Article

Outflow from a Biogas Plant as a Medium for Microalgae Biomass Cultivation—Pilot Scale Study and Technical Concept of a Large-Scale Installation

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Abstract: Microalgae-based technologies have huge potential for application in the environment sector and the bio-energy industry. However, their cost-efficiency has to be improved by drawing on design and operation data for large-scale installations. This paper presents a technical concept of an installation for large-scale microalgae culture on digestate liquor, and the results of a pilot-scale study to test its performance. The quality of non-treated digestate has been shown to be insufficient for direct use as a growth medium due to excess suspended solids, turbidity, and organic matter content, which need to be reduced. To that end, this paper proposes a system based on mechanical separation, flotation, and pre-treatment on a biofilter. The culture medium fed into photobioreactors had the following parameters after the processing: COD—340 mgO_2/dm^3, BOD_5—100 mgO_2/dm^3, TN—900 mg/dm^3, and TP—70 mg/dm^3. The installation can produce approx. 720 kg_{VS}/day of microalgal biomass. A membrane unit and a thickening centrifuge (thickener) were incorporated into the design to separate and dehydrate the microalgal biomass, respectively. The total energy consumption approximated 1870 kWh/day.

Keywords: microalgae; biogas plant; digestate; photobioreactor; large-scale installation

Citation: Zieliński, M.; Dębowski, M.; Kazimierowicz, J. Outflow from a Biogas Plant as a Medium for Microalgae Biomass Cultivation—Pilot Scale Study and Technical Concept of a Large-Scale Installation. *Energies* **2022**, *15*, 2912. https://doi.org/10.3390/en15082912

Academic Editor: Jack Legrand

Received: 14 March 2022
Accepted: 14 April 2022
Published: 15 April 2022

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1. Introduction

Microalgae-based technologies are commonly regarded to have practical applications in the environment sector and the bio-energy industry [1,2]. Microalgal biomass has been found applicable in sewage treatment [3,4], digestate treatment [5,6], waste management [7], carbon dioxide biosequestration [8,9], biogas upgrading, and flue/exhaust gas treatment [10]. Microalgae are also considered to be a sustainable and environment-friendly precursor for energy carriers [11,12] and a source of numerous value-added chemical substances and compounds, which further contributes to the commercial benefits of the technology [13,14]. Microalgae can be grown very quickly, are resistant to pollution, and do not compete with biomass grown for food, which is why they have been attracting growing interest [15,16]. They can also be grown in places that have no other economic use, in the vicinity of conventional energy plants, sewage treatment plants, agricultural biogas plants, and waste-to-biogas plants [17].

At present, the road to large-scale take-up of technological solutions for the production and use of microalgal biomass is fraught with many economic, technological, and legal difficulties [18]. In the temperate zone, unsuitable conditions (temperature and relatively low sunlight) are an additional impediment to the intensive and efficient production of microalgal biomass. This directly prompts the necessity of equipping such facilities with solutions that ensure the proper thermal and light conditions—crucial factors in microalgae

cultivation [19]. However, these additions considerably drive up the associated investment and operational costs [20].

Therefore, new solutions are needed to improve the commercial viability of technologies for producing and utilizing microalgal biomass [21]. One of the prospective avenues of improvement is the development and take-up of technological solutions that use waste substrates as the growth medium [22,23]. Sites of anaerobic reactors can be technologically and commercially viable locations for microalgal biomass production, given the supply of ready-to-use biogenic compounds in post-fermentation effluent and carbon dioxide from combusted biogas [24]. Additionally, such installations can provide heat during cold periods [25]. This approach is fully validated by the fact that the microalgae cultivation systems used thus far in the temperate climate zone are not particularly effective in terms of technology or economy [26]. Therefore, further exploration of novel and alternative solutions is needed to improve the process.

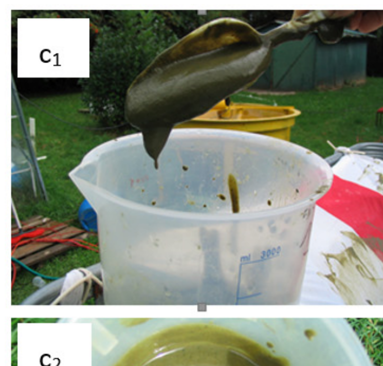
Although the potential of microalgae biomass-based technologies has been well established, the impact of such solutions has been mainly explored in laboratory conditions [27]. The majority of studies on harnessing microalgae in systems for sewage treatment and gas pollutant emission reduction have been carried out in laboratory conditions, with semi-industrial studies being much rarer [28,29]. While these kinds of studies can yield interesting results, they do not provide enough information to properly and exhaustively analyze how such facilities perform in operation. This severely limits the possibility of obtaining reliable data for the comprehensive evaluation of the technological, environmental, and economic efficiency of such solutions [30]. Further complicating such assessments is the fact that researchers have presented contradictory conclusions on the efficiency of microalgal biomass production, as well as the actual technological performance and cost-effectiveness thereof [31]. Janssen et al. (2022) proved that the high costs of microalgae technologies are mainly due to investment and exploitation expenses incurred for harvesting, separation, and biomass dewatering. These unit processes are usually based on the use of energy-intensive centrifuges, membrane separation, or microfiltration [32]. Economically justified solutions such as flocculation and sedimentation carried out in settling tanks are characterized by low technological efficiency related to the degree of concentration and dehydration of microalgae biomass [33]. The same authors believe that the share of expenditure on biomass compaction in the total expenditure will gradually decrease due to the development and widespread access to energy-saving technological solutions [33]. A very important factor that often determines the profitability of microalgae production systems is the need to provide the appropriate intensity and type of lighting [34]. Most photobioreactors require the use of an artificial light source, which directly leads to an increase in energy consumption and operating costs [35,36]. According to Avinash et al. (2020), the efficiency of the photobioreactor increases when the distance between the medium and the light is shorter. However, in practice, it is difficult to achieve [37]. A competitive alternative may be the use of LEDs, a selective and cheap light source emitting a narrow section of the light spectrum, i.e., radiation with a wavelength appropriate for the developing microalgae population [38]. Few reports in scientific and technical journals present technical/technological designs and operational data for large-scale microalgal systems [39]. As it stands, this area of research needs to be developed much further. Therefore, there is a legitimate need for application and verification of the findings obtained in laboratory conditions by launching and operating pilot-scale and full-scale installations.

The objective of this paper is to present selected findings of a pilot-scale study on a system for producing microalgal biomass on digestate sourced from a biogas plant, as well as to present the design for the process. The technology was designed as part of an agricultural biorefinery for the hybrid production of ethanol, biogas, bio-oil, and organo-mineral fertilizers from lignocellulose and microalgal biomass. An application for the system has been filed with the Patent Office of the Republic of Poland (P385950) under the name: “A method of producing electricity, heat, fertilizer and/or feed and ethanol from agricultural crops and post-production waste in the agro/food-processing industry”.

2. Materials and Methods

2.1. Preliminary Study (Pilot-Scale)

Before the full-scale plant was designed, pilot studies were first conducted to assess the viability of using digestate liquor to grow microalgal biomass. To that end, the digestate liquor was assayed and a *Chlorella vulgaris* (UTEX2714) microalgae culture was grown on non-pre-treated (raw) digestate. A raceway hybrid reactor with an active volume of 2.0 m^3 (length—3.5 m, width—1.2 m, active height—0.5 m, total height—1.0 m) was used for the experiment. The photobioreactor was sited at a biogas plant, near the digestate liquor storage. It was fitted with an agitator blade (circulation flow rate 0.5 m/s), an artificial lighting system, valves and ports for supplying digestate/air, drains, a central partition for providing circular flow, and a heating system (Figure 1). The sunlight-permeable reactor surface (transparent covers) had an area of approx. 2.6 m^2. An array of white light-emitting fluorescent lamps was distributed along the central axis of the reactor over approx. 0.6 m^2**.** The effective lighting time was 8 h per day. The light was supplied to the microalgae production system for 12 h of the night in cycles of 2 h light phase/1 h dark phase. An array of white light-emitting fluorescent lamps was distributed along the central axis of the reactor over approx. 0.6 m^2. Fluorescent tubes emitted warm light with a color temperature of 3000 K and electric power of 100 W.



