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USE OF THE WASTE FRACTION FROM BIOETHANOL PRODUCTION FROM SUGAR BEETS FOR THE PRODUCTION OF CHLORELLA VULGARIS SPECIES MICROALGAE BIOMASS

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ABSTRACT: The objective of this study was to determine the possibility of using a liquid waste fraction generated in the process of bioethanol production from sugar beets for biomass production from Chlorella vulgaris microalgae. The process of microalgae culture was conducted in three variants differing in the volume of the liquid phase fed to the technological system. The highest technological effects in biomass growth were noted in the experimental variants in which the distillery stillage constituted 5% and 7% of culture medium volume. Concentration of biomass achieved in these variants reached 1416±45.30 mgo.d.m./dm3 and 1458.3±54.52 $mg_{o.d.m.}/dm^3$, respectively. Increasing the content of the liquid waste fraction in the medium to 10% caused significant growth inhibition of biomass of algae from the species Chlorella vulraris. The use of such a culture medium for microalgae biomass production requires its pre-treatment to remove organic compounds, color and turbidity.

KEY WORDS: microalgae, bioethanol production, photobioreactors, *Chlorella vulgaris*

Introduction

Nowadays, the development and wide-scale implementation of clean, effective, and renewable technologies for energy production become a challenge to scientists and a priority to operators and administrators of energetic systems. It is commonly believed that this goal may in part be achieved through stimulating the development of unconventional methods for energy production based on the use of biomass of various characteristics and origin (Goyal et al., 2008; Börjesson, Berglund, 2006). This concept has however been undermined by some analyses. For example, Fargione et al. (2008) and Searchinger et al. (2008) demonstrated that irrational management of resources of typical energy crops may in fact lead to a negative balance of the volume of greenhouse gases emitted to the atmosphere. In addition, intensive exploitation of arable lands for the culture of crops intended for biofules production is suggested to have adverse impacts on the global food supply and on a significant increase in food prices (Johansson, Azar, 2007).

Therefore, a real need emerges for alternative sources of biomass whose use for energetic purposes would be justified considering both economic and ecological concerns. Taking into account their very high photosynthetic effectiveness, high rate of biomass growth, resistance to various contaminants and possibility of management of area which cannot be used for any other purposes, algae seem to be a perfect alternative to typical energy crops (Shen et al., 2009; Smith et al., 2010).

One of the key elements determining cost-effectiveness of algae biomass production is the use of an inexpensive and available source of nutrients. Many studies conducted so far have investigated possibilities of using wastewater with high concentrations of nitrogen and phosphorus for this purpose (Wang et al., 2008; Li et al., 2008). Dynamic development of bioenergetic systems based on methane fermentation processes in many cases poses difficulties in the management of post-fermentation sludge. After dehydration, the solid phase is applied as a fertilizer or used in co-combustion processes (Holm-Nielsen et al., 2009). In turn, neutralization of the liquid phase is difficult owing to its considerable volume and high concentration of contaminants. The same case is with the currently popular systems for wastewater treatment under anaerobic conditions which allow for efficient biodegradation of organic compounds but not for the removal of biogenes. This fact excludes the possibility of direct discharge of this wastewater to the natural environment (Rajeshwari et al., 2000).

Considering the characteristics of effluents from bioethanol production and algae demands for nutrients, its seems that a substrate of this type may

represent a source of biogenes and microelements. Algae use may affect intensive growth of biomass and allow for simultaneous neutralization of contaminants. Mùnoz et al. (2004) demonstrated that during the photosynthetic process, algae released from 1.50 to 1.92 kg $O_2 \cdot kg^{-1}$ of the produced biomass and that the rate of oxidation achieved during organic contaminants degradation ranged from 0.48 to 1.85 kg $O_2 \cdot m^{-3} \cdot d^{-1}$. Research works conducted so far have proved that a high concentration of CO_2 in the effluents intensifies algae growth, which has a direct effect upon the effectiveness of contaminants degradation (Lundquist, 2008). In systems based on saline water, the use of wastewater or effluents enables balancing the molecular ratio of carbon, nitrogen and phosphorus (C:N:P = 106:16:1), the so-called Redfield's ratio (Lundquist, 2008).

