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Cultivation Parameters Adjustment for Effective Algal Biomass Production

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1. Introduction

Seemingly inevitable energy crisis makes it a high priority for today's society to become independent of already exhausting fossil fuels. Most researched energy sources, regarded as possible alternatives for the currently leading one, are solar, wind, water and biofuels. The latter are produced from high calorific chemical compounds being natural metabolites of living, usually autotrophic, organisms. Although there are many plants that can be used as the biofuel feedstock, it is aquatic organisms, mainly algae, to be the most relevant, since their cultivation involves practically no land requirements and relatively small agricultural input [1,18]. Moreover, their complex biological activity may present algae farming as a beneficial contribution to nutrient cycling and waste water treatment. The considerable potential of algal-based biofuels makes them a subject of thorough investigation. The greatest challenge is to optimize the conditions of algae cultivation.

In the natural, as well as human-created media algae are exposed to a number of environmental factors. Variable chemical and physical conditions influence all the aspects of algal activity, including biomass increase rate and metabolism intensity, thus their efficiency as fuel feedstock. The most important factors are temperature, pH, light intensity, salinity, accessible biogenic elements concentrations and optical activity of the medium. Simultaneously, all of these conditions are constantly

altered by the culture itself, which in turn affects the quantitative and qualitative algae growth etc. Additionally, there is a strong interdependence between particular environmental factors. Therefore the development of algal cultures must be ceaselessly monitored and their properties adjusted, which, combined with our still insufficient understanding of the occurring processes, proves to impede the introduction of algal-based fuels on the global scale [2].

The crucial question concerns the actual impact of previously mentioned environmental conditions on the growth of algae. One of the most vital factors, at the same time one most difficult to control in large-scale cultivation in open ponds, is temperature. Closely related with thermal energy, it determines the enzymatic activity, being the limiting factor for most biochemical processes in the cells. It also affects the physical and chemical properties of the medium, such as density and solubility of most substances, resulting in significant changes within the molecular structure of the algae [1,2]. Other energy-related factor is the culture illumination. It can be modified by either changing the light intensity and spectral composition or the optical activity of the medium. The luminous energy regulates photosynthesis, directly leading to carbon incorporation and biomass increase rate. The metabolic activity of the algae depends also on nutrient availability. The most essential elements that must be supplied are carbon, nitrogen and phosphorus, in the desirable proportions of approximately 100:6:1 [14]. Their accessibility can vary due to environmental factors such as acidity or salinity.

In this project microalgae were grown on pre-treated reject water. This type of wastewater is formed while drainage the digested sewage sludge. As the nutrient concentration in this type of wastewater is comparatively high, effluent cannot be directly discharged, and is normally directed back into the raw sewage for removal of nitrogen and phosphorus. This results in wrong C:N ratio in influent and has a negative influence on microbial processes and structure of biocenoses in biological reactors [9]. From the perspective of microalgae cultivation, the reject water has several advantages: (1) the ratio between inorganically and organically bound nutrients is higher in the reject water than in raw sewage, (2) the nutrient concentration in the reject water is relatively constant as compared to the changing nutrient concentrations in raw sewage, (3) compared to the turbid raw and treated sewage, the reject water is

relatively clear, permitting a higher degree of light availability for algal photosynthesis; and (4) in a working environment perspective, the reject water is safer and more hygienic, since the anaerobic digestion causes sanitation of the pathogens that are inevitably present in raw as well as treated sewage water [19]. Microalgae growing on this type of medium have a dual use – firstly, they purify wastewater from high load of nutrients and heavy metals (on the way of sorption) and secondly, obtained biomass can be used as a biofuel, for example as a feedstock in fermentation chamber and in the production of biogas.

Of all the factors which are growth-limiting for algae, a few can be controlled or adjusted. In order to ascertain their impact on algal biomass increase rate and to analyse physicochemical parameters of a working algal culture, before transferring it to a larger bioreactor, a series of laboratory-scale experiments were conducted. The ultimate target was to intensify the culture growth and receive maximal biomass increase for biomass further usage as an energy source.

