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Keywords: photobioreactor, bio-oil, anaerobic digestate, microalgae

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The Cultivation of Lipid-Rich Microalgae Biomass as Anaerobic Digestate Valorization Technology—A Pilot-Scale Study

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Abstract: The aim of the study was to determine the use of digestate from anaerobic digestion of dairy wastewater as a culture medium for microalgae to obtain bio-oil. The experiments were conducted at a small scale in a closed raceway pond. The efficiency of the microalgae biomass production, the digestate treatment efficiency as well as the content and properties of the bio-oil obtained from the microalgal cells were analyzed. The produced biomass concentration was about 3000 ± 10.5 mg dry biomass/L, with an average growth rate of 160 ± 6.6 mg_{dm}/L·d. The efficiency of organic compound and nutrient removal was above 90%. The bio-oil content in the biomass was about 20%. Based on the results of the study, a concept for technical-scale technology was developed.

Keywords: microalgae; anaerobic digestate; bio-oil; photobioreactor

1. Introduction

Wastewater from dairy processing plants is characterized by a high content of organic compounds and nutrients; thus, there is a potential of its use as a source of nutrients for microalgal biomass production [1]. However, the raw wastewater contains too high a concentration of organic compounds, and is characterized by high turbidity and low transparency. The nutrients are also mainly in the form of complex compounds, difficult to directly use by microalgae [2,3]. When using raw wastewater as a culture medium for microalgae, the high concentration of organic compounds could enhance the growth of detrimental organisms, mainly bacteria, while the turbidity and its dark color could limit the availability of light and inhibit photosynthesis [4]. Therefore, it is important to reduce these factors before using it as a culture medium. One of the methods is wastewater treatment in anaerobic reactors for partial organic compound removal and transformation of biogenic compounds into mineral forms that are easily absorbed by microalgae, as well as reducing turbidity and color [5].

It should also be emphasized that an important factor for effective biomass production is the selection of microalgae species. They should be characterized by high resistance to environmental conditions changes [6]. The green algae of the genus *Scenedesmus* sp. and *Chlorella* sp., as well as cyanobacteria [7] meet this criterion.

Earlier literature presented the technologies for wastewater treatment and pollution degradation using microalgae [8,9]. It has been shown that microalgae offer great potential as a biomass resource for bioenergy production (biohydrogen, biomethane) and other co-products, such as bio-oil [10–14]. Many

microalgae species have the ability to accumulate large quantities of lipids in the cells, amounting to 20–50% of their dry weight, as well as microalgae biomass may contain from 20% to 60% proteins [15,16]. Global demand for biofuels has gradually expanded; thus, there is a need to search for solutions to guarantee energy supply in economically and environmentally friendly ways [17]. Microalgae are a prospective source of organic substrate for bioenergy purposes that compete with typical terrestrial vascular plants, such as rapeseed, soybeans and oil palm [18]. However, an important factor determining the profitability of using algae biomass to produce bioenergy is the choice of their cultivation and separation technology. Today, the only cost-effective technologies for industrial-scale microalgae biomass production are raceway ponds [19,20].

The aim of the study was to determine the use of digestate from anaerobic reactor-treated dairy wastewater to grow mixed-culture microalgae. The results of this small-scale study was the base for developing a novel concept for technical-scale technology.

2. Materials and Methods

2.1. Experimental Design

The experiments were carried out in small-scale photobioreactor (PBR) with a working volume of 2.0 m^3. Digestate from anaerobic digestion of dairy wastewater was a culture medium in PBR. In the study, the efficiency of microalgae biomass growth, digestate treatment efficiency as well as the content and properties of bio-oil obtained from the microalgae cells were analyzed. The study was divided into four stages. In Stage 1, a start-up of the PBR was carried out and the microalgae biomass acclimatization to digestate composition was done. This stage was carried out for 26 days. In Stage 2, the experiments on the efficiency of microalgae biomass production and digestate treatment efficiency were done. This stage was carried out for 65 days. Stage 3 concerned the analyzes of bio-oil obtained from the microalgae biomass. Finally, in Stage 4, the concept of a technical-scale technology for microalgae biomass production was developed.

2.2. Feedstock Origin and Characteristics, and the Microalgae Inoculum

The feedstock for microalgae cultivation was the digestate from an upflow anaerobic sludge blanket reactor (UASB) treating dairy wastewater (Table 1). The UASB reactor was operated at an organic loading rate of 10 kg chemical oxygen demand (COD)/m^3·d and a hydraulic retention time (HRT) of 24 h. Before using as a culture medium, anaerobic digestate was pasteurized (90 °C for 30 min) to ensure purity for microalgae cultures. The quantity of digestate introduced into the PBR was 40.0 L/m^3·d. As the working volume of the PBR was 2.0 m^3, the total quantity of digestate introduced to the PBR was 80.0 L/d. The full hydraulic replacement of the PBR's volume was after 26 days.

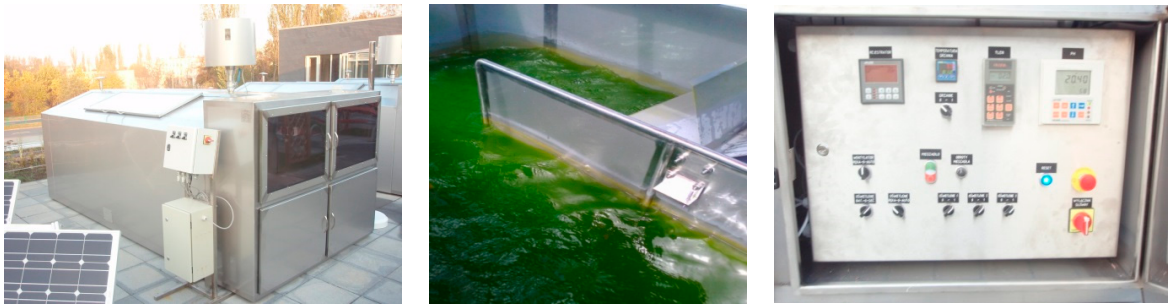
Table 1. Characteristics of the raw anaerobic digestate from the upflow anaerobic sludge blanket (UASB) reactor (RAD) and the pasteurized anaerobic digestate (PAD).