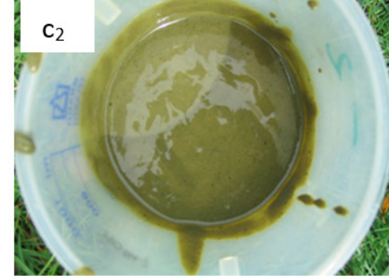


Figure 1. Pilot-scale photobioreactor ((**a**) overview, (**b**) photobioreactor chamber, ($\mathbf{c_1}$,$\mathbf{c_2}$) concentrated microalgal biomass).

In order to assess the extent to which the liquor would have to be pre-treated for the microalgae culture process, the raw digestate was diluted with tap water before being fed into the photobioreactors. Assays indicated that the digestate liquor was of insufficient quality to be used directly as a growth medium for the microalgae biomass. The proportion of raw digestate in this culture medium ranged from 10% to 50%, depending on the variant. The parameters of the digestate solids and raw digestate (liquor) are presented in Table 1. The digestate liquor was fed into the medium at the beginning of the culture cycle, followed by the *Chlorella vulgaris* inoculum (UTEX2714). Initial microalgal biomass concentration in the photobioreactor was approx. 200 mg_{VS}/dm^3. The culture period was 12 days. Samples were taken for analyses every 24 h.

Table 1. Parameters of the digestate and post-separation liquor.

Parameter	Unit	Digestate	Post-Separation Liquor (Liquid Phase)
		Value	
DM	%	4.34 ± 0.83	0.81 ± 0.9
VS	%	72.8 ± 1.8	69.3 ± 1.2
pH	pH	7.4 ± 0.1	7.4 ± 0.1
COD	mg O_2/dm^3	14,280 ± 503	7800 ± 390
BOD_5	mg O_2/dm^3	7070 ± 390	3400 ± 240
TP	mg P/dm^3	205 ± 50	168 ± 44
AN	mg N/dm^3	1550 ± 117	780 ± 90
TN	mg N/dm^3	2080 ± 260	1380 ± 190
Color	Pt-Co units	-	>500

The contents of dry matter (DM) and volatile solids (VS) were determined according to the gravimetric method. Contents of DM in the biomass were determined by drying to a constant weight at 105 °C. Then burning it at 550 °C and loss after combustion was the vs. according to PN-EN 15935: 23022-01 [40]. Chemical oxygen demand (COD), total phosphorus (TP), total nitrogen (TN), and ammonia nitrogen (AN) were determined using a DR 5000 spectrophotometer with an HT200S mineralizer (Hach-Lange, Düsseldorf, Germany). Determination of biochemical oxygen demand (BOD_5) was carried out according to PN-EN 1899-1 [41]. Total alkalinity was determined through titration testing. The pH was determined using a VWR 1000 L pH meter (Gdansk, Poland). The color was measured directly as Pt-Co units by a Hach DR-2000 spectrophotometer (Hach-Lange, Düsseldorf, Germany) according to ASTM D 1209 [42]. The statistical analysis of experimental results was conducted in triplicate using STATISTICA 13.1 PL. One-way analysis of variance (ANOVA) was used to determine significant differences between the groups. Tukey's HSD test was applied for post hoc analysis. Results were considered significant at $\alpha = 0.05$.

2.2. Source Data and Design Guidelines

The digestate liquor was sourced from a 2.1 MW_e agricultural biogas plant used to digest distiller's grains, maize silage, cattle manure, and swine manure. The plant is part of a low-emission agricultural biorefinery for hybrid production of ethanol, biogas, bio-oil, and organo-mineral fertilizers from lignocellulose and microalgal biomass. The plant operated under an organic load rate (OLR) of 3.2 $kg_{VS}/m^3 \cdot d$, hydraulic retention time (HRT) of ≈45 days, a temperature of 42 ± 1 °C, and initial water content in the feedstock of 87%. Digestate was generated in the plant at a rate of Q = 300 m^3/day. Quality parameters for the digestate and the post-separation liquid phase are given in Table 1.

The primary pollutant load indicators were calculated on the basis of characteristic flow and projected pollutant levels (Table 2). At the design stage, it was decided that the post-processing digestate will be subject to treatment until sufficient quality is achieved to discharge it into the receiver. In accordance with Council Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment, the treated liquor was regarded as urban waste-water (with minimum quality requirements adopted for treated effluent). The pollutant limits in the treated effluent were set based on the adopted population equivalent of p.e. = 35,350 (Table 2).

Table 2. Typical pollution loads, pollutant limits in the digestate, and concentration limits at the outflow to the receiver.

Indicator	Concentration	Daily Load	Unit Load	p.e. (Population Equivalent)	Concentration Limits at the Outflow to the Receiver
Unit	g/m^3	kg/d	g/M d	*n*	g/m^3
BOD_5	7070 ± 390	2121 ± 220	60	35,350 ± 515	25
COD	14,280 ± 503	4284 ± 250	90	47,607 ± 665	125
Suspended solids	43,400 ± 630	13,020 ± 460	65	200,307 ± 950	35
TN	2080 ± 260	624 ± 82	12	52,000 ± 720	15
TP	205 ± 50	61.5 ± 15	2.5	24,600 ± 480	2

3. Results

3.1. Pilot Scale

Organic matter in the digestate liquor (as expressed by COD) separated out using a Boerger Bioselect RC 50 solids separator averaged 7800 ± 390 mgO_2/dm^3. TN was approx. 1380 ± 190 mg/dm^3, TP was 168 ± 44 mg/dm^3, suspended solids in the digestate liquor were 6246 ± 998 mg/dm^3, and ammonia concentration was 780 ± 90 mg/dm^3 (Table 1).

The highest performance was achieved with the digestate liquor in the system at 10% reactor active volume. Biomass levels at the end of culture were 1455 ± 52 mg_{VS}/dm^3, with the growth rate being 150 ± 8.4 $mg_{VS}/dm^3{\cdot}d$ (Figure 2). Nitrogen take-up from the medium reached 80 ± 2.8%, whereas phosphorus removal exceeded 81 ± 3.1%. At 15% digestate in the medium, the biomass yield was 1019 ± 25 mg_{VS}/dm^3 with nitrogen and phosphorus removal rates of 66 ± 5.6% and 67 ± 7.1%, respectively. High nitrogen removal was also observed in the 20%-digestate variant, which produced biomass at the level of 1072 ± 31 mg_{VS}/dm^3 at a growth rate of 87.7 ± 3.8 $mg_{VS}/dm^3{\cdot}d$. None of the other series exceeded the biomass concentration of 1000 mg_{VS}/dm^3.

