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Influence of the Light Source on the *Chlorella vulgaris* Biomass Growth in the Culture Medium Supplemented with Anaerobic Digestate

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1. Introduction

The way of providing light is one of the most important elements affecting the effectiveness of production of algae biomass directly. In many studies, it was proven that the type of the light source, wavelength and exposure method affect not only the yield of microalgae production, but also the formation of the taxonomic structure and the chemical composition of the obtained biomass (Schulze et al. 2014). The photosynthetic activity of most microalgae species increases in the range of light intensity at the level of 200-400 μmol E/m^{2}s (Ogbonna & Tanaka 2000), which corresponds to about 10% of the amount of light they can receive directly from the sun. The amount of light needed for microalgae growth is slightly lower than for terrestrial plants. For example, Chlorella vulgaris microalgae can grow under exposure of 50-100 W/m^2, which corresponds to a value of 232 to 465 μmol E/m^2·s of the PAR range. For many algae species, the optimal light exposure conditions range from 5000 lx (90 μmol/m^2·s) to 13000 lx (230 μmol/m^2·s), and a temperature of 17-20°C (Schulze et al. 2014).

Sunlight is undoubtedly the best type of the light source enhancing the growth of microalgae cultures. However, the solar energy is available only during the cloudless days, and in the middle latitudes the intensity of natural sunlight also varies significantly depending on the season. In turn, in the hours around noon when the radiation intensity increases even to 4000 μmol E/m^2·s, a photoinhibition is observed. According to Zhu et al. (2008), photoinhibition of different algae species may occur at a photon flux density ranged from 200 to 800 μmol/m^2·s. Photoinhibition of photosynthesis is very likely to occur in starter

cultures with low concentration of microalgae cells exposed to strong sunlight, where no cellular self-shading is observed (Goksan et al. 2003).

In the closed systems, photoinhibition can be reduced by choosing the proper type and intensity of light, as well as by increasing the contact surface of microalgae cells with light (Torzillo et al. 2003). The best way to eliminate the drawbacks of sunlight for microalgae cultivation is using the hybrid lighting systems in combination with LEDs (Szwaja et al. 2016).

Another method applying molecular tools is genetic reducing of the size of the chlorophyll antennas in microalgae cells. As a result, the efficiency of light energy adsorption is reduced, which allows for the proper and stable process of photosynthesis at a higher light intensity (Melis et al. 1999). In order to ensure the continuous microalgae biomass synthesis, a stable supply of the light energy should be ensured around the clock or in the established photoperiod required by some species of microalgae. Absence of light and inhibition of photosynthesis create anaerobic conditions in photobioreactors, contributing to reducing the rate of biomass growth and concentration of microalgae cells. Thus, in intensive microalgal production, artificial light sources are commonly used.

In the light of the above considerations, there is a need to find technological solutions ensuring optimal lighting conditions for technologically and economically effective production of microalgae biomass. The aim of the study was to determine the influence of the light source on the productivity of *Chlorella vulgaris* biomass cultivating on anaerobic digestate.

2. Materials and methods

2.1. Microalgae inoculum, cultivation in photobioreactors and study organization

The inoculum was a culture of *Chlorella vulgaris* originated from UTEX 2714 Culture Collection of Algae (University of Texas, Austin, USA).

Liquid algal culture was grown photoautotrophically in closed, vertical, tubular photobioreactors with an active volume of 2.5 L (inner diameter 76 mm and 550 mm height) made of transparent plexiglass. An initial concentration of the algae biomass in the photobioreactors was 250±22 mg total solids (TS)/L. Compressed air was delivered continuously at 200 L/h from the bottom of the reactors upwards by peristaltic pumps (Mistral 200). This ensured appropriate mixing of the culture medium, homogeneity of conditions in the entire reactor volume and introduction of atmospheric CO_2 to the culture. The temperature of the culture was maintained at 23.0±2.0°C.

The nutrient medium for *Chlorella vulgaris* cultivation was the mixture of liquid anaerobic digestate, tap water and synthetic medium. The digestate was

