

Inexpensive and universal growth media for biomass production of microalgae

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Abstract

New challenges for the industrial microbiology and biotechnology of algae are to increase the efficiency of microalgal growth rates and to decrease the cultivation costs. Algae could be cultivated in fresh water as well as in salty sea water or wastewater. Microalgal biomass can be used as a sorbent to remove microcontaminants (e.g. heavy metals, biogens) from wastewater. The results proved that it is possible to use an inexpensive and universal growth medium (Bioflorin) together with a popular fertilizer, like Bristol (Br) or Blue Green medium (BG-11), to cultivate *Chlorella vulgaris* and *Scenedesmus armatus*. The mixotrophic condition can be suitable for the cultivation of *Chlorella vulgaris* and *Scenedesmus armatus*. The highest concentrations of algal biomass for both species were achieved on the Bioflorin medium, followed by the BG-11 medium and finally on the Bristol medium in autotrophic as well as mixotrophic conditions. The number of *C. vulgaris* cells under mixotrophic conditions was higher than that of *S. armatus*. The use of inexpensive growth media could lower the production costs of algal biomass on a large scale.

Keywords: *C. vulgaris*, *S. armatus*, decrease in the cultivation costs, microalgae, photoautotrophic and mixotrophic cultivation, physiological activity.

1. Introduction

Microalgal biomass can be used as a sorbent to remove microcontaminants (e.g. heavy metals, biogens) from wastewater as well as a feedstock in different industries, for example to produce biofuels and generate energy, to produce fertilizers, to make cosmetics and pharmaceuticals (Krzywonos *et al.*, 2014; Zabochnicka-Świątek, 2010, 2013; Zabochnicka-Świątek and Malińska, 2012; Zabochnicka-Świątek *et al.*, 2014). Microalgae can also be used for nutritional purpose (Koller *et al.*, 2014). Current studies on the production of microalgal biomass focus on developing and improving the methods for microalgal cultivation that would allow increasing the

efficiency of microalgal growth rates and decreasing the cultivation costs (Becker, 2008; Gouveia and Oliveira, 2009; Nayak *et al.*, 2013).

Algae could be cultivated in fresh water as well as in salty sea water and wastewater. Some researchers have indicated that the most important factor for growing algae is the availability of nutrients [Krzywonos *et al.*, 2014; Krzemińska *et al.*, 2014; Zabochnicka-Świątek, 2017]. The biomass yield during autotrophic cultivation is limited by the growth rate, low light penetration and photoinhibition (Juntila *et al.*, 2015). During the autotrophic growth, microalgae assimilate CO₂ from the atmosphere (Gouveia and Oliveira, 2009; Zabochnicka-Świątek, 2012). Heterotrophic cultivation requires a carbon source with no light, whereas mixotrophic cultivation requires less carbon input than heterotrophy and light is only complementary (Juntila *et al.*, 2015; Kim *et al.*, 2013). However, the high cost of organic carbon is a limiting factor for the heterotrophic cultivation of algae (Zheng *et al.*, 2012).

Regardless of the mode of cultivation, microalgae can be grown in two types of systems, i.e. open (outdoor) and closed systems. Cultivation of microalgae in open systems is performed in artificial (i.e. photobioreactors) or natural aquifers (e.g. ponds) and is cheaper than cultivation in closed systems. The source of light is solar radiation in open systems and artificial light in closed systems (Zabochnicka-Świątek and Krzywonos, 2014). Cultivation of microalgae in closed systems allows one to achieve higher biomass productivity and to control the cultivation conditions more easily.

Numerous studies have demonstrated that heterotrophic cultivation of microalgae helps to obtain higher biomass yields than when they are cultivated under conventional autotrophic conditions (Juntila *et al.*, 2015; Turon *et al.*, 2015). Moreover, some microalgal species can be grown in autotrophic as well as heterotrophic conditions. It was confirmed that *C. vulgaris* can grow