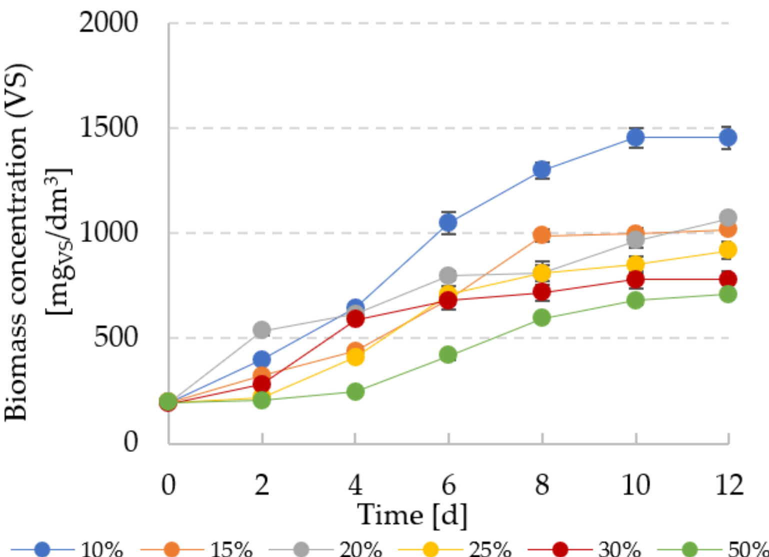


Figure 2. Dynamics of microalgal biomass growth.

There was a noticeable trend of *Chlorella vulgaris* biomass levels dropping as the digestate in the medium increased. An analysis of the relationship between initial levels of the pollution indicators and final *Chlorella vulgaris* biomass levels in the photobioreactors did not demonstrate any highly significant correlations across the tested variants. The coefficients of determination were: R^2 = 0.6683 for the COD/biomass relationship (Figure 3), and R^2 = 0.6522 for AN/biomass (Figure 4). The goodness of fit between TP/biomass was R^2 = 0.7141 (Figure 5). However, a strong correlation was observed between COD, AN, and TP and the growth efficiency of *Chlorella vulgaris* biomass after the removal of

variants in which the share of liquid digestates was 15% and 50% of the active volume of the photobioreactor. In all of these variants, the R^2 was consistently higher than 0.99, regardless of the detected pollution levels (Figures 3–5).

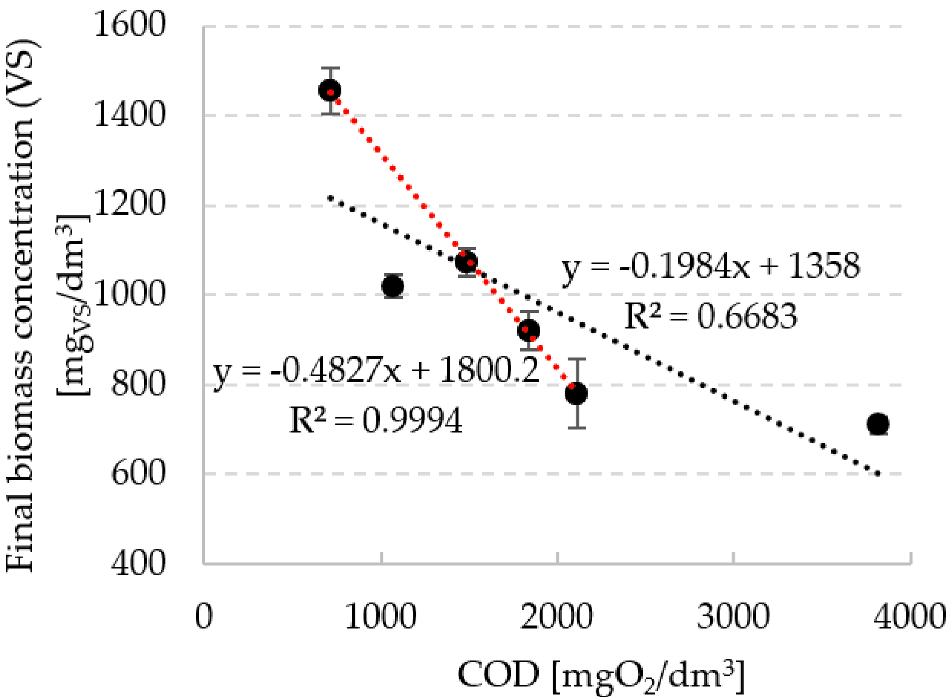


Figure 3. Correlation between initial chemical oxygen demand (COD) concentration and final production of microalgae biomass.

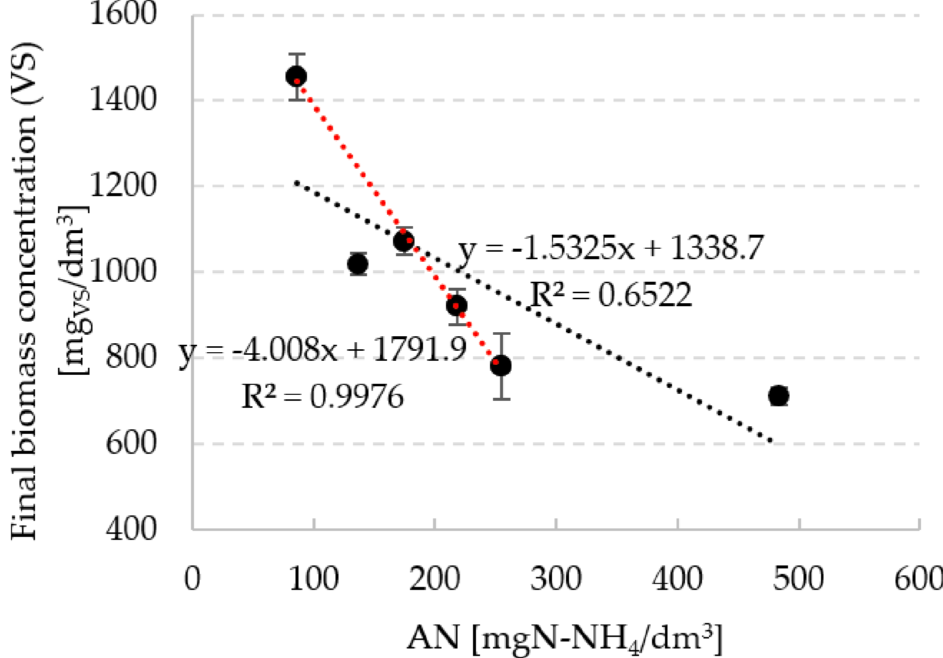


Figure 4. Correlation between initial ammonia nitrogen (AN) concentration and final production of microalgae biomass.

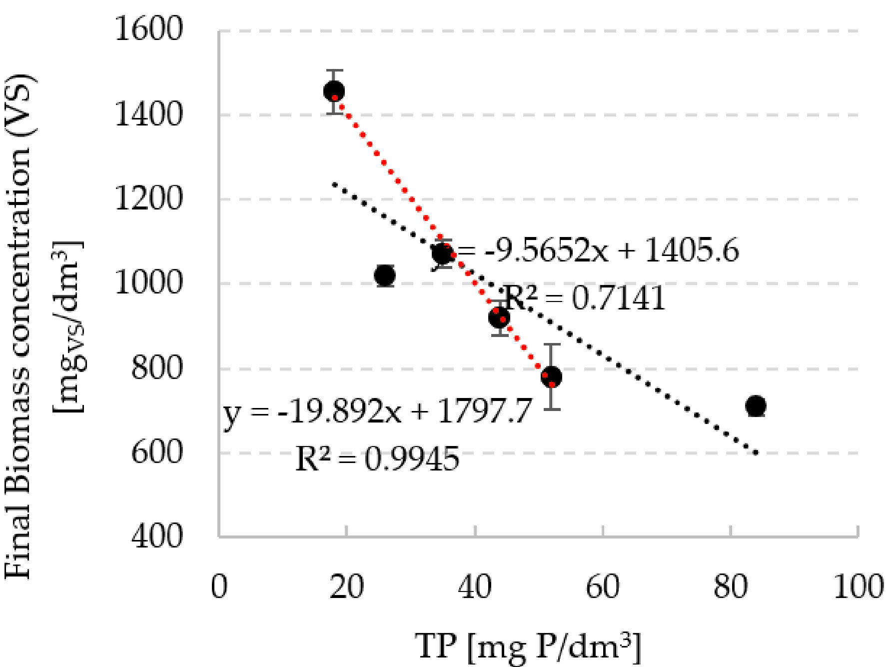


Figure 5. Correlation between initial total phosphorus (TP) concentration and final production of microalgae biomass.