Value	Parameter								
	BOD_5 (mgO_2/L)	COD (mgO_2/L)	BOD_5/COD	pH	TS mg/L	TN mg/L	AN mg/L	TP mg/L	P-PO_4 mg/L
					RAD				
Average	380.4 ± 71.0	799.9 ± 93.6	0.5 ± 0.1	7.4 ± 0.3	112.7 ± 13.4	255.3 ± 64.1	194.9 ± 71.5	60.2 ± 11.0	42.9 ± 13.1
					PAD				
Average	492.0 ± 99.4	891.2 ± 118.1	0.6 ± 0.1	7.2 ± 0.2	98.2 ± 21.2	302.8 ± 99.4	222.0 ± 70.7	61.7 ± 11.9	46.5 ± 12.7

The microalgae biomass used to inoculate the PBR originated from our own culture. Analysis of the microalgae composition indicated that the biomass consisted of 3.0% bacteria, 3.0% Cyanoprocaryota, 72.0% *Chlorella* sp. and 22.0% *Scenedesmus* sp. The lipid content in the cells averaged 17.8% ± 2.1% dry biomass. The initial concentration of microalgae biomass in the PBR was 20 mg_{dm}/L.

2.3. Research Station and Operation Parameters

The experiments were carried out in a closed raceway pond photobioreactor with a working volume of 2.0 m^3 and a depth of 0.4 m. The reactor consisted of a reaction chamber and a control system. The two mechanical stirrers were placed opposite one another at the beginning of the long straight sides of the pond. The stirrer speed was maintained at 30 rpm. (Figure 1). To fulfill the lighting requirements of the microalgae in the pond, three-band fluorescent lamps with narrow-band emission phosphors with a luminous efficiency of 100 lumens per watt were used. The lighting system was powered by solar energy using a set of four monocrystalline solar panels (Celline CL080-12, 80 W). The PBR was covered with a transparent sunlight-permeable cover. The heating system consisted of electric heaters with a heating power of 1.0 kW. The heating system was switched on automatically when the temperature of the culture medium reached 20 °C, while it was switched off at a temperature of 22 °C. The sides and the bottom of the chamber of the pond had a foamed polystyrene insulation with a thickness of about 0.15 m. To provide thermal protection, the transparent cover was double-layered with an internal airbag. In the control cabinet, there was an automatic control system for measurement of the reaction of the culture medium, oxygen content and temperature. The microalgae biomass was thickened with using a 10.0 µm drum microfilter system, and then removed outside the system.



(**a**) (**b**) (**c**)

Figure 1. The photobioreactor: (**a**) a general view, (**b**) a chamber for microalgae cultivation and (**c**) a control system.

2.4. Analytical Methods

The content of dry mass (dm) and volatile solids (VS) were determined according to the gravimetric method at 105 °C. The content of chemical oxygen demand (COD), total phosphorus (TP), orthophosphate (P-PO_4), total nitrogen (TN) and ammonia nitrogen (AN) was determined using a DR 5000 spectrophotometer with an HT 200 s mineralizer (Hach-Lange, Germany). Determination of biochemical oxygen demand (BOD_5) was carried out according to PN-EN 1899-1. Lipid content in the microalgae biomass was determined by Soxhlet extraction (B-323 Büchi, Switzerland). The taxonomic identification of the microalgae was conducted at microscope magnifications of 1.25 × 10 × 40 or 1.25 × 10 × 100 and by using the algae analyzer BBE Moldaenke (Germany). The analyzer allowed to identify algae classes of Chlorophyceae, Bacillriophyceae, Cyanoprocaryota and Phaeophyceae. The bio-oil was extracted directly from the microalgae biomass by pressing with a simple mechanical press. Fuel properties and chemical composition of the bio-oil were analyzed as follows: color (PN-A-86934), peroxide value (PN-84/A-86918), density in 15 °C (PN-EN ISO 3838), viscosity in 40 °C (PN-81/C-04011), impurities content (PN-85/C-04832), water content (PN-ISO 8534), sulfur content (PN-87/C-04288/13), iodine value (PN-ISO 3961), phosphorus content (PN-88/A-86930) and acid value (PN-60/A-86921).

2.5. Statistical Methods

The statistical analysis of the results was carried out with the Statistica 13.1 PL package (Statsoft, Inc., Tulsa, OK, USA). The hypothesis on the 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 the differences between variables. Variance homogeneity in groups was checked with a Levene's test, whereas the significance of the differences between the analyzed variables was determined with a Tukey RIR test. In all tests, the level of significance was adopted at $\alpha = 0.05$.

3. Results and Discussion

3.1. Stage 1

The temperature in the closed raceway pond was maintained at 20 °C, which directly affected the growth of the microalgae biomass and improved the lipid accumulation in the cells. This was also confirmed by studies on *Crypthecodinium cohnii* cultivation [21] and *Nitzschia laevis* growth [22]. Other studies showed that a low cultivation temperature of 20 °C improved the efficiency of fatty acid biosynthesis to maintain the fluidity of the cellular membrane [23]. In addition, a low culture temperature enhanced the intracellular oxygen concentration, which accelerated saturated fatty acid conversion into unsaturated fatty acids by enhancing the enzymatic activity of desaturases. It was confirmed by studies on *Mortierella alpina* 1S-4 [24].

The initial microalgae biomass concentration in PBR was 19.6 ± 1.1 mg_{dm}/L. After 26 days of cultivation, the biomass concentration reached 1999.6 ± 11.0 mg_{dm}/L. It was found that the average biomass growth rate (r) was 76.1 ± 5.3 mg_{dm}/L·d (Figure 2), and the lipid content in the microalgae cells ranged from 14.3% ± 3.8% of dry biomass to 21.3% ± 3.5% of dry biomass, and averaged 17.9% ± 3.7%. The study showed that the nutrients were completely eliminated from the culture medium and used for the growth of algae. The efficiency of the nitrogen and phosphorus compound removal exceeded 97.0%, while the COD and BOD_5 removal efficiencies were about 90%. The lipid content of the microalgae, as well as the concentration of the parameters in the culture medium (raw wastewater) and in the medium after the cultivation time (treated wastewater), are presented in Table 2.