The objective of this study was to determine the possibility of using a liquid waste fraction generated in the process of bioethanol production from sugar beets for biomass production from *Chlorella vulgaris* microalgae.

Research methods

The liquid waste fraction of distillery stillage from the process of alcoholic fermentation of sugar beets served as the culture medium in the experiment. Mean concentrations of the analyzed components in the material after centrifugation (Rotina 380, 3 min., 9000 rpm) and filtration through a blotted paper filter were presented in table 1.

Table 1. Characteristics of the liquid phase of distillery stillage

Parameter	Unit	Value
COD	mgO_2/dm^3	7800±270
$N_{tot.}$	mg/dm^3	257±24.0
NO_3^-	mg/dm^3	130.33±3.06
NO_3^--N	mg/dm^3	29.37±0.65
NO_2^-	mg/dm^3	0.23±0.037
NO_2^--N	mg/dm^3	0.07±0.012
$P_{tot.}$	mg/dm^3	208±2.0
PO_4^{3-}	mg/dm^3	147.33±5.51

Source: author's own work.

Characteristics of the experimental material excluded the possibility of *Chlorella vulgaris* biomass culture with a crude substrate. Therefore the centrifuged fraction had to be diluted to increase culture medium transparency and to decrease concentrations of organic compounds. The process of microalgae culture was conducted in three variants differing in the volume of the liquid phase fed to the technological system: variant 1 – control, in which the culture medium was prepared based on deionized water and pure chemical reagents (table 2); variant 2 – in which the load of the liquid waste fraction fed to the exploited photobioreactors reached 5%, variant 3 – in which the liquid waste fraction load reached 7% load, and variant 4 – in which the liquid waste fraction load reached 10% of the total volume of the culture medium. Increased percentage of the effluent in the culture medium caused complete growth inhibition of microalgae from the genus *Chlorella vulgaris*. Considering the necessity of using a high dilution rate of the tested liquid waste fraction, the concentration of biogens potentially assimilable by the microalgae biomass in the culture medium was too low. It was , therefore, necessary to introduce external sources of nitrogen compounds (table 2).

Table 2. Composition of the synthetic medium used to culture *Chlorella vulgaris*

Component	Unit	Value
$NaNO_3$	g/dm^3	25.0
$CaCl_2 \cdot 2H_2O$	g/dm^3	2.5
$MgSO_4 \cdot 7\ H_2O$	g/dm^3	7.5
$K_2HPO_4 \cdot 3\ H_2O$	g/dm^3	7.5
KH_2PO_4	g/dm^3	17.5
NaCl	g/dm^3	2.5
VB12	mL/dm^3	1.0
VB1	mL/dm^3	1.0
Microelements	mL/dm^3	6.0
Na_2EDTA	mg/dm^3	0.75
$FeCl_3 \cdot 6\ H_2O$	mg/dm^3	97.0
$MnCl_2 \cdot 4\ H_2O$	mg/dm^3	41.0
$ZnCl_2$	mg/dm^3	5.0
$CoCl_2 \cdot 6\ H_2O$	mg/dm^3	2.0
$NaMoO_4 \cdot 2\ H_2O$	mg/dm^3	4.0

Source: author's own work.

Biomass of microalgae of the *Chlorella vulgaris* species was used in the study. The tested culture of algae originated from the Culture Collection of Baltic Algae (CCBA) deposited at the Institute of Oceanography of the University of Gdańsk. These microalgae are widely used in multiple research areas including both pharmacology, dietetics and cosmetology but also in energetic technologies as potential sources of biomass. The culture used in our study was characterized by high resistance to varying environmental conditions and applicability for the culture in media with various physicochemical characteristics. The initial concentration of microalgae in photobioreactors was ca. 50 $mg_{o.d.m.}/dm^3$.

