Influence of carbon dioxide, temperature, medium kind and light intensity on the growth of algae *Chlamydomonas reinhardtii* and *Scenedesmus obliquus*

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Abstract. Microalgae attracts the attention of scientists because of the possibility of using in the energy industry as one of the substrates for the production of renewable energy. So far, the greatest emphasis was put on attempts to obtain strains, and technologies of their culturing, in order to efficiently acquire fat from cells and its further conversion to biodiesel using transesterification reaction. Increasingly, algae are considered also as an efficient biomass producer, which can be used as a substrate for methane production in biogas plants.
In this study the influence of different physical and chemical conditions, on the growth of two algae species: *Chlamydomonas reinhardtii* and *Scenedesmus obliquus* was investigated. Based on the literature and the data obtained for the algae growth on the standard medium and the digestate remaining after fermentation, one may suggest further investigations on the use of other liquid waste from agriculture and industry for algae breeding, including chemical analysis and supplementation of these mediums so as to provide the best conditions for their growth.

1 Introduction

The continuous development of the technologies and ongoing improvement of the financial situation of the society causes the consumption increase. This phenomenon increase demand of fuel and affects the amount of waste. Nowadays more and more emphasis is placed on issues related to waste management and widely understood environmental protection [1,2,3]. European Union inclines its members to invest in renewable energy sources. One of the most stable and promising is biogas production [4,5,6], which like in case of composting process [1,7], not only solves the problem of waste, but also produce energetic fuel. Because the problem is so current the even latest achievements of computer analizys using Artificial Neural Network have been engaged to bring solutions [8,7]

Algae are a very promising alternative for biomass production. They are characterized by the ability to proceed photosynthesis, which is determined by possession of chlorophyll a and other additional photosynthetic pigments, whose presence depend of organis [10]. In addition, they do not produce specialized organs such roots, stems or flowers. These organisms are present mainly in water environment, as well as on a land, in places with high humidity. It is estimated that about 50% of oxygen is produced by algae, mainly in the oceans, which cover about 71% of the Earth [11].

Algae are usually divided on macro- and microalgae. The first group can form a variety of structures, such as leaves or roots, but the cells forming them are not specialized and do not perform specific functions [10]. To this group of organisms belong certain algae species that can form fronds with a length up to 100 meters. Microalgae are, however, single-celled organisms which sometimes are present in a loose cell groups, chains or diplococci. In addition, these organisms are divided to two kingdoms: prokaryotes or eukaryotes. Prokaryotic algae can be called cyanobacteria or blue-green algae. Them excessive growth in water bodies result in water blooms, during which some species can produce cyanotoxins. These compounds are highly toxic and harmful to humans and animals [12]. Some species of microalgae can be used in human nutrition, for example *Spirulina sp*., *Chlorella sp*. or *Nanochloropsis sp*. are commonly used in human nutrition, cosmetic and chemical industry [13].

Microalgae focused attention of scientists because of the potential use of the energy industry as one of the substrates in renewable energy production. So far, the

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greatest emphasis was placed on trying to obtain strains and technologies of their culturing to raise content of neutral fats, which can be convert to biodiesel using transesterification reaction [11,14,15]. Futhermore, algae are considered as an efficient producer of the biomass, which can then be used as substrate for methane production [16,17].

Microalgae present higher degree of utilization solar energy in photosynthesis, compared to plants. They need to grow only inorganic compounds, organic compounds are produced in the process of photosynthesis [18]. That is why scientist are working to make use of its potential [19,20]. An important advantage of algae is can be cultured in many systems: from closed bioreactors, in which is possible to control bioprocess conditions, up to open pools, which are the cheapest solution in algae production. However, there are problems with microbiological contaminations, bacteria and others can freely go into culture from air. Therefore, sometimes it is not required to produce sterile culture, for example in the energy industry with open pools, which are the cheapest solution. Unlike the first-generation fuels, where the substrate are products which can be successfully used in the food industry, algae do not compete with human nutrition, and can be grown on land useless for agriculture. Fuels from algae are cold third generation biofuels [11,18].

2 Materials and methods

In this study two strains of microalgae: *Chlamydomonas reinhardtii* and *Scenedesmus obliquus* were used. The aim of this study was to determine the effects of a few factors: temperature, carbon dioxide concentration, light intensity and type of used medium on growth rate of used strains.

