fermentation

MDPI

Article

Algal Biomass Accumulation in Waste Digestate after Anaerobic Digestion of Wheat Straw

Lyudmila Kabaivanova [1,*], Juliana Ivanova [2], Elena Chorukova [1], Veneren Hubenov [1], Lilyana Nacheva [1] and Ivan Simeonov [1]

[1] The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 26, 1113 Sofia, Bulgaria
[2] Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria
* Correspondence: lkabaivanova@yahoo.com; Tel.: +359-2-9793167

Abstract: Cultivation of microalgae in waste digestate is a promising cost-effective and environmentally friendly strategy for algal biomass accumulation and valuable product production. Two different digestates obtained as by-products of the anaerobic fermentation at 35 °C and 55 °C of wheat straw as a renewable source for biogas production in laboratory-scale bioreactors were tested as cultivation media for microalgae after pretreatment with active carbon for clarification. The strains of microalgae involved were the red marine microalga *Porphyridium cruentum*, which reached 4.7 mg/mL dry matter when grown in thermophilic digestate and green freshwater microalga-*Scenedesmus acutus*, whose growth was the highest—7.3 mg/mL in the mesophilic digestate. During cultivation, algae reduced the available nutrient components in the liquid digestate at the expense of increasing their biomass. This biomass can find further applications in cosmetics, pharmacy, and feed. The nitrogen and phosphorus uptake from both digestates during algae cultivation was monitored and modeled. The results led to the idea of nonlinear dynamic approximations with an exponential character. The purpose was to develop relatively simple nonlinear dynamic models based on available experimental data, as knowing the mechanisms of the considered processes can permit creating protocols for industrial-scale algal production toward obtaining economically valuable products from microalgae grown in organic waste digestate.

Keywords: anaerobic digestion; waste digestate; microalgae cultivation; modeling

Citation: Kabaivanova, L.; Ivanova, J.; Chorukova, E.; Hubenov, V.; Nacheva, L.; Simeonov, I. Algal Biomass Accumulation in Waste Digestate after Anaerobic Digestion of Wheat Straw. *Fermentation* **2022**, *8*, 715. https://doi.org/10.3390/fermentation8120715

Academic Editors: Prashant Praveen and Sheetal Parakh

Received: 4 October 2022
Accepted: 5 December 2022
Published: 7 December 2022

1. Introduction

Microbial fermentation processes in biosphere are responsible for the greater part of the biologically driven hydrogen and methane. Biomethane could be produced by processing various types of waste. Anaerobic digestion (AD) is a well-known biological process used for the utilization of organic waste for green energy production [1]. Increasing biogas production worldwide, rich in biomethane, that could be used for heat and/or electricity generation, will meet the energy supply needs with renewable alternatives [2]. The improvement in various aspects and parameters in AD, such as pretreatments, reactor types, co-digestion, process modeling, and control, promotes reaching a better insight into the process and improving its stability and efficiency [3].

Great quantities of digestate are thus produced after anaerobic digestion of organic wastes, which causes problems related to transport costs, gas emissions, and sludge accumulation. At the same time, nutritional substances, such as nitrogen and phosphorus, remain available therein. It is necessary to find alternative pathways for valorization with the aim of reducing the environmental impact and improving the economic profitability of anaerobic installations [4]. Waste digestate from biogas production can be used for direct treatment of agricultural crops instead of fertilizing, but in most cases, the organic matter

in it is very concentrated, and dilution and storage space are required. The increasing production of digestate causes problems related to transport costs, emissions of greenhouse gases as well as accumulation of increased amounts of nitrogen and phosphorus that can be dangerous. Therefore, it is necessary to find alternative ways of valorization in order to reduce the environmental impact and improve economic profitability. Current management practices exist that involve utilization of digestate for land application either as fertilizer or soil improver [5]. Another approach is to involve the obtained waste digestate as a source of nutrients in a medium for microalgal growth and development. Growth enhancement techniques are sought for cost-effective algal biomass accumulation. Nitrogen and phosphorus are utilized, and no contaminants remain in the waste effluents [6]. Records exist on the traditional and ancient use of wild harvested microalgae as human and animal food, but their cultivation for different purposes started recently [7]. Large-scale microalgae cultivation could decisively contribute to sustainable industrial biomass production for obtaining effective high-value products. Microalgae have attracted much attention recently due to their extensive application potential in renewable energy [8], feed [9], cosmetics [10], biomedicine [11], agriculture [12], etc. Microalgae are considered renewable, sustainable, and economical sources. They can act as bioactive medicinal products and find their place as food additives or natural colorants. Several microalgal species have been investigated for their potential to obtain valuable products with significant pharmacological and biological qualities. Biofertilization is another field of application as a sustainable agricultural practice that applies biofertilizers to increase the soil nutrient content, leading to higher productivity, being at the same time eco-friendly with no pollution. It leads to clean products for clean food and health benefits [13]. Another application of biomass from microbial sources, especially microalgae, is to absorb heavy metal ions as remediation practices [14] or treat municipal, industrial, agro-industrial, and livestock wastewaters [15]. These photosynthetic organisms have their role in atmospheric CO_2 mitigation, which is a major concern related to global warming. Microalgae can be cultivated under various conditions as they adapt easily, using light as an energy source to convert water and carbon dioxide into biomass via photosynthesis [16]. However, the main challenge in the large-scale production of microalgae is to create stable cultures maintained for long periods, for which suitable media and conditions should be established. In order to make algal biomass cheaper so that it can be used for biofuels and other value-added products, it is necessary to reduce the cost of cultivation. Cultivating microalgae in anaerobic digestate as a medium with no addition of fresh water appears as a promising solution for nutrient recovery, pollutant removal, and biofuel production [17]. The digestate represents a mixture of undigested substrates, microbial biomass, and various metabolites. Anaerobic digestate contains excessive amounts of phosphorous and nitrogen that can be utilized in algal biomass accumulation, as for their growth, algae also need nitrogen and phosphorus as major nutrients [18]. Algal growth was also documented in waste water [19]. An innovative approach to wastewater treatment is the cultivation of algae in it, but in many cases, the waters are toxic, and the cultivated algae are unsuitable for use in medicine, pharmacy, and the food industry. In other cases, microalgal cultivation could be carried out in a mixotrophic mode—using extraneous carbon sources, such as glucose and glycerol, thus enhancing the overall biomass concentration and lipid accumulation [20].

The use of algae from two different taxonomic groups enables further production of many secondary metabolites, for the saltwater *Porphyridium cruentum*, large amount of extracellular polysaccharide that is used in cosmetics, pharmacy, etc., and of unsaturated fatty acids and phycobiliproteins, which are also widely used. The green microalga *Scenedesmus*, in turn, accumulates a large amount of protein and green pigments, which are a suitable nutritional supplement for animals, fish, and humans. At the same time, *Scenedesmus acutus* accumulates biomass quickly and is easily adaptable in terms of cultivation conditions. By being able to cultivate algae in waste digestate from different taxonomic groups, growing in different environments (freshwater, saltwater), we show the high adaptability of algae and their potential to grow in an unnatural habitat for them. This fact is a prerequisite for

the creation of circular economy and valorization of processes, and precisely in this, the novelty and significance of this research are expressed.

The aim of this study was to show the possibility of growing red and green microalgae in two types of digestates obtained from anaerobic digestion of wheat straw at mesophilic and thermophilic conditions with stable biomethane production, together with process modeling to predict the dynamics of biomass accumulation and pigment formation as a function of the initial nitrogen and phosphorus values.

2. Materials and Methods

2.1. Anaerobic Digestion

Two different biotechnological processes were performed for anaerobic digestion of wheat straw at mesophilic conditions (35 °C) and at thermophilic conditions (55 °C). Anaerobic digestion processes were performed on a laboratory scale, as previously described [21]. Characteristics of the source digestates—total solids (TS), volatile solids (VS), chemical oxygen demand (COD), nitrogen, phosphorus, and pH are presented in Table 1. Biogas content measurements were performed by "Dräger" (Lübeck, Germany) type specimen X-am 7000.

Table 1. Characteristics of the source digestate.

