Effectiveness of *Scenedesmus sp.* Biomass Grow and Nutrients Removal from Liquid Phase of Digestates

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Abstract: One of the most important factors in determining the profitable production of microalgae biomass is the use of a cost effective growth medium that is rich in nutrients. The objective of the study was to determine the possibility of using digestates from anaerobic digestion of different feedstock mixtures as the media for *Scenedesmus sp.* cultivation. A different liquid digestate composition was obtained in terms of organic compounds, phosphorus, and nitrogen concentrations, depending on the substrates used in the anaerobic digestion. It was found that the highest biomass production was obtained when using digestate from anaerobic digestion of the feedstock mainly composed of microalgae biomass, which was characterized by low organic compounds concentration. In this case, the average biomass concentration reached 2382 mg total solids/L. A lower *Scenedesmus sp.* biomass yield was obtained using digestate from anaerobic digester processing feedstock based on maize silage and cattle menure. In the variants of the study, it was also found that the increase in the initial concentration of ammonia nitrogen in the growth medium up to 160 mg/L significantly reduced the growth of *Scenedesmus sp.* The results indicated the possibility of a high ammonia nitrogen and orthophosphates removal from anaerobic digestates by *Scenedesmus sp.* microalgae. Phosphorus concentration in the cultivation medium is a limiting factor for the growth of *Scenedesmus sp.*, thus phosphorus supplementation should be considered when using liquid digestate as the culture medium. The optimization model indicated that the volume of liquid digestate that was used for preparing the cultivation medium, the initial concentration of organic compounds, and the initial concentration of ammonia nitrogen had a significant impact on the production of *Scenedesmus sp.* biomass.

Keywords: microalgae; liquid digestate; anaerobic digestion; photobioreactor

1. Introduction

Nowadays, the biomass is recognized to have great potential to replace a large fraction of fossil resources due to its high carbon content. In addition, biomass resources are easily locally available, thus their use as an alternative to fossil resources might contribute to reduce dependence on imported fossil fuels in many countries. The biorefinery concept is based on a wide range of technologies that are able to separate any type of biomass resources (wood, grasses, corn, etc.) into particles, such as carbohydrates, proteins, and triglycerides, which can be converted into valuable products, biofuels, and chemicals. In connection with the above, prokaryotic microalgae (e.g., cyanobacteria

Chloroxybacteria) and eukaryotic microalgae (e.g., green algae *Chlorophyta*), red algae (*Rhodophyta*), and diatoms (*Bacillariophyta*) are the main photosynthetic organisms that can be biorefinery feedstock. They can be cultivated in different media (e.g., freshwater, seawater, and wastewater), and they are able to adapt to the variety of environmental conditions (pH, temperature, and light availability).

The scientific literature data shows the details of the operating data, as well as the technologies of nutrient removal and biofuels production using microalgae [1,2]. It has been shown that microalgae biomass is one of the most effective and environmentally friendly source for renewable energy production. Microalgae biomass is now emerging as a potential source for the production of many biorefinery products (biooil) and bioenergy (biomethane, biohydrogen), whose production will contribute to reduced greenhouse gas emissions [3,4].

The profitability of the microalgae biomass production is mostly influenced by the cost of the growth medium used. There are many literature reports that are focused on the use of various wastewaters rich in nitrogen and phosphorus as the cultivation media for microalgae [5,6]. The dynamic development of bioenergetic systems that are based on anaerobic digestion generates large quantities of digestate that need to be utilized in environmental-friendly and economic manner. After dehydration, the solid phase of digestate can be used as a fertilizer or dried and used in biomass co-firing [7]. In contrast, the liquid phase is difficult to utilize due to the large volume and high concentration of nutrients, especially ammonia nitrogen and potassium. Anaerobic wastewater treatment technologies ensure the efficient biodegradation of organic compounds, but they do not provide an effective removal of nutrients. Thus, the digestate from anaerobic reactors has to be treated [8]. The technologies recommended for treating the liquid fraction of anaerobic digestate were membrane filtration, evaporation, and stripping. These methods concentrate the nutrients and remove ammonia nitrogen, however they consume a lot of energy. Liquid digestate can be also mixed with a feedstock for anaerobic digestion, or used to facilitate the composting process. Biological methods with using membrane bioreactors, sequencing batch reactors, and moving bed reactors are also suggested for liquid treatment. Microalgae can assimilate a significant amount of nutrients that are contained in the digestate due to the high requirements of nitrogen and phosphorus for protein biosynthesis. The concentration of protein in microalgal dry matter is, depending on the species, in the range of 20–60%. Nutrients are also used for nucleic acids and phospholipids biosynthesis [9].