2.1 Culturing media

Standard TAP medium was used as control [21] and liquid fraction of digestate from biogas plant as alternative medium. Digestate pulp was centrifugated (4 000 rpm for 10 minutes). For futher research were used supernatant, which were dilluted by distilled water to final concentration 0,5%. All media were sterilized by autoclaving. Cultures were grown in Erlenmeyer flasks of 500 ml capacity, in which were put 100 ml of medium.

2.2 Inoculate preparation

Cultures were inoculated by prepared cells suspensions from *Ch. reinhardtii* and *S. obliquus* cultures from raised in a light chamber (20 ° C, 72 mmol/m^{2}s, 2% CO_2) in 500 ml flasks containing 100 ml of culture, growth on TAP medium. Then, when the cultures raised an absorbance of light with a length of 684 nm of 1-1.5 for *Ch. reinhardtii* and 2-2.5 for *S. obliquus*, cultures were centrifuged (3 000 rpm for 10 min) and resuspended, respectively TAP medium and 0.5% fermentation pulp, in such a volume to concetrate cells ten times. The procedure was carried out again to wash the rest of the pollutants and suspended in the same volume of fluid as after first step. Prepared inoculates were added to relevant flask in a volume of 1 ml.

2.3 Culturing conditions

The cultures were conducted in light chambers that allow you to control the following parameters: light intensity, temperature, relative humidity and carbon dioxide concentration. Cultures were conducted in various combinations of conditions for each combination three times. The study of the following conditions: temperature 10°C and 20°C; the concentration of carbon

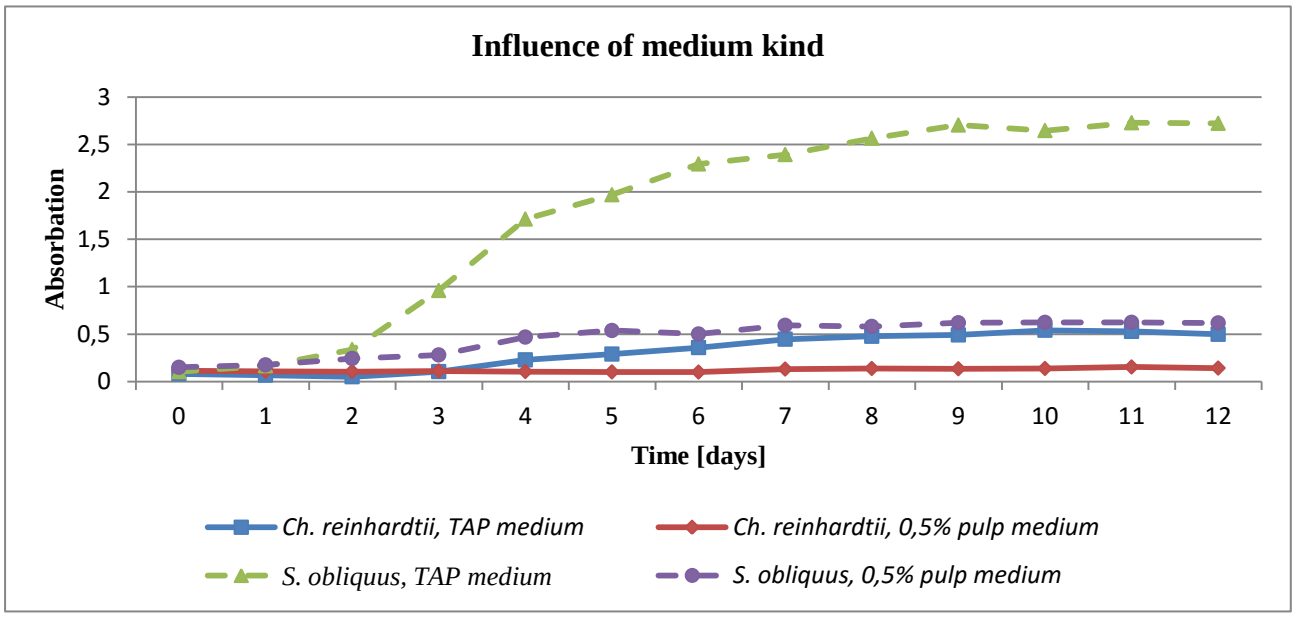

Figure 1. Effect of different medium kind (TAP and 0,5% fermentation pulp) on the cultures of *Ch. reinhardtii* and *S. obliquus* conducted in 2% CO_2 concentration, 72 µmol/m^{2}s light intensity and 20°C.

