

# Study of *Chlorella vulgaris* sedimentation process

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**Abstract.** The paper reports the results of *Chlorella vulgaris* sedimentation process including description of cultivation condition of microalgal biomass. The process of algae cultivation was carried out in photobioreactor comprising systems of carbon dioxide supply, mixing and artificial LED illumination. The growth of microalgae was determined alternatively in three ways by measuring the amount of dry mass over time, counting the cells and measurement of optical density by use of a spectrophotometer. Algae biomass with different concentration was subjected to the separation process by gravity. This led to the determination of the characteristic of sedimentation process for different concentrations and cell sizes. The experimental results indicate that sedimentation process offers a tool with a potential application for microalgae harvesting.

## 1 Introduction

Algal biomass is more and more commonly considered as a raw material with potential application in production of biofuels as well as electricity and heat generation. Due to the depletion of oil resources as well as an increase in environmental pollution, more and more extensive research is being carried out with regard to the potential application of algae biomass as a source of renewable energy [1]. Algae also contain a wide range of nutrients, so they can play the role of a source of food for humans and farm animals. The cultivation of algae does not need to occupy large areas, and thus the production rate of their biomass is much higher than traditional crops. In addition, growing algae in closed photobioreactors can provide high biomass concentrations, as a result of which the cost of algae production can be reduced. Nevertheless, the reactors themselves require the input of relatively expensive materials and consume considerable amounts of energy. The assessment of the economic feasibility of the cultivation also needs to take into account the costs of lighting and CO<sub>2</sub> supply, energy for mixing as well as costs of nutrient. The method applied for separation of this type of biomass, which also determines the efficiency of its cultivation also forms a significant consideration. In spite of this, research works dealing with the production conditions of this type of algae are more numerous, as algal biomass both offers an alternative to renewable energy sources as well as finds a potential application in removing CO<sub>2</sub> from flue gases and in sewage treatment. All of this prove demonstrates the potential applicability of this type of mass in environmental protection.

At present, the separation of algae from cultivation medium and their dewatering form the most energy-

consuming stages of the cultivation. As a result, research focusing on the reduction of the overall costs of their production forms a current need. To support this statement, we can say the energy needed for centrifuging algae from 1 liter of culture medium is equal to 13.8 MJ/kg of dry mass on average, and in the case of algal strains with a high lipid content, it can reach as much as 26.2 MJ/kg of dry matter. The cultivation of algae applied in the production of biodiesel is carried out on a large scale and the costs associated with their isolation can incur considerable costs. It is estimated that the cost of algae dewatering forms a 20-30% of the total expenses needed for the production of biofuels [2]. The whole process is also complex due to the microscopic size of the algae cells (2-200 μm) [3] and it is frequently carried out over several stages, as present by the example in Figure 1.

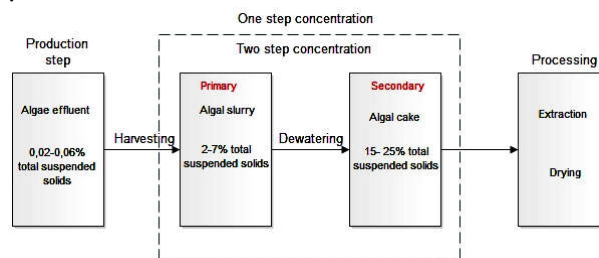


Fig. 1. Diagram of algae cultivation for energy purposes [4].

There are a variety of studies into the extraction of algae accompanying water treatment processes and focusing on the effect of various parameters on this process. Coagulation and flocculation techniques are commonly applied for this purpose. It is also possible to perform the separation of algae from the cultivation medium in a similar manner. The technique applied for