The experiment demonstrated that the cultured *Chlorella vulgaris* biomass removed only a small portion of the organic matter content (as expressed by COD). Depending on the experimental variant, organic matter removal ranged between 9% and 21 %. Post-culture COD in the medium was very high, ranging from 570 mg O_2/dm^3 to over 3480 mg O_2/dm^3 (Table 3). The high organic matter content in the digestate liquor leads to the proliferation of heterotrophic bacteria, inhibiting the growth of the microalgal biomass. The color of the digestate also poses a problem, as it blocks light from penetrating into the culture medium, thus inhibiting photosynthesis.

Table 3. *Chlorella vulgaris* biomass growth rates and trends in pollutant indicators.

Liquor Volume	Day of Culture	COD	TN	AN	TP	Biomass Concentration (VS)	Biomass Growth Rate
% vol.	*n*	mg/dm^3	mg/dm^3	mg/dm^3	mg/dm^3	mg/dm^3	mg$_{VS}$/dm^3·d
10	0 12	720 ± 74 570 ± 31	120 ± 12 24 ± 1.2	86 ± 5.2 18 ± 1.1	18 ± 3.9 3.3 ± 0.4	197 ± 4 1455 ± 52	150 ± 8.4
15	0 12	1070 ± 120 880 ± 62	193 ± 21 66 ± 3.2	137 ± 15 46 ± 2.2	26 ± 2.7 6.0 ± 4.7	197 ± 3 1019 ± 25	82.4 ± 2.1
20	0 12	1490 ± 110 1290 ± 99	239 ± 19 74 ± 4.3	174 ± 20 53 ± 2.8	35 ± 1.9 13.6 ± 2.1	195 ± 7 1072 ± 31	87.7 ± 3.8
25	0 12	1840 ± 190 1630 ± 110	307 ± 17 104 ± 10.7	218 ± 16 72 ± 4.1	44 ± 2.4 21 ± 1.3	195 ± 2 920 ± 42	73.5 ± 5.7
30	0 12	2110 ± 230 1990 ± 90	364 ± 21 249 ± 19	255 ± 19 179 ± 21	52 ± 3.2 42 ± 2.0	190 ± 4 780 ± 77	59.0 ± 3.2
50	0 12	3820 ± 270 3480 ± 110	701 ± 33 590 ± 27	484 ± 25 425 ± 23	84 ± 4.8 61 ± 3.7	196 ± 8 710 ± 19	51.4 ± 4.9

This means that new technologies need to be developed to treat digestate and convert it into a form suitable for microalgal biomass production. The tested digestate is heterogeneous and varies highly in composition, which should also be taken into account. For this reason, the liquor has to be pre-treated before being used to grow microalgae.

This pre-treatment should, in particular, reduce organic matter content and eliminate color/turbidity.

3.2. Technological Concept

The presented technology is designed for the treatment of digestate liquor and the efficient production of microalgal biomass. It is part of an agricultural biorefinery for hybrid production of bio-fuels and organic fertilizers from biomass (Figures 6 and 7). At the start of the process, the digestate sourced from the biogas plant is concentrated in a separator (Figure 8). The process results in dehydrated digestate with a dry matter content of 22–25% and digestate liquor (liquid fraction) containing approx. 1% dry matter. The concentrated digestate is then sent to the storage area, from which it is hauled out to be incinerated or used as a fertilizer. In turn, the digestate liquor is subjected to individual treatment processes to ensure adequate quality for use as a medium to grow microalgae. The second stage consists of a dissolved air flotation process (without amendment with any additional substances), after which the liquor is fed onto a submerged biofilter (Figure 8). In the biofilter, pollutants are biodegraded by microorganisms from settled activated sludge. The liquor (including excess bacterial biomass) is discharged into a vertical sedimentation tank, where the bacterial microflora is separated out and the liquid is clarified. The biomass collected in the settlement section of the tank is periodically cleared and sent to the biogas plant, where it is incorporated into the feedstock mixture.

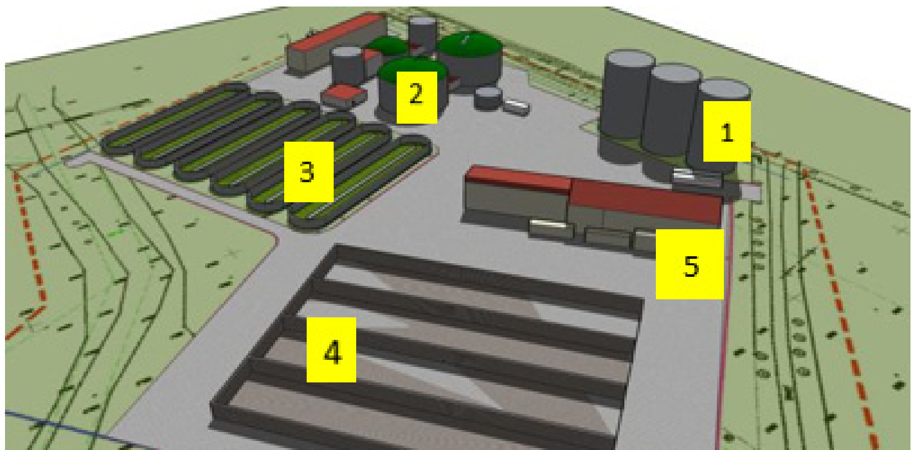


Figure 6. Biorefinery visualization. (1—bioethanol plant, 2—biogas plant, 3—microalgae photobioreactors, 4—feedstock silos, 5—office premises, monitoring and control systems).

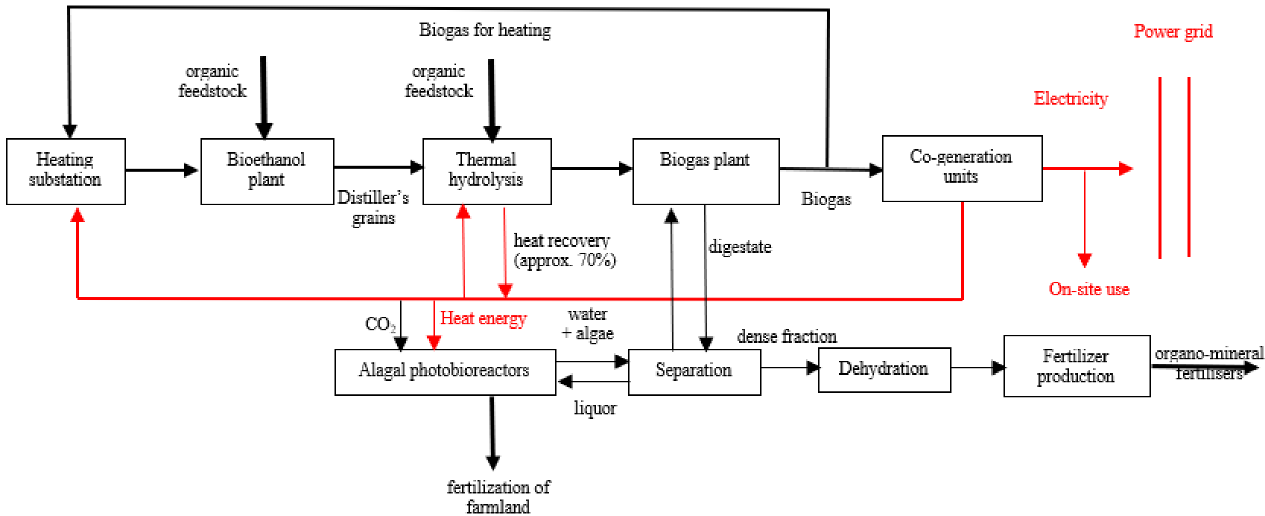


Figure 7. Flowchart of the biorefinery plant.

