

THESIS OF Ph.D. WORK

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**KINEMATICAL SIMULATIONS, MEASUREMENTS IN A TUBULAR
MODEL ALGAE PRODUCTION SYSTEM**

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INTRODUCTION

The challenge of our time is to ensure the future of mankind, which extremely exploits the nature. The use of simple organisms is a possible solution to avoid the approaching, probably catastrophic processes. Microalgae may be used for energy production, neutralization of pollutants, food purposes, or even for preparing different plant protection agents - more recently with the application of genetic engineering.

With the purpose of effective algae production **photo bio-reactors** have been developed. (*Photo Bio-Reactor*, hereinafter: **PBR**) The **PBR** should be constructed based on the needs of the algae to be cultivated. In order to achieve optimal algae growth, appropriate light, gas exchange (CO₂), temperature and pH conditions should be created. My experiments were carried out with a loop reactor of 14 litres. Inter alia, the mixing of gas and liquid phases, the value and variation of light intensity directed on algae and also the build-up on the equipment wall depends on the flow generated by the bubble column.

The primary purpose of my work was to create ideal flow conditions. *Computational Fluid Dynamics (CFD)* software that simulate flow are great help in creating the right flow conditions.

In order to do so, I got to know the **ANSYS FLUENT** simulation environment, where I wanted to model the flow conditions of **PBR** to be modified as accurately as possible. As a result, I have optimized the efficiency of gas input, which determines the flow. By changing flow conditions, the aim was also to facilitate the algae separation. For continuous determination of the algal concentration, I also dealt with methodological development of optical examinations methods.

MATERIALS AND METHODS

1. ALGAE REACTOR

I have performed my measurements with a *Tubular PBR*. It provided an opportunity to maintain the continuous production. For my experiments I have used the *Chlorella Vulgaris* (phylum nr. 116).

The algae flow was provided by the bubble column generated by the inlet gas. The composition of the inlet gas was controlled by a PLC. In the night air was introduced while in the daytime a mixture of CO₂ and air was introduced into the system. A combination of natural light and LED light sources were providing the illumination in the light-isolated room during the daylight.

The water temperature, the pH value and the algae concentration were measured at the upper measuring point of the *PBR*. Transmitted and scattered light were measured in the collateral tube. The temperature of the algae suspension was influenced by the external weather and it varied between 14 and 35 ° C. The temperature below 21 ° C was due to the cooling down in mid-September, which I managed to raise up to 27 ° C with heating.

1.1. REBUILDING OF THE ALGAE PRODUCTION SYSTEM

The new assembly was built in a smaller room with an area of 5 m². The temperature in the room varied from 24 to 33 ° C. In order to minimize the algae cohesion in the tube due to the long, continuous operation over the months, both sides of the O-ring supported the gas inlet (*I01, I02*), making possible the variable algae flow (**figure 1**). Instead of the earlier drain tap, I installed a more efficient drainage (*O02*) based on sedimentation of algae, which is also suitable for separation.



figure 1.: Further developed O-shape displacement

Source: own

Detailed explanation of the new structure:

actuators: X00: control PLC; X01: CO₂ gas reductor; X02 and X03: Rotameter flow regulators (air supply and CO₂); X04: router valve;

out and inflow points: I01: left nozzle; I02: right nozzle; O01: air outlet with expansion tank; O02: drain tap with pre-sedimentator; O03: Moment Sampling chunk;

data logger and sensor: M00: measurement data logger (Almemo 5690); M01: water thermometer (Almemo ZA 930-FS1); M02: air thermometer (Almemo ZA 930-FS1); M03: pressure gauge (Almemo FDA 602 S2K); M04: pH meter (Almemo FY96PHEN); M05:

Global Radiation Meter (Almemo FLA6113-GS); M06: Right Flow Meter (Almemo FLA603-RW4); M07: left illumination sensor; (Almemo FLA603-RW4)

filters: S01: air pre-filter; S02: outlet air filter.

2. CFD MODEL

For the development of fluid dynamics models, together with the partner department of the Budapest University of Technology and Economics we purchased (within a GOP tender) an ANSYS 14.5 FLUENT license. With the attached support service, we created the model of the "O" layout. First, we created a FLUENT project in the ANSYS project manager at WORKBENCH. The applied FLUENT project consists of 3 main parts: geometry, mesh, and solving module.

2.1. 3D MODEL

Boundary conditions and the mesh that can be imported from CAD system into geometric models are very complicated, so we used the *DESIGN MODELER* editor interface built into the *ANSYS* system, where we assembled the model structure from the basic elements.

The first geometric change in our case was the section delimitation, which was implemented by cutting. Cut off parts having the same mesh structure had to be connected before the meshing process.

The second geometric change was the insertion of the inverse pipe end and the drawing of the inflow point (*INLET*) at the end. It is important to mention that, according to our experience, more complex inflow points can be constructed by creating a splitter channel.

The third necessary change was to create an outlet point (*OUTLET*), which was done using a different technique than before. In our case, this meant that after the meshing operation opening of the surface was realized by selecting existing grid points on the meshed surface.

2.2. MESHING

During the meshing, the WALLs and the previously mentioned INLET and OUTLET zones had to be selected. During the meshing of individual sections the appropriate meshing form (which could be: TETRA, HEXA, etc.) and the critical mesh compression close to the wall had to be set. (figure 2).