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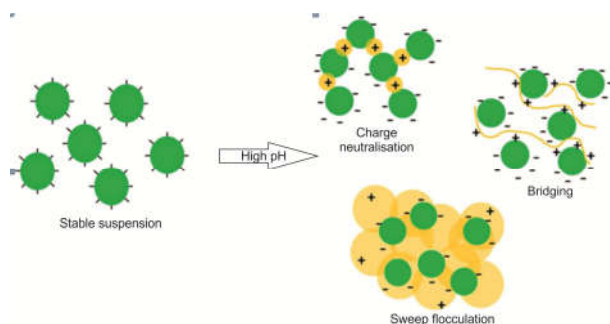
the separation of biomass from liquid depends primarily on the strain of algae applied in the process and the concentration of the suspension. Initial flocculation and coagulation can also be carried out with the purpose of improving the efficiency of the existing types of mechanical processes (centrifugation, filtration, sedimentation) [5,6,7,8]. In the selection of the technique applied to select algae concentration, the aspect of great importance is associated with the quality of the final product. The use of additional chemicals in the production of algae is not recommended e.g. in the food industry, where high product quality is required.

Among the above methods applied for harvesting of algae, sedimentation plays an important role since this method guarantees low operating costs. However, this approach is very slow (the velocity of gravitational settling of algal cells is equal on average to 0.1-2.6 cm/h) and performs better for larger cells, such as *Spirulina* [3]. Nevertheless, the technique is cheap and offers an easy control the process.

A preliminary flocculation process is carried out in order to improve the sedimentation efficiency, resulting in an increase in the size of the settling particles. Flocculation in this case increases cell dimensions, as a result of which better conditions are provided for the settling of algae. Unfortunately, this requires the use of chemical compounds ( $Al_2(SO_4)_3$ ,  $FeCl_3$ ,  $Fe_2(SO_4)_3$ ) [3,4,8]. Many types of algae may also be prone to autoflocculation, the intensity of which depends on the culture conditions. Mechanisms of this are most commonly associated with:

- electrostatic interaction between the negatively charged cell surface and positively charged ions in the culture liquid (neutralization of charges),
- development of salt deposits and extracellular polymers substances (EPS), as well as sweep floc mechanism involving the interaction of microalgae cells with bacteria,
- formation of positive bridge complexes between cells connected by extracellular polymer compounds (bridging).

The potential for the occurrence of various mechanisms of autoflocculation is schematically shown in Fig. 2.



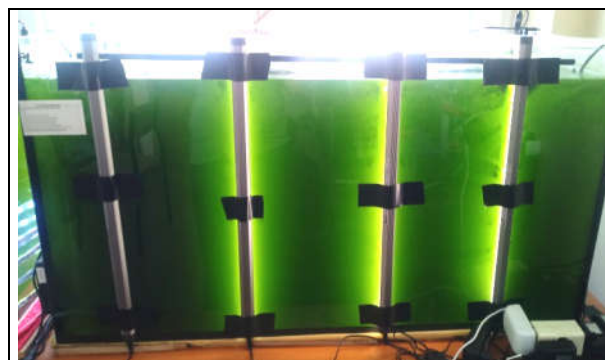
**Fig. 2.** Examples of autoflocculation types: charge-neutralization, bridging and sweep floc mechanism [9].

Other factors that can influence the autoflocculation process are also worth more extensive discussion. Many studies indicate the dependence of the production of

extracellular polymer substances on the level of illumination and temperature [10,11]. Also the concentration of oxygen dissolved in the culture liquid may result in the formation of large and compacted floccoli [12]. The increase in EPS production is also related to the aging of cells in batch cultures, in which the best conditions for flocculation are known to exist at the end of the exponential growth phase and in the stationary phase [13].

## 2 Results and discussion

The study of sedimentation was carried out in laboratory conditions in order to determine the effects of the variable concentrations of microalgae cells on the velocity and efficiency of the settling process. Two strains of single-cell *Chlorella vulgaris* BA 002 and BA 167 algae were used as the study material in the tests (strains were acquired from the Culture Collection of Baltic Algae of the Institute of Oceanography at the University of Gdańsk). These strains differ slightly in their size and morphology. Algae were cultivated in a stirred tank photobioreactor (Fig. 3) using the standard F/2 production medium based on distilled water at the constant temperature of 27°C.