2. Materials and methods

2.1. Growth medium

Algae biomass was suspended in pre-treated reject water, obtained from the first stage of purification – SBR reactor (sequencing batch reactor). In this reactor a partial nitrification – anammox processes occurred. Loads of inorganic nitrogen and phosphorus were partially removed, but still high. $N\text{-}NH_4$ concentration in effluent from SBR reactor was on the level of 60–70 mg/l, $N\text{-}NO_2$ on the level of 8–10 mg/l and $N\text{-}NO_3$ on the level of 14–20 mg/l. Concentration of $P\text{-}PO_4$ in growth medium was variable but also high, reached a level of 30–70 mg/l. Medium was also characterized by pH on the level of 7.4–7.6, COD on the level of 80–180 mg/l and alkalinity on the level of 1.5–3.6 mM/l.

Reject water derives from Gliwice WWTP, from wastewater treatment processes – precisely, they were formed during treatment of sewage sludge. Sludge in first stage was thickened with polymers and then stabilized by biological method – anaerobic digestion in mesophilic conditions. After this process sludge was dewatered by mechanical methods (with usage of filter presses and centrifuges). Reject water

formed in this processes were directed to the test system. Scheme of this system is illustrated at figure 1.

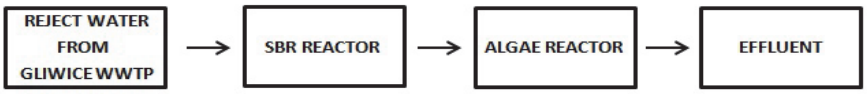

Fig. 1. Test system for treating the reject water in Gliwice WWTP
Rys. 1. Układ badawczy służący do podczyszczania wód osadowych w Centralnej Oczyszczalni Ścieków w Gliwicach

2.2. Influence of microalgae suspension dilution on biomass production

The tested property was relative biomass increase depending on the probe dilution. From a bioreactor 1.5 litres of *Chlorella* sp. culture were taken. The algae were centrifuged at 3500 rpm, after their sedimentation the supernatant was drained off and the algae were suspended in 1.5 litres of effluent from SBR reactor. 100 ml of the resultant suspension was filtered through a previously dried and weighed quality filter paper (medium pore size) and left to dry as a biomass reference. Determination of dry mass was carried out in accordance with PN-75 C-04616/01. 15 flasks were prepared, 3 of every kind:

- control samples with 100 ml of the suspension,
- samples with 80 ml suspension, 20 ml medium (80%),
- samples with 60 ml suspension, 40 ml medium (60%),
- samples with 40 ml suspension, 60 ml medium (40%),
- samples with 20 ml suspension, 80 ml medium (20%).

Flasks were stoppered with cotton wool plugs and incubated on a rotary shaker at 150 rpm for 3 days in ambient temperature (25 ± 2°C). After the incubation period the samples were filtered, dried and weighed. The results were compared with the control samples and the reference. Based on obtained data growth rate of each culture was estimated according to the equation:

$$\mu = \frac{1}{t}\ln\left(\frac{N_t}{N_o}\right) \quad (1)$$

In which:
μ – growth rate [1/day],
t – time of the experiment [day],
N_t – biomass in day 't' [g],
N_0 – biomass in day '0' [g].

2.3. Influence of environmental factors on algae biomass production

The next experiment tested the susceptibility of algae to different environmental factors (N:P ratio, carbonates concentration, optical density of microalgae suspension, photosynthetically active radiation) measured by biomass increase rate. A *Chlorella* sp. suspension was prepared as previously described (100 ml was filtered, dried and weighed as a reference). The algae were placed in 15 flasks with 100 ml suspension each, 3 flasks of following kinds:

- control samples containing the prepared suspension;
- samples with a different optical density of microalgae suspension, achieved through dilution of the culture (50 ml of the suspension, 50 ml of pure medium) – without change in nutrients concentration;
- samples with increased ammonium nitrogen concentration (0.0336 g $(NH_4)_2SO_4$ added to each flask) – reached a value of 160 mg/l, total nitrogen ca. 190 mg/l and phosphates ca. 30 mg/l;
- samples with increased concentration of carbonates (0.1 g Na_2CO_3 added to each flask);
- samples additionally exposed to light of a sodium lamp, surrounded with reflecting foil.

The flasks were stoppered with cotton wool plugs and incubated on a rotary shaker at 150 rpm for 5 days at ambient temperature (25 ± 2°C). After the incubation period the samples were filtered, dried and weighed. The results were compared with the control samples and the reference. Obtained data were used to calculate the growth rate as previously described.

3. Results and discussion

3.1. The first batch test experiment – optical density of microalgae suspension influence

Results of the experiment in which influence of suspension optical density on growth increase was tested are shown in table 1 and as a graph in figure 3. Figure 4 shows a value of growth rate of each culture obtained in this experiment.

