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Cultivation of microalgae *(Chlorella vulgaris)* in laboratory photobioreactor

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Abstract: Algal cultivation fits in sustainable development of natural environment. Their biomass is increasingly regarded as a potential resource with a potential in production of biofuels, electricity and heat. Algae contain a lot of nutrients, so they can be used as food for humans and livestock. Because of their valuable composition (high nutrient content) they are used as supplements of balanced diet, in turn taking into account their biosorption ability they are used to detoxification of human body. Algae cultivation does not require large areas of land to expose cells to sunlight, so their production rate is higher compared to the vascular plants. Moreover, the cultivation in closed photobioreactors leads to high biomass concentration. However, this type of cultivation needs to be performed under strictly observed conditions, which can be evaluated by experiments. This study reports the results of a study involving the development of test stand in which high biomass productivity of *Chlorella vulgaris* can be achieved. This paper focuses on a study including *Chlorella* cultivation and the results of an experiment conducted in a laboratory photobioreactor.

Keywords: algae cultivation, biomass productivity*Chlorella vulgaris*, photobioreactor

JEL codes: Q16, Q21

1. Introduction

Algae form a part of a very extensive group of photosynthetic microorganisms (Fig. 1) with a very high specific growth rate. Algae also form some of the most common organisms on Earth, as they are capable of living in a wide range of conditions. Hence, they are scattered in the entire biosphere. Usually they can be found in water (from sweet to extremely salty waters). They are equally common in water as on land. They differ from other microorganisms due to the presence of chlorophyll and their ability to conduct photosynthesis in each single cell. The separated nucleus, pyrenoid, cellular wall, chloroplasts containing chlorophyll and other colorants, stigma

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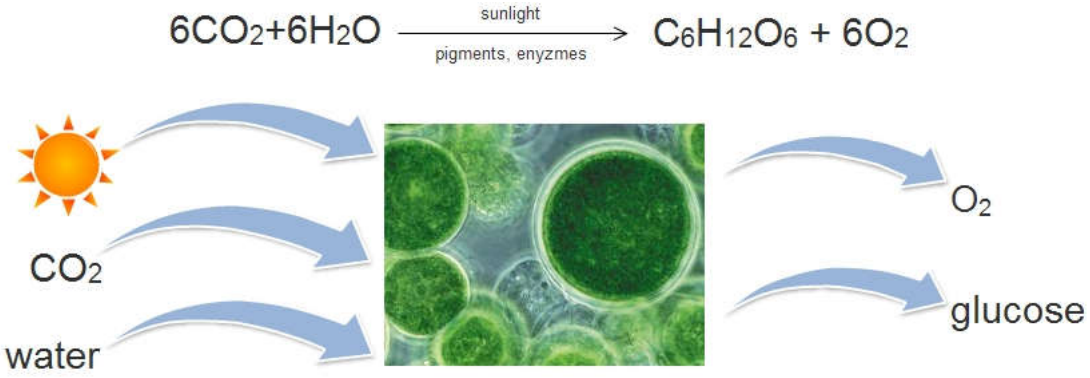
and flagella form the principal components of algal cells (Singh et al., 2012: 2348 ; Mata, 2010: 219). To this time, descriptions have identified over 72,500 algae species and it is estimated that there are from 200,000 to one million of distinct species (Guiry, 2012: 1057). Microalgae forming organisms capable of photosynthesis convert solar energy into the chemical one (Mohsenpour and Willoughby, 2013: 147). They produce valuable biochemical compounds, such as colorants, proteins, carbohydrates and lipids used e.g. for biodiesel production (Mohsenpour and Willoughby, 2013: 147; Chen et al., 2011: 71).

Figure 1. General diagram of photosynthesis performed by microalgal cells



Source: Author's own elaboration.

Algae are also able to absorb volatile organic compounds, remove CO_2, purify wastewater and can find application as a raw material in the production of cosmetics, medical supplies and food components (Borowitzka, 1999: 314; Li et al., 2003: 51; Seo et al., 2012: 230-231).