**Fig.3.** Experimental setup applied for research of productivity of *Chlorella vulgaris* microalgae.

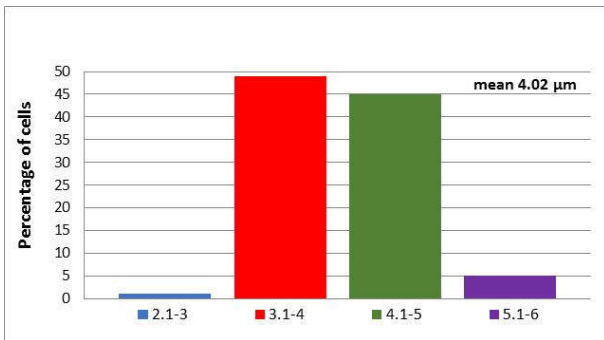
A summary with details of the production medium based on the procedure described in [14] is found in Table 1. The composition of the production medium plays a crucial role since it considerably determines the productivity of the algal culture and the size and composition of the substances found in the algae (such as lipids).

**Table 1.** Composition of cultivation medium used in the study.

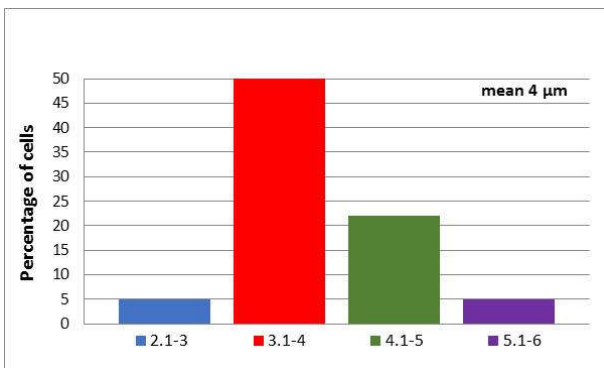
Medium components	Concentration [g/dm <sup>3</sup> ]
FeSO <sub>4</sub> · 7H <sub>2</sub> O	0.042
K <sub>2</sub> HPO <sub>4</sub>	0.53
MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.34
Citric acid · 2H <sub>2</sub> O	1.094
Urea	1.1
CaCl <sub>2</sub> · 2H <sub>2</sub> O	0.08
Na <sub>2</sub> SO <sub>4</sub>	0.2
Glucose	40

The algal slurry samples harvested during the culture provided data needed to determine its productivity and individual cell size distribution. As a result of microscopic tests carried out using the Motic AE2000-T inverted microscope with digital camera Moticom 5+ with image analysis software, the study found that both algae strains have a similar range of individual cell sizes, as shown in Fig. 4 and Fig. 5. The mean size of cells for both suspensions was 4  $\mu\text{m}$ .

The microscope was also used to determine the number of cells per unit of the medium volume. Each sample with a given number of cells was tested simultaneously to determine the absorbance value and the dry mass content. For this purpose, the Thermo Scientific Orion AquaMate 8000 UV-Vis Spectrophotometer and a RADWAG moisture analyzer MA.X2.A with accuracy of mass measurement of 0.1 mg were used.



**Fig. 4.** Distribution of particle size (cells) for *Chlorella vulgaris* BA 002 algae.

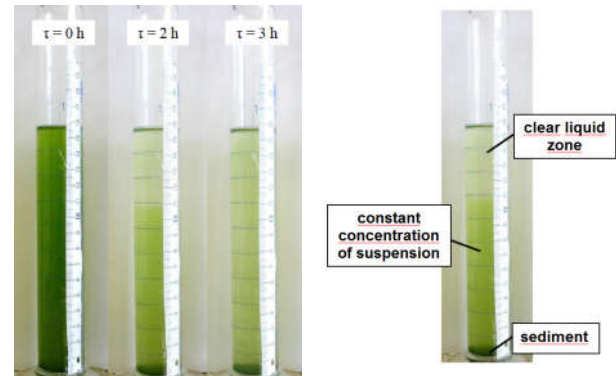


**Fig. 5.** Distribution of particle size (cells) for *Chlorella vulgaris* BA 167 algae.

The sedimentation tests were carried out in measuring cylinders with a capacity of 100  $\text{cm}^3$ . Each cylinder contained a reference scale applied to record the results in millimeters. Tests were carried out for various initial concentrations of the suspension, as shown in Figure 6 for a specific example.