Chlorella vulgaris biomass was grown in vertical tubular reactors with active volume of 2.5 dm^3 (figure 1), under conditions of 24 lighting (intensity of light reaching the photobioreactor's surface was ca. 5.0 klux). The proper process of algae biomass proliferation was ensured by providing indispensable technological conditions concerning culture medium composition and temperature conditions (23°C). Contents of columns were continuously aerated with compressed air delivered from the reactors' bottoms with Mistral 200 peristaltic pumps having the efficiency of 200 dm^3/h. This technological treatment allowed providing carbon dioxide to the system and effective stirring of algae cultures.

Technical parameters of a single experimental installation were as follows:

Total height	H_{tot} = 72 cm
Active height	H_{act} = 66 cm
Internal diameter	D_{int} = 7 cm
Active volume of tank	V_{act} = 2.4 dm^3

Taxonomic analysis of the cultured biomass of algae was conducted under microscope magnifications of: 1.25×10×40 or 1.25×10×10, using an MF 346 biological microscope with Optech 2MP camera and additionally using a BBE Alage OnLine Analyser by Moldaenke. Microalgae biomass used as the inoculum of the exploited bioreactors was subjected to qualitative analyses which included determinations of contents of: dry matter (d.m.), organic dry matter (o.d.m.), and mineral dry matter (m.d.m.), with the gravimetric method acc. to the Polish Standard (PN–75/C–04616.01). Quantification of individual components in the culture medium and characteristics of the tested liquid waste fraction were carried out in samples after filtration using Hach Lange cuvette tests and a UV/VIS DR 5000 spectrophotometer. Light intensity was measured using an HI 97500 luxometer by HANNA.

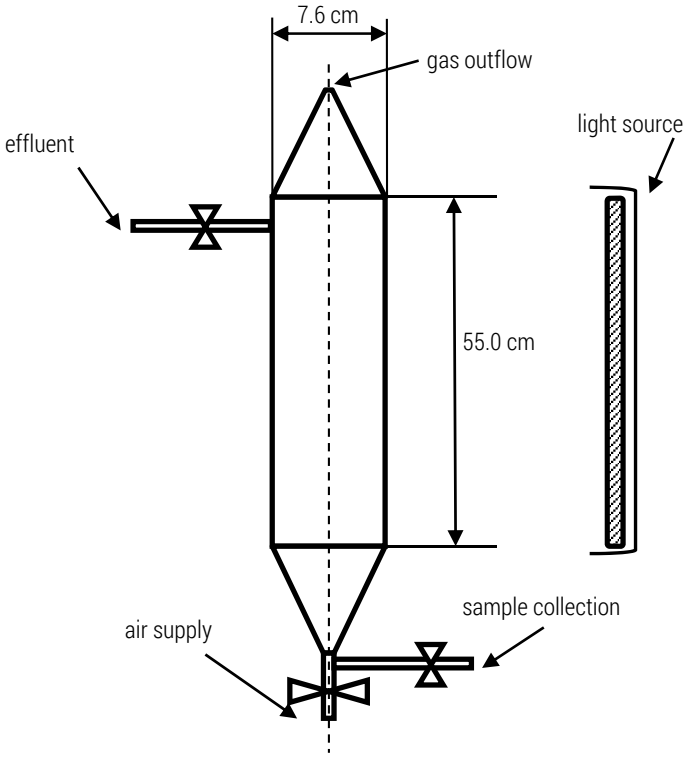

Figure 1. Scheme of a photobioreactor used in the study
Source: author's own work.