obtained from an agricultural biogas plant operated in a technical scale feeding with maize silage and distillery stillage. The concentration of anaerobic sludge in reactor was maintained at the level of 5 g TS/L, the temperature was 40°C and organic loading rate was 2.4 kg of volatile solids (VS)/L·d. Before using as a nutrient medium, digestate was centrifuged (MPW-251 Donserv, 10 min, 5000 rpm) and then autoclaved (30 min, 90°C). The chemical characteristics of anaerobic digestate is shown in Table 1. Due to a high color and the content of organic compounds, the digestate constituted 20% of the active volume of the photobioreactors in series 1 and 10% in series 2. The remaining part of the culture medium was tap water with synthetic medium composed of: $NaNO_3$ 25 g/L, $CaCl_2 \cdot 2H_2O$ 2.5 g/L, $MgSO_4 \cdot 7H_2O$ 7.5 g/L, $K_2HPO_4 \cdot 3H_2O$ 7.5 g/L, KH_2PO_4 17.5 g/L, NaCl 2.5 g/L, VB12 1.0 mL/L, VB1 1.0 mL/L, microelements 6.0 mL/L, Na_2EDTA 0.75 g/L, $FeCL_3 \cdot 6H_2O$ 97.0 g/L, $MnCl_2 \cdot 4H_2O$ 41.0 g/L, $ZnCl_2$ 5.0 g/L, $NaMoO_4 \cdot 2H_2O$ 4.0 g/L, $CoCl_2 \cdot 6H_2O$ 2.0 g/L. The characteristics of the culture medium is shown in Table 2.

Table 1. Characteristics of anaerobic digestate used in the experiment

Parameter	Unit	Concentration	
		Raw digestate	Digestate after centrifugation
Total solids	mg/L	12700±2400	350±34
COD	mg O_2/L	10200±730	7950±370
BOD_5	mg O_2/L	5600±310	3700±190
TN	mg N/L	2750±190	1805±91
$N\text{-}NH_4$	mg $N\text{-}NH_4$/L	2210±130	1300±73
TP	mg P/L	185±22	124±14
$P\text{-}PO_4$	mg $P\text{-}PO_4$/L	158±14	71±9
pH	–	6.9±0.4	7.0±0.2

The photobioreactors were placed in a chamber covered with aluminium foil and were illuminated continuously by different light sources. The experiments in both series were divided into eight variants. The criterion used was the applied light source. Organization of the experimental variants is shown in Table 3.

Table 2. Characteristics of the culture medium used in the experiment

Parameter	Unit	Concentration	
		Series 1	Series 2
Total solids	mg/L	71±21	29±14
COD	mg O_2/L	1590±210	780±113
BOD_5	mg O_2/L	741±39	390±27
TN	mg N/L	361±42	172±35
$N\text{-}NH_4$	mg $N\text{-}NH_4$/L	257±29	129±21
TP	mg P/L	19±6	10±3
$P\text{-}PO_4$	mg $P\text{-}PO_4$/L	12±3	7.1±1.6
pH	–	7.2±0.3	7.1±0.2

Table 3. Organization of the experimental variants depending on the light source used

Vari-ant	Light source	Wavelength (λ) [nm]	Electric power of the light source [W]
1	Fluorescent tube – warm light	Colour temperature: 3000 K	100
2	LED – Warm light	2 local maximum at: 450 nm and 580 nm, colour temperature: 6500K	
3	LED – Red and Blue	640 nm and 470 nm	
4	Fluorescent tube – cold light	Colour temperature: 6500K	
5	LED – Blue	470 nm	
6	LED – Red	640 nm	
7	Daylight	Mean colour temperature: 5900K	Exposed to daylight
8	High pressure sodium lamp	Essential spectrum: 570-620 nm, Colour temperature: 2700K	100

2.2. Analytical methods

The cultivation of the microalgae was carried out for 10 days. After the cultivation process was ended, the obtained algae biomass was concentrated, separated and dehydrated by initial sedimentation and then by centrifugation for 10 min at 5000 rpm (MPW-251, Donserv). Determinations of TS and VS in the solid fraction were carried out by gravimetric analysis. In the supernatant, the concentration of ammonia nitrogen ($N\text{-}NH_4$) was determined with cuvette tests using a DR 5000 spectrophotometer (Hach-Lange, Germany) with an HT 200s mineralizer (Hach-Lange, Germany). At the beginning and end of the experiment, the digestete and the cultivation medium were analyzed for biochemical oxygen demand (BOD_5) with the Oxi-top control system (WTW, Germany) as well as chemical oxygen demand (COD), the concentrations of orthophosphates ($P\text{-}PO_4$), total phosphorus (TP), total nitrogen (TN) with cuvette tests using a DR 5000 spectrophotometer (Hach-Lange, Germany) with an HT 200s mineralizer (Hach-Lange, Germany). The pH value was determined by a digital pH-meter (1000L, VWR). The taxonomic identification of microalgae biomass was conducted at microscope magnifications of: 1.25x10x40 or 1.25x10x100, and with algae analyzer (BB Moldanke, Germany). The light intensity supplied to the photobioreactors was measured using a luxometer NL-100 (Hanna).