Parameter	Digestate From:	
	Mesophilic Process	Thermophilic Process
TS, g/L	9.27 ± 0.04	6.31 ± 0.03
VS, %TS	60.21 ± 0.43	63.46 ± 0.72
pH	7.62 ± 0.01	7.88 ± 0.01
COD, mg/L	668 ± 0.04	591 ± 0.04
NH_4-N, mg/L	177 ± 0.10	175 ± 0.13
PO_4-P, mg/L	3.79 ± 0.06	3.76 ± 0.05

2.2. Microalgal Cultures

Monoalgal, non-axenic cultures of green *Scenedesmus acutus* (Meyen) Puncocharova 1981 (Chlorophyta) and red microalga *Porphyridium cruentum* (AG.) NAG Vischer 1935/107 (Rhodophyta) from the culture collection of the Institute of Botany ASCR, Třeboň, Czech Republic, was used. An initial algal culture density of 0.8 mg/mL dry weight (DW) for *Porphyridium cruentum* and 0.5 mg/mL for *Scenedesmis acutus* was used for all experiments. Cultivation was carried out at 25 °C and continuous illumination (132 µmol photons m^{-2} s^{-1}). Carbon source was provided by bubbling sterile 2% CO_2 (*v*/*v*). Standard culture medium of Setlik [22] was used as control for cultivation of *Scenedesmus* acutus and modified culture medium of Hemerick, [23]—for *Porphyridium cruentum*. 18 g/L of NaCl were added to the medium for *Porphyridium cruentum* from digestate since it is marine.

Growth of algal cultures was measured by dry weight as the algal suspension was centrifuged (Rotofix 32A, Hettich, Tuttlingen, Germany), then supernatant was removed, and cells were dried at 105 °C for 16 h. Dry biomass concentration (mg/mL) was calculated according to Makarevičienė et al. [24].

2.3. Clarification with Active Carbon

A definite quantity (100 mL) of both mesophilic and thermophilic digestates was centrifuged at 15,000 rpm and the supernatant was further decolorized. Adsorption was performed by introducing active carbon (Fluka) with varying concentrations (5–40 g/L) to the liquid digestate taken at the end of the process for biomethane production: C 3345 Fluka ≥ 95% for general laboratory use, Formula: C, MW: 12.01 g/mol, Melting Pt: 3550 °C, Storage Temperature: Ambient. Active carbon was used for clarification as the digestate has to become appropriate for light penetration necessary for cultivation of algae. After 24 h at room temperature, another centrifugation followed, and the obtained supernatant was used as a cultivation medium.

2.4. Nitrogen and Phosphorus Quantity

Uptake of nitrogen and phosphorus from digestate during algae cultivation was monitored, using DR 3900 Spectrophotometer (Hach Lange, GmbH, Munich, Germany) by respective test kits PO_4-P, (LCK 350); TN, (LCK 338); NH_4-N (LCK 302); COD (LCK 314) with RFID technology.

2.5. Pigment Content

Pigment content was measured to prove growth. Pigments—chlorophyll "a", chlorophyll "b" and carotenoids, were measured spectrophotometrically at 665, 645, and 460 nm, respectively, using a T70 UV/Vis (PG Instruments Ltd., Leicester, UK) spectrophotometer after extraction with boiling methanol. Using the absorptions, the pigment content was calculated, employing the Mackiney formulas [25]. Phycobiliproteins were extracted with 0.01 M potassium phosphate buffer (pH 6.7) from homogenized cells (vibrations homogenizator VHG1, City, Germany) at 4 °C for 10 min. The quantities were calculated according to the equations of Siegelman and Kycia [26].

2.6. Mathematical Modeling

The numerical computations were carried out on a PC/Intel Core i5-2320 CPU@2.67 GHz, 4 GB Memory (RAM), Windows 10 (64 bit) operating system. Modeling and numerical experiments were performed using Matlab R2016a. Sequential quadratic programming (SQP) techniques were used as a constrained nonlinear optimization method. The background of the basic SQP algorithm applied here was described in [27].

To quantitatively compare the model with the experimental data, the least squares method was applied, using the formula: $\mathrm{NRMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\frac{\hat{y}_i - y_i}{y_{max} - y_{min}}\right)^2}$, where: y_i—experimental data in hour "i"; model data in hour "i"; y_{max} and value y_{min} are respectively the maximal and minimal value of the corresponding experimental data.

3. Results and Discussion

3.1. Digestate Preparation and Clarification

The liquid fraction of digestate produced is rich in macro and micronutrients, nitrogen, and phosphorous that can be utilized by growing microalgal cultures. Recent studies have focused attention on the possibility of digestate application as a nutrient medium for growing microalgae [28,29]. In this work, liquid digestate from a digester fed on waste wheat straw was collected at the end of the process for biomethane production. Biochemical characteristics of both—mesophilic and thermophilic digestate solutions are given in Table 1. Active carbon was chosen as a suitable adsorbent to reduce the color of liquid digestate in this study. Active carbon is a highly porous carbon, usually obtained from wood or bone charcoal, during the processing of which hydrocarbons have been removed, and its adsorption capacity has been increased. The treatment for clarification of the digestate leads to reducing the turbidity and color of this waste, respectively, increasing the light transmittance and penetration. It is necessary because light, together with carbon dioxide, is needed by algae to carry out the process of photosynthesis—its transformation into the chemical energy of carbohydrates with the release of oxygen which ensures a guaranteed high yield of the microalgae cultivated within this liquid waste. The active carbon was used in this decolorization process to obtain a digestate appropriate for algae cultivation as an alternative medium, and on the other hand, the active carbon involved could be produced from other waste, such as fruit pits, in this way, coping with waste disposal. Next step included microalgal cultivation in the transparent digestate without dilution. The digestate is not diluted because the clarification methodology allows achieving such clarity of the solution that algae can grow well without any need for dilution, thus not reducing the nutrient content without requirements of additional fresh water resources. Algal growth in undiluted digestate is negligible and not economically viable.

Growth in untreated digestate is also negligible as light penetration is lacking (Figure 1, left). The recycling of nutrients from waste digestate into a nutrient medium for algae is a challenge and a new opportunity to ensure a guaranteed high yield of bioresources with low energy, time consumption, and saving pure water.

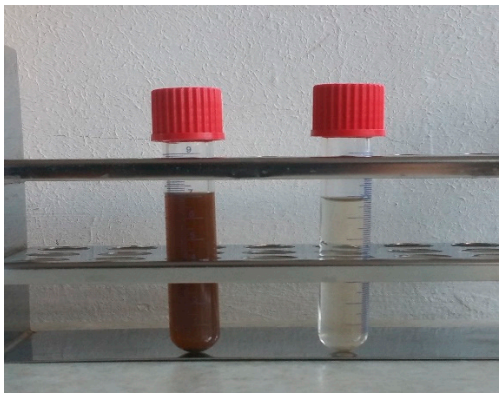


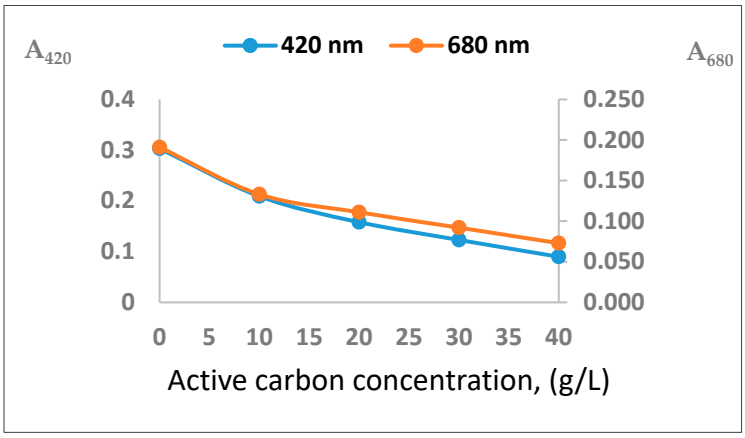
Figure 1. Digestate before treatment (left) and after clarification with active carbon (right).

Optical density at 680 nm and 420 nm was determined to quantify the clarifying effect of the adsorption process with active carbon. Having in mind that the primary pigment involved in the process of photosynthesis is chlorophyll "a"—with strong absorption bands in the regions 400–450 and 650–700 nm [30], tests were conveyed for estimation of the adsorption spectrum at 420 and 680 nm for the anaerobic digestates—mesophilic (Figure 2a) and thermophilic (Figure 2b) in dependence of the quantity of applied active carbon.

A slight difference was observed in the level of adsorption for the two types of digestates at room temperature because they represent by-products of two different anaerobic digestion processes—mesophilic and thermophilic. The similarity is revealed in obtaining an almost transparent medium for cultivation of algae. The values for 680 nm show that the decolorization is more than 10-fold, while for 420 nm, it is in the order of 72–75%.