Statistical analysis of the results and computation of determination coefficients R^2 were made in STATISTICA package 10.0 PL. All physicochemical analyses were carried out in three replications. The hypothesis on distribution of each analyzed variable was verified with the Shapiro-Wilk W test. One-way analysis of variance (ANOVA) was conducted to determine the significance of differences between mean values. Homogeneity of variance in groups was checked with Levene's test, whereas HSD Tukey's test was used to determine the significance of differences between the analyzed variables. Differences were found significant at $p = 0.05$.

Results

The study aimed to determine the feasibility of using a liquid waste fraction from alcoholic fermentation of sugar beets in the process of culture and proliferation of biomass of *Chlorella vulgaris* microalgae.

The rate of biomass growth in photobioreactors and effectiveness of biogens consumption by the algae were monitored throughout the culture period. The highest technological effects were determined in the control variant in which microalgae were cultured on the medium prepared from deionized water and chemical reagents. The final concentration of microorganisms was 2527.33±170.01 $mg_{o.d.m.}/dm^3$ (figure 2), while the effectiveness of nitrogen and phosphorus compounds removal from the culture medium accounted for 76% (figures 3 and 4), and the coefficient of biomass growth for 247.40 $mg/dm^3 \cdot d$ (table 3).

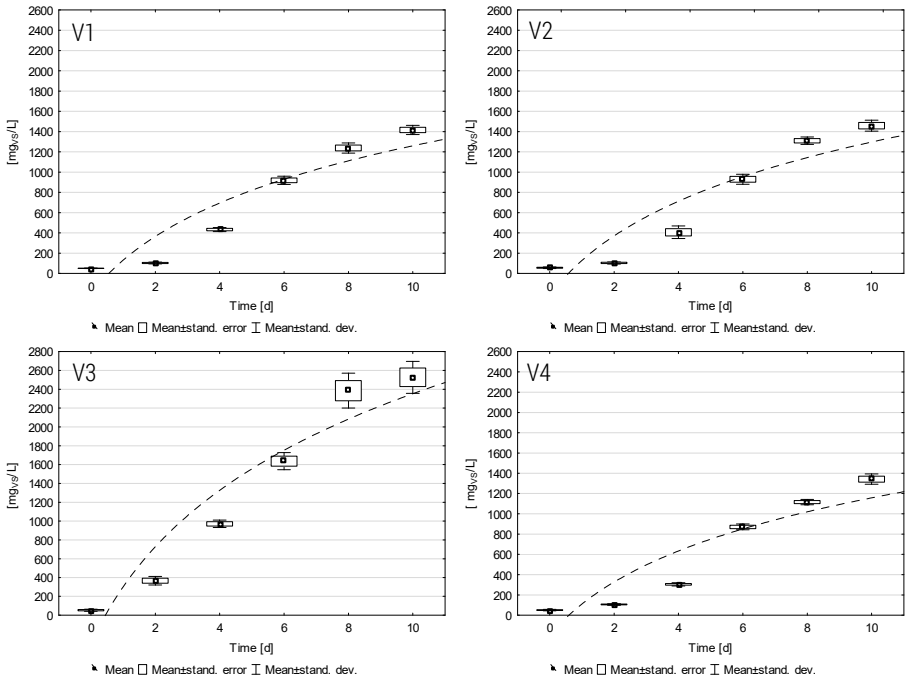

Figure 2. Changes in organic dry matter concentration in the culture medium in the subsequent experimental variants

Source: author's own work.