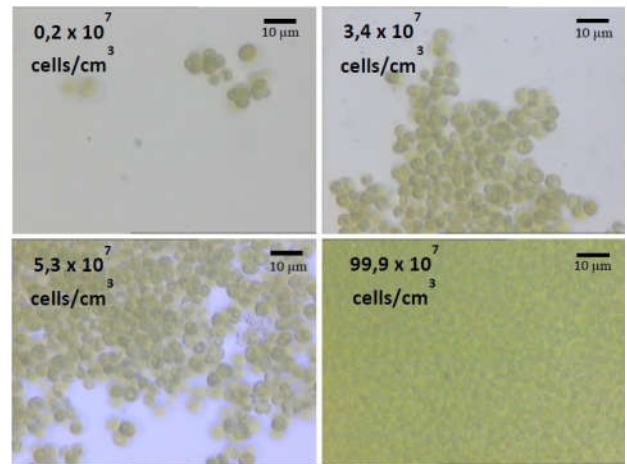
As we can see, the process is accompanied by the occurrence of an interface between the clear liquid and the slurry at the top of the cylinder and between the slurry and sediment in its bottom. In this way, three zones are created with different concentrations of microalgae. During the tests, measurements were made at the time intervals of 5, 15 and 30 minutes depending

on the settling velocity of the suspension. At the same time, samples were taken from each of the analyzed zones (i.e. clear liquid zone, interface and the sediment) with the purpose of determining the concentrations of microalgae by applying microscopic observations.



**Fig. 6.** Suspension with the concentration of  $3.25 \cdot 10^5$  cells/ $\text{cm}^3$  throughout the settling process.

These observations as well as the measurements of dry mass provided the varying concentration of algal cells in each zone throughout the sedimentation process. Examples with microscope slides obtained for suspensions with various concentrations are found in Fig. 7.



**Fig. 7.** Examples of microscopic preparations for *Chlorella vulgaris* BA 002 algal suspensions with various concentrations.

As we can see from the microscope photographs, in each zone there is a different number of cells, and in the sludge interface zone, at the level close to the bottom of the cylinder, a variation in the concentration occurs. On the basis of dewatering and drying of samples with different concentrations of algae, a relation was developed to account for the volumetric concentration of algae with their mass concentration, as well as with the absorbance value. These values were subsequently used in assessing the efficiency of sedimentation. The relation between the various sizes for *Chlorella vulgaris* BA 002 strain is summarized in Table 2.

On the basis of the experimental data, sedimentation curves were developed to determine the variations in the location of specific interfaces. Due to the fact that the

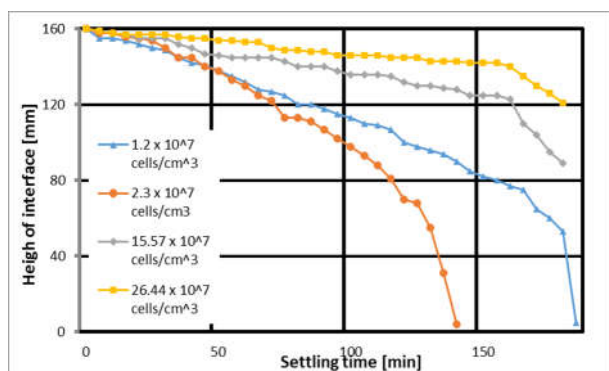


thickness of the sediment was very small, i.e. equal to 1-3 mm on average, a decision was made that the study will only focus on the zone between the clear liquid and suspension with constant concentration (it was done with the purpose of clarity of the drawings).