Due to the high content of micro- and macro-components as well as low calorific value, they form a valuable component with a potential application in the food industry (Pielesz, 2010: 27). Apart from the basic nutrients, algae are also abundant in vitamins and minerals. The highest content of these substances is found in green algae, in particular in respect to such elements as calcium and magnesium (the content of calcium can reach 3.25 g/kg of dry mass). In addition, marine algae contain large amounts of vitamins and iodine. Algae can be consumed in any form and they form a source of vitamins and amino acids. They also affect the reduction of blood pressure and stimulate metabolic processes (Plaza et al., 2008: 11, 25).

The considerable increase in the popularity of algae is associated with the developments in the area of energy production from renewable sources, which takes the form of biodiesel

production based on application of algal biomass. The technology of biodiesel production has been in use for 50 years. This way one can obtain from 10 to 100 times more oil from one hectare in comparison to the oilseed cultivation. An important advantage that makes it possible to consider algae as a raw material for the production of biodiesel is associated with the high oil content in a cells (amounting to 20-77% of the dry mass) (Mata et al., 2010: 220). In addition, algae need lower amounts of water for growth compared to the terrestrial plants (Singh et al., 2012: 2597).

The remains of algae after oil, extraction can find application in animal feed and fertilizers to promote the growth of vascular plants. Microelements such as Zn, Cu, I, Fe and Mn are present in algae in the form of organo-metallic compounds and are more easily absorbed by human organisms than in the inorganic form. A similar relation can be observed for the case of macro-elements (N, P, K, Na). Animal feed is rich in magnesium and calcium. Algae has a potential application in fertilizers and have a stimulating effect on plant growth. Due to the richness of chemical and mineral substances in algal thallus, they can be applied as a component for soil conditioning (Schroeder et al., 2013: 1383). The fertilizers based on algae have a stimulating effect on seed germination and the successive plant growth as well as increase its resistance to pathogens. The most common technique of fertilization involves the application of foliar preparation derived on the basis of algal extraction. This way of fertilization affects the ability of the plant to absorb minerals and in this manner, the quality and volume of the crops (Biłos et al., 2016: 1800).

Algal cultivation can be undertaken both in open and closed systems called bioreactors. The first systems to be applied in algal cultivation on an industrial scale were unenclosed ponds, which first appeared during the Second World War in Germany. Since that time, such bioorganisms are applied as supplements in a diet. In 1950s, it was found that algal cultivation could be additionally enhanced with constant feed of carbon dioxide. The period around 1970 brought a quick development of algal cultivation on an industrial scale in Eastern Europe, Israel, and Japan; still, with the final product designated for use in the food industry.

At present the objective in algal cultivation consists in meeting the specific needs of humans. In the US, the ability of algae to purify water are utilized. In addition, biomethane, biodiesel, bioethanol and biohydrogen are derived from algae (Ugwu et al., 2008: 4021). The literature in the area reports various photobioreactors designs utilized for production of

microalgae, although it is remarked that problems with high energy expenditure and cost of lighting are associated with the algal cultivation on a large scale for practical purposes (Chen et al., 2011: 4021). Bioreactors are designed in accordance with the potential to apply the biomass generated in them. At present, the idea of bioreactors is very broadly discussed. Biomass production in them differs from the processes undertaken in open (conventional) systems in terms of the way in which separation of the algal culture from the surrounding environment is achieved (Holtermann and Madlener, 2011: 1906). An ideal photobioreactor designed for biomass production should be able to absorb the available solar radiation and enable it to be distributed inside the coated vessel containing algal culture with the purpose of producing biomass. High concentration of biomass in photobioreactors is possible only by adequate adaptation of their design and exploitation conditions to match the biological processes occurring inside the culture (Zijffers et al., 2008: 404). It is equally important to feed gas with adequate CO_2 concentration and ensure the extraction of the gas products formed during photosynthesis.

