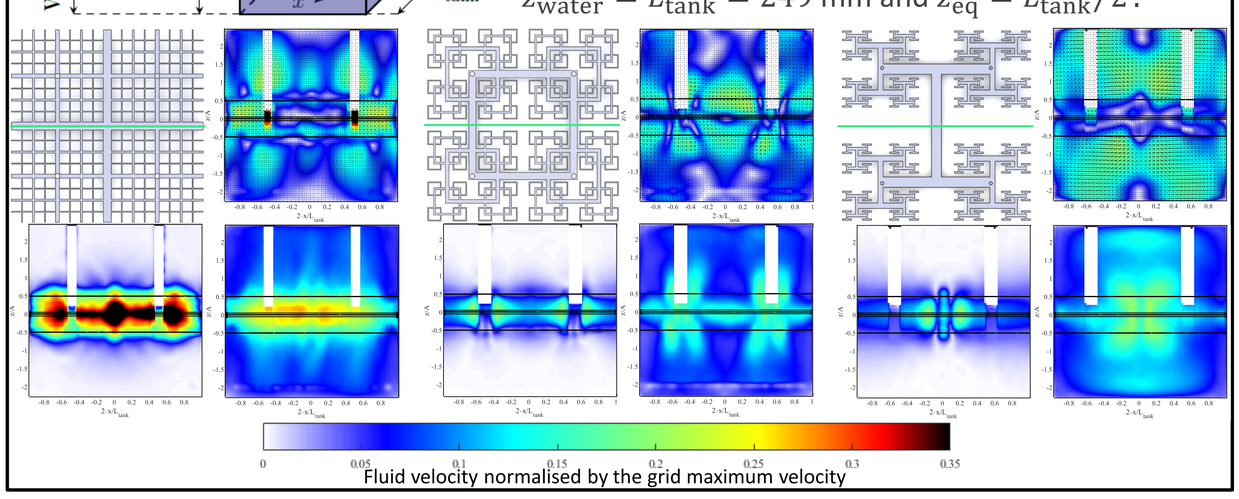
Shear stress sensitivity

Microalgae culture aims: CO_2 capture and absorption, production of interest products as lipids (which can be transformed into biodiesel), photosynthetic pigments, proteins, vitamins or carbohydrates used for therapeutic, food or cosmetic purposes[1,3].

Oscillating grid turbulence and fractal grid designs

Our thesis aim is to **design a PBR stirred by an oscillating grid**, stirring system classically used in fluid mechanics experiments to generate **nearly isotropic and shear-free turbulent flows**. The turbulence generated by an oscillating grid system can be monitored by the oscillation and grid parameters. This kind of stirring system was mainly used to study stratified and interfacial flows and sediments and particles sedimentations[4,5]. Each grid used have a **fractal geometry** and is constructed in an iterative way from a base pattern. In our case, we considered **cross**, **square** or **I-shaped** patterns. Fractal grids were used as fixed grids to generate downstream turbulence in water or wind tunnels[6]. According to the triple Reynolds decomposition, we present some results of the mean (top right), oscillatory (bottom left) and turbulent flows (bottom right) obtained by a **PIV** method for the central plan of each grid (depicted by the green line). We considered $A = 50$ mm, $\nu = 1$ Hz, $z_{water} = L_{tank} = 249$ mm and $z_{eq} = L_{tank}/2$.



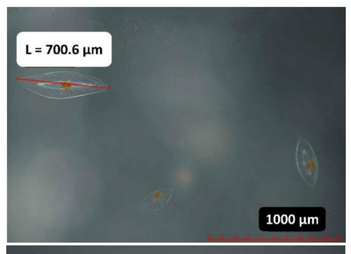


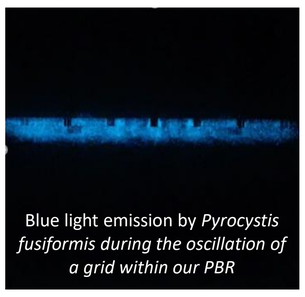
Fluid velocity normalised by the grid maximum velocity

Bioluminescence experiments and results

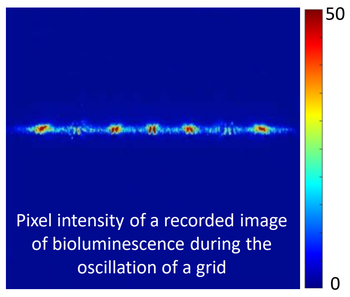
Bioluminescence emitted by *Pyrocystis fusiformis*