Table 1. Dry biomass obtained from the filtrate – experiment with different dilutions (optical density of algae suspension)
Tabela 1. Ilość suchej masy otrzymanej po przefiltrowaniu zawiesiny – eksperyment z różnym rozcieńczeniem próbki (różną zawartością glonów w medium)

	CONTROL SAMPLE [g]	20% [g]	40% [g]	60% [g]	80% [g]
0	0.011	0.0022	0.0044	0.0066	0.0088
1	0.0211	0.0055	0.0124	0.0196	0.0213
2	0.0219	0.0058	0.0126	0.019	0.0206
3	0.0218	0.0054	0.0125	0.0199	0.0211
MEDIUM	0.0216	0.0056	0.0125	0.0195	0.021
INCREASE	0.0106	0.0034	0.0081	0.0129	0.0122
INCREASE [%]	96.36	153.03	184.09	195.45	138.64

The first test, in which the influence of dilution was checked, gave interesting results. Dilution of algae suspension on the level of 40% and 60% was related with the highest biomass increase (184.09% and 195.45%, respectively). Biomass at start day for these dilutions was 0.0044 g and 0.0066 g, after 3 days reached a value of 0.0125 g and 0.0195 g. Growth rate of algae in these cultures was on the level of 0.361 1/day·and 0.348 1/day. Dilution on the level of 80% (80 ml of control culture and 20 ml of growth medium) was too low and gave unsatisfactory results (increase – 138.64%; growth rate – 0.290 1/day). The highest tested dilution (20% – 20 ml of control culture and 80 ml of medium) gave better results of increase than 80%, but was too high for quick period of retention time tested in experiment. Obtained growth rate was on the level of 0.311 1/day. For further tests 50% dilution of algae suspension was assumed.

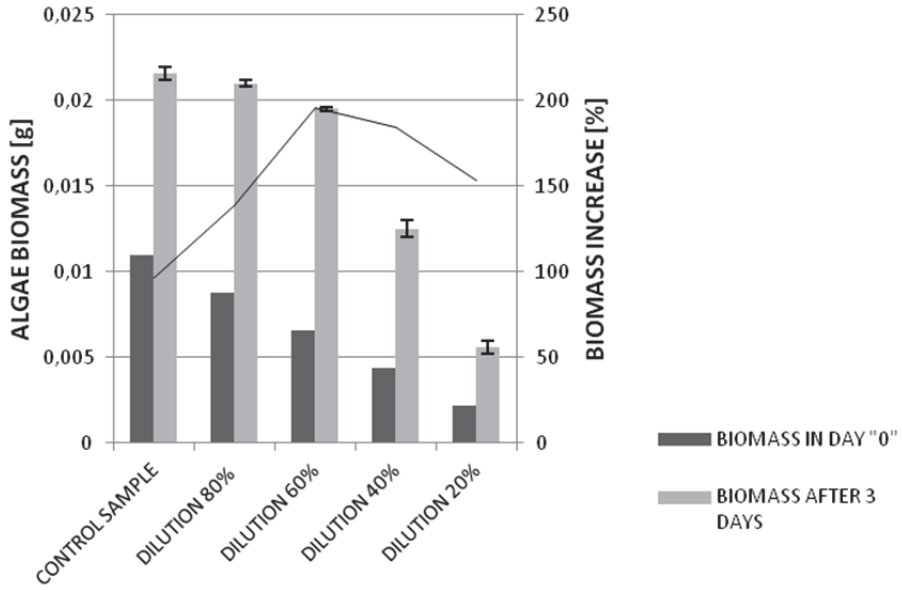

Fig. 3. Results of batch test experiment with different dilutions
Rys. 3. Wykres ilustrujący wyniki eksperymentu z różnym rozcieńczeniem