When considering the composition of liquid digestate and nutrient requirements for microalgal growth, it seems that digestate is a good source of nutrients and microelements for the intensive cultivation of microalgae with simultaneous digestate treatment. Algae have been shown to produce oxygen through photosynthesis in an amount between 1.50 and 1.92 kg O_2/kg of biomass produced. On the other hand, the oxidative degradation of organic compounds ranged from 0.48 to 1.85 kg O_2/m^3·d [10]. High CO_2 concentration in digestate significantly enhanced the growth rate of microalgae, which directly affected the efficiency of nutrient removal, according to the literature [11–13]. In systems using salt water, the Redfield ratio of carbon, nitrogen and phosphorus (C: N: P = 106:16:1) may be balanced by adding wastewater or digestate [14].

The aim of the study was to determine the efficiency of *Scenedesmus sp.* biomass production while using digestate from anaerobic digestion of the mixture of maize silage, cattle slurry and microalgae biomass in different proportions. The data allowed for developing a mathematical formula that can predict the biomass productivity. Nutrient removal efficiency from liquid digestates was also assessed.

2. Materials and Methods

2.1. Experimental Design

The study was carried out on a laboratory scale. Liquid microalgal cultures were grown in vertical, closed photobioreactors (PBRs), with an active volume of 2.5 L (diameter of 7.6 cm, height of 0.55 m), which was made of transparent glass. CO_2 was delivered by a continuous inflow of compressed air (at 250 L/h), which also ensured sufficient mixing of the culture medium and homogeneity of

conditions within the entire PBR's volume. The temperature of the culture was 22.0 ± 2.0 °C, and the thermal conditions were continuously monitored by using the temperature sensors inside the PBRs. Continuous fluorescent lighting (700 lux cool-white light) was used as a constant light source for microalgae. In the experiments, the microalgae of genus *Scenedesmus sp.* were used.

Table 1 shows the study organization. The experimental series differed in the composition of digestate: series 1—digestate from anaerobic digestion of the mixture of 70% maize silage and 30% cattle slurry, series 2—digestate from anaerobic digestion of the mixture of 67% maize silage and 23% cattle slurry and 10% microalgae biomass, series 3—digestate from anaerobic digestion of the mixture of 45% maize silage and 15% cattle slurry and 40% microalgae biomass, and series 4—digestate from anaerobic digestion of the mixture of 15% maize silage and 5% cattle slurry and 80% microalgae biomass. The initial concentration of ammonia nitrogen (AN) in the culture medium ranged from 40 mg N-NH$_4$/L to 160 mg N-NH$_4$/L, depending on experimental variant.

Table 1. Organization of the experiment.

Series		Source of Nutrients			
		Series 1	Series 2	Series 3	Series 4
		Initial concentration of ammonia nitrogen (mg/L)			
	1	40	40	40	40
Variant	2	80	80	80	80
	3	120	120	120	120
	4	160	160	160	160

2.2. Microalgae Inoculum, Nutrient Medium Characteristics and Cultivation

Microalgae inoculum of *Scenedesmus sp.* (EE101) used in the study originated from the UTEX culture collection of algae at the University of Texas at Austin. In all experimental variants, the initial biomass concentration in PBRs was 250 ± 22 mg total solids (TS)/L.

Anaerobic digestates were obtained from continuous lab-scale anaerobic reactors with the working volume of 4.0 L. The operating parameters of the reactors were as follows: hydraulic retention time—40 days, organic loading rate—3.0 kg volatile solids (VS)/m^3·d, and anaerobic sludge concentration—5.0 g total solids (TS)/L. A constant temperature of 37 °C was kept by placing reactors in a thermally insulated chamber that was equipped with a heating system with a warm air circulation system. Before using as the culture medium, anaerobic digestates were distilled (70 °C, 100 rpm) and then pasteurized (30 min., 90 °C) in order to obtain supernatant (liquid digestates) and remove competing microorganisms. Table 2 shows the characteristics of the liquid digestates that were used in series of experiment. Table 3 shows the characteristics of the initial growth media used in each experimental variant.

Table 2. Liquid anaerobic digestate characteristic.