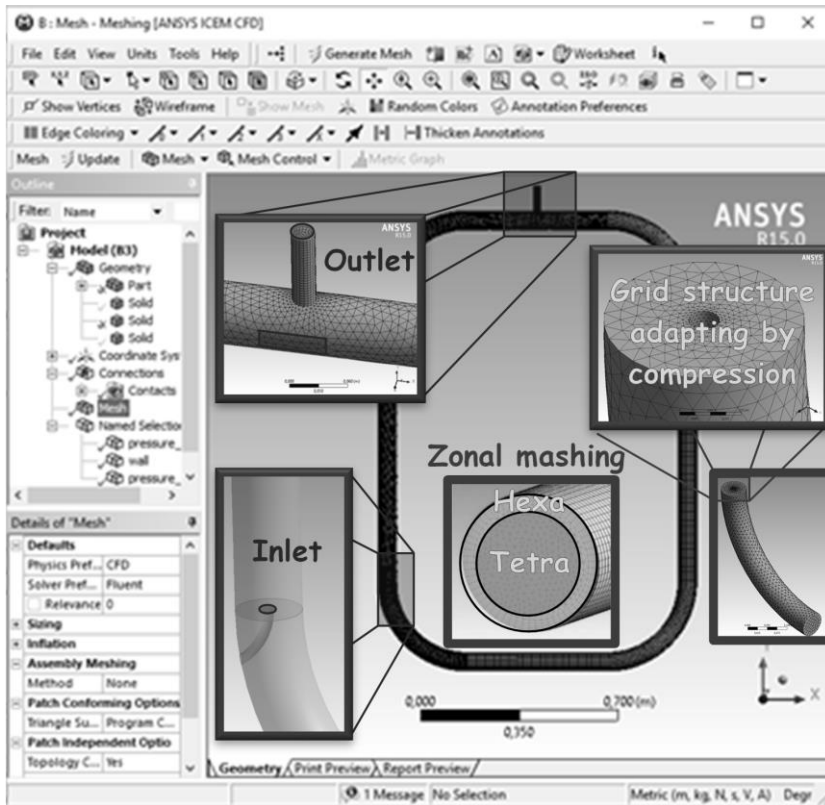


figure 2 Mesh model

Source: own

It is also important to reduce the magnitude of mesh distortion (SKEWNESS), taking into account that it greatly influences the operation of SOLVER. There are a number of options to achieve the favourable SKEWNESS statistics from which we chose the fine-tuning of mesh compression. Deleting or moving grid points would have been unnecessary.

2.3. FLUENT SOLVER

The FLUENT solver parameterization - to test the flow of the suspension - was done according to my own ideas. During the selection, I had to select the appropriate parameters, material and phase characteristics of the model (Mixture and VOF), boundary conditions, run parameters, and the form of displaying the results.

3. ALGAE PRODUCTION AND MEASUREMENT METHODS

Samples of the algae suspension in the device were sampled and / or harvested at scheduled times. From the samples, I obtained information on the concentration of algae in the flow and in the sediment. I harvested the algae if it was necessary.

The sampling was done in two ways: With a pipette at the top of the sampling opening (**figure 1. O03**, labelled with **F (ts)** litre) from the main stream and draining through the drain opening (**figure 1. O02**, designated **Cs**).

Before the necessary harvesting, I also sampled the sediment at all times. After harvesting (1-3 litre), I took a sample from the re-filled BRISTOL system to analyse the biomass growth before and after harvest. The concentration of the samples was measured with a spectrophotometer and validated periodically with dry matter measurements.

The scattered or transmitted and global light intensities from the light source, the pH value, the temperature of the solution and the air, and also the pressure of the feed gas were continuously recorded with an ALMEMO data logger.

The temperature of the air varied between 20 to 31 ° C, which was a great heat fluctuation, but it did not influence my measurements and tests significantly.

2 ml of suspension was used from the samples for the optical examination, which was loaded into the cuvette of the spectrophotometer and diluted as needed in 1/2, 1/4, 1/8, 1/16, 1/3. In case of dilutions I used an automatic (BOECO 100-1000 µl Micropipette) pipette for accuracy and subsequent calibration.

4. DETERMINATION OF ALGAE CONCENTRATION

4.1. DETERMINATION OF DRY MATTER

Based on the accepted method for determining the dry matter of microalgae (Ördög 2014) algae suspension to be measured is filtered on a 2 µm filter paper and after drying the weight of the algae cells remaining on the filter is determined. However, the loss due to small algae pass through the filter paper and also the high price of the filter paper should not be overlooked. That is the reason why I made the gravimetric determination of the algal concentration (MSZ-08 0205-78 1978) as follows.

For the gravimetric determination of the dry matter I used at least 20 cm³, but possibly more than 40 cm³ of suspension or centrifuged concentrate. I applied a 48-hour heat treatment when drying the samples at 80 ° C. The sample holder was closed for a quarter of an hour before returning to the analytical scale. In order to avoid re-moisturizing during the cooling 100 cm³ of airtight containers with a capacity of up to 120 ° C were used instead of using a desiccator. With this method, I was able to perform faster, more efficient, more precise and more accurate measurements. Before the above-mentioned dry matter measurement the concentration increase and the reduction of the alpha suspension volume

were performed by centrifugation. For this purpose, I used – several cycle if need be - a HETTICH Universal 32R type laboratory centrifuge (4 x 150 cm³ suspension at 4000 1/min), so I could compress the suspension for even one hundredth.

When the algae concentration was determined by dry matter measurement, the amount of substances other than the algae in solution was ignored because the dry weight (Wg) of the material in the BRISTOL medium was insignificant compared to the initial weight.

