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Biomass Production and Nutrient Removal by *Chlorella vulgaris* from Anaerobic Digestion Effluents

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Abstract: One of the most important factors determining the profitability of microalgae biomass production is the use of inexpensive and available source of nutrients. The aim of the study was to determine the possibility of using anaerobic digestion effluents (ADE) from the fermentation of distillery stillage, maize silage and bovine slurry as a nutrient in the production of microalgae *Chlorella vulgaris* biomass. The highest biomass production of 2319 mg TS/dm^3 was obtained during the cultivation of microalgae in the medium consisting of the effluents originating from the fermentation with a high share of bovine slurry. Significantly lower *Chlorella vulgaris* biomass growth was noted during cultivation in the medium composed of effluents obtained after dewatering of anaerobic sludge from the methane fermentation reactor fed with distillery stillage. In these series, an increase of the initial concentration of N-NH_4 in the medium to a level of 160 mg/dm^3 significantly reduced microalgae growth. The high efficiency of P-PO_4 removal from 87–100% was noted. The study proved that anaerobic digestion effluents might be used as a nutrient source for efficient biomass production of *Chlorella vulgaris* after optimization of ammonium nitrogen dose.

Keywords: microalgae biomass; post-fermentation effluent; distillery stillage; nutrient removal; *Chlorella vulgaris*

1. Introduction

The development and implementation of efficient and renewable energy technologies on a large scale is becoming a challenge for scientists, as well as a priority for operators and administrators of energy systems. This goal might be partly achieved by stimulating the development of unconventional energy systems, which are based on the use of biomass with different characteristics and origins [1,2]. However, Fargione et al. [3] and Searchinger et al. [4] noted that irrational management of the resources of energy crops could lead to a negative balance of the amount of greenhouse gases introduced into the atmosphere. It is also suggested that intensive use of agricultural land for the production of plants for biofuels' production might negatively affect the global supply of food and result in a significant increase of its prices [5]. Therefore, searching for alternative sources of biomass for energy purposes is justified from an economic and environmental point of view. Taking into account the very high photosynthetic efficiency, the fast rate of biomass growth, the resistance to various types of pollution and the possibility of managing areas that cannot be used for other purposes, a preferred type of biomass might be microalgae [6–9].

One of the most important factors determining the profitability of algae biomass production is the use of inexpensive and available source of nutrients. So far, many studies have been focused on the possibility of using wastewater with a high concentration of nitrogen and phosphorus compounds [10–13]. The dynamic development of bioenergy systems based on methane fermentation in many cases causes difficulties with the management of post-digestion sludge. After dehydration, the solid phase is used as a fertilizer or dried and used in combustion. The liquid phase is difficult to neutralize due to its considerable volume and high concentration of pollutions. The anaerobic wastewater treatment systems are efficient in the biodegradation of organic compounds; however, the biogenic compounds remain in wastewater after purification. This fact excludes the possibility of their direct discharge into the environment [10,14].

Considering the characteristics of anaerobic digestion effluent (ADE) and the nutrient needs of microalgae, it seems that this kind of substrate might be a source of biogenic substances and microelements. Therefore, utilization of ADE as a medium for microalgae might be a solution for intensive biomass growth with simultaneous neutralization of pollutants. During photosynthesis, microalgae produce 1.50–1.92 kg O_2/kg of biomass, while the oxidation rate achieved during the degradation of organic compounds is in the range of 0.48–1.85 kg $O_2/(m^3 \cdot d)$ [15,16]. A high concentration of CO_2 in ADE intensifies the growth rate of microalgae, which affects the efficiency of pollution degradation. In systems based on the use of salt water, utilization of wastewater or leachates balances the molecular ratio of carbon, nitrogen and phosphorus (C:N:P = 106:16:1), the Redfield ratio. The composition of ADE might be different, depending on the substrates used for methane fermentation. The substrates used for methane fermentation are hydrolyzed to various degrees, which might determine nutrients' availability for biomass growth. Additionally, different types of ADE might contain some microalgae growth inhibitors.

The aim of the research was to determine the effect of using anaerobic digestion effluent from reactors fed with maize silage, bovine slurry and distillery stillage on the production of microalgae *Chlorella vulgaris* biomass.

2. Results and Discussion

The effective use of ADE in the intensive production of microalgae biomass depends on factors that can be divided into three groups, namely: properties of the tested species of microalgae, physico-chemical characteristics of the used ADE and the type of photobioreactor and the technological parameters of the process [17].