2.3. Statistical methods

Each experimental variant was conducted in three replications. Statistical analysis of the obtained results was carried out using the Statistica 12.0 PL package (Statsoft, Inc.). Hypothesis on the distributions of the individual studied variables was verified based on the Shapiro-Wilk test. In order to ascertain the significance of the differences between the variables, an analysis of variance (ANOVA) was carried out. Variance homogeneity in groups was confirmed using the Levene test. In order to determine the significance of the differences between the analysed variables, the HSD Tukey test was used. In the tests, a significance level was assumed as $p = 0.05$.

The formulas that can predict the biomass concentration depending on medium characteristic were developed during the study. A multiple regression model using a stepwise progressive regression algorithm was used to identify the relevant predictor variables in the formulas, among the investigated variables by Statistica 12.0 PL package (Statsoft, Inc.). Then, the residual analysis were carried out to validate the regression models.

3. Results and discussion

3.1. Biomass concentration

According to the literature, light-emitting diodes (LEDs) may become the most important light sources around the world and their application in the intensive algal production systems should to be carefully considered. It has been proven, that using the LEDs as a light source in photobioreactors improve the productivity of microalgal biomass. However, only a balanced mix of wavelengths enhanced the growth of algae depending on the photosynthetic pigments composition and concentration found in the cells (Schulze et al. 2014). For this reason, our experiments studied the influence of the light source on the *Chlorella vulgaris* biomass growth. Due to the low photosynthetic quantum yield in natural systems, there is a need to optimize the regime of the light exposition in microalgal biotechnology (Abu-Ghosh et al. 2016, Zhao et al. 2013). The excess of light energy has an adverse impact on photosynthetic apparatus, thus microalgae have some strategies to control the surplus sunlight energy absorbed in photosystems (Wobbe et al. 2016). The photosynthetic conversion process, and hence also the biomass productivity are strongly dependent on the source and amount of light during the culturing (Pruvost et al. 2015, Moheimani et al. 2013).

During the study, the highest concentrations of *Chlorella vulgaris* biomass averaged 1810±77 mg TS/L and 1640±201 mg TS/L were observed in series 2 in variants 1 and 2, respectively (Fig. 1-3). In other variants significantly lower ($p = 0.05$) biomass productivity ranged from 1110±137 mg TS/L in variant 3 to 730±71 mg TS/L in variant 7 were observed (Fig. 1, Fig. 3). In series 2 the lowest biomass production of 440±93 mg TS/L in variant 8 was noted with the high pressure sodium lamp light source (Fig. 1, Fig. 3).

The similar tendency is found in series 1 (Fig. 1, Fig. 2). In variants 1 and 2, the highest biomass concentrations of 1420±159 mg TS/L and 1230±112 mg TS/L were respectively obtained (Fig. 1). The productivity of *Chlorella vulgaris* biomass in other variants was statistically comparable ($p = 0.05$) to obtained in series 2, and the concentration of biomass ranged in the narrow range from 920±21 mg TS/L to 730±77 mg TS/L. Only in variant 8, it was achieved 412±83 mg TS/L (Fig. 1, Fig. 3).

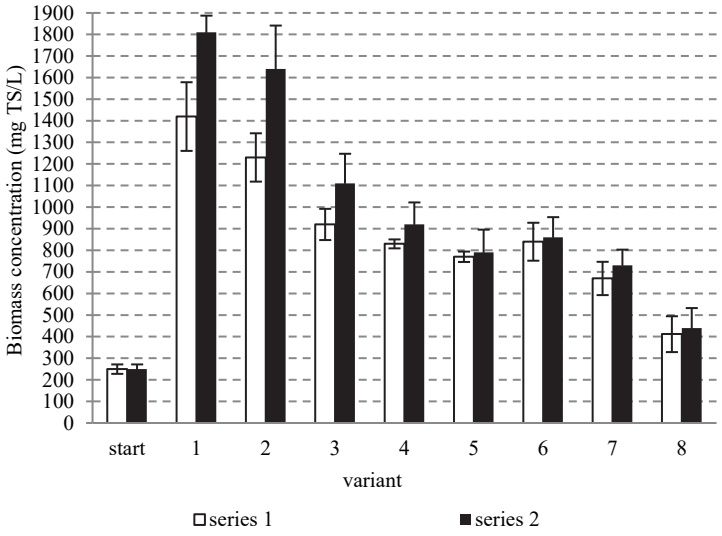

Fig. 1. Final biomass concentration of *Chlorella vulgaris* depending on the experimental variant