Parameter	Unit	Series 1	Series 2	Series 3	Series 4
COD	mg O$_2$/L	9200 ± 470	8730 ± 340	7980 ± 510	7400 ± 220
BOD$_5$	mg O$_2$/L	5400 ± 410	5430 ± 250	4840 ± 330	4130 ± 190
TN	mg N/L	1750 ± 190	1670 ± 130	1420 ± 110	1290 ± 120
AN	mg N-NH$_4$/L	1120 ± 140	1280 ± 90	1010 ± 70	980 ± 80
TP	mg P/L	104 ± 21	99 ± 27	72 ± 17	68 ± 13
P-PO$_4$	mg P-PO$_4$/L	77 ± 14	72 ± 21	51 ± 12	49 ± 11
pH	-	6.9 ± 0.1	6.9 ± 0.2	7.0 ± 0.2	7.0 ± 0.1

Table 3. The initial characteristics of the culture medium.

Variant	Parameter	Unit	Series 1	Series 2	Series 3	Series 4
1	COD	mg O_2/L	319 ± 14	309 ± 32	277 ± 35	269 ± 17
	BOD_5	mg O_2/L	202 ± 9	193 ± 19	159 ± 21	121 ± 19
	TN	mg N/L	52 ± 7	61 ± 16	63 ± 7	54 ± 9
	AN	mg N-NH_4/L	41 ± 5	40 ± 11	42 ± 12	41 ± 7
	TP	mg P/L	4.3 ± 1.2	5.2 ± 1.1	5.6 ± 1.1	4.9 ± 1.0
	P-PO_4	mg P-PO_4/L	3.5 ± 1.1	3.7 ± 1.3	3.4 ± 0.9	3.2 ± 0.8
	pH	-	7.3 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.2 ± 0.1
2	COD	mg O_2/L	607 ± 64	638 ± 48	581 ± 47	544 ± 31
	BOD_5	mg O_2/L	346 ± 31	306 ± 33	302 ± 31	345 ± 16
	TN	mg N/L	103 ± 17	123 ± 19	114 ± 22	109 ± 21
	AN	mg N-NH_4/L	79 ± 12	83 ± 21	82 ± 17	74 ± 11
	TP	mg P/L	7.9 ± 1.4	9.2 ± 1.8	9.3 ± 1.4	8.3 ± 0.1
	P-PO_4	mg P-PO_4/L	6.1 ± 2.0	7.1 ± 1.1	6.1 ± 2.5	5.6 ± 0.9
	pH	-	7.3 ± 0.2	7.2 ± 0.2	6.9 ± 0.1	7.1 ± 1.3
3	COD	mg O_2/L	997 ± 93	1002 ± 93	782 ± 102	738 ± 72
	BOD_5	mg O_2/L	688 ± 44	599 ± 41	361 ± 77	338 ± 38
	TN	mg N/L	161 ± 12	193 ± 26	192 ± 29	170 ± 20
	AN	mg N-NH_4/L	120 ± 19	121 ± 23	119 ± 20	121 ± 13
	TP	mg P/L	9.3 ± 2.0	14.9 ± 3.4	13.9 ± 3.1	11.9 ± 2.6
	P-PO_4	mg P-PO_4/L	7,3 ± 1.7	11.2 ± 3.1	9.1 ± 2.3	8.3 ± 1.9
	pH	-	7.1 ± 0.2	7.2 ± 0.2	7.0 ± 0.2	7.0 ± 0.5
4	COD	mg O_2/L	1204 ± 103	1310 ± 203	1137 ± 194	1040 ± 102
	BOD_5	mg O_2/L	697 ± 59	920 ± 143	591 ± 88	537 ± 57
	TN	mg N/L	214 ± 33	271 ± 41	252 ± 31	232 ± 23
	AN	mg N-NH_4/L	163 ± 28	162 ± 23	160 ± 30	161 ± 12
	TP	mg P/L	17.3 ± 3.9	19.1 ± 4.1	20.3 ± 4.2	18.2 ± 3.8
	P-PO_4	mg P-PO_4/L	13.1 ± 2.6	13.8 ± 3.9	13.1 ± 3.1	11.9 ± 2.2
	pH	-	7.1 ± 0.1	7.0 ± 0.1	7.0 ± 0.2	7.2 ± 0.1

2.3. Analytical Methods

During the study, the samples for the determination of parameters and microalgae concentrations in PBRs were collected once a day in a dose of 50 mL. The loss of the liquid volume in PBRs resulting from sample collection and evaporation was refilled with deionized water to the level of 2.5 L.