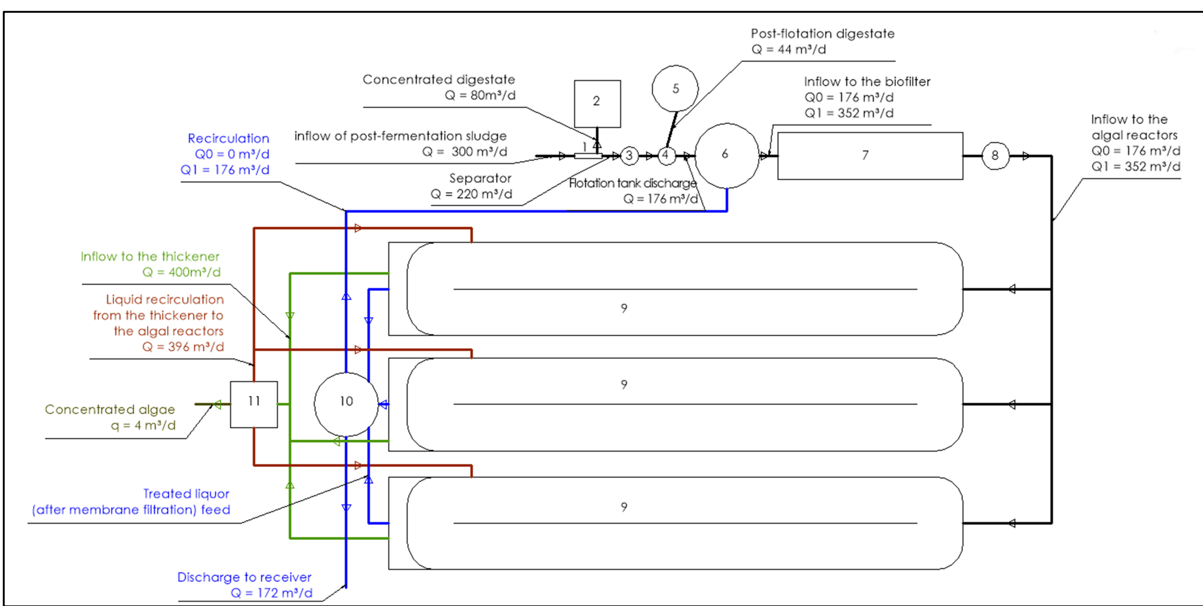


Figure 8. Process diagram of the system for producing microalgal biomass based on digestate (1—separator; 2—field of the thickened sludge; 3—digestate tank; 4—flotator; 5—post-flotation digestate tank; 6—pumping station for post-flotation digestate; 7—biofilter; 8—secondary settlement tank; 9—photobioreactors; 10—draw-off chamber; 11—centrifuge).

The clarified liquor is run off into a photobioreactor for producing microalgal biomass (Figure 8). It is in the photobioreactor where the last step of the treatment process takes place and the microalgal biomass is grown for later use as biofuel, feed, or fertilizer. The growing biomass takes up biogenic substances contained in the liquor as nutrients. The separation of biomass is a crucial step in ensuring sufficient quality of the treated digestate, which is done by means of a membrane unit (Figure 8). The microalgal biomass is separated and concentrated via microfiltration, and the treated liquor is discharged into a receiver. If necessary, the treated digestate can be recirculated back into the biofilter for simultaneous denitrification. There are also input lines running into the photobioreactor to transport liquor directly from the flotation tank and to provide carbon dioxide so that the medium is supplied with nutrients (Figure 8).

3.3. Overview of System Components

3.3.1. Digestate Separator

The digestate from the digester chamber is transported to a Boerger Bioselect RC 50 solids separator. In the separator, a rotary pump draws in the digestate directly from the digester chamber. The solids are then concentrated and separated from the liquid phase via centrifugation and filtration through a perforated cylinder. The digestate fed into the unit contains from 4% to 5% of dry matter. The separator concentrates the digestate biomass to a level of 22–25% dry matter. Given that the capacity of the separator is 50 m^3/h and the amount of solids generated is 300 m^3, the unit is active 6 h/day and produces approx. 80 tons of concentrated solids per day. Approximately 220 m^3/d of the digestate is then sent for further treatment and use in microalgal biomass production.

3.3.2. Dehydrated Digestate Storage Area

The dehydrated digestate from the separator is transported to the storage area by a screw conveyor with a capacity of 14 m^3/h. The outlet of the conveyor hangs above the middle of the storage area. The storage area consists of a concrete tank (walled on three sides) with a drainage system. The open (non-walled) side provides an access driveway, allowing the digestate to be picked up by a backhoe loader. As part of the

process, the concentrated digestate is left in the storage area for three days. The area is 12.0 m long, 12.0 m wide, and 2.0 m high, which translates to an active volume of 288 m^3.

3.3.3. Holding Container for the Liquor

The digestate liquor is conveyed via a drainage system into the treatment process line, specifically to the holding container for the pre-flotation digestate, consisting of a closed, reinforced concrete tank embedded in the ground. The digestate is discharged into the tank by gravity from the Bioselect RC 50 separator. The liquor tank is equipped with a submersible pump with a flow rate of 40 m^3/h. The dimensions of the tank are: diameter—2.0 m, total depth—2.3 m, active depth—2.0 m, and active volume—6.3 m^3. The digestate is fed in from the separator at a rate of 40 m^3/h.

3.3.4. Flotation Tank

The digestate liquor from the separator passes through the digestate holding container into an Inwatec DAF-Nikuni flotation tank (type F, model 40 F). The tank has a capacity of 40.0 m^3/h, a volume of 5.0 m^3, and a pumping rate of 7.0 m^3/h. This is where the digestate is pre-treated by means of flotation. Approx. 95% of total suspended solids are removed in this step. After flotation, the digestate is sent to the digestate storage via a storage tank for post-flotation digestate. The remaining liquor is sent out for further biological treatment. The flotation tank is active for approx. 6 h/day and outputs approx. 45.0 m^3 post-flotation digestate. 176 m^3 liquor is transported for further treatment. This liquor has the following parameters: total suspended solids—approx. 200 g DM/dm^3, organic matter (as expressed by COD)—approx. 1700 mg O_2/dm^3, and BOD_5—nearly 700 mg O_2/dm^3.

3.3.5. Pumping Station for Post-Flotation Liquor

The digestate treated in the flotation tank is transported to the pumping room, and then onto the biofilter. The pumping room contains a holding container made of reinforced concrete with an active volume of 115 m^3 and a pumping system. The system is built around a submergible pump with a flow rate of Q_{max} = 20 m^3/h. The dimensions of the holding container are: diameter—7.0 m, total depth—3.3 m, and active depth—3.0 m.