4.2. HYPERSPECTRAL MEASUREMENT

Optical methods can primarily be used to examine the amount of chemical compounds within the algae suspension based on light transmittance. Liquid samples prepared in cuvettes were analyzed by the spectrophotometer of the Hitachi U-2910 Food Laboratory with three different measuring settings:

1. Total range: 190-1100 nm, spectral resolution: 1 nm
2. Restricted upper range: 550-750nm, spectral resolution: 0.5nm (peak of chlorophyll "a")
3. Reduced lower range: 350-550 nm, spectral resolution: 0.5 nm (β -carotene peak).

The spectrum of each solution was taken in 3 replicates. Considering that the instrument gives each point of the spectrum by measuring and averaging of 10 data this means 30 repetition. That is how I got coherent, machine-noise-filtered data.

4.3. OPTICAL ONLINE MEASUREMENT

In version 2017 of the **PBR** to continuously measure algae concentration light sensors which measured the amount of transmitted and scattered light flux were only located in the left branch.

In order to measure the algae concentration continuously the **PBR** 2018 which I reconfigured has light sensors in both branches, providing a measurable light scattering and transmitting.

5. DATA PROCESSING METHODS

The measurement data were processed under WINDOWS 10 operating system with OFFICE 360 Excel program. Data sorting, regression calculations and editing of illustrative figures were carried out in this software. Calculations with the simulation model were performed with the ANSYS 14.5 FLUENT module.

RESULTS AND DISCUSSION

1. FLOW MEASUREMENTS

The basic criterion of operating an effective *PBR* is to ensure the proper flow conditions;

1. I set the optimal amount of carbon dioxide and air mixture coming from the nozzles for mixing and absorption,

2. I made the flow turbulent to minimize the photosynthetic inhibitory factors, it prevented algae to stay in the light poor (dead) zone near to the axis of the relatively thick (diameter 65 mm) tube.

3. I changed the geometry of the *PBR* to reduce the algae sedimentation. During the rebuilding, I paid special attention to avoid slowing down the flow in the vicinity of the wall.

To assure that the *PBR* complies with the criteria I had to understand the existing flow conditions. In order to clarify the flow conditions, I created a virtual model under ANSYS FLUENT program environment, see **figure 2**. Input data was obtained from preliminary surveys and calculations. In order to design the proper gas flow, I experimented with a variety of nozzle solutions and different input air flow rates. I found that the nozzles performed well even at high input airflows:

- despite the high speed difference, the shear effect was minimal - the algae cells remained intact,
- the mixing effect was adequate for both light quantity and gas exchange,
- wall alignment adhesion has become manageable and minimized.

The model was run in FLUENT with a simpler *Mixture* and later with a *VOF* solving algorithm requiring high computing capacity.

In the vicinity of the nozzles, due to their size and increased flow rate, greater meshing density was necessary to avoid computational errors caused by distortion. In the flattened nozzle simulation, it was a particular challenge to manage the distributed flow, because FLUENT allowed to use one input only. I tried to solve this issue in two ways:

- merging of the inlet channels with a gap
- and by building an undivided inlet point with two channels in the front.

Comparing the *Mixture* simulation with the real flow we can state that the main flow forms appear in the model, but their separation is not possible due to the averaging (figure 3).