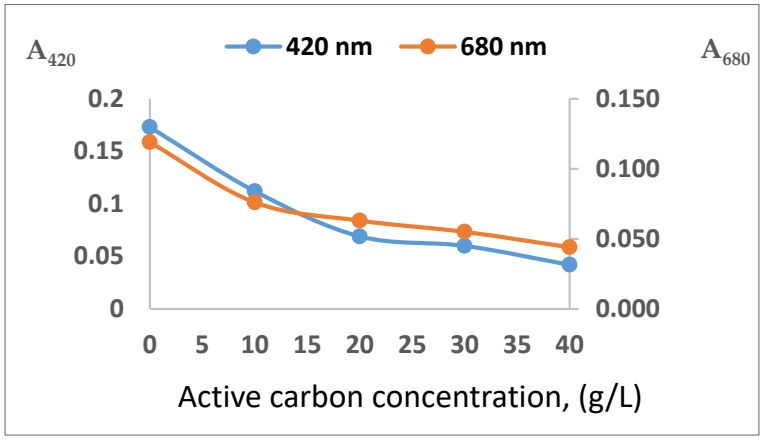
3.2. Algae Cultivation

After clarification, both investigated strains were introduced once in a mesophilic digestate and then in a thermophilic digestate. *Scenesdesmus acutus* growth was highest—7.3 mg/mL in the mesophilic digestate, higher than growth in the control medium (Figure 3a). *Porphyridium cruentum* reached 4.7 mg/mL dry matter when grown in thermophilic digestate (Figure 3b).



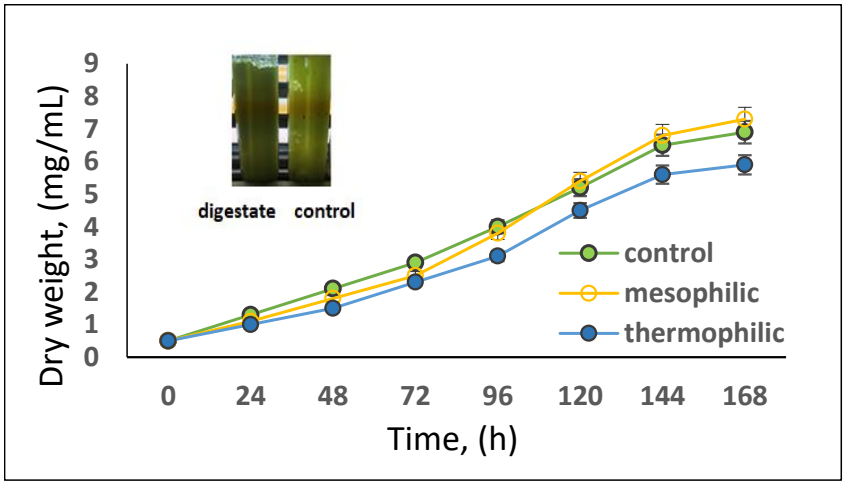
(**a**)

Figure 2. *Cont.*

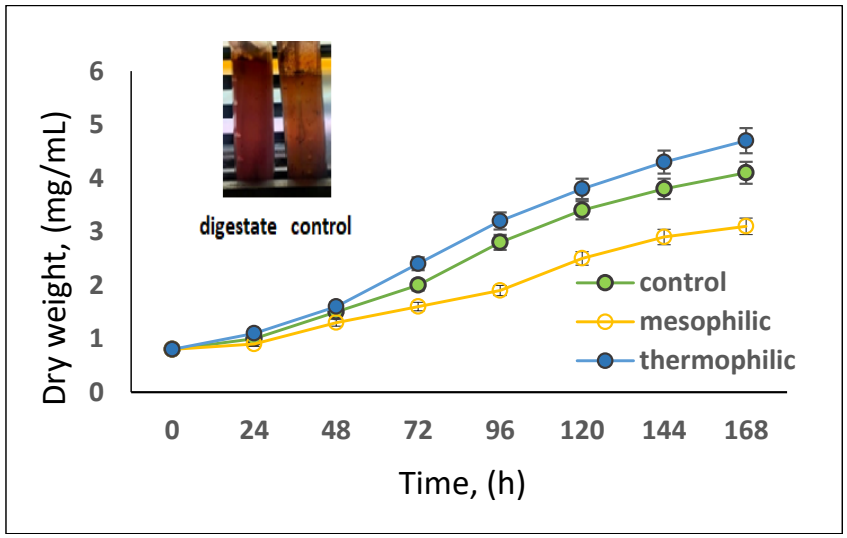


(**b**)

Figure 2. Effect of activated carbon concentration on decolorization rate for mesophilic (**a**) and thermophilic (**b**) digestate.



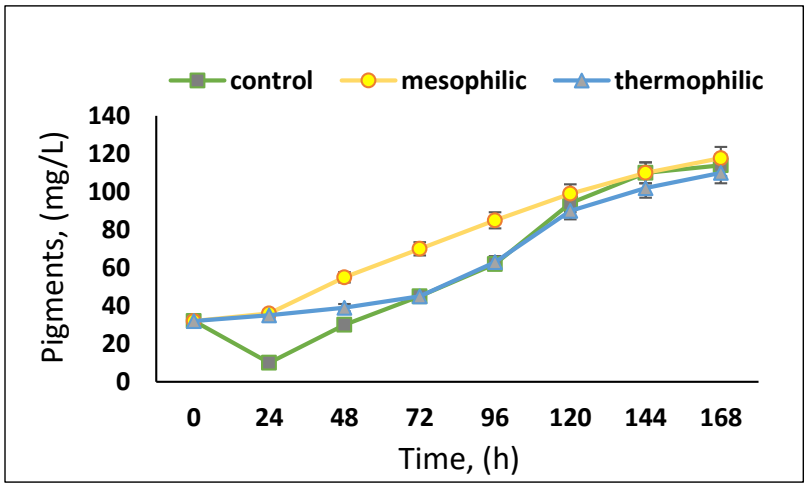
(**a**)



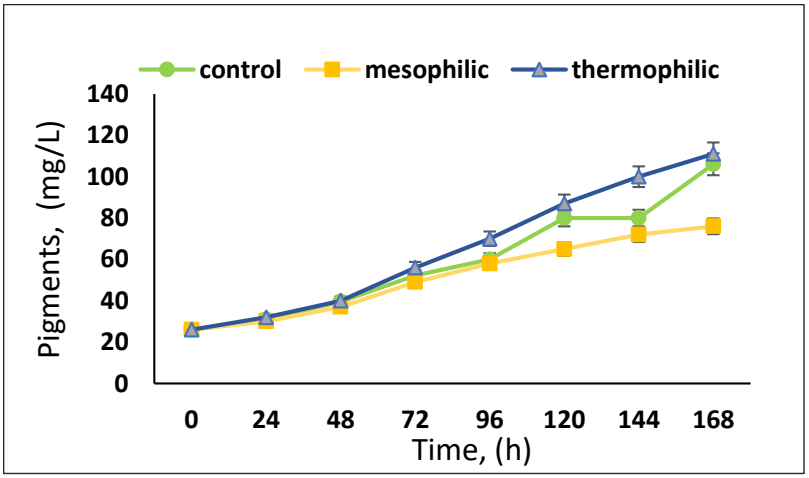
(**b**)

Figure 3. Growth of *Scenesdesmus acutus* (**a**) and *Porphyridium cruentum* (**b**) in the decolorized digestates.

Microalgae productive chains are attracting attention as sustainable alternatives to obtain natural pigments [31]. Pigment content was evaluated to prove growth. Total amount of pigments of *Scenedesmus acutus* at the end of the process was 117 mg/L, which represents a 4-fold increase since the process has begun, showing good growth and pigment synthesis (Figure 4a). For *Porphyridium cruentum* the increase in pigments content was also proved to be about 4-fold since the process beginning (Figure 4b). According to the investigations of Fernandes et al., *Chlorella vulgaris* grew better on all three digestates tested in comparison to the F/2 control medium [32], which complies with the results obtained in this study.



(**a**)



(**b**)

Figure 4. Pigment content during growth of *Scenedesmus acutus* (**a**) and *Porphyridium cruentum* (**b**) grown in digestate.

Indeed, digestate composition shows a vast potential to support microalgal growth, especially in terms of macronutrients, such as phosphorus and nitrogen, and together with remaining micronutrients in the right composition and ratio, it provided good development of algae with production of various products [33]. The characteristic pigments are presented for *Scenedesmus acutus*—chlorophyll a, chlorophyll b, and carotenoids (Figure 5a) and for *Porphyridium cruentum*—phycobiliproteins (PBP), carotenoids and chlorophyll a (Figure 5b).