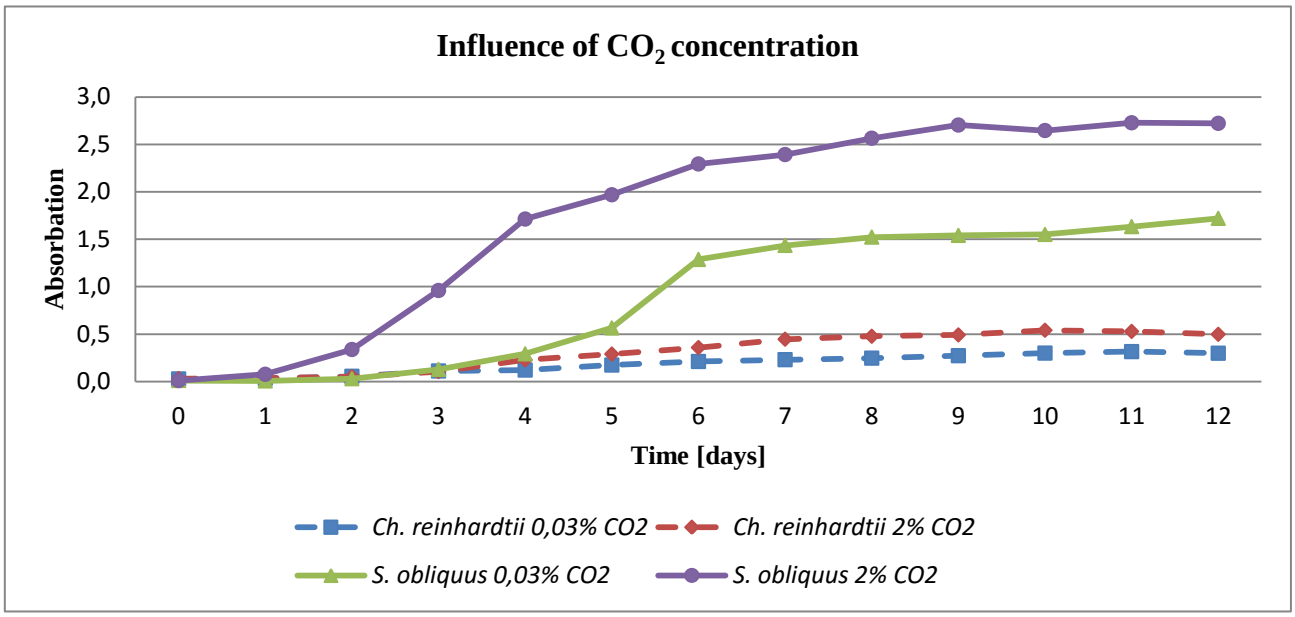

Figure 2. Effect of different CO_2 (0,03% and 2%) on the cultures of *Ch. reinhardtii* and *S. obliquus* conducted on TAP medium in 72 µmol/m^{2}s light intensity and 20°C.

dioxide: atmospheric (about 0.03%) and 2%, the exposure to light 72 and 10 µmol/m^{2}s; all the time kept constant relative humidity of 70%.

The prepared cultures were carried out in the above-mentioned conditions for 12 days, and sample were taken at 24-hour intervals and analyzed using a spectrophotometer Specord PC 205 (Analytikjena company). Measurement of absorbance of 684 nm wavelenght light was measured in the range of 0 to 0.3, otherwise the sample was diluted [22].

3 Results

3.1 Influence of medium type

Both in the case of *Ch. reinhardtii* and *S. obliquus* replacing the TAP medium, which is dedicated to the cultivation of algae, to 0.5% fermentation pulp had a negative impact on the growth of algae. In the case of *Ch. reinhardtii* growth has slowed down up to four times, and in the case of *S. obliguus* five times.

In the literature, there are reports of the use of other liquid wastes as the substrate for growing algae [23]. For some substrates it was observed efficient biomass productivity, as well as highlights the possibility of using algae for the treatment of a variety of liquid wastes, mainly by conversion of inorganic nitrogen compounds and incorporation to algal biomass, which can be further used as starting material in biomethan plants.