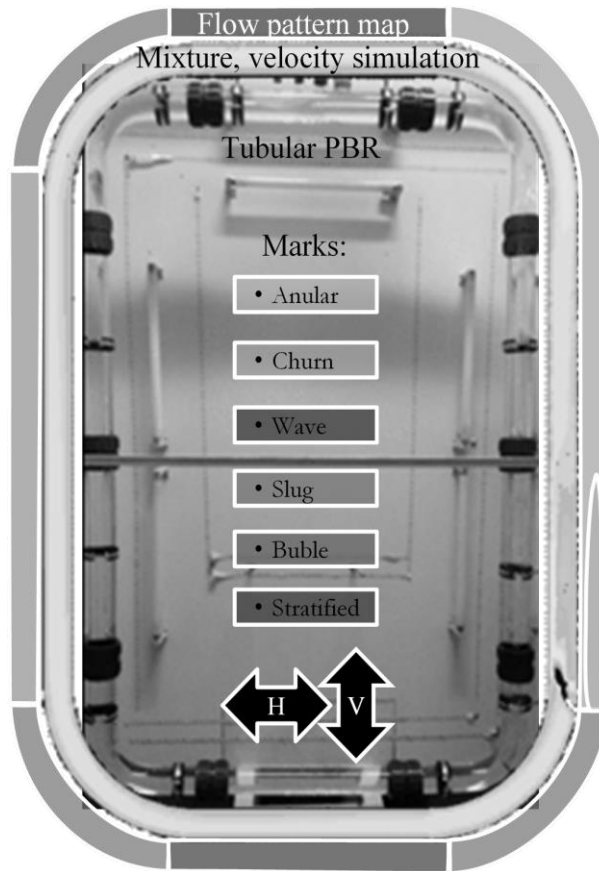


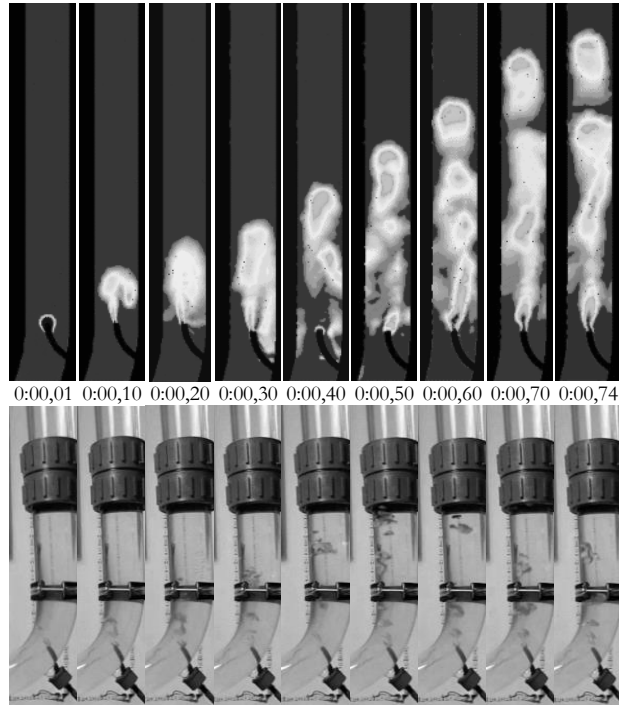
figure 3: Comparison of flow characteristics of flow in the algae production device and in the mixture model

Source: own

The mixture simulation was run on a step-by-step basis, with a snapshot taken of every 25th step. To achieve the convergence of the equation, nearly 10,000 steps were required, which meant more than 3 hours of running time. The average velocity data obtained gives an inaccurate image of the actual flow of each phase.

In order to define processes more precisely, I ran the completed virtual model using **VOF** simulation. Analysing the environment of the outflow point, I simulated a period of 0.74 seconds but even that required nearly two weeks to run. In case of the **VOF** model, we got an accurate flow image, but it requires significantly more calculations (625,000 steps) compared to the **Mixture** model. In case of **VOF** simulation, the counting was done on a time basis, with a one second snapshot of 8.340 steps and 3 hours of running time. The

correct operation of the *VOF* simulation model with predefined starting data was verified by analysing 120 frames / second HDR video recordings (figure 4).



*figure 4: Comparison of the flow conditions in the PBR and mixture model
(upper image row: stages calculated by model, lower image row: stages of the video record)*

Source: own

Since the density of the algae suspension differs to a negligible extent from the density of the water (as verified by the Mixture model running until convergence), it would not influence the flow image, so I tested the VOF simulation model using the water density data.

2. INTERPRETATION OF HYPERSPECTRAL MEASUREMENTS

The algae suspension contains microalgae with high pigment, which according to Becker, Sokolichin, and Eigenberger 1994 consists mainly of chlorophylls and carotenoids, and also contains the growth medium of BRISTOL as a nutrient source. The dissolved matter content of the growth medium was negligible and it did not affect the optical measurement.

The Hitachi's spectrophotometer control program provides the following analysis features:

- which helps to detect peaks and valleys in the spectrum,

- which calculates the difference between the peak and the associated wavelength valley, and
- which defines the area below the peak with a background correction.

In optical examination of the *Chlorella vulgaris* algae that was produced by me, peaks and valleys of the characteristic absorbance were sorted by statistical method and their frequency was also determined (**figure 5**).

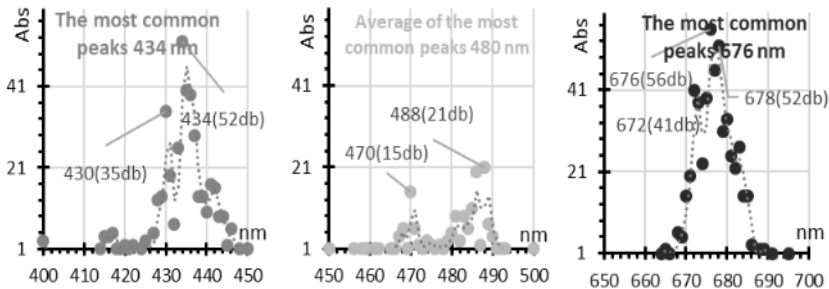


figure 5: Frequency distribution diagrams of peaks (around 434, 480 and 676 nm)

Source: own

There is no consensus among researchers on the characteristic wavelength (400-460 nm and 650-680 nm) of microalgal cultures.

Relationships between the absorbance peaks of algae suspension (434, 480 and 676 nm) and the algae concentration can be seen in **figure 6**.

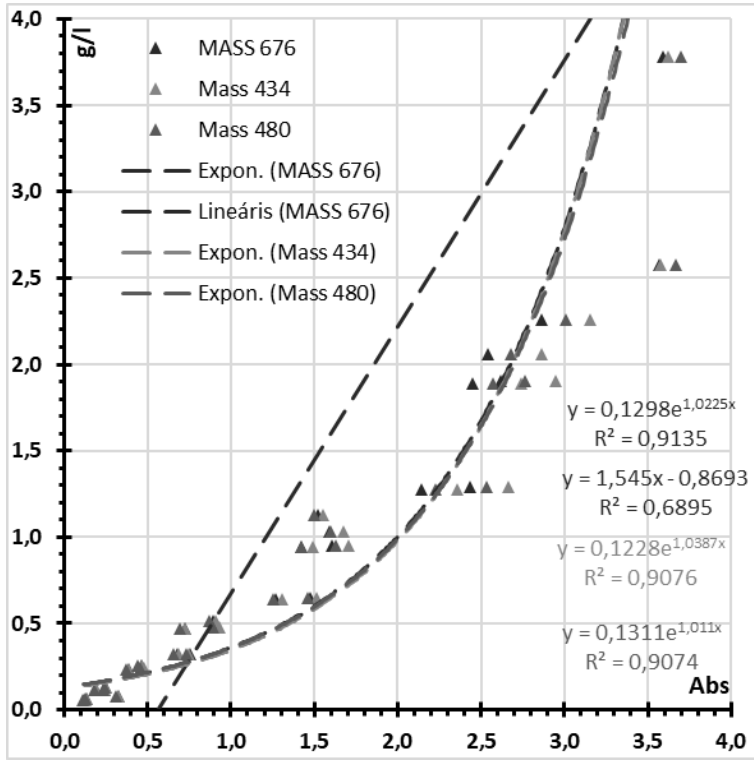


figure 6: Absorbance peaks (434, 480 and 676 nm) and their function of concentration.