3.3.6. Biofilter

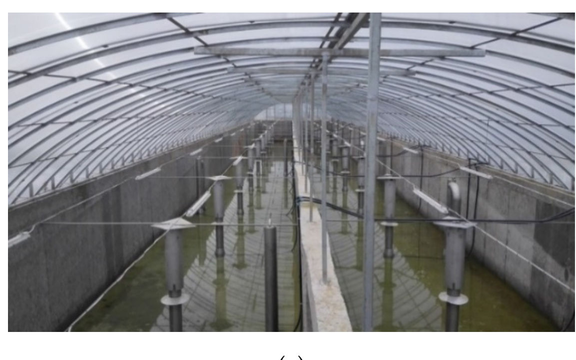
After flotation, the treated liquor is fed onto the biofilter where it is further biodegraded. In the biofilter, the digestate is subject to processes of organic matter removal, ammonification, and denitrification. Prior to the denitrification step, nitrification is primarily conducted in the microalgae-producing photobioreactors. Denitrification is possible by recirculating the medium from the photobioreactors to the biofilter. The bioreactor consists of a biomass-rich submergible bed and is deep-aerated via a fine-bubble grid. High levels of biomass are achieved through the use of packing. The chamber consists of a reinforced concrete tank with the following dimensions: length—20.0 m, width—5.0 m, total depth—3.6 m, active depth—3.3 m, and active volume—330 m^3. The organic matter load in the biofilter is approx. 300 kg O_2/d. With the volume of the biochamber and the biomass concentration being around 10 kg_{DM}/m^3, the load on the biofilter is close to 0.10 kg $COD/kg_{DM}\cdot d$. The hydraulic load on the bed surface is approx. 0.1 m/h if the medium is not recirculated from the photobioreactor chambers. In the recirculated variant, the hydraulic load rises to 0.2 m/h. The hydraulic retention time may range from 1.7 to 0.8 d, depending on how the unit is operated. Overall the hourly capacity of the aeration devices is 19.8 kgO_2/h, compressed air consumption—925 m^3/h, and daily energy consumption—300 kWh/d. The organic removal rate (as expressed by COD and BOD_5) is approx. 80%, whereas the TN removal rate is approx. 40%. The resultant effluent after biofiltration has the following parameters: COD—340 mgO_2/dm^3, BOD_5—100 mgO_2/dm^3, TN—900 mg/dm^3, and TP—70 mg/dm^3.

3.3.7. Secondary Settlement Tank

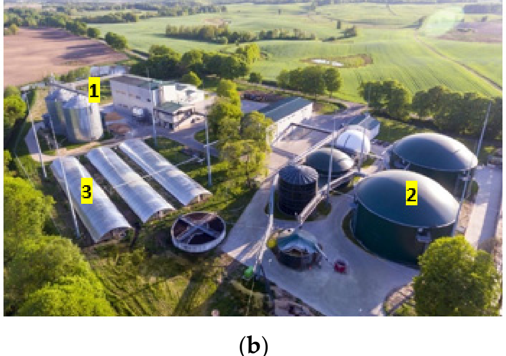
The digestate, mixed with excess biofilm, is discharged from the biofilter into a vertical, circular secondary sedimentation tank with the following dimensions: volume of the flow system—20.0 m^3, area of the sedimentation zone—6.7 m^2, height of the flow system—3.0 m, diameter—3 m^2, and height of the conical section—1.65 m. Approx. 120 kg/d excess digestate is sedimented out in the tank daily, containing approx. 95–94% moisture, which corresponds to a volume of 2.0 m^3/day. This output is used as a feedstock for methane fermentation.

3.3.8. Photobioreactors

The final step of the biological processing system consists of circulation ditch-type photobioreactors for growing microalgal biomass (Figure 9). Three devices can operate simultaneously in the system, provided they are of similar design. Each single photobioreactor has a volume of 1600 m^3 (length—60 m, width—10 m, and active depth—2.5 m), bringing the total volume of the system to 4800 m^3. The photobioreactors consist of concrete tanks with geomembrane lining on their bottoms, sidewalls, and central partitions. The central partition has the following dimensions: length—50 m, base width—2.0 m, crest width—1.0 m, and height—3.0 m. Inside the concrete rim are rollers for the sliding dome. Each photobioreactor is topped with a three-sectioned polycarbonate dome on an openwork support structure (Figure 10). The dome has a base 11.0 m wide and juts out 5.0 m above the crest of the central partition.

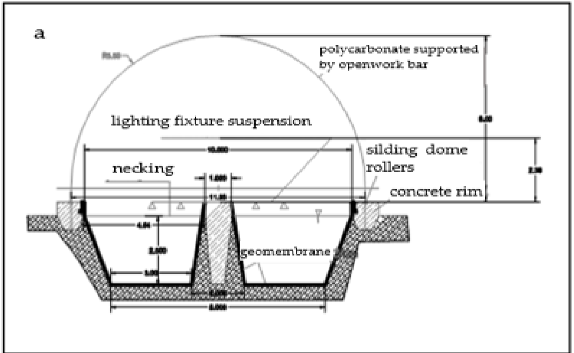


(**a**)



(**b**)

Figure 9. Photobioreactor for growing microalgae based on digestate ((**a**) overview, (**b**) placement in the process flow of the biorefinery: 1—distillery, 2—biogas plant, 3—microalgae photobioreactors).



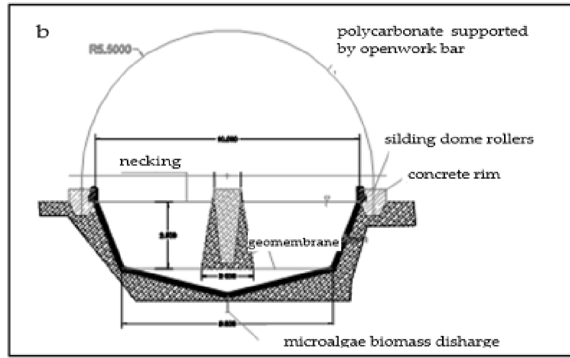


Figure 10. Cross-section views of the hybrid photobioreactor ((**a**)—A-A plane cross-section, (**b**)—B-B plane cross-section).

Stirring in the photobioreactors is provided by mechanical stirrers fitted at the extreme end of the longer straight sides of the reactor and/or through aeration with CO_2-rich gases. Each reactor has two stirrers fitted opposite each other. The medium flow rate in the photobioreactors is maintained at 0.3 to 0.6 m/s.

At the designing stage, the optimal process temperature range in the photobioreactors was assumed to be 20 °C to 30 °C. To maintain these temperatures, a heating system was set up, consisting of high-quality steel piping within the tank, installed with 20 cm clearance from the photobioreactor wall. Each reactor was fitted with a set of 6 heating pipes with a diameter of 20 cm. The rows of heating tubes are spread out with 15 cm spacing between their cross-section axes. The supplied heat is used solely for maintaining the target temperature.

The outlet of the pipe for supplying treated digestate is located downstream of the stirrer. The liquor is transported from the secondary settlement tank to the photobioreactors via gravity. The photobioreactors operate in parallel, which means that each of them is supplied with liquor at equal (two-hour) intervals. A separation chamber is fitted at the outflow from the secondary settlement tank. A digestate supply pipe runs from the chamber to the algae reactors. The separation chamber is equipped with three automatic valves that open sequentially every two hours. Valve 1 opens up the digestate liquor supply to reactor 1. After 2 h, valve 1 closes and valve 2 opens, supplying liquor to reactor 2. The liquor supply pipe is 20 cm in diameter. Carbon dioxide is fed into the gas zone of the algal reactors (the process requires approx. 1400 kg CO_2/day).

To ensure efficient photosynthesis and biomass growth, the light intensity should be maintained at a minimum of 150 µmol Em^2s. Adequate illumination in the photobioreactors is provided by means of tri-band fluorescent lamps with narrowband phosphors with luminous efficacy of 100 lumens per watt of delivered energy (100 lm/W) or 100 lux/W (1 lux = 1 lumen/m^2). The light requirements of the algal biomass are: 0.02 µmol Em^2s = 1 lux, 150 µmol Em^2s = 7500 lux, 7500 lux $\times$ 2000 m^2 = 15,000,000 lux (2000 m^2—surface of algal reactors). The microalgae cultivation system is artificially illuminated for 12 h of the night under a 2 h light/1 h dark regime. The lighting system is thus effectively active for a total of 8 h per day.