when the light and CO_2 are available or in the dark, providing that the source of external organic carbon is available (Becker, 2008). For example, *Chlorella vulgaris* and *Scenedesmus* sp. are classified as the fastest growing microalgae and are rich in valuable constituents that can be later used for production of biofuels, fertilizers, etc. *C. vulgaris* contains 14-22% of lipids, 51-58% proteins, 12-17% of carbohydrates and 4-5% of nucleic acids (Kim *et al.*, 2010). These two species of microalgae are very common in nature, and thus easily available. This is the reason why *C. vulgaris* and *S. armatus* were selected for the present study. The selection of an algal multiplication technology influences the cost-efficiency of algal biomass utilized for energy (Dębowski *et al.*, 2013). Currently, there are a number of studies on the potential of various types of microbial biomass that can be used for a wide range of applications, e.g. for recovery of metals from waste materials (Willner *et al.*, 2015). The application of algal biomass for energy purposes would require a high yield of biomass over time at the lowest operating costs. There are still gaps in our knowledge about inexpensive cultivation methods of microalgae, prior to introducing them on an industrial scale.

In order to reduce the production costs, inexpensive and universal media for cultivation and maintenance of algae are needed. Popular fertilizers for algal cultivation are Bristol or BG-11 (Allen and Steiner, 1968; Bold, 1949). Moreover, the mixotrophic growth of microalgae requires continuous delivery of organic carbon sources, which is usually expensive and hence the use of inexpensive organic carbon compounds will additionally reduce the cultivation costs. Therefore, the overall goal of this study was to select the type of cultivation and growth medium that would enable the highest growth rate of *C. vulgaris* and *S. armatus* as well as lower cultivation costs. This study focuses on the mixotrophic cultivation of microalgae at decreased cultivation costs. The innovative aspect is the possibility of applying inexpensive organic carbon sources during the mixotrophic cultivation and an inexpensive growth medium.

The literature provides descriptions of molasses-based growth and lipid production of several algal strains (El-Sheek *et al.*, 2014; Gaurav *et al.*, 2016; Gautam *et al.*, 2013; Mondal *et al.*, 2016; Mondal *et al.*, 2017). However, there still remains a gap in knowledge on a possible use of an inexpensive and universal growth medium (Bf), together with popular fertilizers: Bristol or BG-11 to increase the physiological activity of the *Chlorella vulgaris* and *Scenedesmus armatus*.

The scope of the study included: (i) an analysis of the effects of a cultivation type and growth medium on selected algal species, (ii) an analysis of the growth rate and cell density, (iii) determination of the effect of cultivation on the production of oxygen and chlorophyll a by microalgae, (iv) an analysis of changes in pH during the cultivation, and (v) an economic analysis.

2. Materials and methods

2.1. Experimental procedure

Batch-fed cultivation of algae was performed in 500 cm³ glass bottles (in static conditions) sterilized at 160°C for 2 h prior to use. The laboratory experiments were conducted for 20 days in autotrophic and mixotrophic conditions. The addition of an external source of carbon allowed us to conduct the process in mixotrophic conditions, where 5 cm³ solution of molasses (m) of a concentration of 0.15 g/L of molasses in the final medium was added to each bottle daily at the beginning of the dark phase. Molasses can be used for enhancing the growth of microalgae in heterotrophic and mixotrophic conditions. Molasses contains 25% of glucose, 25% of fructose and 30% of sucrose (Becker, 2008).

The samples were placed in a phytotron chamber with the temperature set at 29°C during the day and at 25°C during the night. During the day, the light at an intensity of photosynthetically active radiation (PAR) was set at 80 $\mu\text{mol}/\text{m}^2\text{s}$ in cycles of 12 h for a bright phase and 12 h for a dark phase. The algal cultivation was conducted in the batch-fed system. *C. vulgaris* (Cv) and *S. armatus* (Sc) were selected for the study. The strains of unicellular green microalgae *C. vulgaris* and *S. armatus* were obtained from the Culture Collection of Baltic Algae (CCBA), Institute of Oceanography, University of Gdańsk, Poland. Algal species were sampled during the phase of the exponential growth and the initial concentration was 0-3 g/L of Cv and 0.4-0.8 g/L of Sc and cell density was 2.5×10^6 cells/cm³.

2.2. Growth media

The selected growth media were prepared in a laboratory by adding a commonly available fertilizer for green plants, i.e. Bioflorin (Bf) (Tropical, Poland) for general cultivation, to growth media recommended for algae, i.e. Bristol (Br) and Blue Green medium (BG-11). Table 1 presents the chemical composition of the selected growth media.