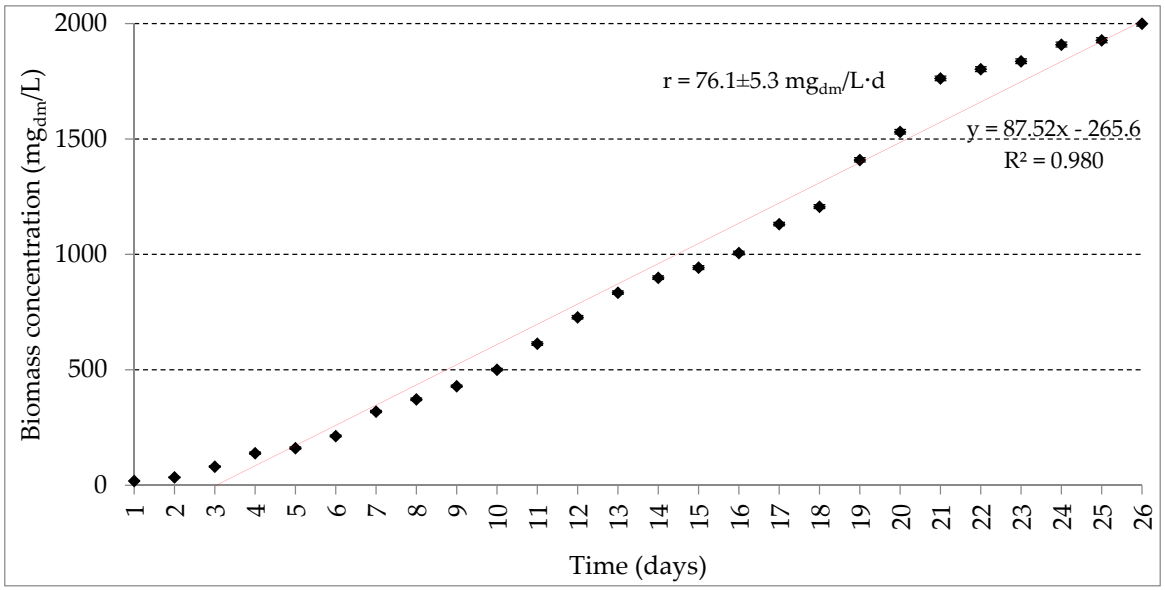


Figure 2. Microalgae biomass production over the cultivation time in stage 1.

Table 2. Lipid content of the microalgae biomass, and characteristics of the culture medium (raw sewage) and the medium after the cultivation time (treated wastewater) in Stage 1.

