Cultivation of Microalgae in Liquid Digestate to Remove Nutrients and Organic Contaminants

Ewelina Sobolewska[1,2] · Sebastian Borowski[1] · Paulina Nowicka-Krawczyk[3]

Abstract

The goal of this research was to assess the efficiency of the liquid digestate treatment conducted with algal, environmental isolates illuminated entirely with sunlight. The photobioreactor was exposed to natural conditions and evaluated based on the reduction of chemical oxygen demand (COD), nitrogen compounds, and soluble phosphates. Microalgal and bacterial communities growing during the treatment process were studied. A high removal rate of soluble COD (=91%) and nutrients (=86%) was achieved. The average concentrations of nitrogen, phosphates, and COD in the reactor effluent were 95 mgN/L, 49 mg/L, and 735 mg O_2/L, respectively. The overall algae-bacteria biomass productivity of 22 mg/L/d, calculated on the total suspended solids (TSS) basis, was recorded. The microbial analysis revealed the dominance of *Tetradesmus obliquus* followed by *Microglena* sp. in the first 14 weeks of the experiment. At the end of the experimental run, *Chlorella sorokiniana* cells appeared as a result of illumination intensity changes. The dominating bacteria belonged to Firmicutes (26.31%), Patescibacteria (17.38%), and Actinobacteriota (14.86%) and could have been responsible for the transformation of nitrogen and oxidation of organic contaminants. The research demonstrated that natural sunlight can successfully be used for efficient liquid digestate treatment with the algae-bacterial community.

Keywords Vegetable waste digestate · Nitrogen and phosphorus removal · Microalgae-bacteria community · Sunlight-operated bioreactor

Introduction

Managing waste streams generated during technological processes is widely recognized as a significant challenge for the industrial sector. Recently, much attention is being paid to the use of anaerobic digestion (AD) for organic waste valorization, biogas production, and digestate generation [1]. Over the last 20 years, the production of biogas obtained in the AD process has increased significantly. According to the European Biogas Association (EBA), in 2020, as many as 18,943 biogas plants and 725 biomethane plants were recorded across Europe, producing around 167 TWh (15.8 bcm) of biogas and 26 TWh (2.5 bcm) of biomethane. The EBA projects production to reach a minimum of 380 TWh by 2030, showing an increasing trend in the near future and indicating an increased interest in the anaerobic digestion of organic waste [2]. However, apart from biogas, the main product of the anaerobic treatment process is digestate, which is rich in burdensome nutrients and organic contaminants [1]. In accordance with OJ L 170, 25.6.2019 [3] post-digestion products obtained, *inter alia* from fresh plants or their parts can be used as fertilizing products in UE. However, such digestate must meet certain criteria, and the anaerobic digestion process has to be carried out in specific conditions [3]. On the other hand, due to a high ammonia content, an uncontrolled management of digestate may lead to water eutrophication disturbing the ecological stability of ecosystems. Moreover, digestate may also enhance biological contamination due to the presence of numerous pathogens and phytotoxins. Additionally, digestate storage may increase greenhouse gas emissions

✉ Ewelina Sobolewska
ewelina.sobolewska@dokt.p.lodz.pl

1 Department of Environmental Biotechnology, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Wólczańska 171/173, 90-530 Lodz, Poland