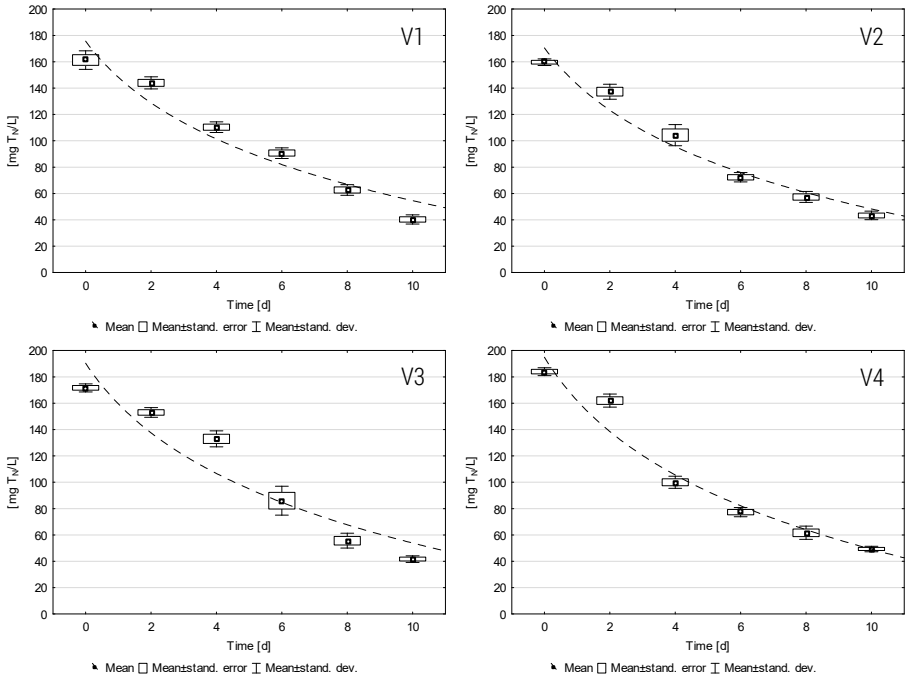

Figure 3. Changes in total nitrogen concentration in the culture medium in the subsequent experimental variants

Source: author's own work.

In variants established to test the liquid phase of distillery stillage in the process of microalgae biomass proliferation, the concentrations of produced biomass were significantly lower. In variant 2, the concentration of *Chlorella vulgaris* was at 1416 ±45.30 $mg_{o.d.m.}/dm^3$ and the effectiveness of nitrogen compounds removal reached 73% (figure 2 and 3). In variant 3 the respective value was at 1458.3 ±54.52 $mg_{o.d.m.}/dm^3$ (figure 2). Similar was also the effectiveness of nitrogen compounds consumption by microalgae which reached on average 76% at the end of the culture (figure 3). Variant 2 and 3 were characterized by a high effectiveness of phosphorus compounds removal from the culture medium, i.e. 91%-92% for each variant (figure 4). The rate of biomass growth was 136.50 $mg/dm^3 \cdot d$ in variant 2 and 140.20 $mg/dm^3 \cdot d$ in variant 3 (table 3). Poorer technological effects were observed in variant 4. The concentration of produced biomass was at 1343.0 ±50.59 $mg_{o.d.m.}/dm^3$ and biomass growth coefficient at 129.20 $mg/dm^3 \cdot d$ (figure 2, table 3). Effectiveness of denitrification reached 73% (figure 3). In turn, effectiveness of phosphorus compounds removal reached barely 63% and their concentration in the culture medium after completed culture accounted for 6.87 ±0.21 mg $P_{tot.}/dm^3$ (figure 4).