Based on these results it is concluded that it is

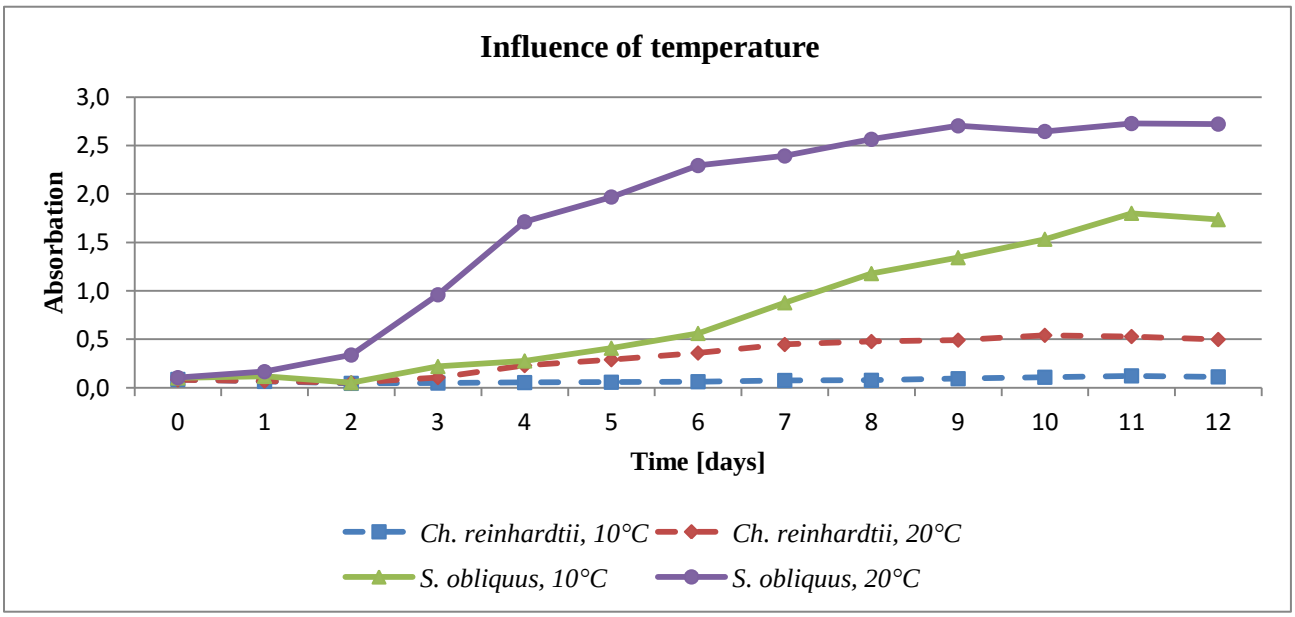

Figure 3. Effect of different temperature values (10 and 20 °C on the cultures of Ch. reinhardtii and S. obliquus conducted on TAP medium with 2% CO_2 concentration and 72 µmol/m^{2}s light intensity.