2 Interdisciplinary Doctoral School, Lodz University of Technology, Żeromskiego 116, 90-924 Lodz, Poland

3 Department of Algology and Mycology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

(N$_2$O, CH$_4$) as anaerobic digestion rarely decomposes all volatile solids. On the other hand, a high concentration of nutrients and macroelements (phosphorus, nitrogen, potassium, sodium, magnesium, sulfur, calcium) and the presence of various trace elements (e.g. iron, copper, nickel, cobalt) make the digestate an attractive material for reuse. Digestate can be separated into a solid (constituting 10–20% by mass) and a liquid (80–90% by mass) fraction, allowing to treat them independently [1, 4]. The most common separation devices for digestates used in full-scale plants are decanter centrifuges, screening drum presses, and screw presses. Compared to liquid digestate, solid digestate has attracted more attention in research and development opportunities due to efficient post-treatment processes and valorization purposes. In recent years, new routes have been proposed for solid fraction valorization, including biochar production, biofuel generation (e.g., for applying in domestic furnaces), and treatments for methane recovery. The liquid fraction is more difficult to manage and also creates greater environmental problems than the solid digestate. This results from a low C/N ratio and a high content of refractory substances, including phosphorus, nitrogen, and often heavy metals. The increased concentration of nitrogen and other compounds can restrict the application of liquid digestate in the soil as described by the European Nitrates Directive [1, 5]. Furthermore, the high water content makes the liquid digestate difficult to store and transport as well as limits its direct use as a soil conditioner or fertilizer due to high costs [6]. So far, several chemical and mechanical techniques have been developed to treat the liquid digestate, including ammonia stripping, reverse osmosis, or struvite precipitation [7]. However, these technologies are characterized by low efficiency, high costs, and a generation of difficult-to-treat by-products. To overcome these problems, new biological methods are being introduced and studied. One of the alternatives is the application of microalgae capable of growing in a low C/N environment typical for liquid digestates. These organisms are known to utilize ammonium nitrogen, nitrates, and phosphates, as well as some organic compounds [8]. Both pure cultures and complex communities have been studied in the literature. They have been successfully applied to treat anaerobic digestates obtained from various waste processing, including food waste[9, 10], food waste and animal manure [11], agricultural waste [12], or piggery waste [4, 13]. However, in contrast to mono- and axenic cultures, the use of microalgae and microalgae-bacteria consortia for digestate treatment is believed to be a more appropriate approach since microbial consortia are considered more stable due to their more diverse metabolism and different light absorption capabilities, which can contribute to the increased nutrient removal efficiency as well as greater biomass productivity. In mixed systems, bacteria produce CO$_2$, which is then consumed by algae and converted to O$_2$ and, in turn, absorbed by ammonium oxidizing and heterotrophic bacteria. In addition, dissolved organic carbon released by microalgae can be used by heterotrophic bacteria. Moreover, nitrifying bacteria oxidize ammonia that could be toxic to algae and produce nitrates better available by the latter microbes [14, 15]. Liquid digestate containing high nutrient contents is therefore a potentially attractive material for the cultivation of microalgae or algae-bacteria communities. With biomass separation, treated liquid digestate could be applied as process water, animal drinking water, or used for irrigation in areas of drought [15]. Additionally, biomass created during the treatment is a promising substrate for a sustainable production of various compounds and value-added products. The bioenergetic potential of algae is currently the main subject of diverse studies for the algal-derived fuels (bioethanol, biodiesel, biomethane, or biohydrogen). Moreover, algal biomass is a rich source of bioactive compounds including lipids, fatty acids, pigments, proteins, amino acids, and polysaccharides. Recent studies have also shown the antibacterial, antiviral, and antioxidant potential of properly processed biomass. This approach is consistent with the overall idea of a zero-waste and circular economy [16].

It should be noted that most of the experiments described in the literature have been performed using preliminarily treated digestate in order to avoid biological contamination of algae consortia and primarily reduce ammonia concentrations and turbidity. High turbidity, elevated ammonia contents, and unfavorable nitrogen-to-phosphorous ratio (N/P) can hinder the development of microalgae despite the high tolerance of many strains to toxic components [17, 18]. High pH of some digestates combined with progressing algal development (which increases pH during respiration processes) favors the formation of the un-ionized form of ammonia, which is more toxic and difficult to uptake than ionized ammonium nitrogen. Therefore, many studies point to the need for pH adjustment in order to improve ammonium intake and boost digestate remediation [19]. Although preliminary treatment of digestate (substrate dilution [17], sterilization [18], pH adjustment [19]) can improve microalgae growth, at the same time, it generates additional costs and could be impractical on an industrial scale [18].

The objective of this research was to assess the efficiency of the non-sterilized and non-filtered liquid digestate treatment conducted with algal, environmental isolates using natural sunlight for illumination. For the treatment process, *Tetradesmus obliquus* and *Microglena sp.* were used, with *Microglena* applied previously only in a few, singular studies [20, 21]. In comparison to analogous research, liquid digestate, as a substrate less optimal for algal growth, was not subjected to sterilization, filtration, or pH correction. A bioreactor, in which the treatment process was conducted, was illuminated only with natural sunlight despite the

experiments performed in a transitional climate zone, where cloudy days prevail. Abovementioned conditions (application of non-commercial, environmental isolates, lack of sterilization, filtration and pH correction, and no additional illumination) were tested for achieving satisfactory treatment efficiency with the potential to reduce operating costs.