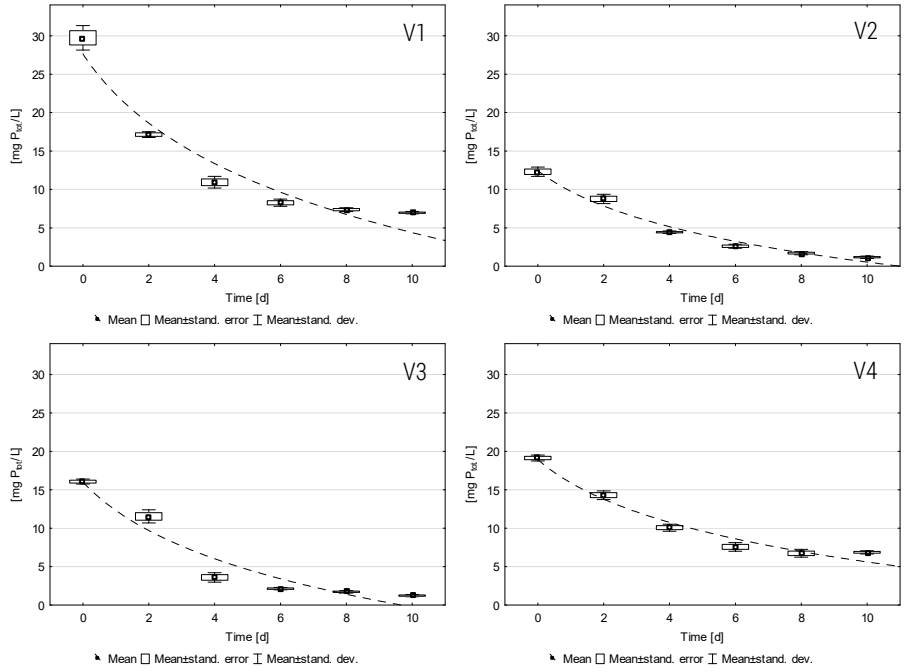

Figure 4. Changes in total phosphorus concentration in the culture medium in the subsequent experimental variants

Source: author's own work.

Discussion

Many literature reports suggest the feasibility of using liquid wastes of various type in the process of microalgae biomass production (Mùnoz, Guieysse, 2006). Their use as a culture medium may directly contribute to reduction of costs incurred on the supply of water and nutrient substances indispensable for the effective growth of microalgae biomass (Wang et al., 2010). Experimental works conducted so far have proved that high concentration of CO_2 in wastewater and effluents intensifies algae biomass growth, which has a direct impact on the effectiveness of contaminants degradation (Lundquist, 2008). In this study, we analyzed the possibility of using a liquid waste phase from bioethanol production from sugar beets in the process of microalgae biomass production.

In the case of using waste substrates, of outmost significance is the choice of an appropriate species characterized by relatively high resistance to substances likely to occur in the applied wastes. It was proved that such requirements were met by the species of *Chlorella sp.* and *Scenedesmus sp*. genera.

Algae from the genus *Chlorella sp.* are resistant to effects of heavy metals, owing to which they may be used in treatment processes of industrial wastewater (Mùnoz et al., 2003). Literature works provides examples of using algae from the genus *Chlorella sp.* in biodegradation of so noxious wastewater like: landfill leachate (Lin et al., 2007), wastewater from the timber and paper industry (Yewalkar et al., 2007), from textile industry (Acuner, Dilek, 2004), from phenolic industry (Essam et al., 2007) or from the production of ethanol and citric acid (Valderramaa et al., 2002). *Chlorella sp.* is used for the treatment of wastewater containing organic compounds, e.g. effluents from fermentation tanks (Ogbonna et al., 2000), or from dairy processing plants (Bernal et al., 2008). Other investigations proved that *Chlorella pyrenoidosa* may be cultured based on effluent from a fermentation tank and contribute to effective removal of organic contaminants and biogenes. In this study, microalgae biomass concentration in the reactor reached 1.25 g d.m./dm^3. It was also found that the effectiveness of contaminants removal by *Chlorella pyrenoidosa* reached 78.76% for nitrogen, 94.78% for phosphorus and 98.34% for COD since day 6 to day 8 of the culture (Su et al., 2012). Considering the aforementioned results, we tested biomass of *Chlorella vulgaris* in our study.