3.3.9. Microalgal Biomass Growth

Based on the pilot study, the biomass growth rate was projected to be 150 $g_{VS}/m^3 \cdot d$. Given the active volume of the photobioreactors (4800 m^3), this should provide for a growth rate of 720 kg_{VS}/day. However, the microalgal biomass growth rate can vary according to the season. This is why the reactors were designed to minimize the impact of external conditions on biomass growth parameters by maintaining stable process temperature and level of illumination.

The daily P load fed into the algal reactors is 21 kg/d. Given the average P demand of 0.028–0.03 kg P/kg_{VS} microalgae and the biomass yield (720 kg_{VS}/day), the estimated consumption will fall within the range of 19.4 to 21.6 kgP. This translates to 1.4 kgP to 0.0 kgP in the outflow, which means that the concentration will be below the target level of 2.0 mg P/dm^3. The daily N load fed into the algal reactors is 90 kgN/d. Given the average N demand of 0.12–0.15 kg N/kg_{VS} microalgae and the biomass production rate (720 kg_{VS}/day), the estimated consumption will fall within the range of 86.4 to 108 kgN. This translates to 3.6 kgN to 0.0 kgN in the outflow, which means that the concentration will be below the target level of 15.0 mg N/dm^3.

3.3.10. Microalgal Biomass Separation

Membrane Modules

The medium mixed with the microalgal biomass is transported from the photobioreactors into a membrane separation unit, with one membrane module for each photobioreactor, as well as a flocculation unit and a centrifuge. Then, 170 m^3 of treated digestate liquor should be discharged into the receiver via the membrane modules. If recirculation is applied, this value should be increased to a maximum of 350 m^3/d. The membrane modules are located in chambers located on the extreme side of each photobioreactor. On the southernmost side of each photobioreactor, the crest of the side-wall is located 0.5 m below the liquid level. This enables the microalgae-containing liquid to flow freely into

the unit. The membrane module chamber is 5.0 m long, 10.0 m wide, and 2.5 m deep, with a volume of 125 m^3. The volume of liquid discharged into the photobioreactors is Q_{liq} = 300 m^3/d, whereas concentrated biomass discharge is Q_{conc} = 12 m^3/d. Transported daily into the receiver or used on-site is 288 m^3 of treated liquor. The system is equipped with 3 membrane modules (Puron Hollow Fiber, model PSH-250), each module having a filtration area of 250 m^2 (bringing the total filtration area to 750 m^2). The operating parameters for the filtration module are as follows: operating temperatures of 5–40 °C, maximum filtration transmembrane pressure of 0.6 bar, maximum backflush transmembrane pressure of 14.5 bar, outside membrane fiber diameter of 2.6 mm, filtration pore diameter of 0.05 µm, required airflow for backflush of 0.4 $Nm^3/h \cdot m^2$, and required blower capacity of 100 Nm^3/h.

The suction pump generates pressure that forces water out of the microalgae chamber, where the algal biomass is dehydrated. Therefore, this is where the inlet for the biomass-thickening centrifuge system (thickener) was situated. The post-filtration liquid is transported into the drain chamber tank. The membrane module is continuously purged with compressed air to prevent biomass build-up. The design provides for using one blower unit for each module (e.g., a 3 kW Rots BAH 10/30 blower with a flow rate of 109.79 Nm^3/h). The membranes are periodically rinsed (backflushed) with pure water collected in the drain chamber. The membrane unit consists of: membrane unit blowers, air pipes, filtrate suction pumps/backflush pumps, metering systems for NaOH, hypochlorite and acetic acid (for membrane rinsing), automation and control units (in-house system-control cabinet with a touch panel), control lines, valves, dampers, process pipes, and recirculation pipes.

After membrane separation, the microalgal biomass is fed into an ALDRUM Drum Thickener, Midi model (manufactured by Alfa Laval), preceded by an ALDRUM Flocculation Reactor, Midi model (also by Alfa Laval). The thickener is active for a total of 6 h/day. The algae for thickening are successively extracted from reactor 1, algal reactor 2, and algal reactor 3 (in this order). After thickening, the liquid is recirculated to the photobioreactors. The treated digestate is discharged into the drain chamber, and from there pumped out to the recirculation system and the receiver. The drain chamber will be equipped with two submergible pumps, the first of which will have a pumping capacity of 20 m^3/h and will be used to transport the liquor to the receiver. The second pump, with a capacity of 10 m^3/h, will be used for recirculation. Recirculation is activated intermittently. The drain chamber is simultaneously used as a tank for clean water (used to rinse the membrane unit).

Energy Requirement

The costs of running the system for producing algal biomass and treating digestate liquor relate mainly to the required power input. An overview of the required energy inputs is presented in Table 4.

Table 4. Energy is required to power the system for producing algal biomass and treating digestate.

No.	Description	Power [kW]	Operation Time [h]	Power Input [kWh/d]
1	Bioselect separator with pump	11.0	6	66
2	Screw conveyor	1.0	6	6
3	Submergible pump	0.5	6	3
4	Nikuni flotation tank	5.5	5.5	30.25
5	Submergible pump	1.0	5.5	5.5
6	Biofilter aeration	12.4	24	297.6
7	Algal reactor stirring	4.8	24	115.2
8	Algal reactor lighting	150	8	1200
9	Thickener	5.5	5.5	30.25
10	Membrane units	18.75	18.5	346.8
11	Circulating submergible pumps	2	8	16
			Total:	**1865.8**

4. Discussion

Every technology has to be tested before deployment—first in pilot-scale installations, then on a semi-industrial scale, and, finally, as prototype full-scale installations [43]. The maturity of new technologies is measured on TRL (technology readiness level), a nine-level scale that can be used to estimate how far a technology is away from being ready for use [44]. This metric provides valuable information for potential investors or buyers. In this light, large-scale studies are particularly important [45]. Given the current level of advancement of microalgae-based technologies, such testing is necessary to accurately identify operational and technological problems [46]. This is particularly important in the case of systems used in climate zones not conducive to such processing (due to temperatures being too high or low, insufficient sunlight, or low water resources) [47].

This pilot-scale study has shown that higher levels of digestate in the medium negatively impact *Chlorella vulgaris* biomass growth rate. This can be attributed to the increased color and turbidity in the medium caused by suspended organic matter, which limits exposure to light and, consequently, inhibits microalgae growth [48]. The literature data indicates that sufficient dilution of digestate liquor is a major factor in microalgae production on digestate [49,50]. In our own study, digestate was diluted in tap water, so that the digestate proportion in the culture medium would range from 10% to 50%. The highest biomass production and pollution removal performance was achieved with 10% digestate liquor in the system. Microalgae growth is also inhibited by high levels of ammonia nitrogen [51]. Literature reports indicate that ammonia is toxic to microalgae at concentrations exceeding 100 mg/dm^3 in the medium [52]. *Spirulina platensis* is completely inhibited at 150 mg $ammonium/dm^3$ [53], and the same is true for *Chlorella sorokiniana* at 210 mg/dm^3 [54]. The present pilot study indicates that AN levels of 137 ± 15 mg/dm^3 inhibit *Chlorella vulgaris* biomass growth.

A comparative study has been conducted to investigate the discrepancy between laboratory data and pilot study data. The study used reactors with volumes of 0.25 dm^3, 100 dm^3, and 1000 dm^3 [55] as well as bacterial and microalgal consortia extracted from fish ponds. Theperformance proved to vary significantly depending on the scale of the reactor. In the laboratory, the net biomass growth rate was 18 ± 21 $mg/dm^3/day$ for 0.25 dm^3 reactors and 4 ± 38 $mg/dm^3/day$ for 100 dm^3 reactors. The TN removal rates were 3.16 ± 2.73 $mgN/dm^3/day$ and 1.82 ± 3.97 $mgN/dm^3/day$, respectively. The pilot-scale experiment (1000 dm^3) produced a net biomass growth rate of 12 ± 24 $mg/dm^3/day$ and TN removal rate of 1.63 ± 2.95 $mgN/dm^3/day$. This shows that laboratory data cannot be used to extrapolate performance for large-scale microalgae-based systems [55].