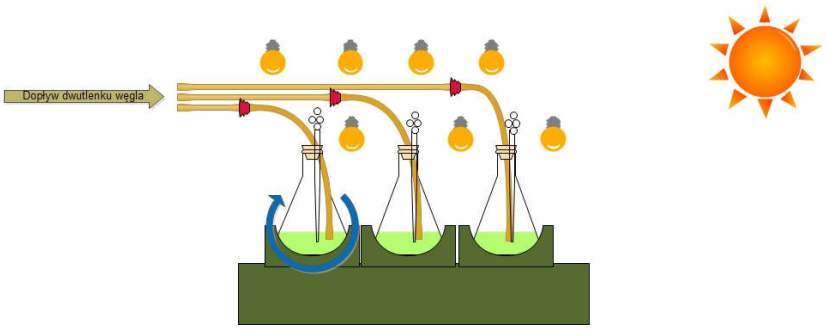
The literature reports a number of experiments demonstrating that the productivity of biomass and carbon absorption coefficient increases along with the higher concentration of carbon dioxide in the atmosphere (Yoo et al., 2010: 73; Jacob-Lopes et al., 2008: 30-33). Another important aspect is associated with mixing bioalgal suspension, which affects the distribution of CO_2 in the suspension along with the enriching substances present in the liquid. A condition that needs to be maintained involves evenly distributed light exposition of the individual cells (Ugwu et al., 2008: 4042; Zhang et al., 2002: 97; Chai and Zhao, 2012: 360). Another important exploitation parameter in each culture is associated with the maintenance of an adequate temperature. All of these parameters, including photobioreactor size, its shape, depth of light penetration, constant CO_2 supply (an important component in photosynthesis) and mechanisms of the mixing process have to be adapted suitably to ensure the maximum growth rate of algae (Seo et al., 2012: 4476-4478).

The objective of this work reported in this paper was to design a setup that enables the production of a maximum possible volume of biomass of the *Chlorella vulgaris* algal species. The presentation also includes selected results of a study involving the cultivation and maximization of the efficiency of the algal mass increase.

2. Materials and methods

The cultivation was performed in an experimental setup composed of 100 ml Erlenmeyer flasks that were previously subjected to autoclave sterilization at a temperature of 120°C for 20 minutes. The diagram of the experimental setup is found in Figs. 2. and 3. Initially, each flask was filled with 50ml of medium and 2ml of Chlorella vulgaris BA 002 strain species containing $2.5 \cdot 10^6$ of cells. The strain used in the study was obtained from *Culture Collection of Baltic Algae* of the Institute of Oceanography at the University of Gdańsk. A photograph with the culture is found in Fig. 4.

Figure 2. Diagram of experimental setup

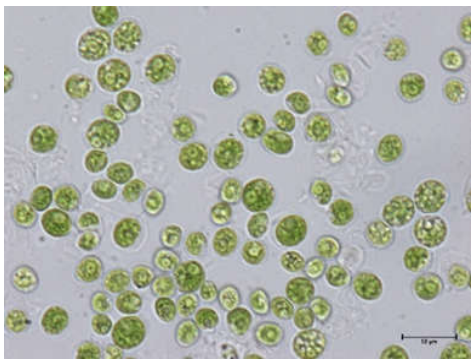


Source: Author's own elaboration.

Figure 3. Photo of the experimental setup

Source: Photo taken by Biłos and Patyna.

Figure 4. Photo of *Chlorella vulgaris* BA 002 culture



Source: Picture taken by The Institute of Oceanography of the University of Gdańsk

Table 1 contains information regarding the chemical composition of the medium dissolved in water along with the concentrations of its components. We can note at this point that the medium formed in the manner described above was subsequently subjected to sterilization in an autoclave.

Table 1. Composition of production medium used in the study

Medium components	Concentration, [g/dm^3]
$FeSO_4$ x $7H_2O$	0.042
K_2HPO_4	0.53
$MgSO_4$ x $7H_2O$	0.34
Citricacid x $2H_2O$	1.094
Urea	1.1
$CaCl_2$ x $2H_2O$	0.08
Na_2SO_4	0.2
Glucose	40

Source: study results.