Table 1. Composition of growth media

Constituents	Bioflorin	BG-11	Bristol
	Final concentration, g/L		
NaNO ₃	0.008	1.5	0.25
CaCl ₂ ·2H ₂ O	-	0.036	0.25
MgSO ₄ ·7H ₂ O	-	0.075	0.075
K ₂ HPO ₄	-	0.04	0.075
K ₂ O	0.0325	-	-
P ₂ O ₅	0.015	-	-
Trace metals			
Mn	-	0.00181	-
Zn	0.0001	0.00022	-
Mo	0.0003	0.00039	-
Cu	0.0005	0.00008	-
Co	0.00005	0.00005	-
B	0.0005	0.00286	-
Fe	0.005	0.006	-

The BG-11 and Bioflorin growth media contained more nutrients than the Bristol one. The latter contained only six general nutrients and showed no content of trace metals which are necessary for microalgal growth (i.e. Zn,

Cu, etc.). Bioflorin is a liquid plant fertilizer with the composition of macro- and microelements required for plant growth. The growth media used in this study differed in the chemical composition. BG-11 contained not only basic nutrients and trace metals but also other compounds (e.g. Na_2CO_3 , EDTA, citric acid) with buffering properties. Also, BG-11 and Bioflorin contained trace quantities of Zn, Mo, Cu, Co and B, which additionally facilitated the growth of microalgal biomass. Moreover, BG-11 contained Mn and Bioflorin contained Fe. Control experiments, which contained only *C. vulgaris* and *S. armatus* with no addition of growth media, were also performed to gain understanding of the influence of growth media on the growth rate of algal biomass.

2.3. Analytical methods

The concentration of biomass was determined from the number of cells counted in a Thoma chamber and as dry matter according to PN-C-04541:1978 (PN-C-04541:1978). The size of algal cells was determined under a microscope (NIKON Eclipse E200) integrated with a colour digital camera (DS-Fi1) and software (NIS Basic Research). The pH was measured with a Cybercan pH11 meter (Eutech Instruments) according to PN-EN ISO 10523:2012 (PN-EN ISO 10523:2012), dissolved oxygen was measured with CO-401 meter (Elmetron) according to PN-EN ISO 5814:2013-04 (PN-EN ISO 5814:2013-04), and the content of chlorophyll *a* was determined according to PN-ISO 10260:2002P (PN-ISO 10260:2002P). All the measurements were conducted in triplicates and the data are presented as arithmetic means calculated from three measurements.

3. Results and discussion

3.1. Effects of growth media on *C. vulgaris* and *S. armatus* growth

In this study, biomass was determined with two methods: (1) measuring dry matter and (2) counting algal cells in a Thoma chamber. The number and size of algal cells were determined under a microscope. Figure 1 shows the biomass concentrations of the investigated microalgae determined from the measurement of dry matter. The concentration of algal biomass grown on a selected medium in mixotrophic condition was lower in each case than the concentration of algal biomass obtained in autotrophic conditions. An increase in the concentration of biomass from both species grown without any growth medium (the control samples) was insignificant during the entire experiment and the biomass concentration did not exceed 1×10^3 mg/L. Similar results were obtained using the Br medium in mixotrophic conditions.

The highest concentrations of algal biomass for both species were determined after the application of the Bf medium, lower after the BG-11 medium and the lowest after the Br medium in autotrophic as well as mixotrophic conditions. Some differences in the total biomass were observed between the selected species. Figure 2 presents the number of microalgal cells (cell density) counted in a Thoma chamber.

An analysis of the effects of the selected growth media on the concentration of biomass (Figure 1) and the number of microalgal cells (Figure 2) showed that in both species the application of Bf was the most beneficial, followed by BG-11. The cultivation method and medium composition have an impact on the number of cells.