Species of microalgae that can be cultivated on the medium consisting of ADE must be characterized by high resistance to changing of environmental conditions and a high concentration of pollutants [18]. *Nannochloropsis salina* has been tested in the previous studies, due to the very high biomass production and high content of fatty substances in the biomass [19,20]. In other experiments, the effectiveness of a *Scenedesmus* sp. biomass production was determined [21].

In the study, the microalgae *Chlorella vulgaris* was tested, due to the eurobiontic character of this species and very high resistance to harmful substances present in the environment. Recently, *Chlorella vulgaris* has been considered as a perspective species that can be cultivated in medium with ADE addition [10,14,22]. *Chlorella* sp. can be used in a biodegradation of hardly biodegradable or toxic pollutants [13,23]. Moreover, *Chlorella* sp. is resistant to heavy metals; therefore, it was used in industrial wastewater treatment [24]. The literature presents research on the use of *Chlorella* sp. for the biodegradation of such wastewater as leachate from landfills [25], wastewater from the wood and paper industry [26,27], textile industry [28], phenolic industry [29,30] or the production of ethanol and citric acid [31]. Additionally, *Chlorella* sp. is used for the treatment of wastewater containing organic compounds, e.g., effluents from fermentation chambers [32]. The microalgae can accumulate heavy metals, i.e., copper, nickel and lead, thus *Chlorella* sp. is used to remove these metals from wastewater [33,34].

2.1. *Chlorella vulgaris* Biomass Production

Due to the composition of ADE from installations operated on a technical scale being able to change within a wide range, which depends on the organic substrate used, difficulties in determining their amount that should be introduced into photobioreactors might occur [17]. The factor that can determine the dose and degree of dilution of ADE introduced into the algae biomass production systems is the concentration of N-NH_4. In the study, the initial concentrations of N-NH_4 ranged from 40–160 mg/dm^3. The initial concentration of N-NH_4 used was due to the high concentrations of ammonium nitrogen in the culture medium inhibiting microalgae culture production [21]. According to literature data, the content of N-NH_4 in raw effluents is usually too high to be tolerated by microalgae, and therefore, dilution is necessary [35]. In the study, the effluents were diluted using deionized water.

In the case of a N-NH_4 concentration of 40 mg/dm^3, the highest productivity of *Chlorella vulgaris* was obtained in the series III and IV. In these series, at the end of the experiments, the concentration of biomass was 788 mg TS/dm^3 and 793 mg TS/dm^3, respectively (Figure 1). In the series I and II, the concentration of biomass in the photobioreactors was 660 mg TS/dm^3 and 696 mg TS/dm^3, respectively. Increasing the dose of ADE to a level that increased the initial N-NH_4 concentration to 80 mg/dm^3 improved the production efficiency of *Chlorella vulgaris* biomass. The use of fermentation effluents in the series III as a cultivation medium resulted in the biomass concentration of 1301 mg TS/dm^3. In the series IV, the biomass concentration in the photobioreactor was 1261 mg TS/dm^3. Significantly lower ($p < 0.05$) productivity was noted in the series I and II. The concentrations of biomass in the culture medium were 1019 mg TS/dm^3 and 899 mg TS/dm^3 in the series I and II, respectively (Figure 1). The increased concentration of ammonium nitrogen in the medium to 120 mg N-NH_4/dm^3 significantly ($p < 0.05$) increased the *Chlorella vulgaris* biomass concentration. In this variant, the highest, statistically comparable ($p < 0.05$) biomass concentrations in the photobioreactors of about 2100 mg TS/dm^3 were observed in the series III and IV. In this variant, the lowest biomass concentration in the photobioreactor of 1570 mg TS/dm^3 was noted in the series I (Figure 1). Increasing the dose of ADE to the level of ammonium nitrogen in the photobioreactor of 160 mg N-NH_4/dm^3 resulted in a significant ($p < 0.05$) reduction of the biomass production to about 1300 mg TS/dm^3 in the series I and II. However, in the case of the series III and IV, an increased concentration of ammonium nitrogen of 160 mg N-NH_4/dm^3 positively influenced the growth of *Chlorella vulgaris* biomass. The highest biomass concentration of 2382 mg TS/dm^3 was noted in the series IV (Figure 1).