Days	Lipid Content (% dm)	Culture Medium (mg/L)—Raw Wastewater						Medium After Cultivation Time (mg/L)—Treated Wastewater					
		COD	BOD_5	TN	AN	TP	$P\text{-}PO_4$	COD	BOD_5	TN	AN	TP	$P\text{-}PO_4$
1	14.3 ± 3.6	799.3 ± 90.1	404.5 ± 71.4	181.3 ± 65.3	144.9 ± 34.1	47.8 ± 11.2	31.6 ± 10.3	81.3 ± 16.8	29.5 ± 5.8	3.7 ± 1.3	1.3 ± 0.4	1.1 ± 0.5	0.4 ± 0.1
2	14.3 ± 3.8	799.3 ± 90.1	404.5 ± 71.4	181.3 ± 65.3	144.9 ± 34.1	47.8 ± 11.2	31.6 ± 10.3	82.5 ± 15.9	33.7 ± 5.4	4.9 ± 1.5	1.7 ± 0.5	0.9 ± 0.3	0.6 ± 0.1
3	15.1 ± 3.4	799.3 ± 90.1	404.5 ± 71.4	181.3 ± 65.3	144.9 ± 34.1	47.8 ± 11.2	31.6 ± 10.3	79.1 ± 14.8	38.2 ± 5.5	3.9 ± 1.1	2.1 ± 0.5	1.2 ± 0.4	0.3 ± 0.1
4	15.3 ± 3.9	799.3 ± 90.1	404.5 ± 71.4	181.3 ± 65.3	144.9 ± 34.1	47.8 ± 11.2	31.6 ± 10.3	88.2 ± 16.1	34.8 ± 5.1	3.1 ± 1.1	2.3 ± 0.4	1.1 ± 0.4	0.3 ± 0.1
5	15.9 ± 4.6	824.3 ± 90.6	419.3 ± 71.1	163.4 ± 64.1	137.1 ± 21.6	64.2 ± 10.4	43.5 ± 9.8	73.7 ± 15.5	37.1 ± 5.4	5.7 ± 1.2	2.0 ± 0.5	1.0 ± 0.5	0.4 ± 0.1
6	14.6 ± 3.7	824.3 ± 90.6	419.3 ± 71.1	163.4 ± 64.1	137.1 ± 21.6	64.2 ± 10.4	43.5 ± 9.8	79.0 ± 14.9	30.7 ± 4.9	6.0 ± 1.3	1.9 ± 0.4	0.9 ± 0.4	0.2 ± 0.1
7	15.7 ± 4.5	824.3 ± 90.6	419.3 ± 71.1	163.4 ± 64.1	137.1 ± 21.6	64.2 ± 10.4	43.5 ± 9.8	81.3 ± 16.7	33.9 ± 5.2	5.9 ± 1.3	2.4 ± 0.5	0.9 ± 0.5	0.3 ± 0.1
8	17.2 ± 4.0	824.3 ± 90.6	419.3 ± 71.1	163.4 ± 64.1	137.1 ± 21.6	64.2 ± 10.4	43.5 ± 9.8	84.6 ± 15.7	29.6 ± 5.1	5.2 ± 1.5	1.7 ± 0.4	1.4 ± 0.6	0.4 ± 0.1
9	18.1 ± 3.7	824.3 ± 90.6	419.3 ± 71.1	163.4 ± 64.1	137.1 ± 21.6	64.2 ± 10.4	43.5 ± 9.8	73.5 ± 14.4	28.1 ± 4.7	7.1 ± 1.4	1.7 ± 0.3	1.5 ± 0.5	0.4 ± 0.1
10	21.3 ± 3.5	824.3 ± 90.6	419.3 ± 71.1	163.4 ± 64.1	137.1 ± 21.6	64.2 ± 10.4	43.5 ± 9.8	69.9 ± 15.1	38.2 ± 5.1	6.8 ± 1.3	1.9 ± 0.4	1.4 ± 0.4	0.5 ± 0.1
11	19.6 ± 4.2	809.7 ± 92.4	399.8 ± 69.4	181.9 ± 60.1	139.7 ± 28.5	59.9 ± 11.5	40.1 ± 9.5	70.3 ± 16.0	41.0 ± 4.3	6.3 ± 1.1	2.0 ± 0.5	1.6 ± 0.5	0.7 ± 0.1
12	19.2 ± 3.8	809.7 ± 92.4	399.8 ± 69.4	181.9 ± 60.1	139.7 ± 28.5	59.9 ± 11.5	40.1 ± 9.5	73.3 ± 15.2	37.2 ± 4.8	6.1 ± 1.2	2.3 ± 0.5	1.2 ± 0.4	0.5 ± 0.1
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14	21.3 ± 3.4	809.7 ± 92.4	399.8 ± 69.4	181.9 ± 60.1	139.7 ± 28.5	59.9 ± 11.5	40.1 ± 9.5	80.2 ± 15.5	35.5 ± 5.9	7.4 ± 1.3	2.1 ± 0.4	1.4 ± 0.5	0.3 ± 0.1
15	19.8 ± 3.1	809.7 ± 92.4	399.8 ± 69.4	181.9 ± 60.1	139.7 ± 28.5	59.9 ± 11.5	40.1 ± 9.5	77.3 ± 16.2	30.0 ± 5.6	7.2 ± 1.3	2.7 ± 0.4	1.6 ± 0.3	0.7 ± 0.1
16	19.6 ± 3.2	809.7 ± 92.4	399.8 ± 69.4	181.9 ± 60.1	139.7 ± 28.5	59.9 ± 11.5	40.1 ± 9.5	83.1 ± 16.0	29.1 ± 5.3	7.8 ± 1.1	3.0 ± 0.5	2.1 ± 0.5	0.6 ± 0.1
17	18.2 ± 3.3	809.7 ± 92.4	399.8 ± 69.4	181.9 ± 60.1	139.7 ± 28.5	59.9 ± 11.5	40.1 ± 9.5	73.1 ± 15.5	31.6 ± 4.9	8.2 ± 1.5	3.1 ± 0.5	1.3 ± 0.3	0.8 ± 0.1
18	18.7 ± 3.8	789.6 ± 94.5	406.3 ± 73.1	203.6 ± 67.2	152.2 ± 36.0	64.2 ± 10.8	47.4 ± 10.4	79.6 ± 15.8	29.1 ± 6.1	6.9 ± 1.1	2.7 ± 0.5	1.7 ± 0.4	0.8 ± 0.2
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20	18.0 ± 3.7	789.6 ± 94.5	406.3 ± 73.1	203.6 ± 67.2	152.2 ± 36.0	64.2 ± 10.8	47.4 ± 10.4	80.8 ± 15.8	34.7 ± 6.4	8.0 ± 1.2	2.8 ± 0.4	1.4 ± 0.4	0.9 ± 0.2
21	18.6 ± 4.1	789.6 ± 94.5	406.3 ± 73.1	203.6 ± 67.2	152.2 ± 36.0	64.2 ± 10.8	47.4 ± 10.4	86.2 ± 15.2	39.8 ± 6.8	7.9 ± 1.1	3.2 ± 0.5	1.4 ± 0.3	0.9 ± 0.2
22	18.9 ± 3.2	789.6 ± 94.5	406.3 ± 73.1	203.6 ± 67.2	152.2 ± 36.0	64.2 ± 10.8	47.4 ± 10.4	81.7 ± 15.6	36.2 ± 5.5	8.1 ± 1.0	3.1 ± 0.3	1.7 ± 0.3	0.8 ± 0.2
23	17.4 ± 3.5	789.6 ± 94.5	406.3 ± 73.1	203.6 ± 67.2	152.2 ± 36.0	64.2 ± 10.8	47.4 ± 10.4	84.3 ± 16.8	40.9 ± 5.0	8.3 ± 1.3	3.3 ± 0.4	1.3 ± 0.4	0.6 ± 0.1
24	18.9 ± 4.3	813.6 ± 93.1	431.7 ± 76.8	201.1 ± 62.8	155.1 ± 38.1	62.8 ± 10.5	38.9 ± 10.1	80.1 ± 17.2	37.1 ± 4.8	9.4 ± 1.3	3.0 ± 0.3	1.2 ± 0.4	0.5 ± 0.1
25	18.3 ± 3.0	813.6 ± 93.1	431.7 ± 76.8	201.1 ± 62.8	155.1 ± 38.1	62.8 ± 10.5	38.9 ± 10.1	76.3 ± 16.1	33.9 ± 4.9	10.2 ± 1.4	2.7 ± 0.4	1.1 ± 0.3	0.6 ± 0.1
26	18.7 ± 4.2	813.6 ± 93.1	431.7 ± 76.8	201.1 ± 62.8	155.1 ± 38.1	62.8 ± 10.5	38.9 ± 10.1	74.9 ± 16.3	35.6 ± 5.1	9.8 ± 1.3	2.4 ± 0.4	1.6 ± 0.5	0.7 ± 0.2

3.2. Stage 2

In Stage 2 of the experiment, the final concentration of microalgae reached 3000 ± 10.5 mg_{dm}/L, with an average biomass growth rate of 160 ± 6.6 mg_{dm}/L·d (Figure 3). Other authors [25], who investigated the columnar photobioreactors for microalgae cultivation, achieved a biomass concentration of 2500 mg_{dm}/L. However, in studies [26] conducted in an airlift tubular reactor with a working volume of 500 mL, the obtained biomass concentration was about 1800 mg_{dm}/L. In other studies [27], the generation time of *Chlorella vulgaris* at a temperature of 27 °C was 8.6 h, while at 5 °C it was extended till 48.5 h. The biomass and lipid productivities were enhanced by mixotrophic cultivation [28]. Bhatnagar et al. [29] studied the growth of *Chlamydomonas globosa*, *Chlorella minutissima* and *Scenedesmus bijuga* under a mixotrophic and heterotrophic mode, and a higher biomass productivity by mixotrophic cultivation was obtained. In these studies, there was a high removal of organic compounds from the culture medium, which could indicate the mixotrophic growth of microalgae.