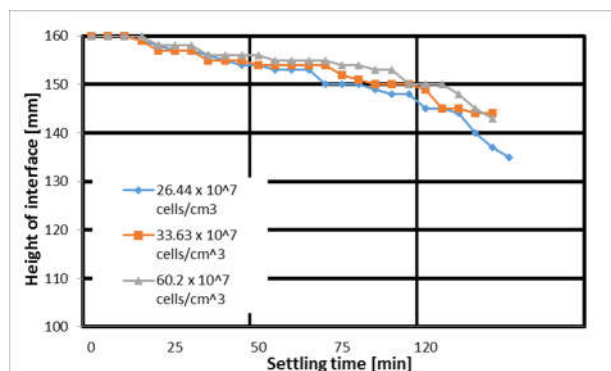
**Table 2.** Measured values of *Chlorella vulgaris* BA 002 algal suspension.

Number of cells in unit volume [cells/cm <sup>3</sup> ]	Concentration [g/dm <sup>3</sup> ]	Absorbance [-]
$0.5 \times 10^7$	0.437	0.185
$1.0 \times 10^7$	0.733	0.456
$10 \times 10^7$	6.063	5.342
$50.0 \times 10^7$	29.750	27.06

Examples with the results of sedimentation process of the *Chlorella vulgaris* BA 002 and BA 167 algae strains are presented in Figs. 8 and 9.



**Fig.8.** Algae settling curves – *Chlorella vulgaris* BA 002.



**Fig.9.** Algae settling curves – *Chlorella vulgaris* BA 167.

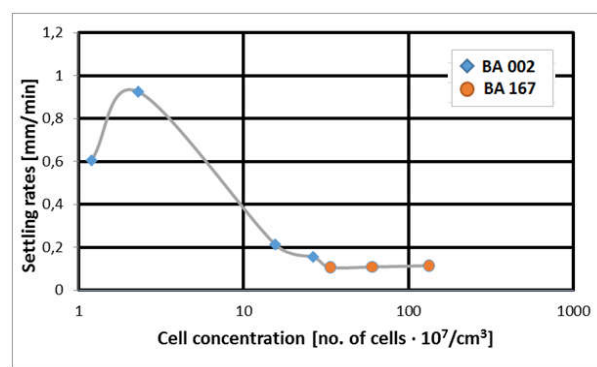
The analysis of the experimental data demonstrates that the velocity of formation of the clear liquid zone is variable over time and is relative to the initial value of the concentration of a specific suspension. Throughout the initial phase of the settling period, the course is linear, but after about 120 minutes the settling rate increases. Microscopic observations of the sizes of particle agglomerates formed as a result of autoflocculation were carried out to determine the variable conditions of the suspension during the settling process. Dino-Lite Edge AM7515MT8A Digital Microscope was used for the study. Examples of the results of the particle size formed are shown in Fig.10. We can also note here that the average size of

agglomerates in this case was 20-50  $\mu\text{m}$  and it was many times greater from individual algal cells, whose size is 3-5  $\mu\text{m}$ . The studies conducted in this area demonstrated that the effect of algae agglomeration does not occur in the conditions when very low concentrations are present in the suspension and is limited in the extent at its high concentrations.



**Fig. 10.** Microscope image of algal suspension – *Chlorella vulgaris* BA 002 with the initial concentration of  $1.3 \cdot 10^7$  cells/cm<sup>3</sup>, (911 mg/dm<sup>3</sup>).

The consequence of the occurrence of different algal cell sizes during settling process is associated with various settling velocities, as shown in Fig.11.



**Fig. 11.** Settling rates of *Chlorella vulgaris* algae for BA 002 and BA 167 species.

From the course of the curve in this figure, as can conclude that algal cells have lower settling velocities at both low and high concentrations compared to the conditions marked by their mean concentrations. The singularities in terms of settling velocities result from the interactions of the algal cells, which could be likely to either repel each other (as the particles can carry a negative electrostatic charge) or agglomerate, depending on the chemical state of the culture medium. The explanation of this phenomenon requires further systematic research.

### 3 Conclusions

The present study confirmed the potential applicability of settling processes for harvesting algae from the cultivation medium. The analysis showed that the

settling velocity is significantly affected by the initial concentration of the suspension and the conditions in which the cultivation is performed. The acceleration of the process can involve the use of specific concentrations of the suspension and the application of the phenomenon of autoflocculation. A more comprehensive description of the process needs further studies in the area.

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