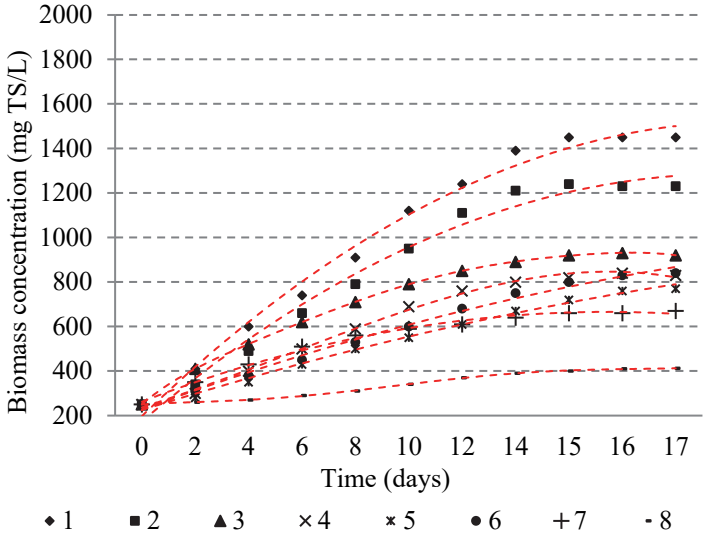

Fig. 2. Changes in biomass concentration of *Chlorella vulgaris* in series 1 depending on the experimental variant

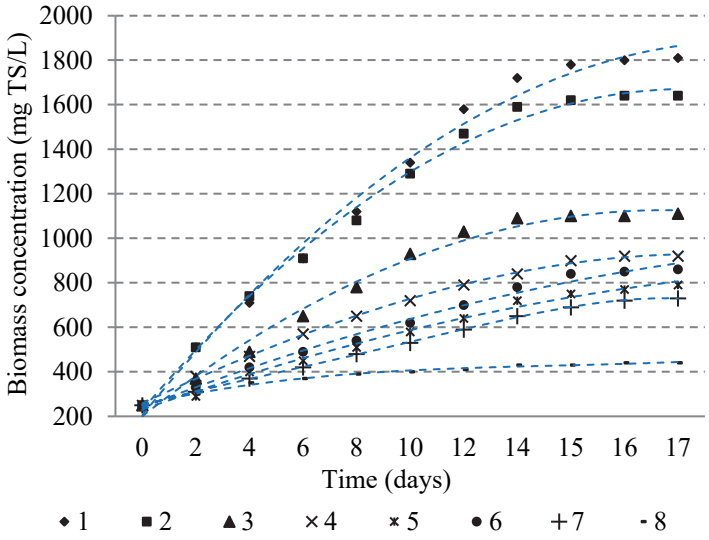

Fig. 3. Changes in biomass concentration of *Chlorella vulgaris* in series 2 depending on the experimental variant

3.2. Organic compounds and nutrient removal from the culture medium

Light spectral quality and intensity significantly influence the microalgal growth, thus it should be considered when choosing a source of light in intensive biomass production and nutrients removal from the culture medium (Wobbe et al. 2016, Baer et al. 2016, Teo et al. 2014). Our studies also confirmed this relationship. The light absorption by microalgae is closely dependent to their chemical composition. The intensity of light should be delivered uniformly to photobioreactors providing a sufficient amount of energy to the cells in the culture (Baer et al. 2016, Teo et al. 2014).

Type of the light source significantly affected the efficiency of COD removal from digestate in series 1 (Fig. 4). The lowest COD concentration at the end of experiment of 903±55 mg O_2/L was noted in variant 1, and the highest of 1610±21 mg O_2/L in variant 8. These values were significantly different ($p = 0.05$) from those obtained in other experimental variants, in which the COD concentration ranged from 1070±230 mg O_2/L to 1273±112 mg O_2/L. In series 2, the concentrations of COD were statistically comparable regardless of the type of light, and they ranged from 519±31 mg O_2/L in variant 1 to 710±91 mg O_2/L in variant 4 (Fig. 4). The lowest BOD_5 concentration in the culture medium was observed in variant 1 and 2, irrespective of its initial concentration in the culture medium (Fig. 5). In other variants, the concentrations of BOD_5 were significantly

higher than those recorded in variant 1 and 2, and amounted to 260±19 mg O_2/L in variant 4 to 330±22 mg O_2/L in variant 5. In series 1, the highest BOD_5 concentration of 699±73 mg O_2/L was found in variant 8 (Fig. 5).