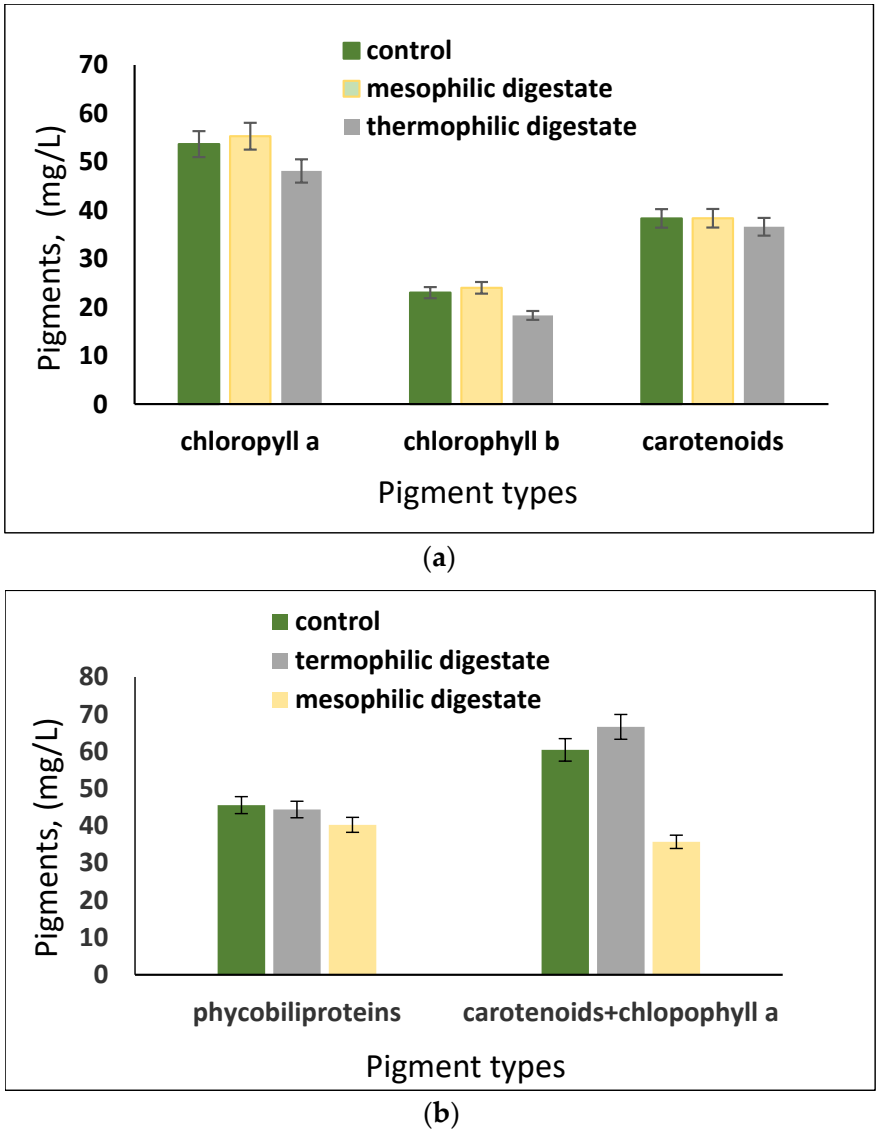


Figure 5. Pigment content of *Scenedesmus acutus* (**a**) and *Porphyridium cruentum* (**b**) at the end of cultivation.

3.3. Nitrogen and Phosphorous Uptake

Nitrogen and phosphorus are essential macronutrients needed to promote algal growth and metabolic activities [34]. The uptake of nitrogen and phosphorus from digestate necessary for growth during algae cultivation was monitored. Nitrogen decreased 3.5-fold during growth in thermophilic digestate of *Porphyridium cruentum* and 4.3-fold when *Scenedesmus acutus* grew in mesophilic digestate (Table 2).

During growth of *Scenedesmus acutus* in the mesophilic digestate, phosphorous was completely utilized. After growth of *Porphyridium cruentum* in the thermophilic digestate, the remaining quantity was only 0.12 mg/L (Table 3).

The high mineral content of digestate is the reason to try its direct application as a fertilizer in agriculture [35], but in recent years, after such fertilization, cases of vegetable production contaminated with pathogens have been identified [36]. It could also be easily washed away during rain falls. Application of microalgae as biofertilizers is beneficial as they introduce carbohydrates, as well as nitrogen and phosphorous that are being released gradually. Circular economy has appeared as a challenge with dual purposes implementation—to improve the production of economically valuable products together with a reduction of the environmental impact by decreasing the inflow of resources and

waste generation [37]. After the accumulation of algal biomass, value-added products could be obtained from microalgae that could find further applications. The remaining part of it could be returned back into the anaerobic bioreactor working on lignocellulosic substrates as a co-substrate for enhancing biogas and biomethane production [38] or used as food supplements. Microalgae also favor soil nutrient cycling and promote plant growth by improving nutrient availability for plants [39]. The produced by algae exopolysaccharides also act to improve soil structure and contribute to the stabilization of soils by the formation of biological soil crusts [40], together with the algal cell itself, containing high levels, from 30 to 50% sulfated polysaccharides of its dry matter [41]. Thus, they appear as promising sources of plant biostimulant development [42].

Table 2. Nitrogen uptake during growth for the two investigated strains.

	Scenedesmus acutus			*Porphyridium cruentum*		
Time	**Control**	**Mesophilic Digestate**	**Thermophilic Digestate**	**Control**	**Mesophilic Digestate**	**Thermophilic Digestate**
	N (mg/L)	**N (mg/L)**	**N (mg/L)**	**N (mg/L)**	**N (mg/L)**	**N (mg/L)**
0 h	200	176	173	280	139	140
24 h	170	134	168	260	130	123
48 h	156	98	160	248	124.8	116
72 h	148	92.3	154.1	225	112.1	109
96 h	127	84	130	219	109	92
120 h	114	77	117	212	108	70.2
144 h	100.6	60.2	90.6	200	100	55.6
168 h	94	41	88	180	99	40

Table 3. Phosphorus uptake during growth for the two investigated strains.

	Scenedesmus acutus			*Porphyridium cruentum*		
Time	**Control**	**Mesophilic Digestate**	**Thermophilic Digestate**	**Control**	**Mesophilic Digestate**	**Thermophilic Digestate**
	P (mg/L)	**P (mg/L)**	**P (mg/L)**	**P (mg/L)**	**P (mg/L)**	**P (mg/L)**
0 h	42.5	3.88	3.52	38.75	3.77	3.98
24 h	37	3.42	3.46	29	3.65	3.62
48 h	25	2.6	2.8	18	3.51	3.1
72 h	18	2.1	2.4	12.2	3	2
96 h	11.4	1.8	2.1	8.3	2.1	1.78
120 h	8	0.87	2	5.9	1.9	0.6
144 h	4.1	0.3	1.1	3.2	0.66	0.33
168 h	3.63	0	0.3	1.3	0.18	0.12

3.4. Modeling of the Obtained Experimental Data

To our knowledge, mathematical models related in a dynamic way to the concentrations of nitrogen (N) and phosphorus (P) as substrate with biomass concentration and pigment content does not exist. The purpose of modeling in this work was to develop relatively simple nonlinear dynamic models based on the available experimental data because many of the mechanisms of the considered processes are unknown or poorly studied.

The analysis of the obtained experimental data for the two investigated strains of microalgae in this study led us to the idea of nonlinear dynamic approximations with an exponential character. They were created in two stages based on the available results:

1. Modeling of nitrogen (N(t)) and phosphorus (P(t)) uptake;
2. Modeling of biomass accumulation (X(t)) and pigments (Pigm(t)) based on the first stage.