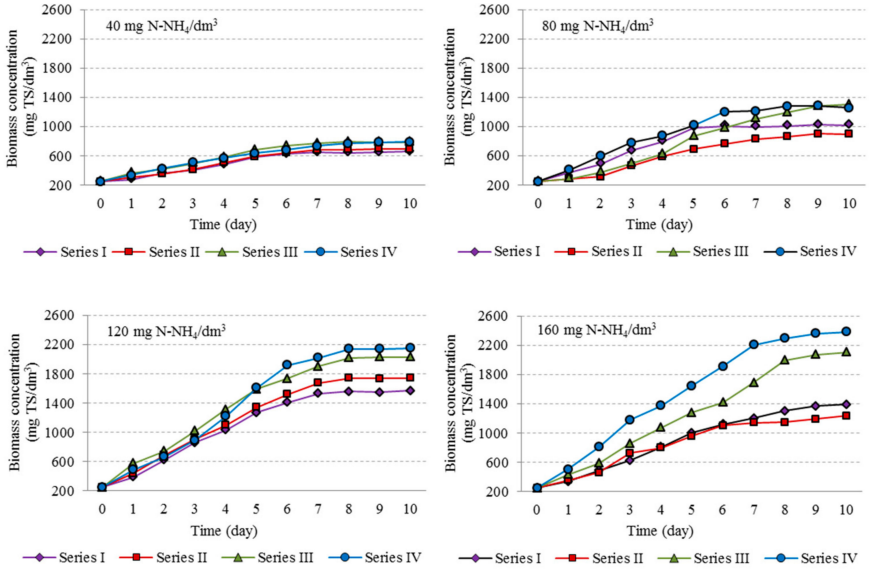


Figure 1. The biomass concentration of *Chlorella vulgaris* depending on the variants and series of the experiments.

The obtained results suggest that the highest doses of ADE limited the growth of *Chlorella vulgaris* biomass. This phenomenon could be related to too high a concentration of organic compounds introduced into the culture medium or a limitation of the light penetration and thus reduced photosynthesis due to the turbidity of the tested effluents. Moreover, lower biomass production in the series I and II might be a result of different ADE origination. In the series I, the substrate of distillery stillage was probably not completely hydrolyzed, hence the nutrients were not available for the algae's growth. Similarly, in the series II, effluent originating from fermentation of substrates with a high content of distillery stillage hampers algae growth. The results showed that effluent from the fermentation of maize silage and bovine slurry promoted high biomass production due to readily available nutrients.

The limitation of *Chlorella vulgaris* biomass growth in the medium with 160 mg N-NH_4/dm^3 (the variant IV) could also be associated with too high of a concentration of free ammonia in the culture medium. Wang et al. [10] cultivated *Chlorella* sp. in medium with effluent from a reactor with fermentation of bovine manure. The authors noted that the efficiency of biomass production, lipid content in microalgae cells and efficiency of pollutant removal were correlated with the dilution level of the fermentation effluents. In other studies, it was proven that the factor influencing the rate of biomass production of microalgae from the genus *Scenedesmus* was the initial concentration of N-NH_4 in the culture medium prepared on the basis of effluents from the wastewater treatment plant [21]. The range of ammonium nitrogen concentration was from 50 mg N-NH_4/dm^3–260 mg N-NH_4/dm^3. The highest biomass concentration was 2600 mg TS/dm^3. Another reason for the limited biomass growth could be the limitation of light access resulting from the turbidity and the color of ADE. The introduction of high doses of ADE into the photobioreactors had a direct impact on the limitation of the light transmittance of the environment. Detailed biomass production results from three replicates are additionally presented in Table S1.