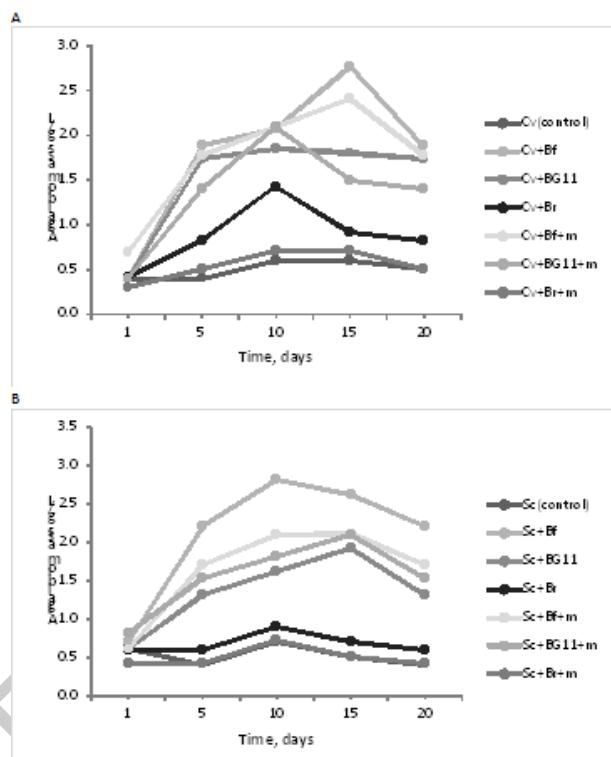


Figure 1. Changes in the concentration of algal biomass of (A) *C. vulgaris* (B) *S. armatus*

During the growth of the microalgal populations in a batch-fed system, the following phases can be distinguished: adaptive (i.e. lag, induction), exponential (i.e. logarithmic), declining growth, stationary and death (Mikscha and Sikora, 2010). Both microalgal species grown with the addition of the selected media and without any media (the control) entered the declining growth and stationary phases from day 6. In the control, the algal culture entered the death phase from day 12-13. The growth media caused diverse effects on the occurrence of the microalgal growth phases. The number of algal cells in the control was significantly lower than after the application of any of the selected growth media. Until day 10 in the case of *C. vulgaris* and until day 12 in the case of *S. armatus*, the population growth was similar for the algal culture grown with and without the addition of the selected media. After the application of BG-11, the first phase of logarithmic growth, which lasted from day 1 to day 5, was followed by the declining growth and stationary phases (from day 5 to day 10). Then, another phase of logarithmic growth (from day 10 to day 13) occurred and was followed by the declining growth phase. It has to be pointed out that some differences between the species were observed. The maximum number of algal cells, i.e. 21×10^6 cells/cm³, was observed for *C. vulgaris* on day 20 after the addition of BG-11 in the autotrophic and

mixotrophic conditions. This growth medium contributed to the highest increase in the population of *S. armatus*, so that on day 20 the number of algal cells was about 16×10^6 cells/cm³.

Slightly lower values of algal cell numbers were observed after the application of Bf, and then following Br. However, an increase in the microalgal population of *S. armatus* was similar for both BG-11 and Bf media during the entire experiment. Based on the results presented in Figures 1 and 2, the least beneficial effect on the biomass growth was observed for Br. All the time, the concentration of microalgal biomass for *C. vulgaris* was higher than the concentration of microalgal biomass for *S. armatus*. The total number of cells was consistently higher for *C. vulgaris* than for *S. armatus*.

As shown in Table 2, the maximum growth rate and algal cell density were observed on different days. *C. vulgaris* and *S. armatus* showed the maximum growth rate before the maximum cell density was reached. *C. vulgaris* reached the highest growth rate and cell density of 0.59 mg/L day⁻¹ and $\sim 21 \times 10^6$ cells/cm³, respectively. Compared with the mixotrophic growth, the autotrophic culture of *C. vulgaris* showed higher growth after the application of Bf and Br. The growth rate of *S. armatus* was higher in all autotrophic cultures compared to mixotrophic conditions. *S. armatus* reached the highest growth rate and cell density of 0.84 mg/L day⁻¹ and 16×10^6 cells/cm³.