The samples of cultivation media, anaerobic digestates, as well as samples from PBRs, were centrifuged at 5000 rpm for 10 min. In supernatants the following analyses were determined: biochemical oxygen demand (BOD_5) using Oxi-Top Control system, chemical oxygen demand (COD), total phosphorus (TP), orthophosphate (P-PO_4), total nitrogen (TN), and ammonia nitrogen (AN) while using a spectrophotometer with a mineralizer. The gravimetric method was used to determine the

total solids (TS) and volatile solids (VS) in the retentate. The pH of aqueous solutions was determined with a pH-meter. Luxmeter was used to measure light intensity in photobioreactors.

The taxonomic identification of microalgae biomass was conducted at microscope magnifications of: $1.25 \times 10 \times 40$ or $1.25 \times 10 \times 100$ and with using algae analyzer.

2.4. Statistical Methods

Each experimental variant had three replications. The statistical analysis of the experimental results was carried out with statistical package for data processing. The hypothesis on distribution of each analyzed variable was verified with a Shapiro–Wilk W-test. One-way analysis of variance (ANOVA) was applied to determine the significance of difference between variables. The variance homogeneity in groups was checked with a Levene's test, whereas the significance of differences between the analyzed variables was determined with a Tukey RIR test. In all tests, the level of significance was adopted at $\alpha = 0.05$.

The model that can estimate the microalgae biomass production, depending on the initial characteristics of the culture medium and the amount of digestate in its preparing was 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. The relevant predictor variables in the formula among the investigated variables were determined. Fitting a model to the experimental data was assessed while using determination coefficients. The significance of polynomial regression model was verified using F-statistic and referenced to critical values. A lack-of-fit test was performed to assess whether the model fitted well. The test involved comparing the model with the model containing the remainder of the explanatory variables omitted in it. The model was subjected to an estimation test. Subsequently, the residual analysis was carried out to validate the regression model with statistical package for data processing.

3. Results and Discussion

3.1. Microalgae Biomass Production

Factors that affect algal production using anaerobic digestate as growth medium are the genus and species of microalgae, digestate characteristic, as well as the type and operational parameters of photobioreactors [15]. Microalgae species that can be used in intensive biomass production while using anaerobic digestate should be characterized by the high resistance to the changing of environmental conditions due to various characteristics of wastewaters and harmful contaminations [16]. *Nannochloropsis salina* [17] and *Scenedesmus sp.* [18] have met these requirements, according to the literature.

In this study, *Scenedesmus sp.* microalgae was used due to its eurybionic nature and high resistance to harmful substances that are present in the environment. Many data indicate that it is one of the most promising species for effective cultivation on various anaerobic digestion effluents [19,20]. Reports in the literature indicate the possibility of using microalgae in the biodegradation of hard-degradable substances, as well as toxic pollutants [12,21]. Green algae show a high resistance to heavy metals that are present in many industrial wastewaters [22]. The genus of *Scenedesmus sp.* has been successfully used in landfill leachate treatment [23], wastewater treatment from paper-pulp and electroplating industry [24], treatment of dye from textile industry [25], detoxification of phenolic substances [26], and wastewater from ethanol and citric acid production [27]. Biosorption of metal ions i.e., copper (II), cadmium (II), and lead (II) by green algae has been also proven by [28].

There are difficulties in determining their amount due to the fact that the composition of digestate discharging from anaerobic reactors operating in technical scale may vary within wide limits, which should be introduced into the PBRs [15]. Anaerobic digestates are mostly concentrated, thus the dilution is necessary before using as a culture medium. It seems that the factor that can determine the

degree of dilution is the concentration of ammonia nitrogen. The AN concentration was ranged from 40 to 160 mg/L in our study.

During the study, the analyzed parameters were organic compounds concentration (COD, BOD_5) and nutrients (total nitrogen, ammonia nitrogen, total phosphorus, and orthophosphates), which mostly influenced the biomass growth. A different composition of the liquid digestates were obtained, depending on the proportion of substrates in the feedstock for anaerobic digestion. For example, the differences in the COD values ranged from 9200 ± 470 mg O_2/L in series 1 to 7400 ± 220 mg O_2/L in series 4, which is close to $20 \pm 2\%$. Higher differences were observed in the total phosphorus concentration ($35 \pm 4\%$) and orthophosphates ($27 \pm 3\%$). The concentration of the total nitrogen in series 1 was nearly $27 \pm 4\%$ lower than in series 4, while the highest ammonia nitrogen concentration was found in series 2 and was by $24 \pm 4\%$ higher than in series 4.