Source: own

In case of all three wavelengths I got a very close exponential correlation. Parameters of correlations are very similar, so the graphs show practically fitting curves. The calculation was also done by taking into account the data measured at all three wavelengths (434, 480, 676 nm) and I found that their calibration curves can also be described by the same model ($y = 0.128 * e^{1.024 \cdot x}$, $R^2 = 0.9093$).

3. MEASUREMENT OF TRANSMITTED AND REFLECTED RADIATION TO CONTINUOUSLY MEASURE THE ALGA YIELD

3.1. INTERPRETATION OF DATA MEASURED AT THE FIRS VERSION OF THE LOOP REACTOR (2017)

Continuous changes in the algae production were detected by measuring the intensity of the direct or scattered light coming from the light source and passing through the cross section of the left side of the tube of the **PBR**. The results of the flux meter show that the

algae grew significantly in 5 days as indicated by a significant decrease in the intensity of the transmitted light to about thirtieth (**figure 7**).

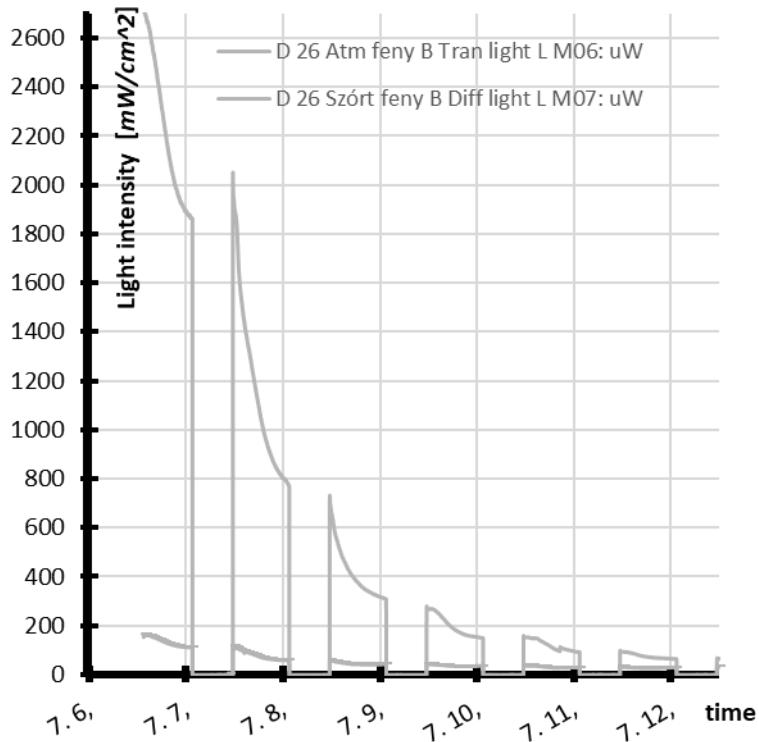


figure 7: Transmitted (higher) and reflected (lower) light intensity in the PBR- after the starting of the experiment on 05.07.2017 in the period of 06.07-12.07

Source: own

The interruptions in the figure show the periods of daylight: illuminated days and dark nights (without measurement). The scattered light intensity also decreased in a similar way, but the change started from a much smaller value and the steepening was lower. No correlation was found between the intensity of the scattered light and the algae concentration ($R^2 = 0.0644$). Due to the inconsistency of the measurements with the scattered light, I decided to move the detector in the reconstructed **PBR** and then use it to detect the transmitted light. The relationship between the transmitted light ("transmitted (left)" sensor) and the algae concentration shows a medium power function correlation ($R^2 = 0.6999$) (**figure 8**).