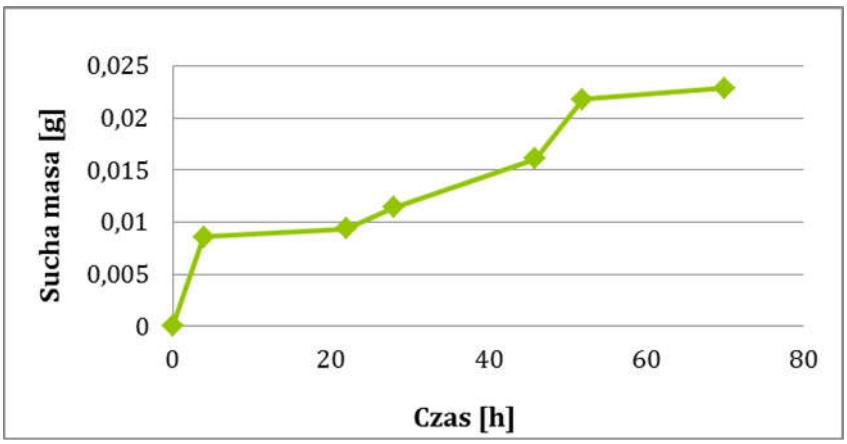
The cultivation was undertaken in the conditions of artificial lighting. The culture was illuminated by flourescent light in cycles consisting of 15 minutes of light followed by 15 minutes of dark. The capacity of the light source was equal to 1500 lux. The scope of the study also included the measurement of the pH rate profile. The pH measurements were performed with the use of Elmetron CP-401 pH-meter, and the values gained were equal to 7 to 8 on average. All tests were performed in the ambient range of temperatures, i.e. 22-24°C. The samples used for the study of the concentration of microalgae in the suspension and testing concerning the content of dry mass were performed on a daily basis, at constant time intervals. The microscopic

observations regarding the state of the suspension were performed with the aid of Jenamed 2 Fluorescence microscope.

3. Discussion of the results

Determination of dry mass content involved taking a sample of algal suspension in and placing it in a tube that was previously weighed. The centrifugation of the suspension was performed in a laboratory centrifuge running at a speed of 5,000 rpm. Subsequently, the tubes containing algae were subjected to drying a laboratory oven with forced air circulation at a temperature of 100°C for 2 hours. After the tube was removed from the oven, it was placed in a desiccator where it was subsequently weighed and the increase of the dry mass in relation to the result of the earlier test was established. Fig. 5 illustrates the increase of the dry mass of *Chlorella vulgaris* algae in relation to the specific volume of the suspension taken for the analysis. The algal cells increased their mass by nearly 25 times.

Figure 5. Increase in weight of *Chlorella vulgaris* algae over time



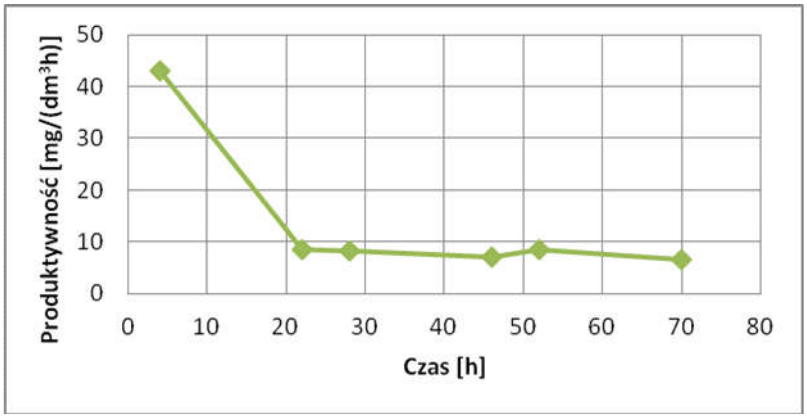
Source: study results.