The initial concentrations of AN were associated with the fact that high concentrations of ammonia in the culture medium may directly affect the microalgae growth [18]. The content of AN in digestate is usually too high to be tolerated by microalgae according to literature and, therefore, it is necessary to dilute them [29]. In our experiments, the digestate was diluted with deionized water. The lowest AN concentration of 40 mg/L in variant 1 ensured the highest productivity of *Scenedesmus sp.* biomass in series 3 and 4, reaching 903 ± 78 mg TS/L and 868 ± 92 mg TS/L, respectively (Figure 1). In series 1 and 2, the biomass concentration in PBRs was, respectively, 735 ± 27 mg TS/L and 811 ± 45 mg TS/L (Figure 1). Higher initial AN concentration of 80 mg/L improved the efficiency of biomass production. In series 4, the microalgal biomass productivity reached 1480 ± 73 mg TS/L, while, in series 2, it was 1163 ± 44 mg TS/L (Figure 1). Significantly lower ($p < 0.05$) biomass yield was recorded in series 1 and series 3 (Figure 1). Significantly ($p < 0.05$) higher final *Scenedesmus sp.* biomass production was found when the AN in the culture medium was 120 mg/L. In series 3 and 4, the highest and statistically comparable ($p < 0.05$) effects of biomass productivity was observed. The final concentration of *Scenedesmus sp.* biomass in PBRs was 2017 ± 92 mg TS/L in series 3 and 2254 ± 107 mg TS/L in series 4 (Figure 1). The lowest biomass yield was noted in series 1 (Figure 1). In variant 4 (160 mg $N-NH_4$/L), no significant changes ($p < 0.05$) in biomass production were found. The highest final biomass concentration of *Scenedesmus sp.* reached 2232 ± 149 mg TS/L in series 3 and 2360 ± 106 mg TS/L in series 4 (Figure 1).

The inhibiting effect of high ammonia nitrogen concentration of 160 mg/L on the biomass growth of *Scenedesmus sp.* could be associated with a high concentration of free ammonia. Wang et al. [20] studied the possibility of using digestate from anaerobic digestion of cattle manure as a growth medium for green microalgae cultivation. They found that the biomass yield, lipid content in algae cells, and the nutrient removal form digestate were correlated with the rate of digestate dilution. In other studies, it was found that *Scenedesmus sp.* growth was affected by the initial concentration of AN that ranged from 50 mg/L to 260 mg/L in the culture medium based liquid digestate [18]. The highest microalgae biomass production was 2600 mg TS/L with a growth rate of about 114 mg TS/L·d [18]. Cho et al. confirmed the negative effects of high concentration of free ammonia on algal biomass productivity in 2013 [30]. In our study, the results indicated that using the most concentrated digestates limited the growth of *Chlorella sp.* This phenomenon could be also associated with high concentration of organic compounds, as well as light limitation of photosynthesis due to high turbidity of the culture medium.

The mathematical formula of *Scenedesmus sp.* biomass productivity while using anaerobic digestate as a culture medium is characterized by a standard error of 248.57 and it represents about 81.14% of changes observed in the microalgae growth (coefficient of determination $R^2 = 0.8114$, F = 63.101, $p < 0.001$).