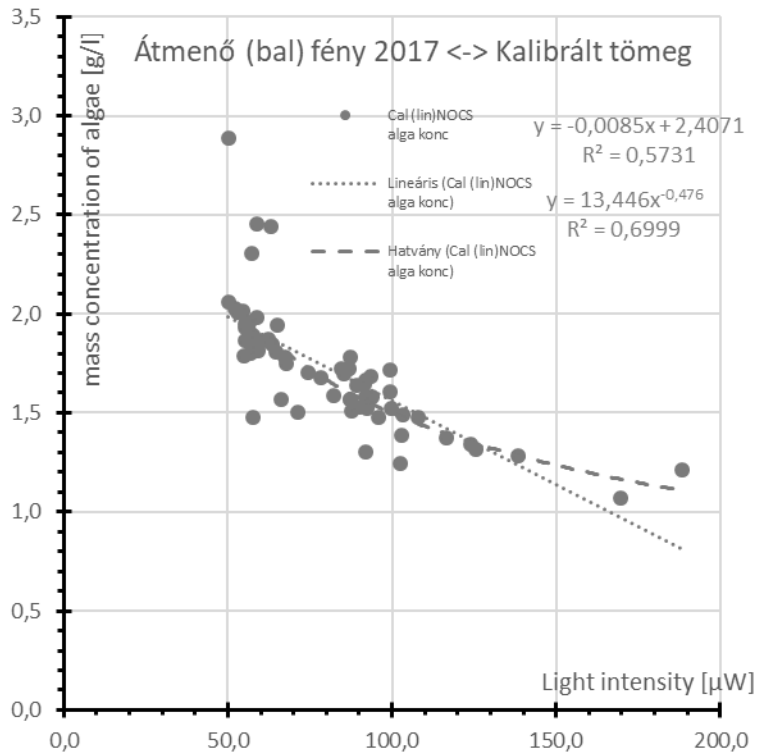


figure 8: The function of light intensity and yield (left detector) in 2017

Source: own

Some points in the medium power function (dashed) curve are remarkably distant. The mistake was probably caused by the algae sediments in the left tube branch, because at the time of the measurement the gas inlet was only provided in the right tube branch.

3.2. INTERPRETATION OF DATA MEASURED BY MODIFIED VERSION OF THE LOOP REACTOR (2018)

With the gas injection capability on both sides of the loop reactor, alternating flow of algae became feasible. Minimizing the adherent algae mass also made the operation of continuous light detectors more reliable. With the modified version I made algae production in the period between 16.01.2018 and 23.12.2018. I investigated the correlation between the light intensities measured by the detectors at the time of sampling and the algae concentrations determined in the samples.

The relation between transmitted light in the left branch tube ("transmitted (left)" detector and the algae concentration shows a very high power function correlation ($R^2 = 0.9286$) (figure 9).

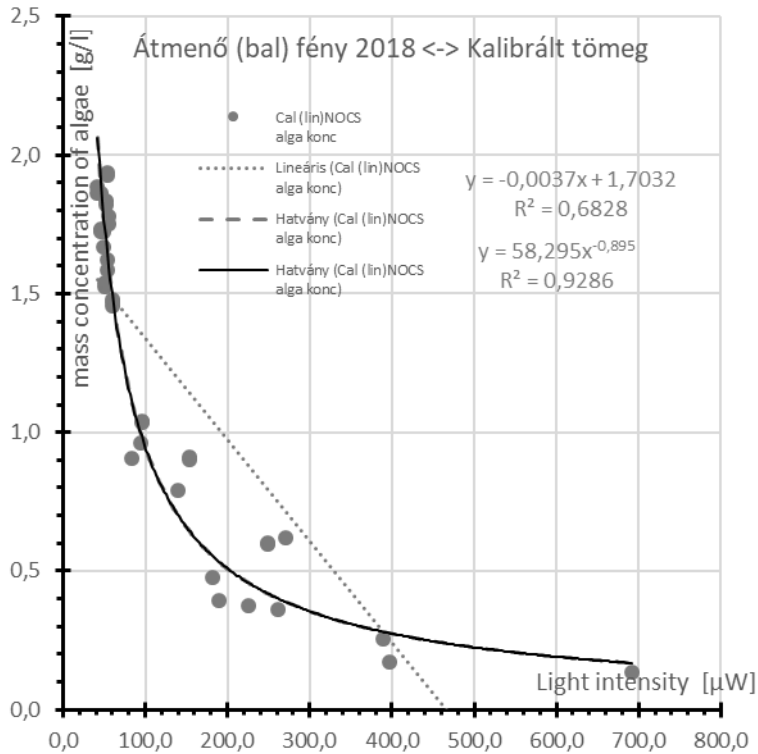


figure 9: Correlation between transmitted light and the algae concentration (left detector) in 2018.

Source: own

The power correlation is not only high but also the value of the exponent is close to -1 (-0,895). Exposure -1 is a reciprocal correlation, so I also examined the relationship between the reciprocal of light intensity and the algae concentration. There is a linear relationship between reciprocal light intensity and algae concentration ($R^2 = 0.9363$). If the section of the axis is selected as 0, the relationship becomes a simple proportion ($y = 87,463 * x$, $R^2 = 0,9179$). After the conversion, I also got a very strong power function correlation ($R^2 = 0.9351$) between the transmitted light and the algae concentration measured with the detector in the right tube branch. In this case, the reciprocal of light intensity and the algae concentration could also be expressed in a simple proportion ($y = 105.72 * x$, $R^2 = 0.9332$).

4. RELATION BETWEEN FLOW SPEED AND ALGAE REMOVAL

During the 2018 rebuilding of the **PBR**, thanks to the adequate flow rate adjustment and alternating gas injection I managed to prevent the formation of algae sediments on the wall at the ascending and descending branches. With the help of an algae trap located at the bottom of the device, the dense algae suspension could be regularly removed.

5. TEMPORAL CHANGES IN ALGAE PRODUCTION

Based on calibration correlations I determined the algae concentrations of both periods as a function of time.

5.1. INTERPRETATION OF DATA MEASURED WITH THE FIRST VERSION OF THE LOOP REACTOR (2017)

In the first version of the *PBR*, there was also a smaller sampling chunk, which is suitable for collecting algae. During the sampling, algae concentration was also determined at the sampling chunk. After the first week, in addition to the chunk sample, 3 litres of sample were harvested. **figure 10** shows algae concentrations at sampling different times.