Table 2. Autotrophic and mixotrophic culture of *C. vulgaris* and *S. armatus* under different conditions. Data are represented as the average \pm standard deviation of three independent replicate cultures

Cultivation	Sample	Growth rate (mg/L day ⁻¹)	Culture time (day)	Cell density ($\times 10^6$ cells/cm ³)	Culture time (day)
<i>C. vulgaris</i>					
Autotrophic	Cv (control)	0.10	11	8	15
	Cv+Bf	0.54	17	16	20
	Cv+BG11	0.52	10	20	20
	Cv+Br	0.37	10	13	20
Mixotrophic	Cv+Bf+m	0.41	17	17	20
	Cv+BG11+m	0.59	10	21	20
	Cv+Br+m	0.14	10	13	20
<i>S. armatus</i>					
Autotrophic	Sc (control)	0.13	10	6	15
	Sc+Bf	0.84	10	16	20
	Sc+BG11	0.42	13	16	20
	Sc+Br	0.39	17	12	20
Mixotrophic	Sc+Bf+m	0.46	13	16	20
	Sc+BG11+m	0.14	10	16	20
	Sc+Br+m	0.10	17	12	20

The microscopic analysis performed on day 20 of the experiment (not presented here) provided evidence for an evaluation of the number and size of algal cells, and thus allowed us to link the counts of algal cells with the measured concentrations of biomass and the number of cells counted in a Thoma chamber. The algal cultures without growth media showed a low number of algal cells for both algal species. Generally, algal cells exposed to the growth media yielded more numerous populations of both algal species. In addition, the number of algal cells was

10^6 cells/cm³, respectively. *S. armatus* showed the higher growth after the application of Bf and then after BG-11

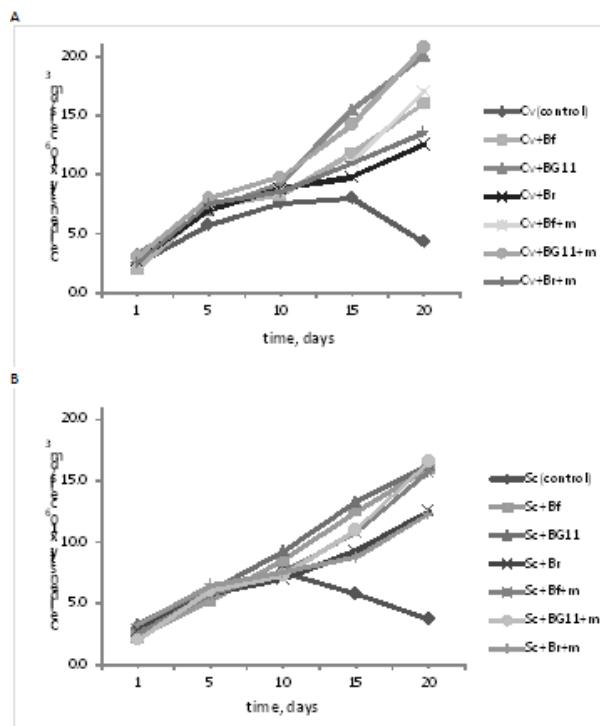


Figure 2. Increase in the population of microalgae from the selected species (A) *C. vulgaris* and (B) *S. armatus*

higher for all the growth media applied than that *S. armatus*. This is confirmed by the data presented in Figures 1 and 2.

The cells of *C. vulgaris* grown in the mixotrophic conditions were bigger than those grown in autotrophic conditions. This phenomenon was not observed for the cells of *S. armatus*.

The results indicate that by growing microalgae of *Chlorella vulgaris* and *Scenedesmus armatus* species with

the addition of various growth media and under different cultivation conditions (autotrophic and mixotrophic) it was possible to evaluate the effects of the composition of a growth medium and a cultivation type on the biomass growth rate.

Microalgae are often grown in phototrophic and heterotrophic conditions. However, it has to be pointed out that microalgae grow better in photoheterotrophic/mixotrophic conditions (Yeh *et al.*, 2012). The current results have confirmed the findings reported in the literature. An increase in the cell counts of *C. vulgaris* and *S. armatus* was observed in all the growth media and under both cultivation conditions (autotrophic and mixotrophic conditions). The results prove that microalgae of *C. vulgaris* and *S. armatus* can use an external source of organic carbon, which contributed to an increase in the biomass growth.