$$BP(mg\ TS/L) = 5.008D - 2.2092COD + 11.5390AN + 398.5987 \tag{1}$$

where *BP* is the predicted biomass yield in mg TS/L, *D* is the volume of digestate in mL used to prepare the culture medium, *COD* is the initial COD concentration in the culture medium in mg/L, and *AN* is the initial ammonia nitrogen concentration in the culture medium in mg/L. The formula allow for the basic prediction of biomass yield when using digestate as a growth medium.
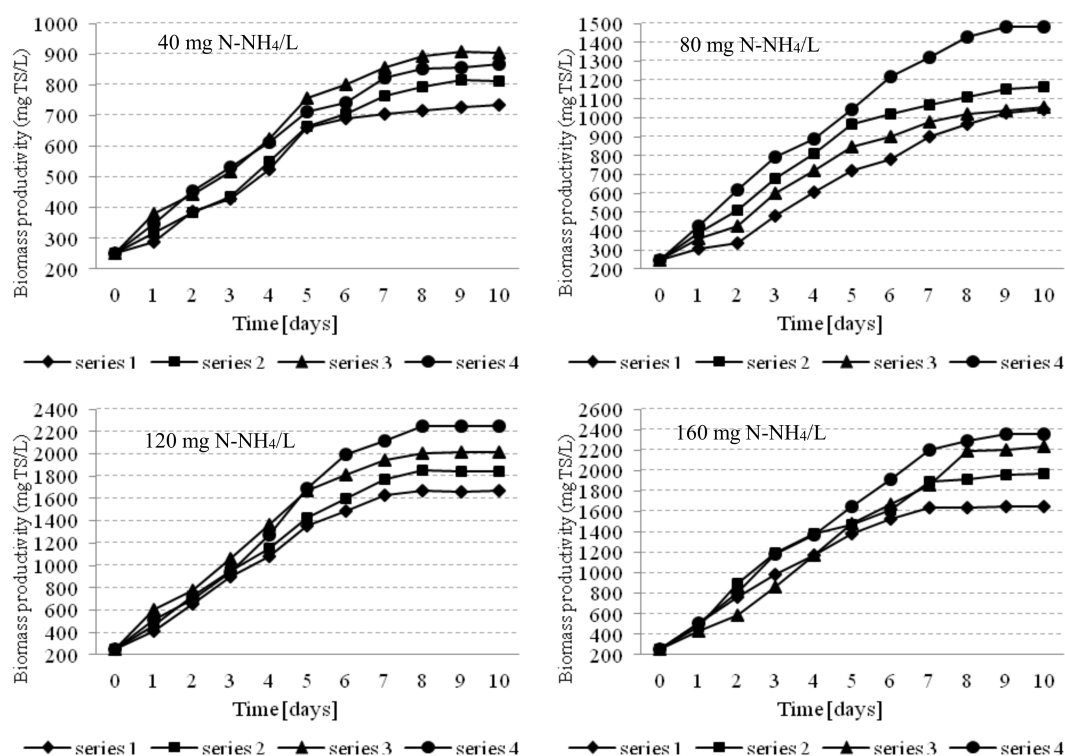
Figure 1. *Scenedesmus sp.* biomass productivity in experimental variants during the experimental period.

3.2. Effectiveness of Nutrients Removal

The nutrient removal efficiency from a growth medium was assessed during the study. In experimental variant that was associated with the initial ammonia nitrogen concentration of 40 mg/L, the efficiency of AN removal was over 96% in all series. The lowest concentration of AN in the culture medium at the end of the experiment was 0.21 ± 0.1 mg/L in series 4. A significantly higher value was observed in the growth medium in series 1 (1.47 ± 0.2 mg/L) and 2 (0.96 ± 0.2 mg/L) (Figure 2). Similar effects of ammonia nitrogen removal (over 95%) were noted in variant, in which the initial concentration of AN was 80 mg/L. In series 2, the concentration of AN in a growth medium at the end of experiment was as low as 1.77 ± 0.2 mg/L. In series 1, the highest concentration of AN was observed (5.02 ± 0,6 mg/L), (Figure 2). In variant 3 (120 mg N-NH$_4$/L), the concentration of AN in the culture medium at the end of the cultivation time ranged from 7.04 ± 0.3 mg/L in series 4 to 17.71 ± 1.4 mg/L in series 1. Similarly, the high concentrations of AN in the culture medium were observed in variant 4, in which the residual AN concentration ranged from 17.69 ± 2.7 mg/L in series 4 to 43.2 ± 3.2 mg/L in series 2 (Figure 2).