2.2. Efficiency of Nitrogen Ammonium Removal

In the variant I (initial concentration of nitrogen ammonium 40 mg N-NH_4/dm^3), more than 95% efficiency of ammonium nitrogen removal in the medium was noted. The lowest concentration of N-NH_4 in the medium at the end of the experiment (0.11 mg/dm^3) was observed in the series IV. A significantly higher concentration of N-NH_4 was noted in the post-production medium in the series I, 1.29 mg/dm^3, and the series II, 0.77 mg/dm^3 (Figure 2). Similarly, high efficiency ammonium nitrogen utilization for biomass growth was observed in the variant II (initial concentration of nitrogen ammonium of 80 mg N-NH_4/dm^3). In the series III, the concentration of N-NH_4 in the medium at the end of the cultivation was the lowest (1.11 mg N-NH_4/dm^3). The highest concentrations of ammonium nitrogen in the medium of 4.89 mg N-NH_4/dm^3 and 4.26 mg N-NH_4/dm^3 were observed in the series I and II, respectively. In the variant III (initial ammonium nitrogen concentration of 120 mg N-NH_4/dm^3), the concentration of N-NH_4 in the medium at the end of the cultivation ranged from 33.12 mg N-NH_4/dm^3 in the series II to 6.98 mg N-NH_4/dm^3 in the series IV. The highest concentrations of ammonium nitrogen in the medium after 10 days of the cultivation were noted in the variant IV (initial concentration of ammonium nitrogen of 160 mg N-NH_4/dm^3).

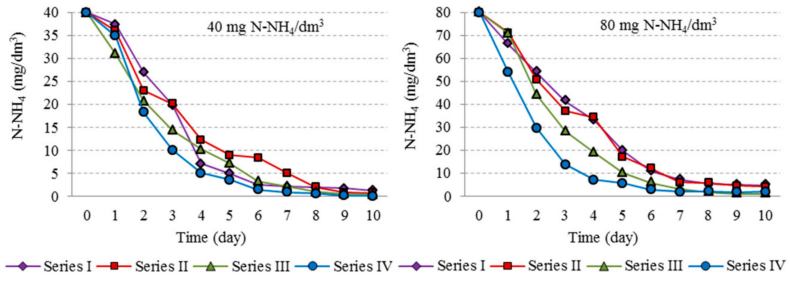


Figure 2. *Cont.*

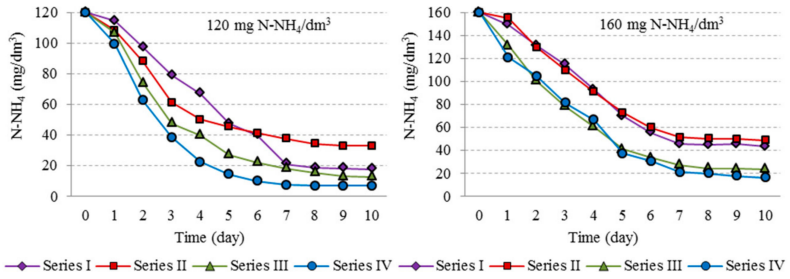
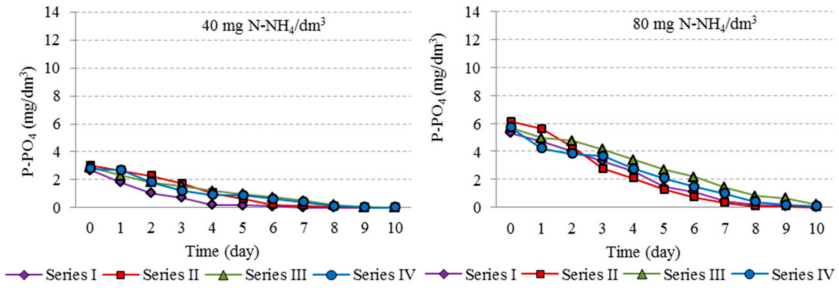


Figure 2. The changes of concentration of ammonium nitrogen in the cultivation medium depending on the variants and series of experiments.

The concentration of N-NH_4 in the medium might influence the concentration of free ammonia, which has a negative effect on the microalgae biomass production. Uggetti et al. [21] observed that the increase of ammonia concentration from 2 to 9 mg NH_3/dm^3 in the medium decreased the rate of biomass production by 18%. The increase of ammonia concentration from 9 to 34 mg NH_3/dm^3 reduced the microalgae growth rate by 77%. Cho et al. [36] also proved the inhibitory effect of ammonia content on microalgae growth. High concentrations of free ammonia in many cases eliminate the possibility of use of ADE as a cultivation medium for microalgae. However, control of the concentration of N-NH_4 by dilution of the substrate enables efficient biomass growth. It should be noted that also other compounds in ADE could limit the growth of microalgae, including calcium, magnesium, potassium and sodium; however, their concentrations are usually below the inhibition threshold [37].