As a result of the analysis of the experimental data, the following structure of the model is proposed:

$$N(t) = N_0 e^{-K_N t} \quad (1)$$

$$P(t) = P_0 e^{-K_P t} \quad (2)$$

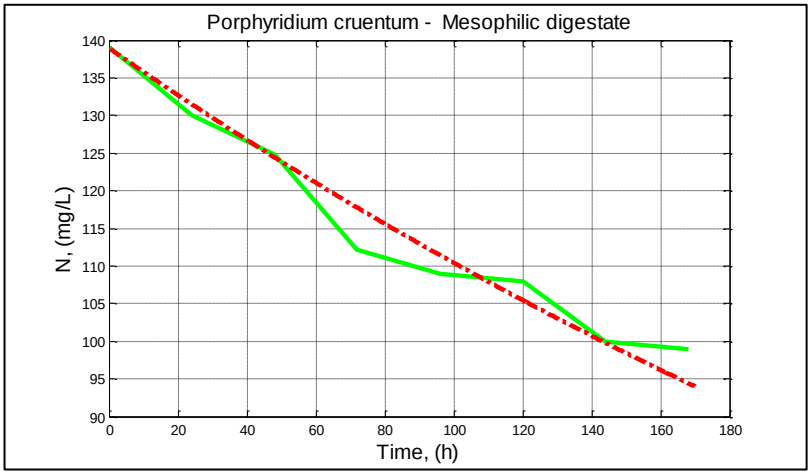
$$X(t) = A_0 + A_1 e^{bN(t)+cP(t)} \quad (3)$$

$$Pigm(t) = B_0 + B_1 e^{dN(t)+fP(t)} \quad (4)$$

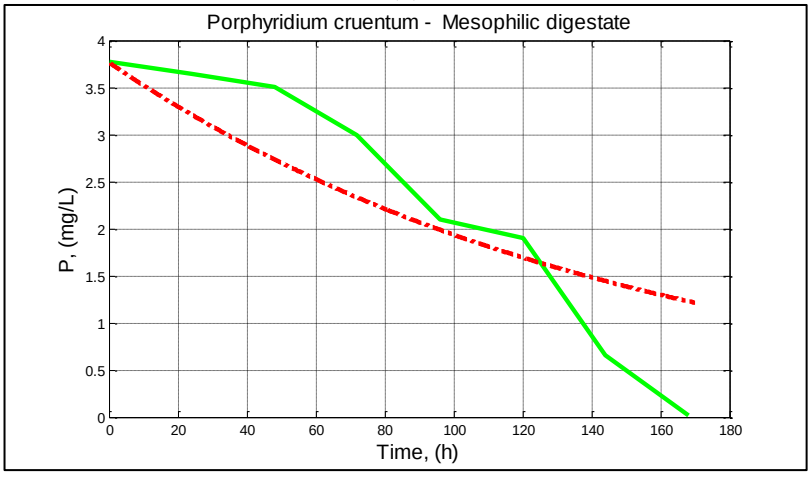
Equations (1) and (2) correspond to the first stage (consumption of N and P in time). In them, N_0 and P_0 are the initial conditions of the variables (they are known from experimental data), and K_N and K_P are unknown coefficients that must be identified based on experimental data.

As a result of the performed parametric identification for the digestate from a mesophilic AD process and strain *Porphyridium cruentum*, the following optimal coefficients were obtained: $K_N = 0.0023$, $K_P = 0.0067$.

Grafical comparisons of results obtained in the first stage (for N and P) are presented in Figure 6a,b.



(**a**)



(**b**)

Figure 6. Experimental data (in green) and model approximation (in red) for: (**a**) N(t), $N_0 = 139$, $K_N = 0.0023$; (**b**) P(t), $P_0 = 3.77$, $K_P = 0.0067$.

Visually, it was revealed that the model fits quite well with the experimental data for N(t) and P(t). Quantitative verification was done for the final form of the model by the method of calculating the normalized root mean square error.

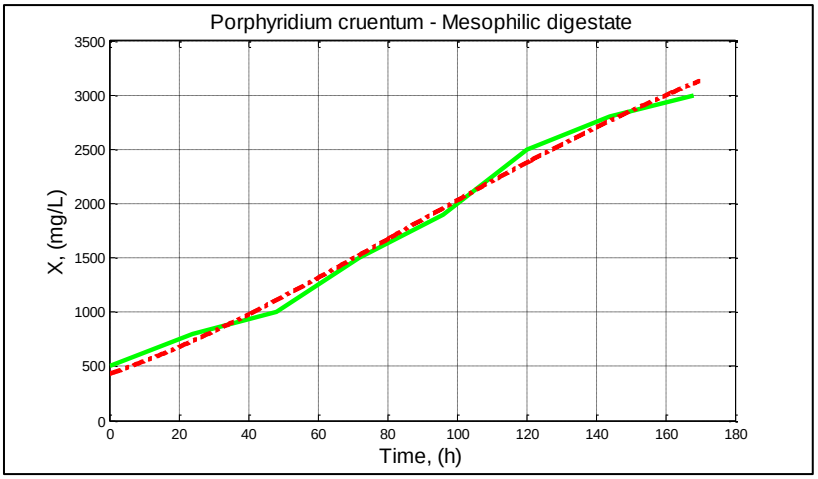
Equations (3) and (4) correspond to the second stage. In them, N(t) and P(t) are the functions found during the first stage (Equations (1) and (2)), and A_0, A_1, B_0, B_1, b, c, d, and f are unknown coefficients, which must be identified on the basis of experimental data.

In order to reduce the number of unknown coefficients, we started with the following simpler solution: We assume that $A_0 = B_0 = 1$, where $A_1 = [X(\infty) - 1]$, $B_1 = [Pigm\ (\infty) - 1]$. X(∞) and Pigm (∞) are taken from the experimental data (the attained final values). Under this assumption, only the following four coefficients: b, c, d, and f remain for identification.

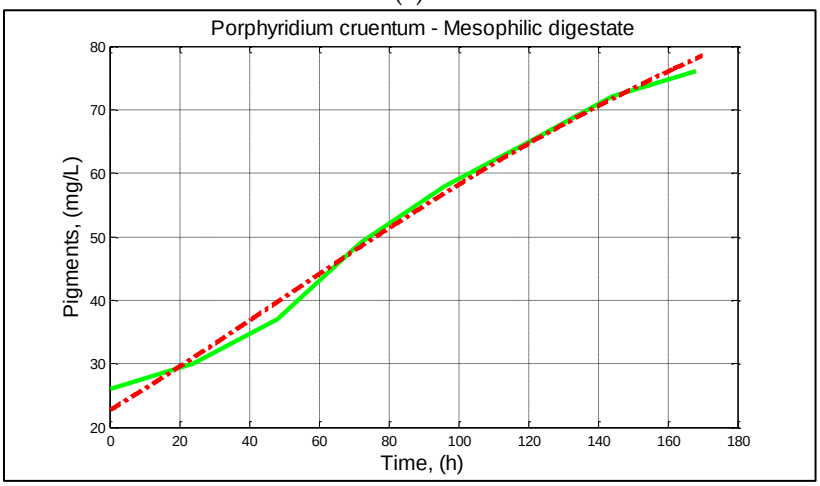
After performing the identification, the results obtained in the second stage are shown in Table 4, Figure 7a,b.

Table 4. Coefficients identification.

Coefficient	A_1	b	c	B_1	d	f
Coefficient lower bound	0	−1	−1.5	0	−1	−2
Coefficient upper limit	6000	1	1	500	1	1
Coefficients identified values	5015.42	−0.002	−0.6	129.55	−0.004	−0.32



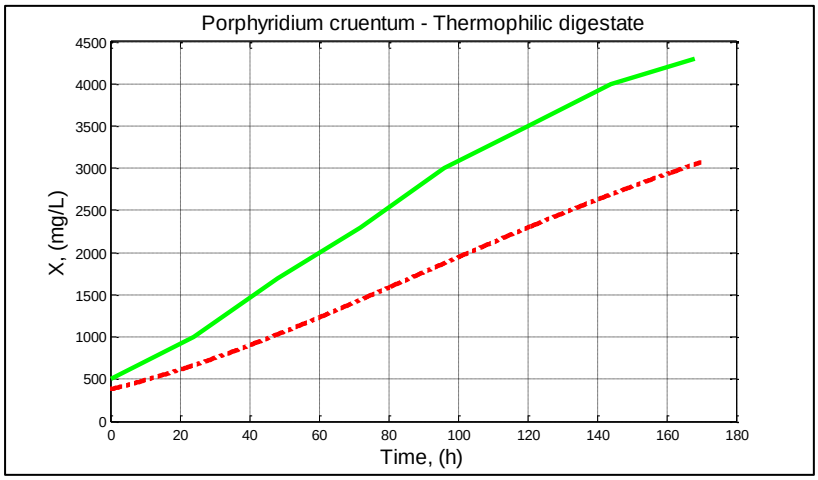
(**a**)