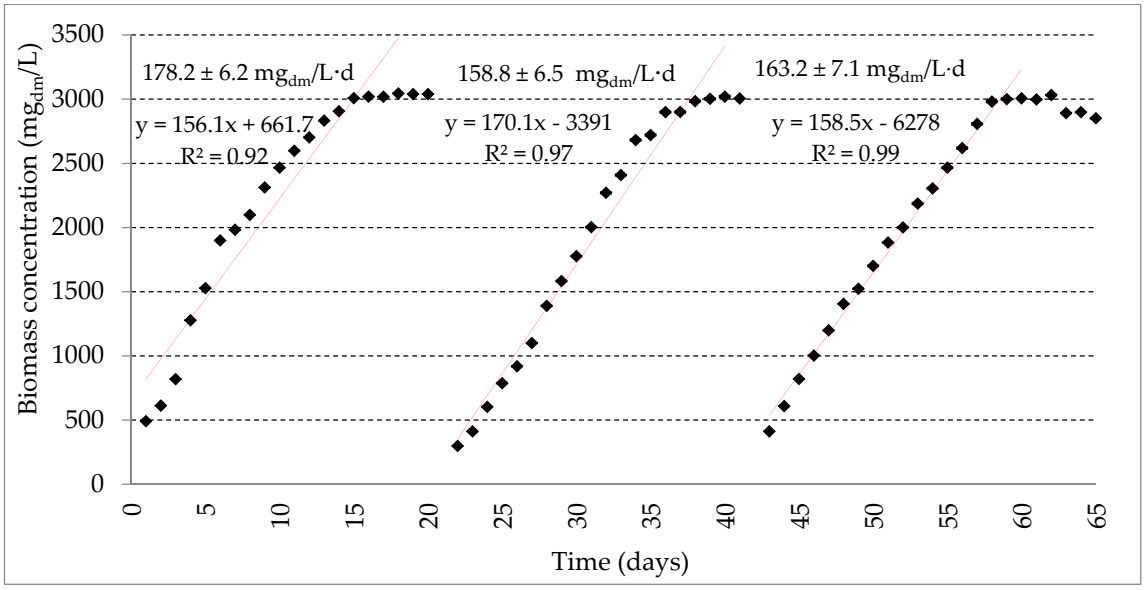

Figure 3. Microalgae biomass production over the cultivation time in Stage 2.

There are strategies for enhancing lipid accumulation in microalgae, including a combination of stress factors (nitrogen limitation, light regime, temperature, CO_2 concentration), co-culturing with other microorganisms, addition of phytohormones and chemical additives [30,31]. In the present study, the lipid content in the microalgae biomass was about 20% of the dm. Such a low lipid content was probably due to a high concentration of nitrogen in the culture medium, mainly in the form of ammonia nitrogen. Other studies confirmed that nitrogen limitation in the culture medium strongly affected the lipid accumulation in the microalgae cells and lipid quality, mainly in terms of triglyceride content [32,33]. The results of other work on *Chlorella vulgaris* ESP-31 cultivation under photoautotrophic, heterotrophic and mixotrophic modes showed that nitrogen-limiting conditions enhanced the lipid accumulation, regardless of the cultivation mode, and that the total lipid content in the biomass ranged from 20% to 53% [34]. Other authors [35] studied the effect of salinity, nitrogen concentration and light intensity on the lipid biosynthesis efficiency. The results showed that, in certain conditions, the lipid concentration in cells was over 76.0%, which was higher than in biomass grown on conventional medium. However, it was found that a low amount of nitrogen in the culture medium directly influenced the increase in lipid accumulation, but also reduced the biomass growth rate [36].

In this study low concentrations of organic compounds and nutrients in the medium after the cultivation process (wastewater treated) were found (Table 3), where the COD and BOD_5 concentrations were in the range of 68.7 ± 14.1 to 84.6 ± 15.0 mg O_2/L and 13.9 ± 4.9 to 38.2 ± 5.8 mg O_2/L, respectively,

and TN and AN ranged from 2.1 ± 1.3 to 8.0 ± 1.6 mg/L and from 1.2 ± 0.3 to 3.0 ± 0.6 mg/L, respectively, while the concentrations of TP and orthophosphates ranged, respectively, from 1.1 ± 0.4 to 1.7 ± 0.5 mg/L and from 0.4 ± 0.1 to 0.9 ± 0.2 mg/L. Thus, the organic compounds removal was about 90% for COD and averaged 95% for BOD_5 (Table 4). Regarding nutrients, the total nitrogen and ammonia nitrogen removal achieved 99%, while the efficiencies of total phosphorus and orthophosphate removal were, respectively, 98% and 99%. Other authors showed that cultivation of a mixed culture of *Chlorella vulgaris* and *Scenedesmus obliquus* in aquaculture wastewater allowed to reduce the nitrogen concentration up to 86.1% and the phosphorus concentration up to 82.7%, achieving a final nitrogen and phosphorus concentration in the culture medium of 1.30 mg/L and 0.12 mg/L, respectively [37]. Cultivation of *Platymonas subcordiformis* in aquaculture wastewater removed nutrients efficiently (87.0–95.0% nitrogen removal and 98.0–99.0% phosphorus removal) [38]. Sawayama et al. [39] found that a high efficiency of nutrient removal from wastewater used as a culture medium by *Botryococcus braunii* resulted in the production of biomass with a high carbohydrate content. The phosphorous removal efficiency by *Scenedesmus obliquus* cultured in urban wastewater was 98% [40].