2.3. *Efficiency of the Orthophosphates' Utilization*

Depending on the variant of the experiments, the concentration of orthophosphates in the medium ranged from about 3.0 mg P-PO_4/dm^3 in the variant I to nearly 12.0 mg P-PO_4/dm^3 in the variant III (Figure 3). Irrespective of the series, very efficient use of orthophosphates by growing biomass of microalgae *Chlorella* sp. was observed. In the series I of variant I, on the seventh day of cultivation in the medium, no mineral phosphorus compounds were noted. In the other series, the concentration of orthophosphates was also close to zero. Increasing the doses of ADE introduced into the medium influenced the orthophosphates' utilization. The orthophosphates were observed until the end of the experiment. The concentrations of P-PO_4 in the medium at the end of the experiments were very low. This indicates that orthophosphates are a limiting factor and might reduce the growth rate of algae biomass. Thus, probably, supplementation of ADE with phosphorus compounds would be necessary.



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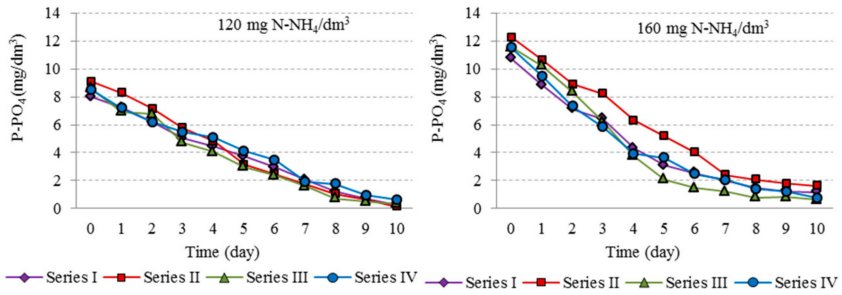

Figure 3. The changes of P-PO_4 concentration in the medium during cultivation of *Chlorella vulgaris* in the variants and series of experiments.

2.4. Utilization of ADE for Microalgae Cultivation

In the study, a significant ($p < 0.05$) decrease in concentrations of biogenic compounds (N-NH_4, P-PO_4) in the medium after cultivation of *Chlorella vulgaris* was observed. The highest technological effects were observed in the series III and series IV, in which the effluents originated from the fermentation of the substrates with a high share of bovine slurry (from 40%–80%); while medium composed of the effluents originating from the fermentation of the substrates with a high share of distillery stillage lowered the biomass production and nutrients' removal. The literature data present different systems using microalgae in wastewater treatment. Sawayama et al. [38] used microalgae *Botryococcus braunii* for removal of N_{tot} and P_{tot} from effluent obtained from a reactor with activated sludge. Yun et al. [39] used microalgae *Chlorella vulgaris* in the removal of ammonia from wastewater that was obtained in a steel mill. The ammonia assimilation rate was at the level of 0.022 g $NH_3/(dm^3 \cdot d)$. Microalgae *Chlorella pyrenoidosa* was cultivated in medium consisting of post-fermentation effluent and efficiently removed nutrients and organic compounds [40]. The concentration of microalgae biomass in the reactor was at the level of 1.25 g TS/dm^3. The authors obtained an efficiency of nitrogen removal of 78.76%, phosphorus removal of 94.78%, COD removal of 98.34% between six and eight days of cultivation. Martinez et al. [41] observed significant reduction of nitrogen and phosphorus compounds' concentration by *Scenedesmus obliquus* during municipal wastewater treatment in open reactors. The authors obtained the efficiency of phosphorus compound removal at a level of 98% and the total elimination of ammonium nitrogen from treated wastewater. Gomez Villa et al. [42] cultivated *Scenedesmus obliquus* on the fractional-technical scale in a medium composed of synthetic wastewater. The removal of nitrogen compounds was 53% in the summer and 21% in the winter. The phosphorus removal was observed only during the day and was reduced during the night. The efficiency of phosphorus removal was 73% in the summer and 45% in the winter. Hodaifa et al. [43] used *Scenedesmus obliquus* for treatment of industrial wastewater obtained during olive oil extraction. The authors noted a reduced BOD concentration of 67.4%. In order to improve the efficiency of the removal of organic compounds from industrial wastewater, Mùnoz et al. [44] used a technological solution in which the microalgae *Chlorella vulgaris* was immobilized as a biofilm on the elements of a photobioreactor. Depending on the experimental variant, the efficiency of organic compounds' removal was higher in the range from 19%–40%, in comparison to a typical photobioreactor with suspended microalgae biomass.