The efficiency of nitrogen removal was directly related to the biomass productivity. The lowest concentrations of N-NH_4 (below 50 mg/L) and TN in the culture medium at the end of experiment were observed in variants 1 and 2, irrespective of the series (Fig. 6-7). In variants 3 to 8 significantly ($p = 0.05$) higher concentrations of N-NH_4 were noted (Fig. 6). In series 1, they ranged from 121±26 to 182±32 mg N-NH_4/L, while in series 2 from 72±5 to 94±21 mg N-NH_4/L (Fig. 6). A similar tendency was observed for TN removal (Fig. 7). In series 1, the lowest TN concentrations of 73±39 mg TN/L in variant 1 and 117±21 mg TN/L in variant 2 were recorded ($p = 0.05$). In other variants, TN concentration was about 250 mg TN/L. In series 2, the lowest TN concentration of 21±9 mg/L was obtained in variant 1, which was significantly higher by 66±19 mg TN/L than noted in series 2. In other variants, the amount of TN was above 100 mg/L (Fig. 7).

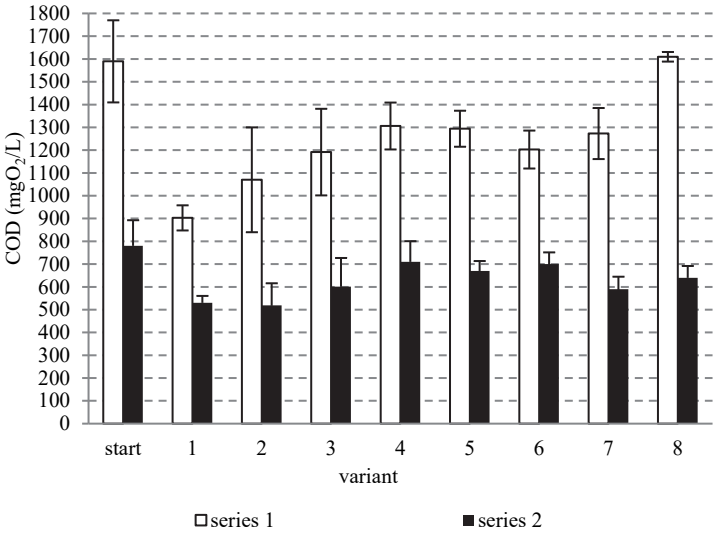

Fig. 4. Final COD concentration in the culture medium depending on the experimental variant

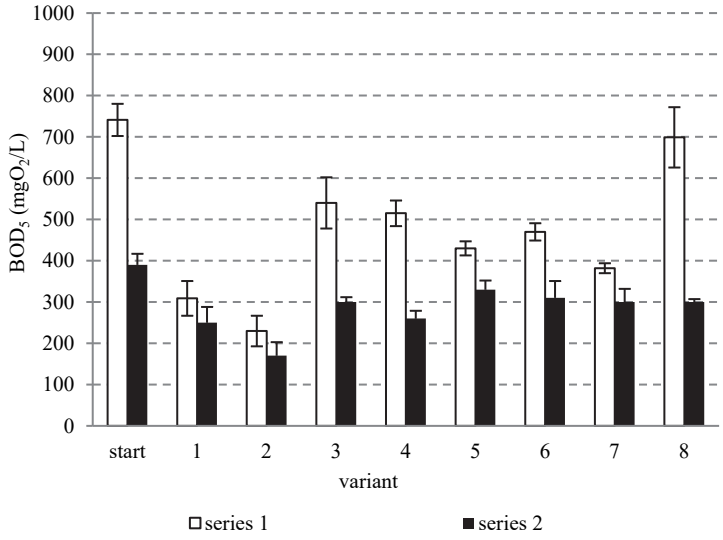

Fig. 5. Final BOD_5 concentration in the culture medium depending on the experimental variant

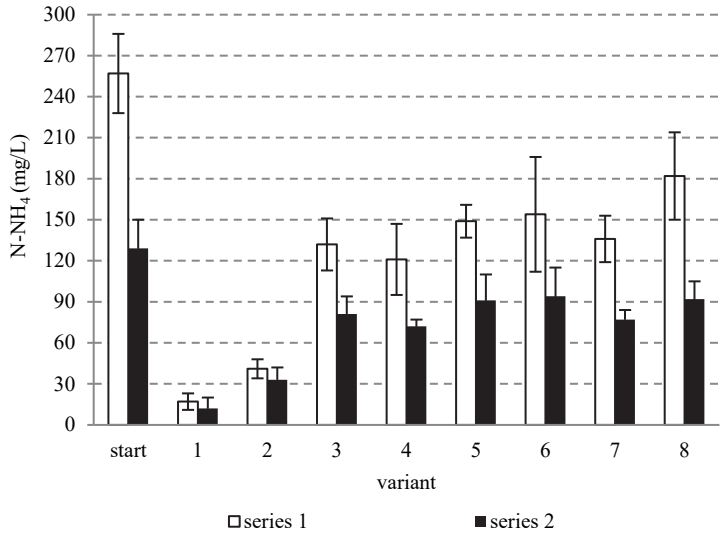

Fig. 6. Final $N\text{-}NH_4$ concentration in the culture medium depending on the experimental variant