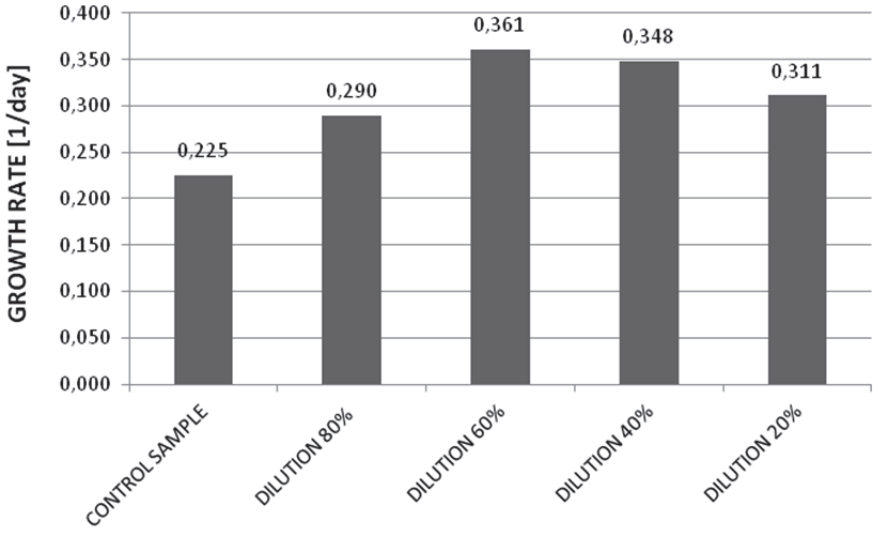

Fig. 4. Growth rate of *Chlorella* sp. depending on culture dilution
Rys. 4. Szybkość wzrostu *Chlorella* sp. w zależności od rozcieńczenia zawiesiny wyjściowej

3.2. The second batch test experiment – environmental factors influence

Results of batch-test with different environmental conditions are shown in table 2 and illustrated as a graph in figure 5. Figure 6 shows growth rate of each algal culture obtained in this test.

Table 2. Biomass obtained after drying the filtrate from flasks – batch test with influence of environmental factors
Tabela 2. Ilość suchej masy otrzymanej po przefiltrowaniu zawiesiny – eksperyment z różnymi czynnikami środowiskowymi

	CONTROL SAMPLE [g]	DILUTION [g]	$(NH_4)_2SO_4$ [g]	Na_2CO_3 [g]	PAR [g]
0	0.0314	0.0157	0.0314		
1	0.0523	0.0514	0.057	0.0678	0.0646
2	0.0545	0.0509	0.0583	0.0677	0.072
3	0.0573	0.0457	0.055	0.0649	0.0646
MEDIUM	0.0547	0.0493	0.0568	0.0668	0.0671
INCREASE	0.0233	0.0336	0.0254	0.0354	0.0357
INCREASE [%]	74.2	214.23	80.79	112.74	113.59

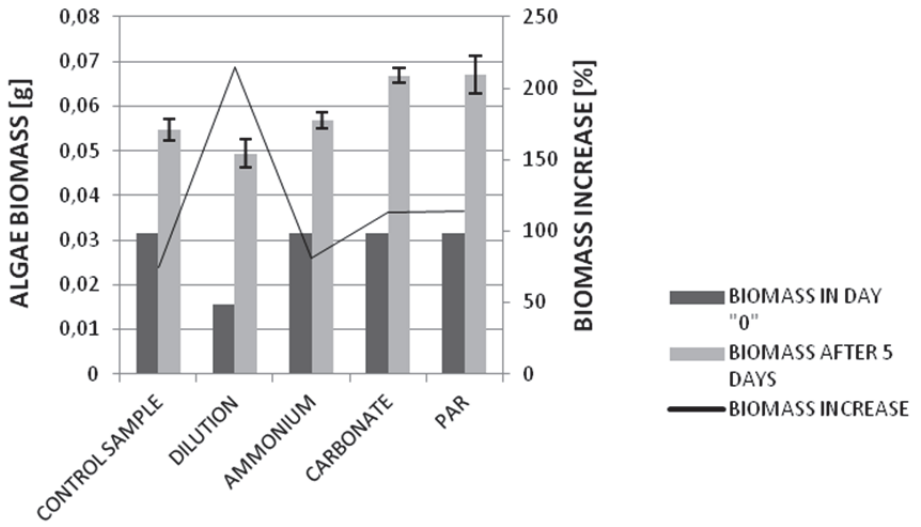

Fig. 5. Results of batch test experiment with different environmental factors
Rys. 5. Wykres ilustrujący wyniki eksperymentu z różnym czynnikami środowiskowymi