During a student project, we proposed to design experiments at the interface between fluid mechanics measurements and microalgae cultures: take advantage of the **bioluminescence** ability of a **phytoplankton** specie when it is mechanically stressed by a flow to identify the **high shear-stress area** within our oscillating grid stirred photobioreactor. We decided to use the **dinoflagellate** specie ***Pyrocystis fusiformis*** which is non-toxic unlike the dinoflagellate well known for this kind of bioluminescence experiments. The bioluminescence is due to the enzymatic oxidation of the **luciferin** by the **luciferase** when the cell membranes are deformed and their permeability is modified by the action of shear-stress. The light is emitted when the oxidation product returns to its ground state. *Pyrocystis fusiformis* follows a **circadian cycle** with 12 hours of light and 12 hours of dark. The bioluminescence only occurs during the **dark phase**[7,8].





Blue light emission by *Pyrocystis fusiformis during the oscillation of a grid within our PBR*

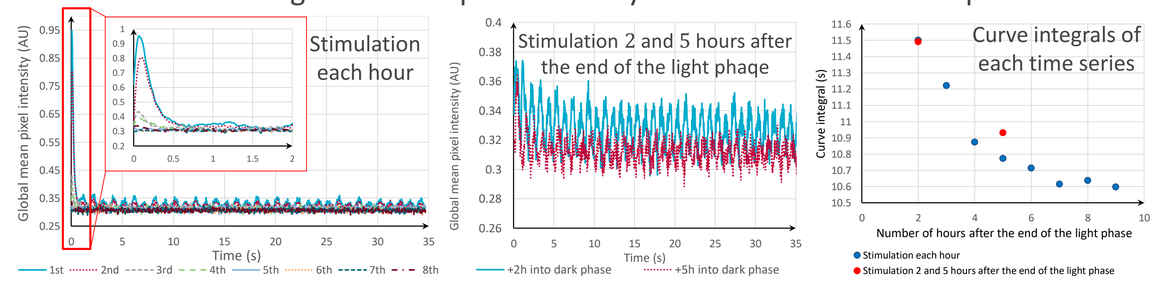


Pixel intensity of a recorded image of bioluminescence during the oscillation of a grid

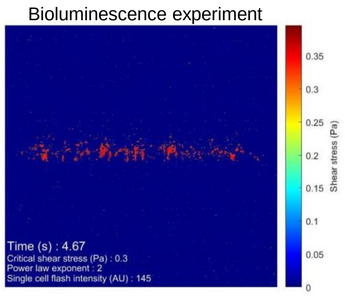
For our experiments, we considered the PBR in the same configuration as for the PIV experiments with a simple cartesian grid, $A = 50$ mm and $\nu = 0.5$ Hz. The bioluminescence images were recorded with a CMOS camera LaVision Imager M-lite 5M.

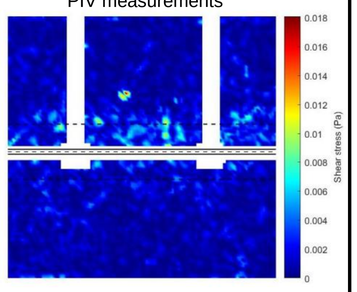
Schedule-dependent light emission and shear-stress estimation

We stimulated the phytoplanktons each hour by starting the oscillation of the grid during 1 minute from the second hour after the end of the light phase (the light emission optimum lies between 2 and 6 hours after the end of the light phase). Between each stimulation, the culture was left to rest. To evaluate a potential tiredness of the culture due to previous stimulations, the same protocol was applied but only 2 and 5 hours after the end of the light phase. For each stimulation, the light emission was recorded and the global mean pixel intensity of each frames was computed.



Based on an **empirical** and **probabilistic** model[7] involving a critical shear-stress value beyond which the light emission can occur, we linked the pixel intensity to the local shear-stress. On the right, we present a comparison between bioluminescence and PIV experiments for shear-stress maps at similar and comparable moments.





Project conclusions and perspectives

Our results show a clear dependence of the light emission intensity on the hours in the circadian cycle leading to challenging reproducibility and repeatability. In addition to this cyclic behaviour, the bioluminescence intensity depends on the previous stimulations too. These information lead us to conclude that only one measurement per day can be performed to estimate the shear-stress with an appropriate calibration. The model used to estimate the shear-stress does not consider this time and previous stimulations dependence. In addition, the model used was established with simple laminar flows while oscillating grid flows are turbulent. All that being mentioned, the feasibility of such bioluminescence experiments in a complex flow configuration and with for now a simple optical setup highlight the potential for the use of *Pyrocystis fusiformis* as local flow shear-stress indicators for experimental investigations at the crossroad between fluid dynamics and bioprocess engineering.

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