Barceló-Villalobos et al. (2019) explored changes in culture parameters (lighting, temperature, pH, and dissolved oxygen) in a pilot-scale thin-layer reactor and their impact on *Scenedesmus almeriensis* culture productivity. The 120 m^2 thin-layer reactor was run continuously. The results of the pilot study were used to identify culture inhibitors and to devise a model for assessing the impact of culture conditions on photosynthesis efficiency and *Scenedesmus almeriensis* cell proliferation. The authors stated that this represents the first step in optimizing and scaling up this reactor design for industrial applications [56].

Morillas-España et al. (2021) assessed *Anabaena* sp. and *Dolichospermum* sp. production capacity (with wastewater and swine manure used as feedstock) in a 1.04 m^3 raceway reactor located outdoors and operated at a culture depth of 0.12 m. With a dilution ratio of 0.3/day, biomass yields peaked at 20.9 $g/m^2{\cdot}day$ for *Anabaena* sp. and 28.0 $g/m^2{\cdot}day$ for *Dolichospermum* sp. The highest rates of TN removal for *Anabaena* sp. and *Dolichospermum* sp. were 2471 and 3621 $mg/m^2{\cdot}day$, respectively. Maximum $P\text{-}PO_4{}^{3-}$ removal for the *Anabaena* sp. and *Dolichospermum* sp. cultures was 81.5 and 87.1 $mg/m^2{\cdot}day$, respectively [57].

Oostlander et al. (2020) were successful in growing *Rhodomonas* sp. on a pilot scale in three identical tubular photobioreactors with a working capacity of 200 dm^3 each, in the sunlight conditions of the Netherlands from February to July. This is the first recorded pilot-scale culture of *Rhodomonas* sp. z o.o. using natural sunlight. The biomass productivity

was two to five times higher than in previous pilot-scale experiments with *Rhodomonas* sp. in stable, artificial lighting [58].

Growing microalgae on an industrial scale is often a long-term process, susceptible to culture failure and unwanted contamination. A two-step scale-up process has been proposed to address this issue. *Chlorella vulgaris* cells grown heterotrophically in 0.2 and 5 m^3 reactors (with yields averaging 27.54 ± 5.07 and 31.86 ± 2.87 $g/dm^3/day$, respectively) have been used to directly inoculate several industrial 100 m^3 tubular PBRs for autotrophic production of microalgae. All heterotrophically inoculated cultures in the photobioreactors reached standard levels of protein and chlorophyll, i.e., 52.18 ± 1.30% DW and 23.98 ± 1.57 mg/gDW, respectively. Apart from providing inoculum of consistently high quality, the two-step approach also led to a fivefold reduction in scale-up time and a 12-fold reduction in the area needed for industrial production [59].

Pereira et al. (2018) grew *Tetraselmis* sp. CTP4 in 35 m^3 and 100 m^3 tubular photobioreactors (PBRs) on an industrial scale for 60 days. In the course of the optimization process, it was shown that maintaining culture rates of 0.65–1.35 m/s and a pH of 8.0 produced better yields. The best performance in terms of volume (0.08 ± 0.01 $g/dm^3/day$) and area (20.3 ± 3.2 $g/m^2/day$) was achieved in the 100 m^3 PBR, with the 35 m^3 PBR producing inferior yields (0.05 ± 0.02 $g/dm^3/day$ and 13.5 ± 4.3 $g/m^2/day$). CO_2 sequestration monitoring in the 100 m^3 PBR, showed that CO_2 emissions were reduced by 65% on average [60].

5. Conclusions

The qualities of digestate liquor and raw liquor preclude them from being used directly as a growth medium for the microalgae. It is therefore necessary to reduce suspended solids, turbidity, and organic matter content. In this pilot-scale study, this was achieved by diluting the digestate in tap water so that the proportion of digestate in the culture medium would range from 10% to 50%. The highest biomass production and pollution removal performance was achieved with 10% digestate liquor in the system. Biomass levels at the end of culture were 1455 ± 52 mg_{VS}/dm^3, with the growth rate being 150 ± 8.4 $mg_{VS}/dm^3 \cdot d$. Nitrogen take-up from the medium was around 80 ± 2.8%, whereas phosphorus removal exceeded 81 ± 3.1%.

To ensure that the digestate processed in the large-scale installation is of adequate quality, a digestate pre-treatment system consisting of mechanical separation, flotation, and pre-treatment on a biofilter was proposed. The resultant effluent after biofiltration had the following parameters: COD—340 mgO_2/dm^3, BOD_5—100 mgO_2/dm^3, TN—900 mg/dm^3, and TP—70 mg/dm^3. The design can produce approx. 720 kg_{VS}/day of microalgal biomass. A membrane unit and a thickener were used to separate and dehydrate the microalgal biomass, respectively. The process requires a total power input of approx. 1866 kWh/day. Photobioreactor illumination is responsible for the bulk of the energy consumption, requiring 1200 kWh/day.

An important stage in the development of each technology is its achievement of the level of advancement, which allows for the development of a project, cost estimate for investor works, and the economic balance of operating activities. Innovative, high-risk solutions, must be associated with obtaining funding from various programs supporting the development of innovation. This significantly reduces the costs incurred and the investor's risk but allows the weakest points of the technology to be found in the conditions of full-scale operation. Research works on a pilot scale, developing a technical concept, building a large-scale facility, and carrying out a technological start-up are only the beginning of data collection, their verification and indication of directions for changes, modification, and technological and cost optimization of the presented microalgae biomass production technology. It should be emphasized that this is an object included in the bio-refinery complex, which should be considered as a whole. Nevertheless, it can already be stated that the greatest challenges relate to the reduction of costs related to the separation and thickening of microalgae biomass, and above all, to the reduction of expenditure on

the illumination of photobioreactors. It is necessary to search for a cheap light source, perhaps based on the use of efficient selective LEDs, or to seek conditions for effective micotrophic or heterotrophic microalgae cultivation. An important aspect is also the selection of the best, from the economic point of view, the method of valorization of the obtained microalgae biomass.

According to the authors, minimizing costs and improving economic efficiency should be based on a comprehensive approach to the technology of microalgae biomass production. It should take into account the use of cheap heating, nutrients, lighting, and a source of carbon dioxide. The technological solution presented in the manuscript includes just such an integrated approach to the economics of microalgae biomass production. Photobioreactors have been designed as an integral element of a low-emission agricultural biorefinery based on a hybrid system for the production of ethanol, biogas, bio-oil, and organic and mineral fertilizers from lignocellulose and microalgae biomass.

Author Contributions: Conceptualization, M.Z. and M.D.; Methodology, M.D.; Validation, M.Z.; Formal analysis, M.Z. and M.D.; Investigation, M.Z., M.D. and J.K.; Resources, M.Z., M.D. and J.K.; Data curation, M.Z.; Writing—original draft preparation, M.D. and J.K.; Writing—review and editing, M.Z., M.D. and J.K.; Visualization, M.D. and J.K.; Supervision, M.Z.; Project administration, M.Z.; Funding acquisition, M.Z., M.D. and J.K. All authors have read and agreed to the published version of the manuscript.

Funding: The manuscript was supported by Project financially supported by Minister of Education and Science in the range of the program entitled "Regional Initiative of Excellence" for the years 2019–2022, project No. 010/RID/2018/19, amount funding 12,000,000 PLN.

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.

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