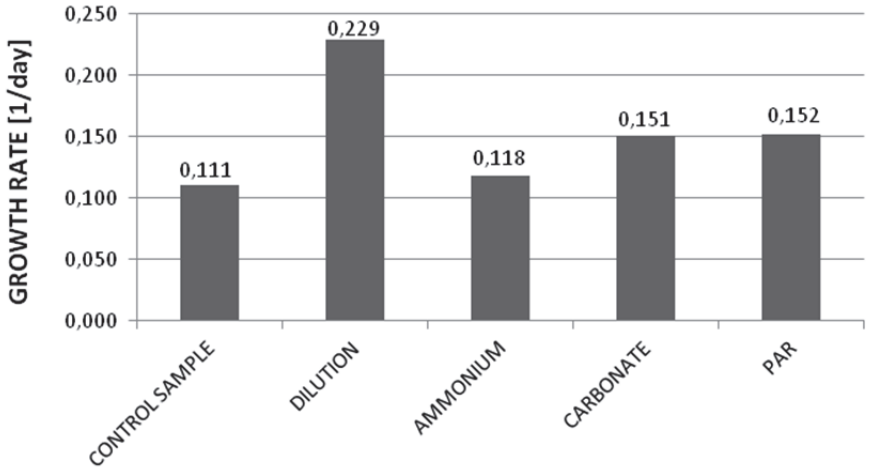

Fig. 6. Growth rate of *Chlorella* sp. depending on influence of environmental factors
Rys. 6. Szybkość wzrostu *Chlorella* sp. w zależności od badanego czynnika środowiskowego

Results of second batch-tests showed that reducing the concentration by half caused in more than a double increase (214.23%) of obtained dry biomass, in comparison with control samples. Growth rate was on the level of 0.229 1/day, and was the highest of other tested factors. Addition of sodium carbonate as a carbon dioxide source and lightening by PAR also resulted in higher biomass growth in comparison with control samples (increase on the level of 112.74% and 113.59% respectively). N/P ratio change from 3.5 to 6.5 had no significant impact on the amount of biomass, but this result must be subjected to further analysis. In this case biomass increase and growth rate was at similar level as in control cultures (80.79%; 0.118 1/day). The highest growth increase was obtained for diluted samples. It showed that the optical density of algae suspension was a major factor which limited biomass increase in the test flasks.

3.3. Discussion

As literature sources and preliminary tests showed, microalgae are undemanding in their cultivation. These aquatic organisms only need the access to basic nutrients and sunlight (or artificial photosynthetically active radiation – PAR) for growth and multiplication. Environmental conditions, like pH or temperature are also very important (too low value of

these parameters can be growth limiting) but in general – microalgae cultivation is an uncomplicated and relatively cheap procedure. It is also a carbon-neutral process because for each kilogram of algal biomass produced, about 1.88 kilograms of CO_2 are sequestrated from atmosphere [10]. Among the requirements for algal growth the most expensive are nutrients – if their production involves natural resource and energy utilization. Optimization of this factor can mitigate biomass production costs and improve process economics [3]. For their cultivation different types of wastewater can be used – also reject water, which are difficult to treat [19]. Additionally – obtained biomass can be used as a bioenergy source, for the production of different biofuels, including biodiesel, bio-oil, biogas and biohydrogen [5,7,12,15]. This is the reason why they are interesting for researchers. The most important problem in applicable solutions is how to increase the growth and nutrients uptake in short period of time. In our experiment we have done a series of batch tests in laboratory conditions to check, which of the most important environmental factors can influence on the growth rate. Similar studies have been done by several research groups interested in this topic. Blair et al. [3] analysed the impact of different light wavelength on the algal growth rate and volumetric biomass productivity. Their study shown that clear and blue light wavelengths had a positive impact on the growth rates, compared to the red and green light. Shriwastav et al. [17] studied adaptability of growth and nutrient uptake potential of *Chlorella sorokiniana* with variable nutrient loading. Researchers harvested algae in mineral, diluted BG11 media with different concentrations of nitrate (as $NaNO_3$) and phosphate (as K_2HPO_4) to achieve required nutrient levels. Results shown the ability of *C. sorokiniana* to regulate its nutrient uptake in accordance with the available nutrients levels in the surrounding without any harmful effect on growth or productivity until either nutrient became rate limiting. These results indicate that the typical Redfield molar ratio of 16N:1P [16] in algae could be variable. Deviations of this ratio have been also reported by Hullat et al. [8] and Klausmeier et al. [11]. Effect of algal density on growth and nutrient removal as other important parameter was investigated by Tam et al. [20]. High algal density lead to self-shading, reduction in photosynthetic efficiency and accumulation of auto-inhibitors [4,6], so there is a great need to indicate optimal level of this factor. Value of this parameter obtained in this study was similar to growth rates of

algae harvested in commercial Bristol medium (0.3664 1/day), however inoculum size was characterized as a number of cells of *Chlorella vulgaris*, not as a dry weight [13].