3. Materials and Methods

3.1. Experimental Setup

The efficiency of production of *Chlorella vulgaris* biomass on a medium with the addition of ADE was investigated in closed, vertical photobioreactors at the laboratory scale. Due to the type of substrate used, a series of experiment was distinguished: series I, effluent obtained after dewatering of anaerobic sludge from the methane fermentation reactor fed with distillery stillage (DS) and maize

silage (MS) (in a ratio of 70% VS:30% VS); series II, effluent obtained after dewatering of anaerobic sludge from the methane fermentation reactor fed with DS, MS and bovine slurry (BS) (in a ratio of 67% VS:23% VS:10% VS); series III, effluent obtained after dewatering of anaerobic sludge from the methane fermentation reactor fed with DS, MS and BS (in a ratio of 45% VS:15% VS:40% VS); series IV, effluent obtained after dewatering of anaerobic sludge from the methane fermentation reactor fed with DS, MS and BS (in a ratio of 15% VS:5% VS:80% VS). Additionally, the efficiency of biomass production depending on the concentration of ammonium nitrogen in the substrate in each series was tested. The variants of the experiments were obtained by dilution of the substrate and obtaining the following initial concentrations of ammonium nitrogen of 40, 80, 120 and 180 mg $N\text{-}NH_4/dm^3$.

3.2. Materials

Microalgae *Chlorella vulgaris* was obtained from culture collection UTEX 2714 (University of Texas at Austin). Anaerobic digestion effluent was obtained from the laboratory reactors with an active volume of 4 dm^3 (total volume of 5 dm^3) with an anaerobic sludge concentration of 5 g TS/dm^3. The reactors were operated at a temperature of 37 °C. The organic loading rate in the reactors was 3 kg $VS/(m^3 \cdot d)$. The ADE before cultivation was centrifuged with a rotational speed of 5000 rpm for 10 minutes and then sterilized at 90 °C for 30 min (Tuttnauer 2840 EL–D, Tuttnauer Europe B.V., Breda, The Netherlands). The aim of this pretreatment was to remove suspended solids, obtain supernatant-containing substances in the dissolved phase and disinfect the medium, which protected the purity of microalgae cultures. The characteristics of the ADE used in the experiments are presented in Table 1.

Table 1. Values of the basic indicators of the anaerobic digestion effluents (ADE) used in the experiments.

Indicator	Unit	Series I	Series II	Series III	Series IV
COD	mg O_2/dm^3	7800 ± 520	7150 ± 670	6420 ± 390	5200 ± 530
BOD	mg O_2/dm^3	3400 ± 390	3100 ± 450	2840 ± 370	2770 ± 470
N_{tot}	mg N/dm^3	1400 ± 230	1420 ± 170	1290 ± 140	1130 ± 270
$N\text{-}NH_4$	mg $N\text{-}NH_4/dm^3$	1150 ± 210	1010 ± 140	970 ± 130	910 ± 160
P_{tot}	mg P/dm^3	74 ± 16	68 ± 17	59 ± 21	61 ± 13
$P\text{-}PO_4$	mg $P\text{-}PO_4/dm^3$	49 ± 10	52 ± 11	48 ± 13	43 ± 10
pH	-	6.8 ± 0.3	7.1 ± 0.2	7.0 ± 0.2	7.2 ± 0.3

In all variants of the experiment, in order to ensure the initial concentration of $N\text{-}NH_4$ and a similar initial concentration of algae biomass, to the photobioreactors was introduced 1540 cm^3 of the culture of microalgae from self-cultivation with a concentration of 401 ± 42 mg TS/dm^3 and supplemented to the level of 2500 cm^3 with tested substrates and deionized water. The volume of substrates and deionized water in subsequent variants of the experiment is presented in Table 2. The initial *Chlorella vulgaris* biomass concentration was about 250 mg TS/dm^3. The characteristics of the culture medium at the beginning of the experiment are shown in Table 3.

Table 2. The volume of ADE and deionized water introduced to the photobioreactors.

Variant	Concentration of $N\text{-}NH_4$ (mg $N\text{-}NH_4/dm^3$)	Series I		Series II		Series III		Series IV	
		ADE (cm^3)	Water (cm^3)	ADE (cm^3)	Water (cm^3)	ADE (cm^3)	Water (cm^3)	ADE (cm^3)	Water (cm^3)
1	40	87	873	100	860	103	857	130	830
2	80	174	786	200	760	206	754	260	700
3	120	261	699	300	660	309	651	390	570
4	160	348	612	400	560	412	548	520	440

Table 3. The initial concentrations of indicators in the medium depending on variant and series of the experiment.