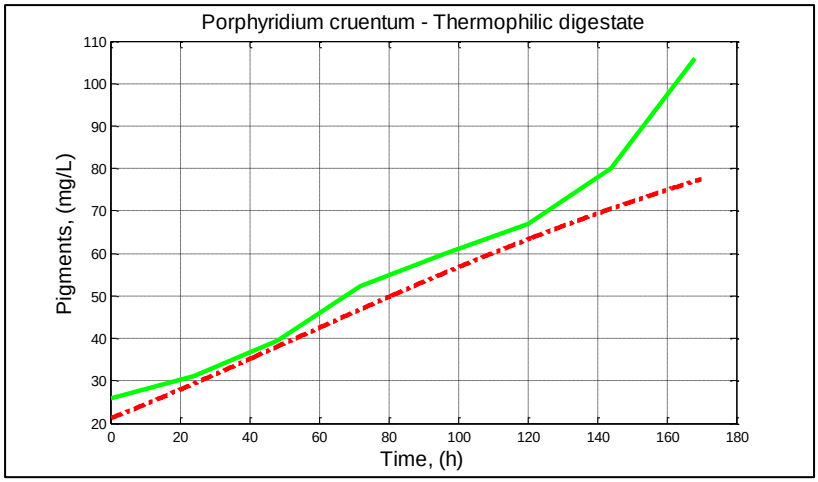
(**b**)

Figure 7. Experimental data (in green) and model approximation (in red) for digestate from mesophilic AD process for: (**a**) X(t); (**b**) Pigm (t); ($N_0 = 139$; $K_N = 0.0023$; $P_0 = 3.77$; $K_P = 0.0067$).

The same model was applied for the process with the digestate from a thermophilic AD process. Results are shown in Figure 8a,b.



(**a**)



(**b**)

Figure 8. Experimental data (in green) for digestate from thermophilic and model approximation (in red) for: (**a**) X(t); (**b**) Pigm (t); ($N_0 = 139$; $K_N = 0.0023$; $P_0 = 3.77$; $K_P = 0.0067$).

Quantitative comparison of the model with the experimental data was carried out by the widely used least squares method, which was applied by the formula:

$$\mathrm{NRMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\frac{\hat{y}_i - y_i}{y_{max} - y_{min}}\right)^2} \quad (5)$$

where: y_i—experimental data in hour "i"; $\hat{y}_i$—model data in hour "i"; y_{max} and value y_{min} are respectively maximal and minimal value of the corresponding experimental data.

The normalized root-mean-square error (NRMSE) for the biomass in the mesophilic digestate is 0.0261, and for the thermophilic—0.2587, respectively. The values for NRMSE for the pigments in mesophilic and thermophilic digestates are 0.0257 and 0.0808.

It is generally accepted that an NRMSE of less than 5% is acceptable. Considering the relatively small number of experimental data in our case, the found values of root mean square errors in terms of biomass and pigmentc are completely acceptable. As pointed out by Alvarez-Garreton et al. [43], the NRMSE provides information about the spread of the ensemble and the performance of the ensemble mean, which can be considered the best estimate of the ensemble prediction [44].

It was calculated that NRMSE for X is 10-fold greater and for Pigm—even more than 3-fold, when the obtained model for the digestate from termophilic AD process was applied. Finally, qualitatively and quantitatively, it could be concluded that the same model is not appropriate, and new coefficients are identified for this case.

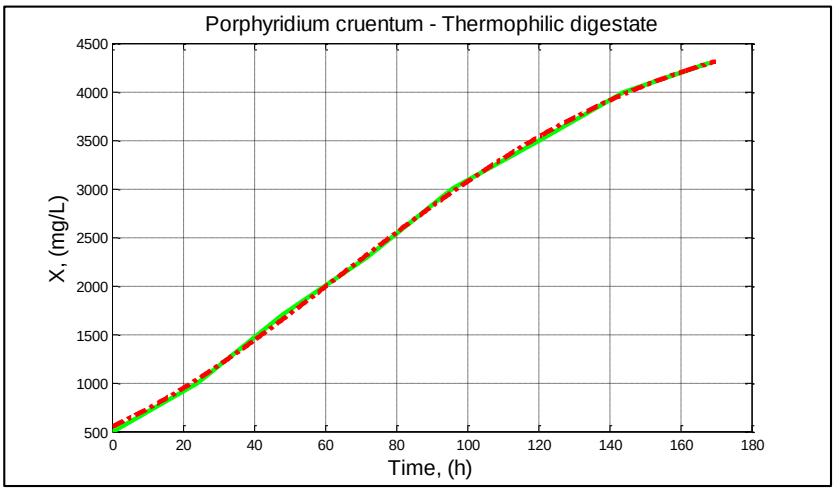
Based on the optimal coefficients from the first stage for the digestate from the thermophilic AD, the following ones were obtained:

$$K_N = 0.0034,\ K_P = 0.0109 \quad (6)$$

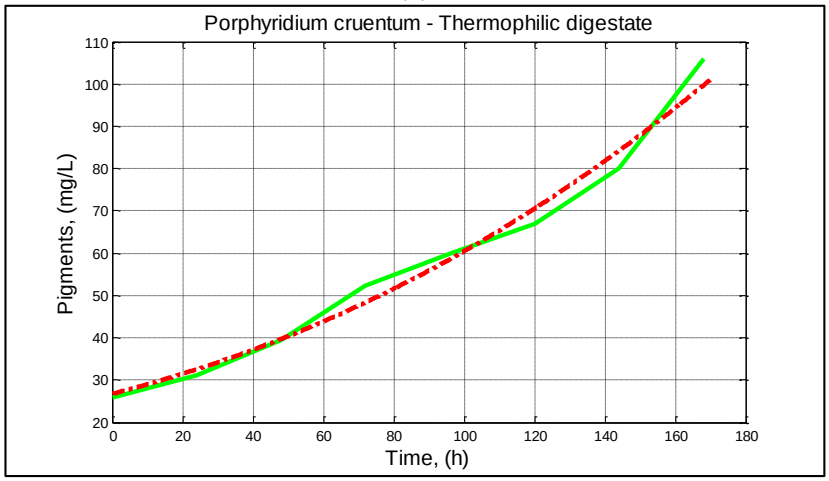
After performing the second stage of identification for the digestate from thermophilic AD process, the results obtained are shown in Table 5 and Figure 9a,b.

Table 5. Coefficient identification for the thermophilic digestate.

Coefficient	A_1	b	c	B_1	d	f
Coefficient lower bound	0	−1	−1.5	0	−1	−2
Coefficient upper limit	6000	1	1	1000	1	1
Coefficients identified values	2743.33	0.012	−0.84	889.15	−0.029	0.12



(**a**)

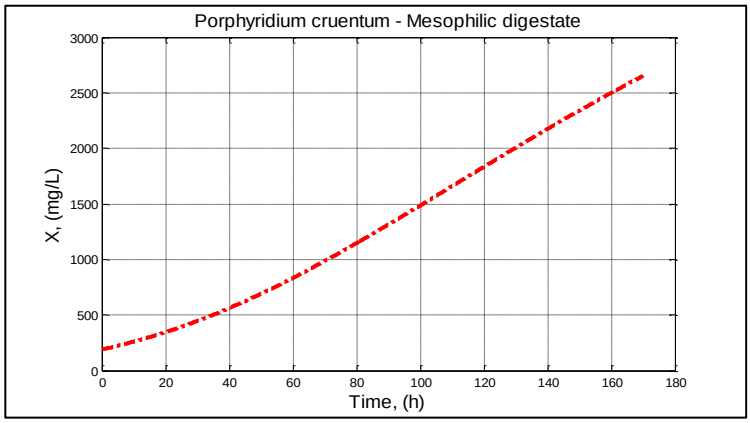


(**b**)

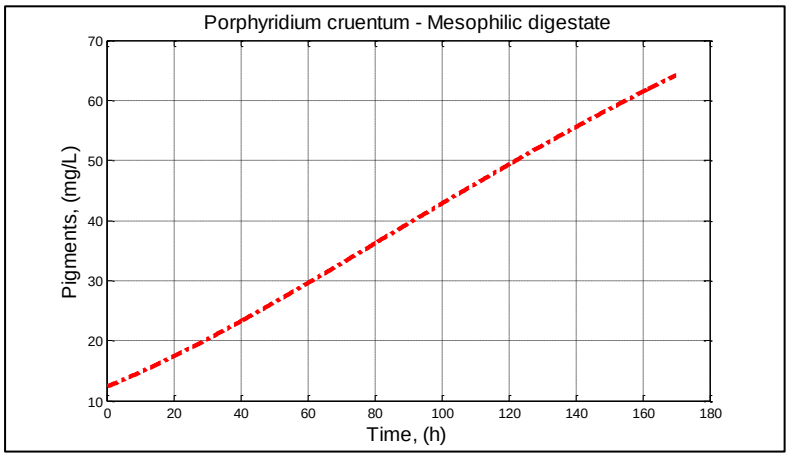
Figure 9. Experimental data (in green) and model approximation (in red) for for digestate from thermophilic AD process for (**a**) X(t); (**b**) Pigm (t); ($K_N = 0.0034$, $K_P = 0.0109$).