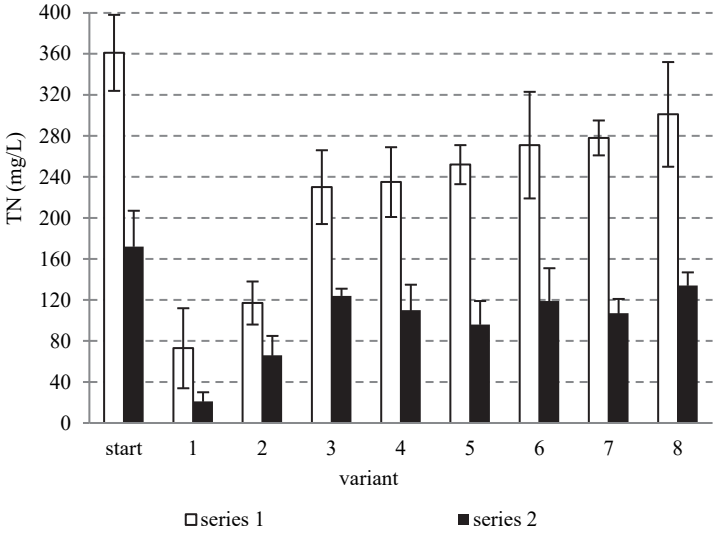

Fig. 7. Final TN concentration in the culture medium depending on the experimental variant

The most important advantages of using artificial light sources are stability and easy controlling, as well as simply incorporation into photobioreactors design. According to the literature, microalgae biomass production efficiency and organic compounds removal from the culture medium were greater with using artificial light sources comparing to sunlight (Wobbe et al. 2016, Baer et al. 2016, Kim et al. 2014, Hashimoto et al. 2015). The presented studies also confirmed this dependence.

Regardless of the experimental series and variant, the effective phosphorus removal from the culture medium was observed. The initial concentrations of phosphates in series 1 and 2 were respectively 12±3.0 mg $P\text{-}PO_4$/L and 7.1±1.6 mg $P\text{-}PO_4$/L, while total phosphorus concentrations were 19±6.0 mg TP/L in series 1 and 10±3.0 mg TP/L in series 2 (Fig. 8-9). In series 1, phosphates concentration at the end of the experiment ranged from 0.9±0.2 mg $P\text{-}PO_4$/L in variant 1 to 2.4±0.3 mg $P\text{-}PO_4$/L in variant 5, while in series 2, it was in the range of 0.7±0.3 mg $P\text{-}PO_4$/L in variant 1 to 1.4±0.3 mg $P\text{-}PO_4$/L in variant 6 and 7 (Fig. 8). The lowest concentration of TP was noted in variant 1, series 1 (2.6±0.7 mg/L) and in variant 1, series 2 (0.8±0.1 mg/L), (Fig. 9).

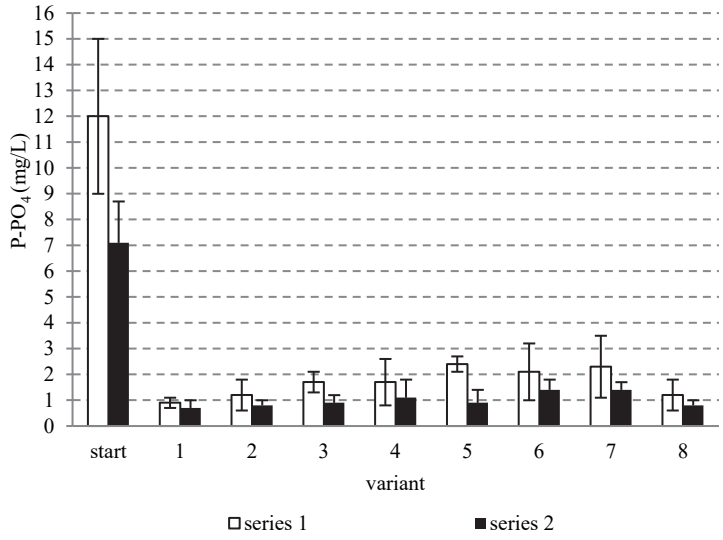

Fig. 8. Final P-PO_4 concentration in the culture medium depending on the experimental variant