The phosphorus removal was strongly affected by the initial orthophosphate concentration in the growth medium. It was noted that the lowest and the highest concentrations of orthophosphate in the medium at the end of experiment were, respectively, 3.5 mg/L in variant 1 and nearly 13.0 mg/L in variant 4 (Figure 3). Regardless of the experimental series, the efficient use of orthophosphates by *Scenedesmus sp.* microalgae was observed. In variant 1, series 1 no mineral phosphorus was found on the eighth day of cultivation. In the remaining series, the concentration of orthophosphates was also close to zero (Figure 3). When the dose of digestate in preparing the culture medium increased, the concentration of orthophosphates in the growth medium at the end of experiment was also increased. However, these concentrations were very low, which could state that phosphorus is a limiting factor that affects the biomass growth.
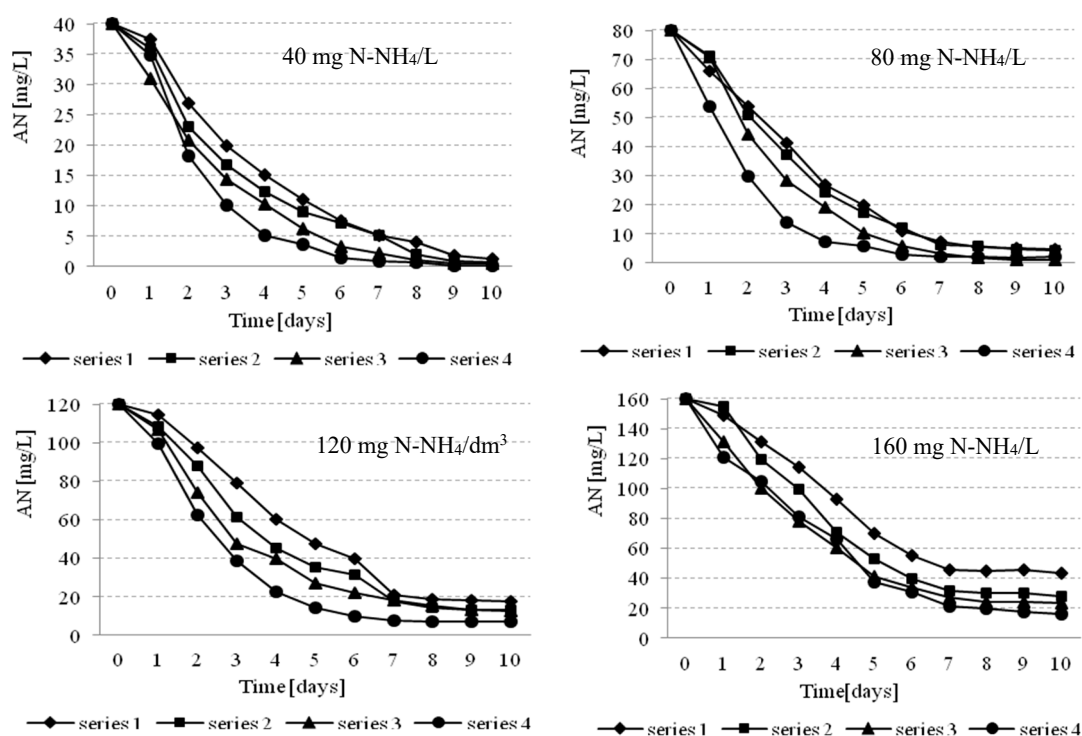
Figure 2. Changes in ammonia nitrogen (AN) concentration in experimental variants during the experimental period.
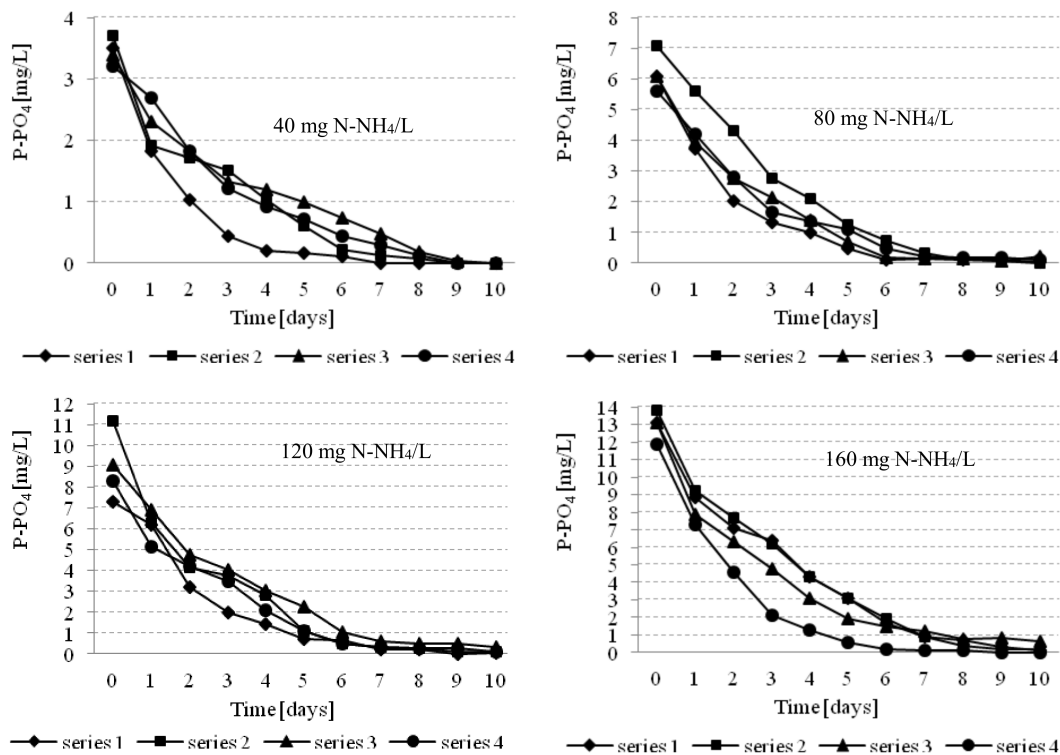