The value for NRMSE for the biomass in the thermophilic digestates is 0.0058 and for the pigments—0.0321. Qualitatively and quantitatively, the obtained coefficients are appropriate for the thermophilic case.

Models were simulated under different initial conditions for nitrogen and phosphorus, and predictive results for biomass and pigment were obtained, which show the trend of the experimental results and provide a basis for future work (Figure 10a,b).



(**a**)



(**b**)

Figure 10. Model prediction for digestate from mesophilic AD process for different initial conditions for (**a**) X(t); (**b**) Pigm (t); ($N_0 = 200$; $K_N = 0.0023$); (**b**) P(t) ($P_0 = 5$; $K_P = 0.0067$).

Microalgal biomass production requires nutrients like nitrogen, carbon, phosphorus, and traces of metals. In industry, creating a cost-effective and stable supply of essential nutrients is still challenging. Microalgal biomass production is costly due to the necessity of nutrients and harvesting, which remains a significant barrier to their larger-scale utilization [45]. Estimation of nitrogen and phosphorous content for microalgal growth is of utmost importance. These essential macronutrients play a role in microalgal metabolism [46]. Depletion or excessive sources of these nutrients might affect the quality of biomass [34]. Higher concentrations though could be toxic to microalgae [47]. The presented vision for utilization of anaerobic digestate with its components for algal biomass production may be considered as one of the options for recycling and can be included in waste management. Another is involving the phytoremediation ability of algae to purify contaminants from water bodies and wastewater [48]. Finally, it is a cost-effective strategy, supporting the conservation of the environment and energy security as critical challenges in the global economy.

4. Conclusions

Cultivation of microalgae in waste digestate is a promising cost-effective strategy for valuable algal product production with various applications. This approach encourages circular economy and leads to saving fresh water and nutritional supplements for the growth medium preparation. The proposed nonlinear dynamical models are "black box" type. They reflect the dynamics of biomass accumulation and pigment formation as functions of nitrogen and phosphorus consumption and can serve to predict the dynamics of these variables as a function of the initial nitrogen and phosphorus values.

Author Contributions: Conceptualization, writing—original draft preparation, project administration, L.K.; data curation, J.I.; validation, E.C.; methodology, investigation, V.H.; formal analysis, L.N.; software, I.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Bulgarian National Science Fund, Grant KP-06-N-26/5.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ngan, N.V.C.; Chan, F.M.S.; Nam, T.S.; Thao, H.V.; Maguyon-Detras, M.C.; Hung, D.V.; Cuong, D.M.; Hung, N.V. Anaerobic digestion of rice straw for biogas production. In *Sustainable Rice Straw Management*, 4th ed.; Gummert, M., Hung, N., Chivenge, P., Douthwaite, B., Eds.; Springer: Cham, Switzerland, 2020; pp. 65–92. [CrossRef]
2. Tasmaganbetov, A.B.; Ataniyazov, Z.; Basshieva, Z.; Muhammedov, A.U.; Yessengeldina, A. World practice of using biogas as alternative energy. *Int. J. Energy Econ. Policy* **2020**, *10*, 348–352. Available online: https://econjournals.com/index.php/ijeep/article/view/9805 (accessed on 3 October 2022). [CrossRef]
3. Anwar, N.; Wang, W.; Zhang, J.; Li, Y.; Chen, C.; Liu, G.; Zhang, R. Effect of sodium salt on anaerobic digestion of kitchen waste. *Water Sci. Technol.* **2016**, *73*, 1865–1871. [CrossRef] [PubMed]
4. Jafar, R.; Awad, A. State and development of anaerobic technology for biogas production in Syria. *Clean. Eng. Technol.* **2021**, *5*, 100253. [CrossRef]
5. Logan, M.; Visvanathan, C. Management strategies for anaerobic digestate of organic fraction of municipal solid waste: Current status and future prospects. *Waste Manag. Res.* **2019**, *37* (Suppl. 1), 27–39. [CrossRef] [PubMed]
6. Dalrymple, O.K.; Halfhide, T.; Udom, I.; Gilles, B.; Wolan, J.; Zhang, Q.; Ergas, S. Wastewater use in algae production for generation of renewable resources: A review and preliminary results. *Aquat. Biosyst.* **2013**, *9*, 2. [CrossRef]
7. García, J.L.; de Vicente, M.; Galán, B. Microalgae, old sustainable food and fashion nutraceuticals. *Microb. Biotechnol.* **2017**, *10*, 1017–1024. [CrossRef]
8. Anto, S.; Mukherjee, S.S.; Muthappa, R.; Mathimani, T.; Deviram, G.; Kumar, S.S.; Verma, T.N.; Pugazhendhi, A. Algae as green energy reserve: Technological outlook on biofuel production. *Chemosphere* **2020**, *242*, 125079. [CrossRef]
9. Mendes, M.C.; Navalho, S.; Ferreira, A.; Paulino, C.; Figueiredo, D.; Silva, D.; Gao, F.; Gama, F.; Bombo, G.; Jacinto, R.; et al. Algae as food in Europe: An overview of species diversity and their application. *Foods* **2022**, *11*, 1871. [CrossRef]
10. Thiyagarasaiyar, K.; Goh, B.-H.; Jeon, Y.-J.; Yow, Y.-Y. Algae metabolites in cosmeceutical: An overview of current applications and challenges. *Mar. Drugs.* **2020**, *18*, 323. [CrossRef]
11. Nikolova, B.; Semkova, S.; Tsoneva, I.; Antov, G.; Ivanova, J.; Vasileva, I.; Kardaleva, P.; Stoineva, I.; Christova, N.; Nacheva, L.; et al. Characterization and potential antitumor effect of a heteropolysaccharide produced by the red alga *Porphyridium sordidum*. *Eng. Life Sci.* **2021**, *19*, 978–985. [CrossRef]
12. Ammar, E.E.; Aioub, A.A.A.; Elesawy, A.E.; Karkour, A.M.; Mouhamed, M.S.; Amer, A.A.; EL-Shershaby, N.A. Algae as bio-fertilizers: Between current situation and future prospective. *Saudi J. Biol. Sci.* **2022**, *29*, 3083–3096. [CrossRef] [PubMed]
13. Abdel-Raouf, N.; Al-Homaidan, A.; Ibraheem, I.B.M. Agricultural importance of algae. *Afr. J. Biotechnol.* **2016**, *11*, 11648–11658. [CrossRef]
14. Kabaivanova, L.; Chernev, G.; Ivanova, J. Construction of inorganic and hybrid biosorbents for heavy metal ions removal. *Int. J. Bioautomation* **2015**, *19*, 473–482.
15. Abdel-Raouf, N.; Al-Homaidan, A.A.; Ibraheem, I.B.M. Microalgae and wastewater treatment. *Saudi J. Biol. Sci.* **2012**, *19*, 257–275. [CrossRef]
16. Ozkurt, I. Qualifying of safflower and algae for energy. *Energy Educ. Sci. Technol. Part A* **2009**, *23*, 145–151.
17. Ayre, J.M.; Mickan, B.S.; Jenkins, S.N.; Moheimani, N.R. Batch cultivation of microalgae in anaerobic digestate exhibits functional changes in bacterial communities impacting nitrogen removal and wastewater treatment. *Algal Res.* **2021**, *57*, 102338. [CrossRef]