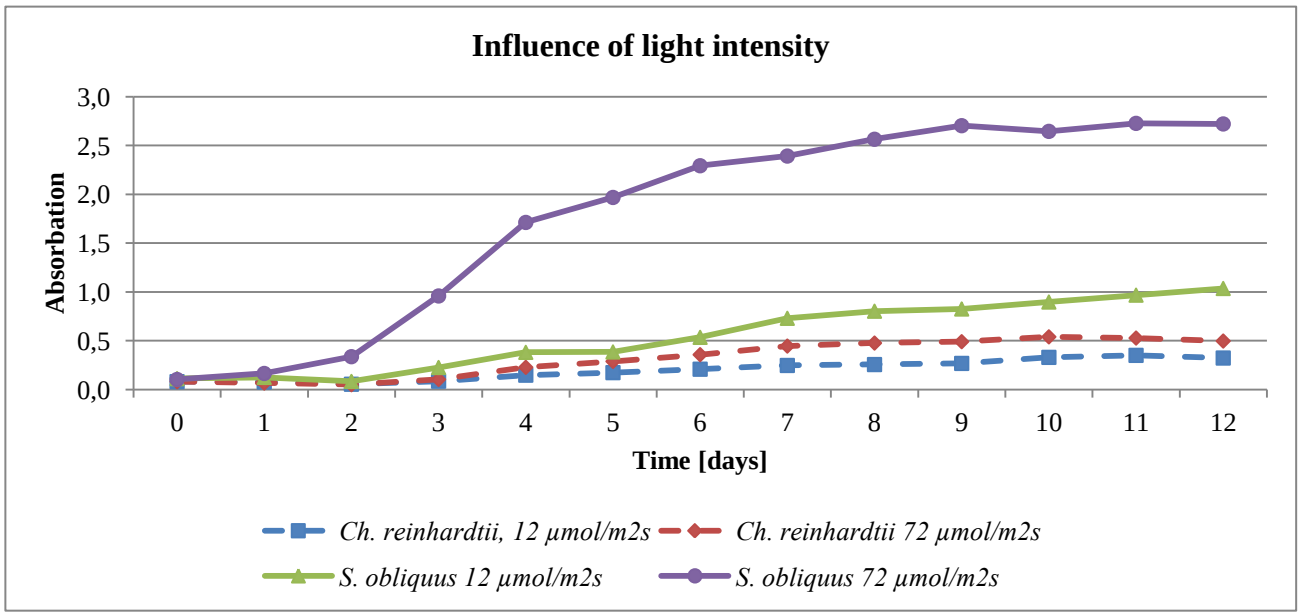

Figure 4. Effect of different temperature values 10 and 20 °C on the cultures of *Ch. reinhardtii* and *S. obliquus* conducted on TAP medium with 2% CO_2 concentration and 72 µmol/m^2s light intensity.

necessary to analyze a larger number of substrates from different places, as well as analysis of received materials and testing their supplementation with certain chemicals to create a suitable environment for algae to develop.

3.2 The impact of the carbon dioxide concentration

Supplementation of the culture using carbon dioxide had positive impact to the growth of both the substrates a standard (TAP) and 0.5% digestate pulp. These results are confirmed in the literature (Anderson, 2005; Singh and Singh 2014). Supplementing cultures with carbon dioxide is a commonly used technique to increase the rate of cell growth and to increase their final concentrations in relation to the volume of the culture.

Supplementation with carbon dioxide have also aspects related to environmental protection - the use of waste gas containing a high concentration of carbon dioxide in breeding algae can have a positive impact on reducing emissions of this gas by industrial plants. This possibility is also part of the European Union's current policy aimed to reducing carbon dioxide emissions by member states [24].

3.3 Effect of light intensity

It was found that the lighting at the level of 72 µmol/m^2s greatly accelerates the growth of both species of algae.

Interestingly, the comparison of the growth curves for *S. obliquus* culture, carried out at 20 °C on TAP medium with light intensity of 12 µmol / m^2s and different concentrations of carbon dioxide (0.03 and 2%), were not shown any major difference in speed growth. Probably light intensity is limiting factor for cell grow. The data obtained correspond to the information literature on the impact of light on the growth of algae [25,26].

3.4 Effect of temperature

In the case of temperature impact on the growth of algae were not shown any differences which might be different from the data in the literature [18,25,26]. Temperatures closer to the optimum temperature for the used strain (about 20 °C), caused the faster growth. However, the low temperature turns out to be a strong factor limiting the growth of tested species, even when others remain at an optimum level (high light intensity and carbon dioxide concentration and growth in the medium TAP).

4 Discussion

In the above study the effect of various physical and chemical parameters on the growth of microalgae from species *Ch. reinhardtii* and *S. obliquus* were investigated. Based on the literature and obtained data about the growth of algae on standard TAP medium and diluted digestate pulp can suggest needed of further studies on the use other liquid wastes from agriculture and industry to breeding algae. It can be needed to characterized chemical composition of used wastes and its supplementation in appropriate substances.

Furthermore, it seems reasonable to investigate the effect of feeding cultures by wastes gas reach in carbon dioxide from different processes, such as combustion or various types of fermentation. The development of efficient procedures of this procedure can significantly increase the rate of algae growth and also allows minimizing the process costs. Additionally use of such procedures seems to be beneficial to the environment. Nowadays when research on alternative energy sources seems to be more urgent and a different scientists get involved using various techniques from Internet to process control [27] to Artificial Neuron Networks [28]

research on algae as energetic biomass of the future seems to be a promising solution for energetic stability of the World.

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