3. Conclusion

The experiment showed that among the studied factors (in the tested range) the most important for the algae growth intensification is the density of the culture suspension. Too high density results in poor access to sunlight and high competition. In effect low removal of nutrients from reject water is bound to occur. Too low density may create a necessity to extend the hydraulic retention time in bioreactor and cause technological problems (very low flows). The best results of biomass increase of *Chlorella* sp. in pretreated reject water correspond with the 0.045–0.067 g/l of microalgae suspended in the medium. Growth rates related with these values were on the level of 0.348 and 0.361 1/day, respectively.

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Określenie optymalnych warunków hodowlanych dla efektywnej produkcji biomasy glonowej

Streszczenie

Mikroglony są niewielkimi organizmami wodnymi o bardzo dużym potencjale w zakresie oczyszczania ścieków. Ma to związek z faktem, że są mało wymagające w hodowli, a do wzrostu i rozmnażania mogą z powodzeniem wykorzystywać związki biogenne zawarte w ściekach. Dodatkowo, powstająca biomasa może posłużyć jako substrat energetyczny – może zostać wykorzystana do produkcji biopaliw, takich jak biodiesel, bioetanol czy biobutanol lub jako wsad do komory fermentacji, czyli do produkcji biogazu. Ze względu na rosnące zainteresowanie pozyskiwaniem znacznych ilości biomasy glonów podjęto próbę zoptymalizowania warunków hodowlanych pozwalających na pozyskanie największej ilości surowca przetwórczego. W eksperymencie przeprowadzono w warunkach laboratoryjnych serię analiz w zawiesinie, w których jako medium wzrostowe dla mikroglonów z rodzaju *Chlorella* wykorzystano wody z odwadniania przefermentowanych osadów ściekowych, pochodzące z Centralnej Oczyszczalni Ścieków w Gliwicach. Wody osadowe charakteryzują się wysokim stężeniem azotu nieorganicznego w postaci jonów amonowych, które z powodzeniem wykorzystywane są przez mikroglony do wzrostu. Dodatkowo ścieki takie, w porównaniu z surowymi ściekami komunalnymi, są relatywnie klarowne, dzięki czemu możliwe jest przenikanie światła w głąb medium hodowlanego. W przeprowadzonym eksperymencie wody osadowe w pierwszym etapie poddane zostały podczyszczaniu w reaktorze typu SBR, a następnie skierowane jako dopływ do reaktora glonowego. Celem badań było określenie wpływu podstawowych czynników środowiskowych na tempo wzrostu glonów, a tym samym na przyrost biomasy. W pierwszym teście analizowano wpływ gęstości optycznej hodowli na szybkość przyrostu biomasy. Zawiesinę glonów jednokomórkowych rozcieńczano medium wzrostowym odpowiednio w stosunku 1:5, 2:5, 3:5 oraz 4:5 w odniesieniu do hodowli kontrolnych. Uzyskane wyniki pozwoliły określić optymalną gęstość zawiesiny mikroglonów w reakto-

rze (odpowiadającą największym przyrostom biomasy, a tym samym wysokiemu usunięciu związków biogennych). Początkowe stężenie zawiesiny mikroglonów na poziomie 0,045–0,067 g/l odpowiadało największym przyrostom biomasy w próbach (tempo wzrostu odpowiednio 0,348 i 0,361 1/dzień). W drugiej serii testów analizowano dodatkowo wpływ takich czynników jak: stosunek N:P, dodatek węglanów jako źródła dwutlenku węgla oraz dodatkowe oświetlanie promieniowaniem aktywnym fotosyntetycznie (falą świetlną o długości w zakresie absorpcji chlorofilu a oraz b), w odniesieniu do próby kontrolnej, a także do uzyskanej wcześniej optymalnej gęstości zawiesiny mikroglonów. W eksperymencie wykazano również, że wody osadowe, charakteryzujące się wysokim ładunkiem nieorganicznych związków azotu i fosforu, a także obecnością metali ciężkich mogą być z powodzeniem wykorzystywane jako medium wzrostowe dla glonów z rodzaju *Chlorella*.

Słowa kluczowe:
mikroglony, wody osadowe, przyrost biomasy

Keywords:
microalgae, reject water, growth rate