18. Grobbelaar, J.U. Algal nutrition. In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, 1st ed.; Richmond, A., Ed.; Blackwell Science Ltd.: Oxford, UK, 2004; pp. 97–115. Available online: https://dl.icdst.org/pdfs/files/8a0e128e46252f218192dc8d9cfa090e.pdf (accessed on 3 December 2022).
19. Shahid, A.; Malik, S.; Zhu, H.; Xu, J.; Nawaz, M.Z.; Nawaz, S.; Alam, A.M.; Mehmood, M.A. Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; A review. *Sci. Total Environ.* **2020**, *704*, 135303. [CrossRef]
20. Rana, M.S.; Prajapati, S.K. Stimulating effects of glycerol on the growth, phycoremediation and biofuel potential of *Chlorella pyrenoidosa* cultivated in wastewater. *Environ. Technol. Innov.* **2021**, *24*, 102082. [CrossRef]
21. Kabaivanova, L.; Petrova, P.; Hubenov, V.; Simeonov, I. Biogas production potential of thermophilic anaerobic biodegradation of organic waste by a microbial consortium identified with metagenomics. *Life* **2022**, *12*, 702. [CrossRef]
22. Zachleder, V.; Šetlik, I. Effect of irradiance on the course of RNA synthesis in the cell cycle of *Scenedesmus quadricauda*. *Biol. Plantarum* **1982**, *24*, 341–353. [CrossRef]
23. Hemerick, G. *Handbook of Physiological Methods: Culture Methods and Growth Measurements*, 1st ed.; Stein, J.R., Ed.; Cambridge University Press: New York, NY, USA, 1973; pp. 259–260.
24. Makarevičienė, V.; Skorupskaitė, V.; Andrulevičiūtė, V. Biomass and oil production of green microalgae *Scenedesmus sp.* using different nutrients and growth. *Environ. Res. Eng. Manag.* **2012**, *62*, 5–13. [CrossRef]
25. Mackinney, G. Criteria for purity of chlorophyll preparations. *J. Biol. Chem.* **1940**, *132*, 91–109. [CrossRef]
26. Siegelman, H.W.; Kycia, J.H. Algal biliproteins. In *Handbook of Phycological Methods, Physiological and Biochemical Methods*, 2nd ed.; Hellebust, J.A., Craigie, J.S., Eds.; Cambridge University Press: Cambridge, UK, 1978; pp. 71–79. [CrossRef]
27. Nocedal, J.; Wright, S.J. *Numerical Optimization*, 2nd ed.; Springer Series in Operations Research; Springer: New York, NY, USA, 2006. [CrossRef]
28. Monlau, F.; Sambusiti, C.; Ficara, E.; Aboulkas, A.; Barakat, A.; Carrère, H. New opportunities for agricultural digestate valorization: Current situation and perspectives. *Energy Environ. Sci.* **2015**, *8*, 2600–2621. [CrossRef]
29. Uggetti, E.; Sialve, B.; Trably, E.; Steyer, J.P. Integrating microalgae production with anaerobic digestion: A biorefinery approach. *Biofuels Bioprod. Biorefin.* **2014**, *8*, 516–529. [CrossRef]
30. Marazzi, F.; Sambusiti, C.; Monlau, F.; Cecere, S.E.; Scaglione, D.; Barakat, A.; Mezzanotte, V.; Ficara, E. A novel option for reducing the optical density of liquid digestate to achieve a more productive microalgal culturing. *Algal Res. Part A* **2017**, *24*, 19–28. [CrossRef]
31. Silva, S.C.; Ferreira, I.C.F.R.; Dias, M.M.; Barreiro, M.F. Microalgae-derived pigments: A 10-year bibliometric review and industry and market trend analysis. *Molecules* **2020**, *25*, 3406. [CrossRef] [PubMed]
32. Fernandes, F.; Silkina, A.; Gayo-Peláez, J.I.; Kapoore, R.V.; de la Broise, D.; Llewellyn, C.A. Microalgae Cultivation on Nutrient Rich Digestate: The Importance of Strain and Digestate Tailoring under PH Control. *Appl. Sci.* **2022**, *12*, 5429. [CrossRef]
33. Bauer, L.; Ranglová, K.; Masojídek, J.; Drosg, B.; Meixner, K. Digestate as Sustainable Nutrient Source for Microalgae—Challenges and Prospects. *Appl. Sci.* **2021**, *11*, 1056. [CrossRef]
34. Yaakob, M.A.; Mohamed, R.M.S.R.; Al-Gheethi, A.; Aswathnarayana Gokare, R.; Ambati, R.R. Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: An overview. *Cells* **2021**, *10*, 393. [CrossRef]
35. Barzee, T.G.; Edalati, A.; El-Mashad, H.; Wang, D.; Scow, K.; Zhang, R. Digestate Biofertilizers Support Similar or Higher Tomato Yields and Quality Than Mineral Fertilizer in a Subsurface Drip Fertigation System. *Front. Sustain. Food Syst.* **2019**, *3*, 58. [CrossRef]
36. Nag, R.; Whyte, P.; Markey, B.K.; O'Flaherty, V.; Bolton, D.; Fenton, O.; Richards, K.G.; Cummins, E. Ranking hazards pertaining to human health concerns from land application of anaerobic digestate. *Sci. Total Environ.* **2020**, *710*, 136297. [CrossRef] [PubMed]
37. Velasco-Muñoz, J.F.; Aznar-Sánchez, J.A.; López-Felices, B.; Román-Sánchez, I.M. Circular economy in agriculture. An analysis of the state of research based on the life cycle. *Sustainab. Product. Consumpt.* **2022**, *34*, 257–270. [CrossRef]
38. Hubenov, V.; Carcioch, R.A.; Ivanova, J.; Vasileva, I.; Dimitrov, K.; Simeonov, I.; Kabaivanova, L. Biomethane production using ultrasound pre-treated maize stalks with subsequent microalgae cultivation. *Biotechnol. Biotechnol. Eq.* **2020**, *34*, 800–809. [CrossRef]
39. Marks, E.A.N.; Montero, O.; Rad, C. The biostimulating effects of viable microalgal cells applied to a calcareous soil: Increases in bacterial biomass, phosphorus scavenging, and precipitation of carbonates. *Sci. Total Environ.* **2019**, *692*, 784–790. [CrossRef] [PubMed]
40. Colica, G.; Li, H.; Rossi, F.; Li, D.; Liu, Y.; De Philippis, R. Microbial secreted exopolysaccharides affect the hydrological behavior of induced biological crusts in desert soils. *Soil Biol. Biochem.* **2014**, *68*, 62–70. [CrossRef]
41. Domozych, D.S.; Ciancia, M.; Fangel, J.U.; Mikkelsen, M.D.; Ulvskov, P.; Willats, W.G.T. The cell walls of green algae: A journey through evolution and diversity. *Front. Plant Sci.* **2012**, *3*, 82. [CrossRef]
42. Rachidi, F.; Benhima, R.; Sbabou, L.; El Arroussi, H. Microalgae polysaccharides bio-stimulating effect on tomato plants: Growth and metabolic distribution. *Biotechnol. Rep.* **2020**, *25*, e00426. [CrossRef]
43. Alvarez-Garreton, C.; Ryu, D.; Western, A.W.; Su, C.-H.; Crow, W.T.; Robertson, D.E.; Leahy, C. Improving operational flood ensemble prediction by the assimilation of satellite soil moisture: Comparison between lumped and semi-distributed schemes. *Hydrol. Earth Syst. Sci.* **2015**, *19*, 1659–1676. [CrossRef]

44. Massari, C.; Brocca, L.; Tarpanelli, A.; Moramarco, T. Data Assimilation of Satellite Soil Moisture into Rainfall-Runoff Modelling: A Complex Recipe? *Remote Sens.* **2015**, *7*, 11403–11433. [CrossRef]
45. Nazifa, R.; Forruque, A.S.; Anjum, B.I.; Mofijur, M.; Sarfaraz, K. Strategies to Produce Cost-Effective Third-Generation Biofuel from Microalgae. *Front. Energy Res.* **2021**, *9*, 749968. [CrossRef]
46. Zarrinmehr, M.J.; Farhadian, O.; Heyrati, F.P.; Keramat, J.; Koutra, E.; Kornaros, M.; Daneshvar, E. Effect of nitrogen concentration on the growth rate and biochemical composition of the microalga, *Isochrysis galbana*. *Egypt. J. Aquat. Res.* **2019**, *46*, 1687–4285. [CrossRef]
47. Procházková, G.; Brányiková, I.; Zachleder, V.; Brányik, T. Effect of nutrient supply status on biomass composition of eukaryotic green microalgae. *J. Appl. Phycol.* **2013**, *26*, 1359–1377. [CrossRef]
48. Ummalyma, S.B.; Singh, A. Biomass production and phycoremediation of microalgae cultivated in polluted river water. *Bioresour. Technol.* **2022**, *351*, 126948. [CrossRef] [PubMed]