3.3. Stage 3

In this study, the bio-oil produced from the microalgae biomass was transparent and had a yellow-green color. The peroxide value reached 0.5, the overall content of the impurities was 2.0 ± 0.1 mg/kg, the residual carbon was 0.1% (m/m), the sulfur content was 2.0 ± 0.1 mg/kg, the acid value was 0.4 ± 0.1 mg KOH/g and the iodine value was 75 ± 5 g iodine/100 g (Table 5). These values were lower than those given in PN-EN-14214 for fatty acid methyl esters (FAME) for their use in car engines and heating applications (0.5 mg KOH/g for the acid number and 120 g iodine/100g for the iodine number). The low acid value of petroleum products is beneficial for internal combustion engines; the low iodine value of the fuel is also an advantage. Moreover, unsaturated fatty acids are highly reactive, and thus could polymerize and react with oxygen or other chemical compounds to form hazardous substances in fuels. Petroleum fuels are characterized by a maximum iodine value of 120 g iodine/100g or by modified products with a high content of oleic acid (C18:1) [41]. The bio-oil obtained in these studies contained a high content of oleic acid (C18:1), amounting to 63.15% ± 5.55%, while the linoleic acid (C18:2) concentration was 20.57% ± 2.82%. The characteristic of the FAME in the bio-oil is showed in Table 6. The viscosity and density of the bio-oil provides evidence of the purity of the FAME [42], and in the present studies these values exceeded the recommended values (Table 5).

3.4. Stage 4

The technical concept and the selection of accompanying equipment were made for the dairy processing plant treating wastewater in a UASB reactor with a daily wastewater volume of Q = 300 m^3/d. The characteristics of the anaerobically treated wastewater along with the required effluent quality are presented in Table 7.

Table 3. Characteristics of the culture medium (raw wastewater) and the medium after the cultivation time (treated wastewater) in Stage 2.

Days	Culture Medium (mg/L)—Raw Wastewater						Medium After Cultivation Time (mg/L)—Treated Wastewater					
	COD	**BOD_5**	**TN**	**AN**	**TP**	**P-PO_4**	**COD**	**BOD_5**	**TN**	**AN**	**TP**	**P-PO_4**
1	780.2 ± 9.5	401.5 ± 9.4	281.3 ± 0.3	244.9 ± 5.1	68.8 ± 15.2	51.6 ± 10.2	84.2 ± 15.8	19.5 ± 6.5	3.4 ± 1.2	1.2 ± 0.3	1.2 ± 0.4	0.5 ± 0.1
5	820.3 ± 90.2	418.1 ± 68.5	165.4 ± 62.3	134.2 ± 55.2	66.1 ± 14.1	40.5 ± 11.4	68.7 ± 14.2	38.2 ± 5.8	6.8 ± 1.5	1.9 ± 0.5	1.4 ± 0.5	0.5 ± 0.1
10	801.2 ± 84.5	398.7 ± 67.7	286.4 ± 68.5	242.71 ± 70.2	66.2 ± 16.8	42.3 ± 9.8	68.7 ± 14.1	17.6 ± 4.9	3.3 ± 1.2	2.2 ± 0.6	1.5 ± 0.5	0.5 ± 0.1
15	805.4 ± 88.2	399.9 ± 70.4	282.4 ± 70.3	239.3 ± 68.8	66.0 ± 15.5	42.1 ± 10.5	73.2 ± 15.1	17.2 ± 6.3	2.1 ± 1.3	1.7 ± 0.6	1.2 ± 0.4	0.4 ± 0.1
20	789.6 ± 80.4	406.3 ± 75.4	203.6 ± 63.1	152.2 ± 65.4	64.2 ± 15.7	47.4 ± 11.9	80.8 ± 14.3	34.7 ± 7.1	8.0 ± 1.6	2.8 ± 0.5	1.4 ± 0.3	0.9 ± 0.2
25	789.6 ± 85.5	406.3 ± 77.2	303.6 ± 74.3	252.2 ± 70.3	74.2 ± 17.9	57.4 ± 10.7	79.6 ± 13.4	19.1 ± 5.9	3.9 ± 1.4	2.7 ± 0.5	1.7 ± 0.5	0.8 ± 0.1
30	813.6 ± 86.4	431.7 ± 79.5	301.1 ± 70.1	255.1 ± 71.1	72.8 ± 18.1	58.9 ± 12.8	80.1 ± 15.2	27.1 ± 6.6	3.4 ± 1.3	3.0 ± 0.6	1.2 ± 0.4	0.5 ± 0.1
35	806.1 ± 88.7	400.7 ± 72.3	296.2 ± 69.3	271.2 ± 90.1	69.1 ± 14.3	56.3 ± 11.3	84.6 ± 15.0	19.6 ± 6.0	2.2 ± 1.3	1.7 ± 0.4	1.4 ± 0.5	0.4 ± 0.1
40	813.9 ± 88.4	411.8 ± 76.4	301.2 ± 70.6	269.4 ± 80.5	72.3 ± 16.5	49.9 ± 11.4	73.5 ± 14.1	18.1 ± 6.2	2.1 ± 1.2	1.7 ± 0.3	1.5 ± 0.4	0.4 ± 0.1
45	802.2 ± 89.2	399.2 ± 70.1	288.9 ± 70.1	243.5 ± 82.4	70.9 ± 14.8	58.2 ± 10.7	69.9 ± 14.2	18.2 ± 5.5	2.8 ± 1.1	1.9 ± 0.4	1.4 ± 0.4	0.5 ± 0.1
50	793.4 ± 84.1	417.3 ± 72.2	295.7 ± 71.2	260.6 ± 89.5	68.3 ± 17.2	44.4 ± 12.5	79.6 ± 14.8	19.1 ± 5.4	2.9 ± 1.2	2.7 ± 0.5	1.7 ± 0.3	0.8 ± 0.2
55	799.1 ± 82.5	397.7 ± 69.7	299.9 ± 67.4	277.2 ± 90.4	69.9 ± 17.3	50.8 ± 11.4	73.3 ± 14.3	17.2 ± 5.2	3.1 ± 1.1	2.3 ± 0.4	1.2 ± 0.5	0.5 ± 0.1
65	804.5 ± 86.3	399.8 ± 70.4	281.9 ± 65.2	239.7 ± 87.4	59.9 ± 13.7	40.1 ± 10.6	76.1 ± 14.2	13.9 ± 4.9	3.4 ± 1.0	2.2 ± 0.4	1.1 ± 0.4	0.4 ± 0.1

Table 4. The efficiency of compounds removal and their use for the microalgae biomass growth.