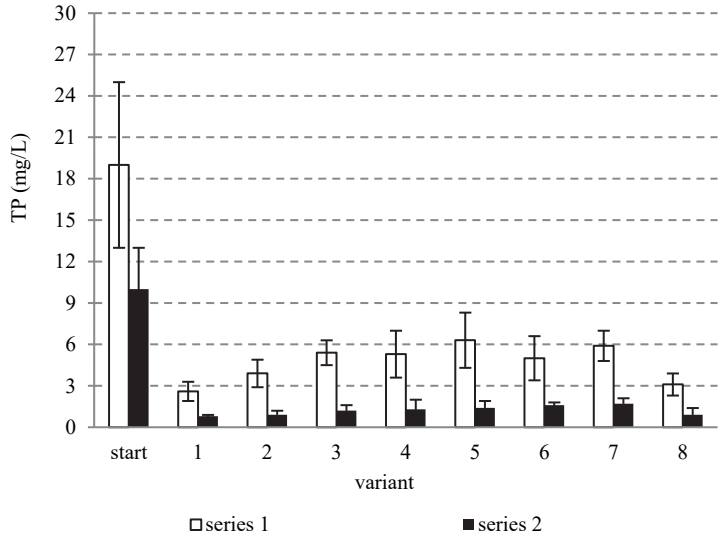

Fig. 9. Final TP concentration in the culture medium depending on the experimental variant

It has been found, that the quantity of direct and diffused light irradiation significantly affect microalgal biomass productivity. However, the light energy limitation is one of the main problems in outdoor photobioreactors exploitation (Pawar 2016, Pruvost 2016). In the intensive microalgal cultivation, maximizing the light absorbed by the cells is a critical factor, because low light conditions may lead to inhibit the biomass growth. The highest biomass productivity can be achieved through continuous algal cultivation in photobioreactors exposed to the constant artificial lighting (Pruvost 2016, Vitova 2015). However, the continuous supplying of light is energy intensive (Wobbe et al. 2016, Kim et al. 2014, Wang et al 2014). Light emitting diodes (LEDs) have been widely used as an alternative to conventional fluorescent and halogen lamps (Liu et al.2017, Wobbe et al. 2016, Singh et al. 2016, Baer et al. 2016) and they are characterized by narrow-band emission spectrum (Vadiveloo et al. 2015, Baer et al. 2016). It has been proved that microalgae cultivated under blue or red monochromatic light enhanced their growth rates as compared to cultures grown under multi-chromatic white light (Liu et al. 2017, Vadiveloo et al. 2015, Baer et al. 2016).

The multiple regression models were developed to indicate variables statistically significantly affecting the microalgae biomass production, and analysis showed that they are COD, N-NH_4 and P-PO_4 concentrations. The estimated values of biomass productivity in the equations in relation to the results obtained in the experimental works are very high, which indicates the correctness of the assumptions made and the practical value of the optimization procedure. The regression formulated models equations for the estimation of biomass production, with their determination coefficients and standard errors are presented in Table 4.

Table 4. Regression equation for the estimation of *Chlorella vulgaris* biomass concentration (BC) with determination coefficient (R^2) and standard error (SE)

Variant	Formula	R^2	SE
1	$BC = 1{,}042COD - 13{,}241N - NH_4 + 100{,}218P - PO_4 + 1994{,}013$	0,9634	28,744
2	$BC = 1{,}186COD - 15{,}080N - NH_4 + 114{,}137P - PO_4 + 1849{,}570$	0,9377	24,989
3	$BC = 0{,}550COD - 6{,}988N - NH_4 + 52{,}893P - PO_4 + 1207{,}118$	0,9155	19,555
4	$BC = 0{,}2604COD - 3{,}3103N - NH_4 + 25{,}0545P - PO_4 + 966{,}0033$	0,9267	16,792

Table 4. cont.

Variant	Formula	R^2	SE
5	$BC = 0{,}0579COD - 0{,}7356N - NH_4 + 5{,}5677P - PO_4 + 800{,}2230$	0,9274	12,772
6	$BC = 0{,}0579COD - 0{,}7356N - NH_4 + 5{,}5677P - PO_4 + 870{,}2230$	0,9441	15,716
7	$BC = 0{,}1736COD - 2{,}2068N - NH_4 + 16{,}7030P - PO_4 + 760{,}6689$	0,9252	10,593
8	$BC = 0{,}0810COD - 1{,}0299N - NH_4 + 7{,}7947P - PO_4 + 454{,}3121$	0,9389	7,554

BC – biomass concentration [mg/L]
COD – initial COD concentration in the culture medium [mg O_2/L]
$N - NH_4$ – initial $N - NH_4$ concentration in the culture medium [mg/L]
$P - PO_4$ – initial $P - PO_4$ concentration in the culture medium [mg/L]

4. Conclusions

The studies proved that the light source significantly enhanced the efficiency of *Chlorella vulgaris* growth on anaerobic digestate. The highest biomass production was observed in variants with the fluorescent tube-warm light (color temperature 3000K) and the LED-warm light with 2 local maximum at 450 nm and 580 nm (color temperature 6500K). In variants with the pressure sodium lamp with the essential spectrum 570-620 nm (color temperature 2700K), the production of microalgae biomass was the lowest.