The phenomenon of *Chlorella sp.* growth inhibition in the subsequent experimental variants could be due to a high concentration of organic compounds in the culture medium. Wang et al. (2010) analyzed the possibility of using effluent from bovine manure fermentation as a source of nutrients for *Chlorella sp.* They demonstrated that the effectiveness of biomass production, content of lipids in algae cells and effectiveness of contaminants removal were correlated with the dilution rate of the post-fermentation effluents. Other investigations proved the initial concentration of COD originating from wastewater treatment plant in the culture medium to be the factor determining growth rate of biomass of microalgae from the genus *Scenedesmus sp*. (Uggetti et al., 2014). High concentrations of organic compounds increase the growth of bacterial biomass competitive to microalgae (Szwaja et al., 2016). Bacteria use nutrients from the culture medium, which additionally reduces the effectiveness of microalgae biomass growth.

Another reason may be restricted access of light resulting from turbidity and coloration of the liquid waste fraction generated in the process of bioethanol production from sugar beets. Feeding high doses of this substrate to photobioreactors had a direct effect on reduced light permeability of the medium.

Table 3. Analysis of the culture medium and algae biomass

Variant	Day of culture	COD	T_N	$P_{tot.}$	NO^{3-}	NO^{3-}-N	NO^{2-}	NO^{2-}-N	PO_4^{3-}	d.m.	m.d.m.	o.d.m.	Biomass growth coefficient
		mg/dm³	mg/dm³	mg/dm³	mg/dm³	mg/dm³	mg/dm³	mg/dm³	mg/dm³	mg/dm³	mg/dm³	mg/dm³	mg/dm³·d
V1	0	41.1±2.74	161.33±7.02	29.13±1.60	77.2±5.5	14.4±0.7	0.038±0.12	0.011±0.01	101±4.38	265±52.12	205±25.41	53.33±11.24	247.40
	2	57.8±4.62	144.0±4.58	17.13±0.38	649±12.1	147±12.4	0.791±0.13	0.241±0.02	41.4±1.29	684±104.89	284±12.35	366.67±45.54	
	4	36.7±7.52	110.0±4.04	10.93±0.75	382±11.0	86.4±5.4..	1.37±0.22	0.417±0.09	32.1±2.47	1298±214.84	275±12.47	971.0±39.51	
	6	74.5±6.94	90.67±4.04	8.27±0.47	648±14.8	146±12.5	1.87±0.20	0.569±0.15	23.0±3.21	1884±389.32	220±24.62	1636.33±91.05	
	8	81.2±8.98	62.67±4.05	7.37±0.25	394±14.3	112±8.62	2.57±0.24	0.846±0.18	24.6±2.15	2699±264.52	275±24.34	2386.0±185.98	
	10	83.8±5.84	40.33±3.51	6.97±0.15	368±9.5	83.1±9.5	3.88±0.35	1.18±0.28	25.3±1.98	2857±285.41	290±13.25	2527.33±170.0	
V2	0	2212±62.0	159.67±2.52	12.3±0.61	85.14±10.2	13.56±2.6	0.05±0.005	1.21±0.06	37.4±2.5	252±47.34	199±24.6	51±2.0	136.50
	2	1238±24.0	137.33±5.68	8.77±0.60	274.25±22.5	121.3±15.4	0.284±0.006	1.78±0.07	9.6±3.1	322±38.41	215±41.3	104±8.89	
	4	353±14.6	104.33±8.02	4.43±0.15	155±14.2	35.9±8.6	6.79±0.6	2.07±0.9	16.5±2.5	723±62.47	287±28.6	433±19.67	
	6	553±26.3	72.33±3.51	2.6±0.26	47.1±8.6	10.6±3.6	20.2±2.4	6.15±1.4	9.32±1.9	1564±51.4	607±15.7	919±40.73	
	8	608±19.4	57.33±4.16	1.7±0.2	81.4±4.9	18.4±2.4	21.6±6.2	6.59±1.6	8.5±0.7	1897±47.2	679±61.3	1238±49.16	
	10	616±31.0	43.33±3.21	1.17±0.15	92.9±12.4	21.0±3.7	22.1±3.7	6.74±2.0	22.1±4.6	2112±28.4	702±22.2	1416±45.30	