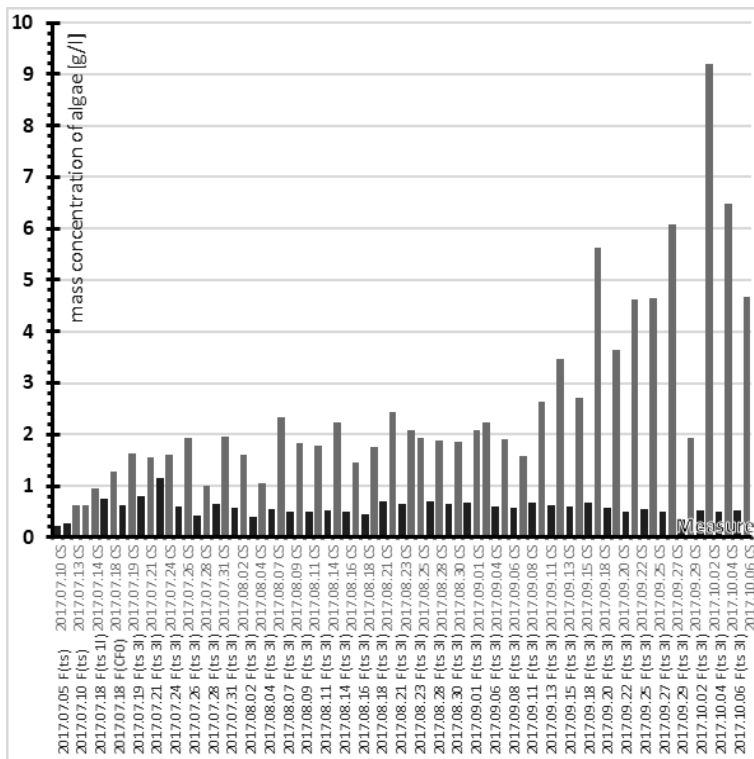


figure 10: Algae concentrations measured from the main flow (black) and the sampling chunk (grey) (2017)

Source: own

The concentrations of the main flow fluctuate around 0.5 g / l, which is visible in figure 10 and identical with concentrations usual mentioned several times in the literature. Algae concentrations collected from the sampling chunk fluctuate around 2 to 8 g / l, depending primarily on the time elapsed since the previous sampling.

figure 11 shows the cumulative amount of algae removed over three and a half months of operation.

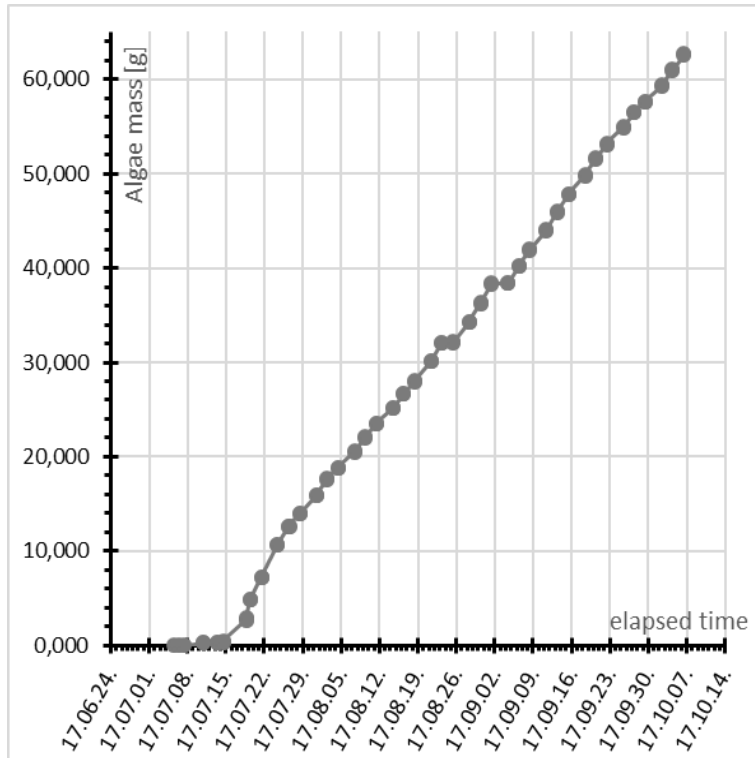


figure 11: Total removed algae mass (g) in case of the original design of loop reactor (2017) until the given day.

Source: own

The detected amount of algae dry matter during the experiment was obtained by sampling constant quantity of algae suspension. The total yield achieved during the whole period was 64 g.

5.2. INTERPRETATION OF DATA MEASURED WITH AMENDED VERSION OF THE PBR (2018)

In the reconstructed **PBR**, I installed a drainage chunk that is more efficient than the previous version and suitable for sedimentation. The idea was given by the algae sedimentation observed in the previous drain version. The newly built chunk together the new method developed in parallel (mainstream samples were taken through the upper sampling point) allowed the sampling of the sedimented algae at all times. On the 13th day (26.01.2018) and on the 41st day (23.02.2018) algae harvesting was performed instead of sampling (**figure 12**).