Days	Removal Efficiency (%)					
	COD	**BOD_5**	**TN**	**AN**	**TP**	**P-PO_4**
1	89.21 ± 0.71	95.14 ± 0.66	98.79 ± 0.10	99.51 ± 0.01	98.26 ± 0.16	99.03 ± 0.01
5	91.63 ± 0.73	90.86 ± 0.10	95.89 ± 0.47	98.58 ± 0.15	97.88 ± 0.25	98.77 ± 0.08
10	91.43 ± 0.77	95.59 ± 0.41	98.85 ± 0.12	99.09 ± 0.01	97.73 ± 0.14	98.82 ± 0.03
15	90.91 ± 0.79	95.70 ± 0.70	99.26 ± 0.22	99.29 ± 0.04	98.18 ± 0.15	99.05 ± 0.01
20	89.77 ± 0.70	91.46 ± 0.14	96.07 ± 0.33	98.16 ± 0.32	97.82 ± 0.05	98.10 ± 0.04
25	89.92 ± 0.55	95.30 ± 0.47	98.72 ± 0.12	98.93 ± 0.08	97.71 ± 0.10	98.61 ± 0.07
30	90.15 ± 0.74	93.72 ± 0.31	98.87 ± 0.14	98.82 ± 0.07	98.35 ± 0.11	99.15 ± 0.01
35	89.51 ± 0.64	95.11 ± 0.52	99.26 ± 0.21	99.37 ± 0.05	97.97 ± 0.25	99.29 ± 0.03
40	90.97 ± 0.68	95.60 ± 0.58	99.30 ± 0.19	99.37 ± 0.06	97.93 ± 0.06	99.20 ± 0.01
45	91.29 ± 0.72	95.44 ± 0.49	99.03 ± 0.12	99.22 ± 0.07	98.03 ± 0.13	99.14 ± 0.01
50	89.97 ± 0.73	95.42 ± 0.43	99.02 ± 0.14	98.96 ± 0.12	97.51 ± 0.15	98.20 ± 0.04
55	90.83 ± 0.76	95.68 ± 0.47	98.97 ± 0.11	99.17 ± 0.10	98.28 ± 0.23	99.02 ± 0.02
65	90.54 ± 0.68	96.52 ± 0.52	98.79 ± 0.06	99.08 ± 0.12	98.16 ± 0.20	99.00 ± 0.01

Ratio of Microalgae Biomass to Introduced Organic Compounds and Nutrients (kg microalgae biomass/$kg_{int.}$)						Ratio of Microalgae Biomass to Removed Organic Compounds and Nutrients (kg biomasy/$kg_{rem.}$)					
COD	BOD_5	TN	AN	TP	P-PO_4	COD	BOD_5	TN	AN	TP	P-PO_4
0.007 ± 0.001	0.013 ± 0.003	0.019 ± 0.002	0.022 ± 0.002	0.081 ± 0.004	0.127 ± 0.008	0.007 ± 0.001	0.014 ± 0.003	0.019 ± 0.002	0.023 ± 0.002	0.083 ± 0.005	0.128 ± 0.009

Table 5. The fuel properties of the bio-oil obtained from the microalgae biomass.

Properties	Unit	Fatty Acid Methyl Esters (FAME) According to the PN-EN 14214 Norm		Bio-Oil
		Min.	Max.	
Density in 15 °C	kg/m^3	860	900	912 ± 35
Viscosity in 40 °C	cSt	3.50	5.00	33.2 ± 5.1
Flash point	°C	120	-	-
Water content	mg/kg	-	500	140 ± 20
Acid value	mg KOH/g	-	0.5	0.4 ± 0.1
Iodine value	g iodine/100g	-	120	75 ± 5
Phosphorus content	mg/kg	-	10.0	4.9 ± 2.2

Table 6. The characteristics of the fatty acid methyl esters (FAME) in the bio-oil obtained from the microalgae biomass.

Fatty Acid	Content in Bio-Oil (%)
Lauric (C12:0)	0.06 ± 0.01
Palmitic (C16:0)	5.27 ± 0.5
Oleopalmitic (C16:1)	0.28 ± 0.01
Heptadecenoic (C17:1)	0.04 ± 0.01
Stearic (C18:0)	1.68 ± 0.25
Oleic (C18:1)	63.15 ± 5.55
Linoleic (C18:2)	20.57 ± 2.82
Linolenic (C18:3)	6.20 ± 0.50
Arachidic (C20:0)	0.56 ± 0.10
Arachidonic (C20:1)	1.25 ± 0.50
Behenic (C20:0)	0.51 ± 0.15
Cervic (C22:1)	0.38 ± 0.11

Table 7. Characteristics of the effluent from the UASB reactor.

Parameter	Unit	Effluent from UASB Reactor	Required Effluent Quality
Total solids	mg/L	100	35
pH	pH	7.4	6.5–9
COD	mg O_2/L	800	125
BOD_5	mg O_2/L	400	25
TP	mg P/L	60	2
AN	mg NH_4/L	200	10
TN	mg N/L	250	30

The three raceway-pond PBRs with a total working volume of 4800 m^3 (1600 m^3 each) will operate under the following conditions. The pool dimensions will be as follows: length—60.0 m, width—10.0 m and active height—2.5 m. The central baffle of the PBR will have the dimensions as follows: length—50.0 m, bottom width—2.0 m, tank wreath width—1.0 m and height—3.0 m. The cover will have a base width of 11.0 m and a height above the wreath of the central partition of 5.0 m.