Concentration of $N\text{-}NH_4$ (mg $N\text{-}NH_4/dm^3$)	Indicator	Unit	Series I	Series II	Series III	Series IV
40	COD	mg O_2/dm^3	272.4 ± 11.1	287.0 ± 12.5	265.5 ± 9.9	229.7 ± 19.8
	BOD	mg O_2/dm^3	119.3 ± 8.9	125.0 ± 7.5	117.9 ± 6.7	122.8 ± 7.7
	N_{tot}	mg N/dm^3	49.7 ± 7.8	57.8 ± 5.6	54.1 ± 4.4	50.7 ± 5.2
	$N\text{-}NH_4$	mg $N\text{-}NH_4/dm^3$	41.0 ± 2.3	41.4 ± 3.1	40.9 ± 1.9	41.0 ± 3.8
	P_{tot}	mg P/dm^3	3.5 ± 1.5	3.7 ± 1.6	3.3 ± 1.4	3.6 ± 1.3
	$P\text{-}PO_4$	mg $P\text{-}PO_4/dm^3$	2.7 ± 0.9	3.0 ± 1.1	2,9 ± 1.2	2.8 ± 1.1
	pH	-	7.2 ± 0.2	7.2 ± 0.2	7.1 ± 0.2	7.0 ± 0.1
80	COD	mg O_2/dm^3	544.8 ± 23.1	573.9 ± 21.1	530.9 ± 19.2	459.5 ± 29.2
	BOD	mg O_2/dm^3	238.6 ± 10.9	249.9 ± 13.1	235.9 ± 9.9	245.7 ± 10.0
	N_{tot}	mg N/dm^3	99.4 ± 8.9	115.5 ± 6.6	108.2 ± 7.8	101.4 ± 6.6
	$N\text{-}NH_4$	mg $N\text{-}NH_4/dm^3$	82.0 ± 1.9	82.7 ± 0.9	81.8 ± 0.5	82.0 ± 0.6
	P_{tot}	mg P/dm^3	7.1 ± 1.1	7.4 ± 0.8	6.6 ± 0.9	7.3 ± 1.2
	$P\text{-}PO_4$	mg $P\text{-}PO_4/dm^3$	5.3 ± 0.6	6.1 ± 0.9	5.7 ± 0.7	5.7 ± 0.8
	pH	-	7.2 ± 0.2	7.3 ± 0.2	7.2 ± 0.2	7.2 ± 0.3
120	COD	mg O_2/dm^3	817.2 ± 33.9	860.9 ± 34.6	796.4 ± 28.9	689.3 ± 24.5
	BOD	mg O_2/dm^3	357.9 ± 12.3	374.9 ± 13.9	353.9 ± 15.5	368.5 ± 14.7
	N_{tot}	mg N/dm^3	149.1 ± 18.9	173.3 ± 9.7	162.3 ± 21.1	152.0 ± 17.9
	$N\text{-}NH_4$	mg $N\text{-}NH_4/dm^3$	123.0 ± 2.1	124.1 ± 2.8	122.7 ± 3.0	123.0 ± 2.2
	P_{tot}	mg P/dm^3	10.6 ± 1.7	11.0 ± 2.0	9.9 ± 0.9	10.9 ± 1.2
	$P\text{-}PO_4$	mg $P\text{-}PO_4/dm^3$	8.0 ± 0.7	9.1 ± 1.0	8.6 ± 0.5	8.5 ± 0.8
	pH	-	7.0 ± 0.1	7.0 ± 0.2	7.3 ± 0.3	7.1 ± 0.3
160	COD	mg O_2/dm^3	1089.6 ± 156.7	1147.8 ± 99.1	961.8 ± 109.1	919.0 ± 181.1
	BOD	mg O_2/dm^3	477.1 ± 39.8	499.8 ± 28.5	471.9 ± 41.1	491.3 ± 35.6
	N_{tot}	mg N/dm^3	198.7 ± 18.9	231.0 ± 26.7	216.4 ± 15.8	202.7 ± 19.9
	$N\text{-}NH_4$	mg $N\text{-}NH_4/dm^3$	163.9 ± 2.1	165.4 ± 3.0	163.7 ± 2.8	164.0 ± 1.9
	P_{tot}	mg P/dm^3	14.2 ± 1.2	14.7 ± 1.5	13.3 ± 1.5	14.6 ± 1.1
	$P\text{-}PO_4$	mg $P\text{-}PO_4/dm^3$	10.7 ± 2.5	12.2 ± 2.3	11.5 ± 2.1	11.4 ± 2.0
	pH	-	7.3 ± 0.1	7.2 ± 0.1	7.0 ± 0.1	7.2 ± 0.2