3.2. Effects of growth media on *C. vulgaris* and *S. armatus* photosynthetic activity

The type of cultivation had an effect on the production of oxygen and chlorophyll a by the selected algae species. Like pH, the production of oxygen released into the environment is an indicator of the efficiency of photosynthesis (Figure 3). During the cultivation of microalgae in autotrophic conditions, irrespective of the selected growth media, the curves presenting changes in the concentrations of dissolved oxygen showed a similar trend. In comparison to autotrophic conditions, mixotrophic conditions had an effect on changes in the physiological activity of the microalgal populations, which is illustrated by all the curves plotted for both species. In the case of *C. vulgaris* grown with the addition of BG-11 or Br, mixotrophic conditions resulted in two peaks of dissolved oxygen: the first occurred on day 10 and the second on took place on day 20. In the cultivation of both species with the addition of Bf in mixotrophic conditions, the peak of dissolved oxygen was observed on day 10 of the experiment. These tendencies reflected changes in the pH of the investigated samples taking place at approximately the same time. A gradual decrease in dissolved oxygen was observed from day 5 in all samples with the supplemented growth media in autotrophic conditions, whereas in the samples without the growth media (grown in water) such a gradual decrease occurred on day 1. Higher concentrations of oxygen in the cultures grown on the BG-11 (14–15 mgO₂/L) and Br (11 mgO₂/L) media in mixotrophic conditions observed on the last day of the experiment indicated that the addition of an external source of carbon enhanced the physiological activity of the investigated microalgal populations. This shows that an algal culture in mixotrophic conditions can be kept in the growing phase for longer time than cultures grown in autotrophic conditions. The increase in the oxygen concentration resulted from the photosynthetic processes and proceeded in the algal cultures that were supplied with all nutrients, thus contributing to the increased population growth of the investigated microalgae.

The lowest concentration of dissolved oxygen was observed after adding the Bf medium in mixotrophic conditions. The concentration of dissolved oxygen was < 2 mgO₂/L from day 15 for *C. vulgaris* and from day 18 for *S. armatus*. These values were lower than in the control sample.

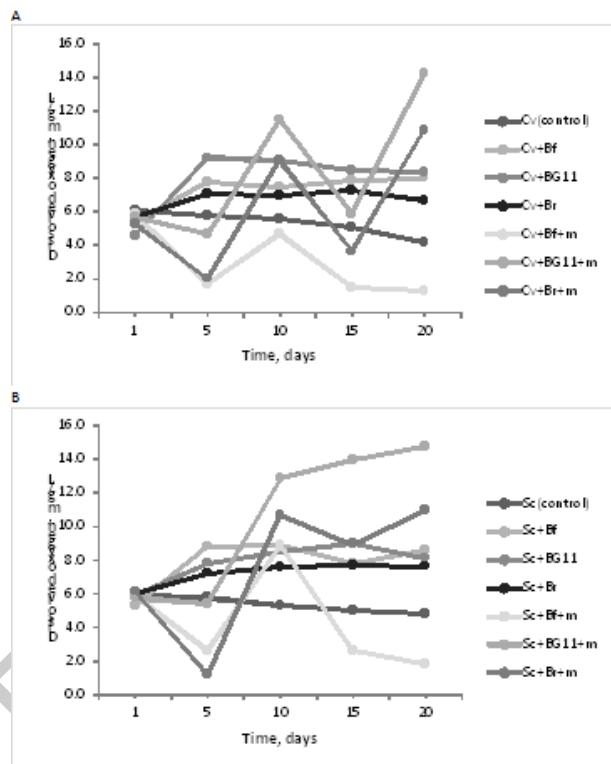


Figure 3. Changes in the concentrations of dissolved oxygen (DO) observed during the cultivation of (A) *C. vulgaris* and (B) *S. armatus*

The mixotrophic cultivation had an effect on some changes in the physiological activity of microalgae. The higher concentrations of oxygen reported on the last day of the experiment in the mixotrophic cultures with the BG-11 and Br media proved that the addition of an external source of organic carbon had a beneficial effect on the physiological activity of the investigated algal species. However, the lowest concentration of dissolved oxygen was determined after the addition of Bf in mixotrophic conditions. Irrespective of the growth media, autotrophic cultivation showed a similar tendency of changes in concentrations of dissolved oxygen.

Figure 4 presents changes in the content of chlorophyll a observed during the experiment. Mixotrophic cultivation stimulated an increase in the concentrations of chlorophyll a. The highest concentration of chlorophyll a was reported for both species with the addition of the BG-11 and Bf growth media. The highest content of chlorophyll a was observed in the culture of *Chlorella vulgaris* with the addition of the BG-11 in mixotrophic conditions (5.2 mg/L) and Bf in mixotrophic conditions (4.2 mg/L). A similar tendency was observed for the cultures of *Scenedesmus armatus*. The highest content of chlorophyll

a was observed for the cultures with the addition of the Bf (4.4 mg/L) and BG-11 (4.1 mg/L) media in mixotrophic conditions.