On the basis of the measurements of the increase of the dry mass of microalgae during their cultivation, it was also possible to determine the productivity of the culture. A relation in the following form was used for this purpose:

$$P(\tau) = \left| C_k - C_p \right| / \Delta\tau ,$$

in which the parameters: C_k, C_p denote the initial and final dry mass content in g/dm^3 measured in a given time interval $\Delta\tau$. The values that were derived with regard to a given sample are summarized in Fig. 6.

Figure 6. Productivity in the subsequent hours of the experiment



Source: study results.

We can note the constant decrease in the productivity in terms of algal mass. The greatest productivity was equal to above 40 mg/(dm^{3}h) and it was achieved in the third hour of the duration of the experiment. After 20 hours, this productivity dropped to 9 mg/(dm^{3}h). For the case of constant cultivation, it can be associated with depletion of the components of the medium. Nevertheless, we can note that the stabilization of the speed, in which the algal mass grew, occurred after a short time. The mean productivity was in this case equal to around 8 mg/(dm^3·h) and this data is derived on the basis of several measurements.

4. Conclusion

The setup used in this research has the potential to produce biomass in the volume comparing to literature data and enabled sterile conditions to be maintained during the cultivation. In this manner, the projected goal of the research was achieved. This was also confirmed by microscopic observations, which do not reveal any traces of contamination. The algal cells increased their mass by around 25 times during the experiment lasting for 70 hours. The productivity in the first hours was equal up to 40 mg/(dm^{3}h), whereas after 20 hours it fell by nearly 4.5 times. This is a

normal process that is associated with the depletion of the nutrients in the medium by the algal cells.

The results of the research also enabled the authors to establish the schedule for the further research plans.

At present the study is concerned with determination of a wider range of parameters which affect the productivity of this type of biomass. In particular, the studies deal with the directions and ways of determining the manner and intensity of light supplied to the culture, ways of suspension mixing, concentration of carbon dioxygen in the feed air, changes in the composition of the medium, etc. The development of the existing database of experimental data will lead to a list of statements regarding the beneficial conditions of producing this type of algae, as they form an alternative to other types of renewable energy sources and fit well in the sustainable growth strategy.

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Hodowla mikroglonów Chlorella vulgaris w fotobioreaktorze laboratoryjnym

Streszczenie

Hodowla glonów, z uwagi na ich właściwości i sposób wykorzystania, od wielu lat wpisuje się strategię zapewnienia zrównoważonego rozwoju. Związane to jest z tym, że biomasa glonów coraz częściej uważana jest za potencjalny surowiec mogący stanowić alternatywne źródła energii odnawialnej, a w szczególności służyć do produkcji biopaliw oraz energii elektrycznej czy cieplnej. Dodatkowo algi zawierają całe bogactwo substancji odżywczych, mogą więc stanowić źródło pożywienia dla ludzi i zwierząt hodowlanych. Ich właściwości biosorpcyjne sprawiają, że działają oczyszczająco na organizm i dlatego są przyjmowane w celu detoksykacji lub jako suplementy zróżnicowanej diety. Hodowla alg nie wymaga dużych powierzchni, a ponadto wskaźnik produkcji ich biomasy jest dużo wyższy niż roślin naczyniowych. Wymaga to jednak prowadzenie jej w ściśle określonych warunkach procesowych, których zakres zmian określa się na drodze doświadczalnej. Celem eksperymentu opisanego w artykule było skonstruowanie stanowiska badawczego pozwalającego na wyprodukowanie jak największej ilości biomasy alg *Chlorella vulgaris*. Uwzględniając konieczność ustalenia odpowiednich warunków procesowych dla ściśle określonych rodzajów mikroalg, w pracy dokonano przeglądu literatury z zakresu warunków hodowli mikroglonów z gatunku *Chlorella* oraz przedstawiono wyniki badań własnych przeprowadzonych w fotobioreaktorze laboratoryjnym.

***Słowa kluczowe**: Chlorella vulgaris*, fotobioreaktor, hodowla alg, produktywność biomasy.