The efficiency of organic compounds expressed as COD and BOD_5 removal, as well as $N\text{-}NH_4$ and TN removal was dependent on the final concentration of microalgae biomass in the culture medium. In variants with the highest *Chlorella vulgaris* biomass production, the lowest concentrations of these indicators in the culture medium were observed at the end of the experiment. In turn, phosphorus compounds were removed effectively irrespective of the light source used and the concentration of microalgae biomass.

It was found, that anaerobic digestate characteristics influenced the efficiency of *Chlorella vulgaris* growth. Higher amount of digestate in the culture medium (20% by volume) significantly reduced biomass productivity. It was associated with increasing in color intensity, turbidity and suspension solids concentration in the culture medium, which reduced light penetration. The multiple regression analysis showed that the initial concentrations of COD, $N\text{-}NH_4$ and $P\text{-}PO_4$ statistically significantly affected the biomass production efficiency.

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Abstract

Many studies have proven that the type of light source and the lighting regime have significantly influenced the efficiency of microalgae biomass production and the taxonomic composition of the cultivated biomass, as well as its chemical composition. The aim of the study was to determine the influence of the light source on the productivity of *Chlorella vulgaris* biomass cultivating on anaerobic digestate. The criterion for dividing experiment into variants was the light source used.

It was found that the light source significantly influenced the *Chlorella vulgaris* growth. The highest biomass production was observed in variants with the fluorescent tube-warm light (color temperature 3000K) and the LED-warm light with 2 local maximum at 450 nm and 580 nm (color temperature 6500K). In variants with the pressure sodium lamp with the essential spectrum 570-620 nm (color temperature 2700K), the production of microalgae biomass was the lowest.

The removal efficiency of organic compounds expressed as COD and BOD_5 as well as ammonium and total nitrogen were dependent on the final concentration of biomass in the culture medium. In variants with the highest biomass production, the lowest concentrations of these indicators in the culture medium were observed. Phosphorus compounds were removed effectively from the culture medium regardless of the light source and the concentration of microalgae biomass.

Keywords:
microalgae, light source, anaerobic digestate, biomass production

Wpływ źródła światła na wzrost biomasy *Chlorella vulgaris* w medium hodowlanym opartym na wykorzystaniu odcieków pofermentacyjnych

Streszczenie

W wielu badaniach udowodniono, iż zastosowany rodzaj źródła światła i sposób oświetlenia ma wpływ na wydajność produkcji mikroglonów oraz na kształtowanie się składu taksonomicznego populacji, skład chemiczny i właściwości uzyskiwanej biomasy. Celem badań było określenie wpływu rodzaju stosowanego światła na efektywność przyrostu biomasy mikroglonów z gatunku *Chlorella vulgaris* w medium hodowlanym opartym na wykorzystaniu odcieków pofermentacyjnych. Kryterium podziału prac badawczych na warianty stanowiło zastosowane źródło światła.

Przeprowadzone prace badawcze udowodniły istotny wpływ stosowanego źródła światła na proces przyrostu biomasy *Chlorella vulgaris*. Najwyższą efektywność przyrostu biomasy *Chlorella vulgaris* obserwowano w wariantach, w których źródło światła stanowiły świetlówki z ciepłym światłem (3000K) oraz diody LED (ciepłe światło o długości fali 450 nm i 580 nm, 6500K). Najniższe efekty technologiczne związane z przyrostem biomasy mikroglonów obserwowano w wariancie, w którym fotobioreaktory oświetlane były wysokoprężną lampą sodową o długości fali 570-620 nm (2700K).

Efektywność usuwania związków organicznych wyrażona wskaźnikami ChZT i BZT_5 oraz azotu amonowego i całkowitego były zależne od końcowego stężenia biomasy mikroglonów w medium hodowlanym. W wariantach, gdzie populacja *Chlorella vulgaris* przyrastała najwydajniej obserwowano najniższe stężenia tych wskaźników w medium hodowlanym na zakończenie cyklu produkcyjnego. Związki fosforu usuwane były skutecznie niezależnie od stosowanego źródła światła oraz koncentracji biomasy mikroglonów w układzie technologicznym.

Słowa kluczowe:
mikroglony, źródło światła, odcieki pofermentacyjne, produkcja biomasy