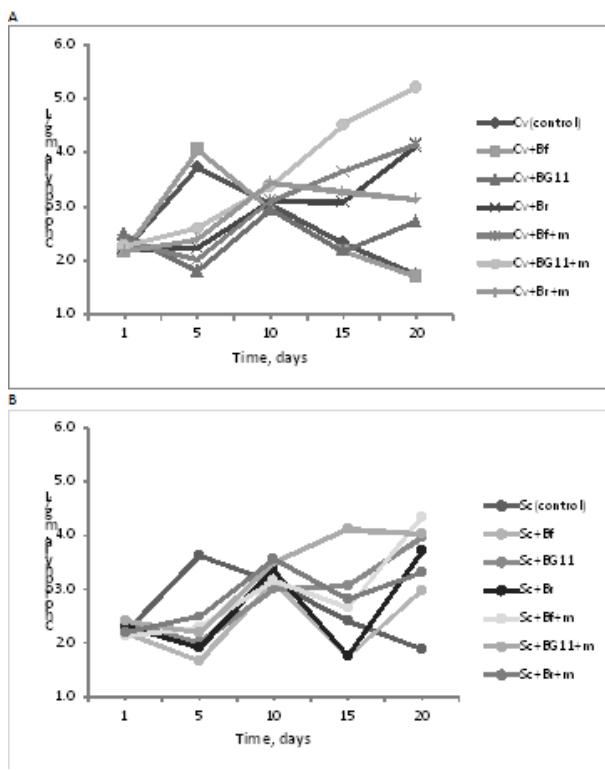


Figure 4. Changes in the content of chlorophyll a observed during the cultivation of (A) *C. vulgaris* and (B) *S. armatus*

The number of cells differed between the investigated species. Generally, the concentrations of biomass (determined from dry matter) obtained from growing *C. vulgaris* were higher than the ones achieved by *S. armatus*. As for the selected growth media, the concentrations of biomass were lower in mixotrophic than in autotrophic conditions. The application of the Bf medium was the most beneficial for both species, with the BG-11 being the second best in this respect. The addition of the Br medium showed the least beneficial effect under both autotrophic and mixotrophic conditions.

The dynamics of cell growth and division are governed by typical cell metabolism and physiological processes such as photosynthesis, respiration, ion assimilation, etc. (Koziel and Włodarczyk, 2011). The reported changes in pH, concentrations of oxygen and chlorophyll a corresponded to the phases of microalgal growth. The supply of an additional source of carbon had an effect on the production of chlorophyll a. Regarding the cultivation in autotrophic conditions, the curves of changes in dissolved oxygen and pH showed similar trends, irrespective of the applied growth media.

3.3. pH variation

One of the indicators of biomass growth is CO₂ assimilation during photosynthesis. The assimilation of CO₂ is indirectly affected by pH. The higher the pH, the more efficient the CO₂ assimilation. This results in

decreasing acidity of an algal culture. Figure 5 presents the pH variations that were observed during the cultivation of microalgae grown on the selected growth media.

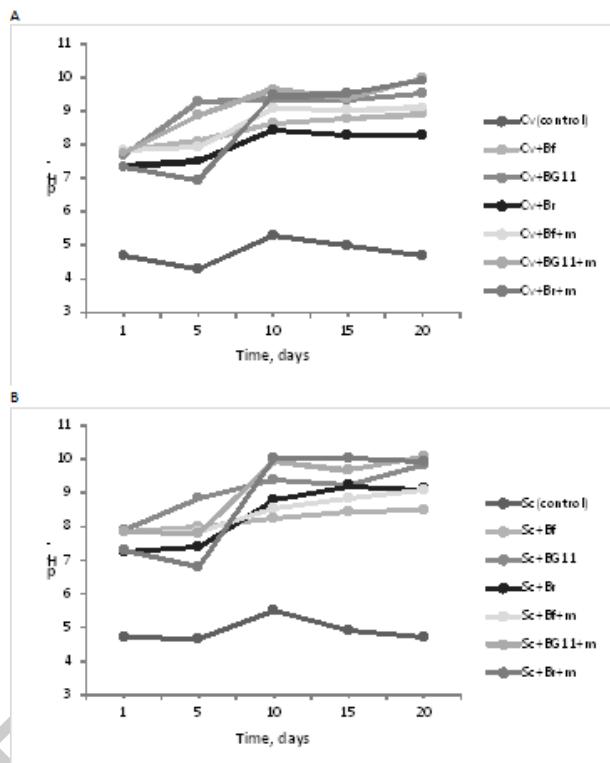


Figure 5. Changes in pH observed during the cultivation of (A) *C. vulgaris* and (B) *S. armatus*