Figure 3. Changes in orthophosphate concentration in experimental variants during the experimental period.

The presented studies proved that the high concentration of organic compounds in the liquid digestates mainly influenced the chemical and physical properties of the culture medium. Moreover,

other microorganisms, such as bacteria, were also grown in that medium, which might compete for nutrients with microalgae, and contribute to increase the turbidity of the medium which limits the light permeability of the medium. These phenomena limited the possibility of the efficient cultivation of *Scenedesmus sp.*, which was proven during the experimental variants. It was also found that a deficiency of phosphorus in digestate mgiht be a factor limiting the intensive production of microalgae biomass.

According to the literature, *Chlorella pyrenoidosa* can be grown on anaerobic digestate with the subsequent removal of nutrients and organic compounds [31]. The authors obtained the concentration of microalgae biomass in photobioreactor on the level of 1.25 g TS/L, and found that the removal efficiency of nitrogen, phosphorus, and COD achieved, respectively, 78.76%, 94.78%, and 98.34% during 6–8 days of cultivation. In turn, the studies on cultivation of microalgae biomass in which the predominant species was *Scenedesmus obliquus* conducted in semi-technical scale demonstrated, where the nitrogen removal from a culture medium based on wastewater averaged 53.0% in the summer, while the efficiency was decreased to 21.0% in the winter [32]. It was also found that the phosphorus removal took place only during the day, and the efficiency ranged from 45.0% in the winter to 73.0% in the summer. Yun et al. [33] used *Chlorella vulgaris* to remove ammonia nitrogen from steel processing wastewater. The rate of ammonia assimilation by microalgae was 0.022 g/L·d.

4. Conclusions

The composition of the substrates that were used in anaerobic digestion influenced the composition of the liquid digestates. The differences in parameters were significant, e.g. the COD concentration ranged from 9200 ± 470 mg O_2/L in series 1 to 7400 ± 220 mg O_2/L in series 4, which was approximately 20%. Higher differences were observed in phosphorus and nitrogen concentrations, which was from 24% to 35%.

It was found that anaerobic digestate can be a proper source of nutrient for the intensive production of *Scenedesmus sp*. biomass. Using the most concentrated digestate in series 1 and 2 had an adverse impact on microalgae biomass productivity. It could be associated with the high concentration of ammonia nitrogen and organic compounds in the culture medium, as well as high turbidity decreasing the rate of photosynthesis. Additionally, other microorganisms, in particular, bacteria, are also grown in that medium, competing for nutrients with microalgae. These phenomena limited the possibility of efficient cultivation of *Scenedesmus sp.*

The highest biomass productivity was obtained in series 3 and 4 while using digestete from anaerobic digestion of the mixture contained a high proportion of microalgae biomass. A high proportion of microalgae biomass in the feedstock for anaerobic digestion mostly influenced the liquid digestate characteristic, where a lower concentration of organic compounds was noted, which had a direct impact on the increase in the rate of growth of microalgae biomass in photobioreactors. Lower *Scenedesmus sp.* biomass yields were obtained when using digestate from the digestion of typical substrates in agricultural biogas plants: maize silage and cattle manure.

The study showed that *Scenedesmus sp.* microalgae can be used for the effective nutrient removal from the liquid digestate. An increase in digestate concentration influenced the presence of orthophosphates in the growth medium at the end of experiment, which allow for stating that the phosphorus content in anaerobic digestate is a limiting factor for the growth of *Scenedesmus sp.* Phosphorus supplementation of the liquid digestate should be considered when using it as a culture medium.

On the basis of the optimization model, it was found that the volume of liquid digestate used for preparing the cultivation medium, the initial concentration of COD, and the initial concentration of ammonia nitrogen had a significant impact on the production of *Scenedesmus sp.* biomass.

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