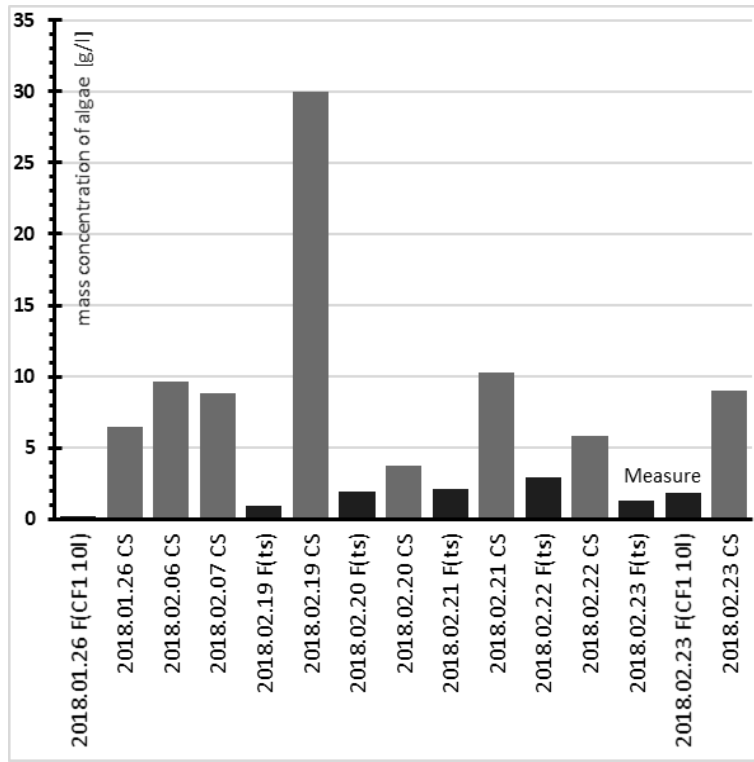


figure 12: Algae concentrations measured from the main flow (black) and from the improved drain chuck (grey) (2018)
Source: own

Concentrations of samples settled in the drainage chunk fluctuated in the range of 5-10 g / l. Samples of 10 ml were taken periodically from the main flow. In the 14 litre device the sampling did not decisively changed the concentration of remaining algae. **figure 13** shows the amount of total algae mass removed during the 41 days of operation.

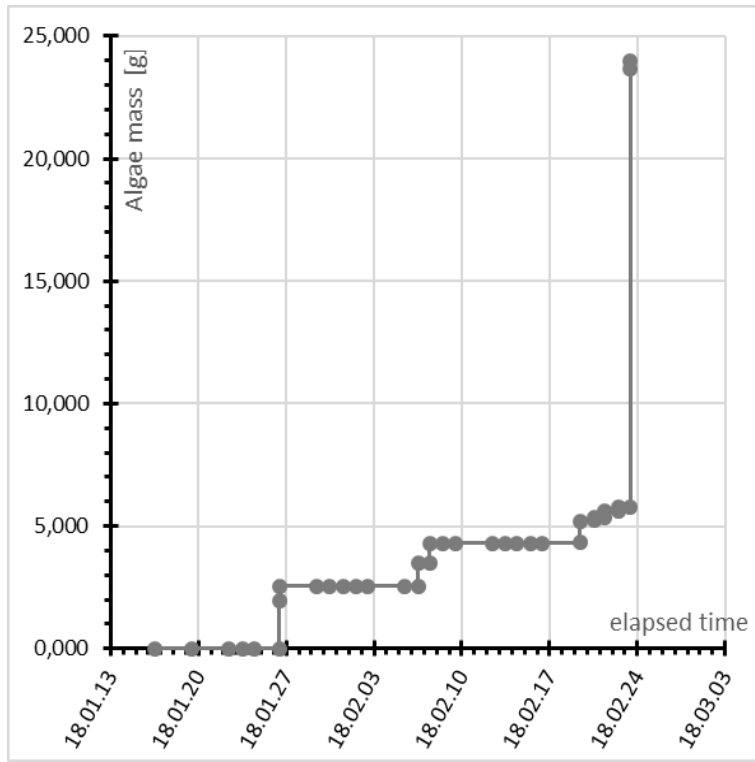


figure 13: The total algae mass (g) removed from the modified loop reactor until the given date.

Source: own

The significant leap in quantity seen on day 13 (26.08.2018) shows the harvesting a quantity of 10 l, that is almost the amount of the entire main flow. On day 41 (23.02.2018) the system was emptied. The total algae yield achieved during the whole period was 24 g.

NEW SCIENTIFIC RESULTS (THESES)

1. I have investigated the injected gas stream generated impact on the flow in a **PBR** used for algae production. The flow was simulated with **Mixture** and **VOF** software. The model calculation results were consistent with the measurement results in case of **VOF** software. The flow forms were not separable with calculations made with the **Mixture** model but the turbulence points were well approximated
2. I have proved that the peak both of β -Carotene at 434.480 nm and chlorophyll 'a' at 676 nm can be used for the optical determination of algae concentration. Their calibration curves can also be described by a common model ($y = 0.128 * e^{1.024 \cdot x}$, $R^2 = 0.9093$).
3. The algae concentration can be continuously monitored by measuring the intensity of transmitted light. According to the high reliability function, the algae concentration is in inverse ratio to this detected light. In the suitable concentration range of the algae production, there is no any significant correlation between the intensity of scattered light and the algae concentration.
4. With suitable modification of the **PBR** the correct gas stream was solved. With it and setting the suitable flow rate, the algae sedimentation was avoided on the **PBR** tube wall.
5. The newly mounted outlet chunk with combined algae trap on the **PBR** could solve the algae extraction from the suspension in a simple gravity way. The algae trap on the bottom of the device produce algae suspension, which is about 10 times (5-10 g / dm³) higher concentration, than it is in main flow.

PUBLICATIONS

I. ARTICLES IN FOREIGN LANGUAGE PUBLISHED IN REVIEWED JOURNALS

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