V3	0	4330±74.6	171.67±3.05	16.07±0.32	82.71±124	17.22±7.6	0.07±0.06	0.83±0.07	50.21±5.8	212±12.4	154±14.9	56±8.19	140.2
	2	2870±51.2	153.0±3.61	11.53±0.85	185.43±14.6	136.24±12.6	0.412±0.04	0.94±0.04	12.47±0.8	347±33.1	244±21.5	104±10.54	
	4	611±24.6	133.0±6.08	3.6±0.62	29.5±8.4	6.66±1.7	3.85±0.6	1.17±0.06	16.32±2.4	887±62.4	420±23.4	407±62.65	
	6	803±18.6	86.0±11.0	2.13±0.15	3.31±0.5	0.75±0.05	0.31±0.01	0.01±0.01	19.24±2.6	1578±42.7	626±24.7	929±49.44	
	8	882±24.6	55.67±5.68	1.73±0.15	4.0±1.3	0.90±0.04	0.56±0.08	0.17±0.01	13.62±2.9	2010±24.6	695±33.5	1310±36.76	
	10	889±19.7	41.67±2.52	1.23±0.15	4.30±0.98	0.97±0.07	0.45±0.05	0.14±0.04	24.4±4.7	2183±44.3	723±41.3	1458±54.52	
V4	0	5755±61.4	184.0±3.0	19.13±0.38	96.1±18.4	22.44±4.6	0.09±0.001	1.16±0.01	63.14±10.6	210±14.7	158±14.6	51±7.02	129.2
	2	4330±34.9	162.0±5.0	14.3±0.56	214.72±12.4	151.18±9.1	0.617±0.07	1.21±0.02	16.32±5.6	319±21.8	221±21.4	106±8.0	
	4	1215±41.3	100.0±4.58	10.07±0.45	38.15±6.4	36.21±6.3	5.84±0.8	1.97±0.01	19.4±4.6	700±19.7	385±14.8	304±15.14	
	6	1281±21.8	77.33±3.51	7.57±0.57	7.21±2.3	15.84±4.5	23.14±2.4	4.18±0.4	18.3±3.1	1438±51.4	591±24.5	871±29.41	
	8	1312±33.9	61.67±5.03	6.73±0.50	8.14±2.7	16.21±3.7	24.63±6.1	6.51±1.3	15.6±3.4	1805±37.4	684±46.1	1115±26.41	
	10	1330±14.52	49.33±2.08	6.87±0.21	8.05±1.98	24.18±6.8	22.51±4.7	7.44±0.9	25.4±2.8	2095±61.4	775±40.8	1343±50.59	

Source: author's own work.

Conclusions

The conducted study demonstrated a very limited possibility of using the tested liquid phase in the proliferation process of *Chlorella vulgaris* microalgae owing to a high concentration of organic compounds, low transparency of the medium, and low concentrations of nitrogen and phosphorus. The highest technological effects in biomass growth were noted in the experimental variants in which the distillery stillage constituted 5% and 7% of culture medium volume. Concentration of biomass achieved in these variants reached 1416±45.30 $mg_{o.d.m.}/dm^3$ and 1458.3±54.52 $mg_{o.d.m.}/dm^3$, respectively. Increasing the content of the liquid waste fraction in the medium to 10% caused significant growth inhibition of biomass of algae from the species *Chlorella vulraris*. The use of such a culture medium for microalgae biomass production requires its pre-treatment to remove organic compounds, color and turbidity.

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The contribution of the authors

Marcin Dębowski – development of research methodology, supervision of experimental work, analyses and presentation of results – 25%
Marcin Zieliński – literature review, construction and operation of photobioreactors – 25%
Magda Dudek – experimental works and analyses of results – 25%
Paulina Rusanowska – operation of photobioreactors, draw conclusions and summary – 25%

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