The photobioreactors will be in the form of earth-concrete tanks, in which the bottom, the walls and the central baffle will be sealed with a geomembrane. The baffle will be a concrete core panel with an earth foundation. The wreath of the ponds will be made of concrete in which there will be rollers for the mobile dome. The PBRs will have three-part polycarbonate domes. Mixing in the PBRs will be done by mechanical stirrers located at the beginning of the long straight sides of the reactor, and each reactor will be equipped with the two mixers opposite to each other. The liquid velocity in the reactors should be ensured at the level of v = 0.3–0.6 m/s.

The temperature in the raceway ponds should be maintained at 20 °C, even in winter. A heating system in the form of high-grade steel pipes inside the tank will be used. A set of a minimum of six pipes with a diameter (d) of 20 cm and with a distance between the cross-sectional axes of the pipes of 20 cm will be installed. The pipes should be placed 20 cm from the tank walls. The heat supplied will only be used to maintain the set temperature. Since the anaerobic digestate supplied into the PBRs has a temperature of 35 °C, it is assumed that the excess temperature is sufficient to heat the culture medium, and the additional heat supplied will only have to cover the losses through the tank walls and covers of the PBRs.

The outlet of a gravity pipeline (diameter of 200 mm) supplying the anaerobic digestate to the PBRs will be localized behind the stirrer in the direction of the liquid movement. The three PBRs will work in parallel, so that each of them will be fed with digestate at regular intervals, every two hours. A separation chamber localized at the outflow of the UASB reactor will be the beginning of a pipeline supplying sewage to the photobioreactors. The distribution chamber will be equipped with the three automatic valves sequentially opened every two hours in the following mode: opening of valve no. 1 will supply digestate into the first PBR, after 2 h the valve no. 1 will close and at the same time valve no. 2 will supply digestate into the second PBR, etc.

After cultivating, the liquid from the PBRs (300 m^3/d, 100 m^3/d from each PBR) will flow into the drum filters. The CO_2 pipelines will supply the carbon dioxide into the gas zone of the PBRs. The biomass production will be around 768 kg/d, which indicates that about 1400 kg CO_2/d should be delivered to the PBRs.

The light energy supply at a level of 150 μmol $Em^{-2}s^{-1}$ day for the efficient growth rate of microalgae biomass should be ensured. The three-band fluorescent lamps with narrow-band emission phosphors with a luminous efficiency of 100 lumens per watt will be used. The light regime for the night period lasting 12 h will be as follows: 2 h light phase/1 h dark phase. The effective time of lighting will be set to 8 h per day.

Before using as a culture medium, the anaerobic digestate from the UASB reactor will be hygienized by UV radiation. The hygienization system will include medium-pressure UV lamps (1000 W) for water disinfection, a stainless-steel chamber and pipes made of quartz.

The effluent parameters from the UASB reactor and from the PBRs with the efficiency of component removal are presented in Table 8.

The growth rate of the microalgae biomass is 160 ± 6.6 mg/L·d. Thus, in the three PBRs (4800 m^3) the rate is 768 kg/d. The total amount of microalgae biomass is calculated based on the concentration of the algae biomass in the PBRs (3000 ± 10.5 mg dm/L) and the PBR volume (4800 m^3), and it will reach 14,400 kg.

The microalgal demand for nitrogen and phosphorus is shown in Table 9. The result is that the demand for nutrients may be higher than their concentrations in the digestate, and a full usage of nutrients should be expected. In case of need, it will be necessary to introduce additional nutrient sources, e.g., in the form of ammonium sulfate or ammonium phosphate. Anaerobic digestate from agricultural biogas plants can also be an additional source of nutrients, where the concentration of nitrogen and phosphorus is high.

Microalgae will be pre-concentrated by using drum filters. The separation system will work about 8 h a day, assuming cyclical work. The algae concentration will be taken sequentially from PBR 1, then from PBR 2 and finally from PBR 3. The supernatant will be returned to the PBRs. The effluent will

be collected in an effluent chamber, from where it will be pumped to the water receiver. The effluent chamber will be equipped with a submersible pump with a capacity of 15 m^3/h, and also will serve as a tank with water to clean the drum filters.

Table 8. Characteristics of the effluents from the UASB reactor and photobioreactor.

Parameter	Value in the Effluent from the UASB Reactor (mg/L)	Efficiency of Removal (%)	Value in the Effluent from the Photobioreactor (mg/L)
ChZT	800	90	80
BZT_5	400	95	17.5
$N_{og.}$	250	99	2.5
P_{og}	60	98	1.2

Table 9. Nutrient requirements in the microalgal photobioreactors.

Parameter	Specific Nutrient Requirement (kg/kg dm·d)	Nutrient Requirement (kg/kg dm·d)	Daily Load (kg/d)
TN	0.1	1440	75
TP	0.01	144	18

4. Conclusions

The maximum concentration of microalgae biomass in the PBR over the cultivation time was about 3000 ± 10.5 mg_{dm}/L. It was found that the average biomass growth rate was 160 ± 6.6 mg_{dm}/L·d. The lipid content in the obtained biomass was about 20%.

The treatment efficiency of the digestate was very high. The removal of organic compounds, such as COD, was close to 90%, and that of BOD_5 averaged 95%. The total nitrogen and ammonia nitrogen removal achieved approximately 99%. The efficiency of the total phosphorus removal was on the level of 98%, while the reduction of orthophosphate concentration in the digestate reached 99%.

The bio-oil obtained from the algae cells was characterized by a high content of oleic acid (C18:1), which amounted to 63.15%. The bio-oil viscosity was 33.2 ± 5.1 cSt, with the permissible value being 5.0 cSt, while the density was 912 ± 35 kg/m^3, exceeding the maximum value of 900 kg/m^3. Thus, it is necessary to clean the obtained bio-oil to improve its fuel properties.

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