3.3. Experimental Stand

Cultivation of microalgae was performed in photobioreactors of an active volume of 2.5 dm^3 (Figure 4), diameter of 7.6 cm and height of 55 cm. Carbon dioxide was introduced to the cultures by air aeration with an intensity of 250 dm^3/h. The cultures were illuminated at 700 lux by cool-white light. The temperature of the culture was 22 ± 2 °C.

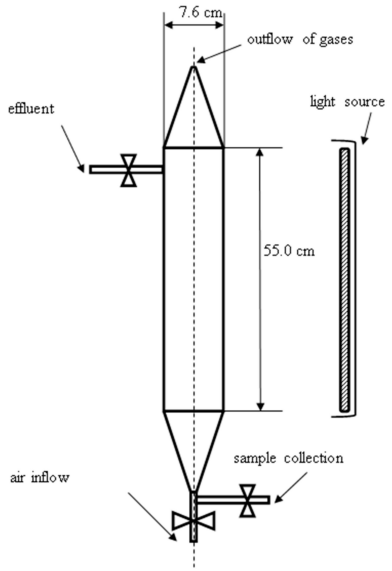

Figure 4. Scheme of the photobioreactor.

3.4. Analytical Procedures

Analyses of the microalgae biomass concentration and nitrogen ammonium concentration in the medium were done once a day. The volume of the collected samples was 50 cm^3, and the medium was refilled with deionized water to a volume 2500 cm^3. The samples were centrifuged in laboratory centrifuge MPW-251 (MPW Med. Instruments, Warsaw, Poland) with a rotational speed of 5000 rpm for 10 min. Then, in the retentate, the concentration of total solids using a gravimetric method and in the supernatant the concentration of ammonium nitrogen N-NH_4 using a cuvette tests and the spectrophotometer DR 5000 (Hach-Lange, London, ON, Canada) were determined. In the effluent and in the culture medium, at the beginning and end of the experiments, the BOD was determined using an Oxi-Top control system (WTW, Germany) and the COD, P-PO4, P_{tot} (total phosphorus) and N_{tot} (total nitrogen) were determined using the spectrophotometer DR 5000 with the mineralizer HT 200s (Hach-Lange). The pH was measured using the pH meter 1000L (VWR International, Radnor, PA, USA). The light intensity was measured with the Luxmeter HI 97500 (Hanna Instruments, Woonsocket, RI, USA). Online detection of chlorophyll concentration, algae classes and photosynthetic activity was done with BBE AlgaeOnLine Analyser (Moldaenke, Schwentinental, Germany).

3.5. Statistical Analysis

Each variant of the experiment was carried out in triplicate. The statistical analysis of the obtained results was done using the STATISTICA 10.0 PL package (StatSoft, TX, USA). The verification of the hypothesis concerning the distribution of each tested variable was determined on the basis of the W Shapiro–Wilk test. In order to determine the significance of differences between variables, a one-way analysis of variance (ANOVA) was carried out. After testing for homogeneity of variance with Levene's test, the significance of differences between variants was tested with Tukey's HSD test. Differences were considered significant at $p < 0.05$.

4. Conclusions

The study proved that anaerobic digestion effluent might be used as a nutrient source for efficient biomass production of *Chlorella vulgaris*. The cultivated microalgae biomass efficiently utilized N-NH_4 and P-PO_4 from the medium. It was found that the high content of the effluent obtained after dewatering of anaerobic sludge from the methane fermentation reactor fed with distillery stillage (series I and series II) decreased the growth rate of microalgae biomass. The effect could be related to too high of a concentration of organic compounds in the medium or limited light penetration by the turbidity of the medium and, thus, reduced photosynthesis.

Supplementary Materials: The following are available online at http://www.mdpi.com/1996-1073/11/7/1654/s1: Table S1: Changes in *Chlorella vulgaris* biomass concentration in cultivation medium depending on the variant and series of the experiment.

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