In the samples with the growth media, the pH values were much higher than in the control sample (without any growth medium). The addition of growth media was beneficial as no significant fluctuations in pH were observed. In contrast, fluctuations in pH were observed in the control samples. This indicates that CO₂ assimilation in the process of photosynthesis was low in the control samples because of the lack of micro- and macronutrients in the cultivating solution.

From day 10 of the experiment, changes in pH were not as significant as during the first few days. After 10 days, the pH values in all samples with *Scenedesmus armatus* ranged from 8.3 to 10.4, whereas the pH in the samples with *Chlorella vulgaris* varied from 7.9 to 10.2. The lowest values of pH were observed in the control (Cv and Sc). During the growth of both populations, the observed pH increase in time indicated that CO₂ assimilation and photosynthesis processes occurred continuously. During the autotrophic growth, the increased consumption of CO₂ in the photosynthetic process resulted from the growth of microalgal biomass, leading to the formation of OH⁻ ions and consequently a higher pH of the solution (Becker, 2008; Kim *et al.*, 2013). Initially, the values of pH could be affected by the type of a growth medium because on day 1 the pH was lower in the culture with the Br medium (7.3) than in the samples with the other media. Then, pH

increased in all samples due to the photosynthetic processes. Similar results were obtained by Nayak *et al.* (2013), who observed a relationship between pH and time assigned to growing microalgae of *Scenedesmus* sp. species (Nayak *et al.*, 2013). These researchers grew microalgae in a photobioreactor at continuous light regime for 12 days and observed that pH of the microalgal culture increased from 6.5 to 7.5 with time. Tam and Wong (1996) reported lower pH during the logarithmic phase and higher pH during the lag and stationary phases. These findings support the results obtained in the present study. It has to be pointed out that excessively high values of pH can impair the mechanisms in algal cells on the biochemical level, causing some disturbances in the metabolism of sugars in *C. vulgaris* (Mayo, 1997).

The lowest pH values of 6.92 (Cv – control sample) and 6.78 (Sc – control sample) were observed under mixotrophic conditions. During the mixotrophic growth, inorganic and organic carbons are metabolized and hydroxyl ions and CO₂ are generated as metabolites, while the pH variation is irregular (Kim *et al.*, 2013).

3.4. Economic analysis

According to the literature, the production cost of algal biomass produced in the PBR (tubular) system is ~9-10€/1 kg (Slade and Bauen, 2013). The Bioflorin growth medium is very cheap –(8€/L) and this commonly available fertilizer for green plants, as mentioned above, contains more nutrients than Bristol. The Bristol medium is much more expensive: ~ 50€/L and the price for the BG-11 medium is ~ 312€/L. Molasses is also a inexpensive source of carbon (~8€/1 kg) for mixotrophic cultivation of algae, and then the cost of biomass production is ~3-4€/1 kg.

4. Conclusions

The results confirmed that it is possible to use an inexpensive and universal growth medium (Bf) and a carbon source (molasses) in place of the popular fertilizers: Bristol or BG-11, to cultivate *Chlorella vulgaris* and *Scenedesmus armatus*. The use of inexpensive growth media will reduce the production costs of algal biomass on a large scale.

The results from the present study justify the following conclusions: the highest concentrations of algal biomass for both species were obtained after the application of the Bf medium, lower after the BG-11 medium and the lowest after the Br medium in autotrophic as well as mixotrophic conditions. Hence, further research will focus on the selection of an efficient method for the production of microalgal biomass depending on time constraints, calorific value of biomass and the investment costs. Microalgal biomass can be used as a sorbent for removal of microcontaminants (heavy metals and/or biogens) from wastewater.

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