Materials and Methods

Liquid Digestate and Microorganisms

The digestate used in the study was obtained from the Warmia Fruit and Vegetable Processing Company Ltd. (Kwidzyn, Poland), treating vegetable wastes (corn and green peas). The substrate originated from the bioreactor working under an organic loading rate equal to 1.5 kgVS/m^3/d. The digestate was subjected to centrifugation (10 min, 4800 G) using Rotina 420 (Hettich, Switzerland) to obtain the liquid fraction further used for the research (Table 1). The liquid digestate was not subjected to other pre-treatments such as sterilization or filtration. Apart from initial, gradual introduction of digestate to the bioreactor until the set HRT was achieved, the substrate was not diluted.

A nonaxenic consortium containing *Tetradesmus obliquus* and *Microglena* sp. green algae (each with an initial cell density of 1.0×10^4 cells/mL) was applied for the experiment. These algae were isolated and identified in the previous study showing the ability to grow in the liquid digestate [20]. Microalgae were initially cultivated using a modified BBM (Bold's Basal Medium) with a threefold nitrogen concentration (3N-BBM) prepared in accordance with Andersen [22].

Experiment Design

A 350 mL volume photobioreactor working in semi-continuous mode was used in the study (Fig. 1). The experiment was conducted from the end of May to the beginning of September in laboratory conditions. The bioreactor was illuminated with natural sunlight of an average intensity of 6.0 kLux. The natural cycle of day and night was therefore kept. The average temperature of 30 ± 2 °C was registered throughout the experimental run. The bioreactor was supplied with the constant airflow maintaining the microalgal cultures in suspension. Air was provided with a capacity of 0.7 L/L/min using a membrane blower, model AP-80 H (Thomas, Yamasuga, Japan). A consortium of algae was initially cultivated in a 3N-BBM medium for 4 weeks, after which the digestate started being introduced to the bioreactor for another 112 days. Hydraulic retention time (HRT) equal to solid retention time (SRT) was set at 30 days. Specifically, twice a week, 39 mL of the effluent was withdrawn from the bioreactor, and the same volume of fresh

Table 1 Physicochemical characteristic of the liquid digestate fraction

Indicator	Average value ± standard deviation
pH	7.80 ± 0.09
Total solids [g/kg]	3.20 ± 0.02
Volatile solids [g/kg]	1.66 ± 0.01
Volatile solids [%TS]	51.33 ± 0.54
Total suspended solids [mg/L]	789.99 ± 111.23
Turbidity [FAU]	480.32 ± 96.38
Apparent color [mg/L Pt–Co]*	$12{,}099.31 \pm 2231.12$
True color [mg/L Pt–Co]**	$10{,}987.22 \pm 2021.41$
Total chemical oxygen demand [mg O_2/L]	7920.00 ± 535.00
Soluble chemical oxygen demand [mg O_2/L]	7840.00 ± 477.00
Total volatile fatty acids [mg/L]	3201.71 ± 978.44
Ammonium nitrogen [mg/L]	578.88 ± 78.11
Nitrates [mg/L]	1009.99 ± 100.21
Nitrites [mg/L]	9.41 ± 2.82
Orthophosphates [mg/L]	349.22 ± 15.21
Zinc [mg/L]	14.99 ± 1.49
Aluminum [mg/L]	10.21 ± 8.47
Copper [mg/L]	5.66 ± 0.39
Iron [mg/L]	4.11 ± 2.15
Chlorides [mg/L]	215.22 ± 28.89
Sulfides [μg/L]	1549.22 ± 608.22
Sulfates [mg/L]	0.00 ± 0.00

*determined in unfiltered samples

**determined in filtered samples

liquid digestate was introduced. Feeding and discharge of the bioreactor were performed manually using a syringe. Total and soluble chemical oxygen demand (tCOD/ sCOD), microalgal biomass parameters defined by chlorophyll *a* concentration, optical density, the number of algal cells, as well as light intensity were measured once a week. Other indicators such as pH, nitrates, nitrites, ammonium nitrogen, orthophosphates, turbidity, and total suspended solids (TSS) were determined twice a week. Microbiological parameters, turbidity, TSS, and tCOD were measured directly in the reactor effluent, whereas the remaining indicators were determined after filtration. Treatment efficiency (the removal rate of nutrients and COD) was calculated for the steady state period, which was established between the 56th and 112th day of the run.

Analytical Methods

Physicochemical Analysis

A DR6000 spectrophotometer and the following HACH-Lange tests: PhosVer 3 (no. 8048), NitraVer 5 (no. 8039),

Fig. 1 Laboratory installation used for semi-continuous experiment (**1** bioreactor, **2** air outlet, **3** sample collection port, **4** liquid digestate inlet, **5** source of light, **6** air inlet, **7** rotameter, **8** membrane air pump)
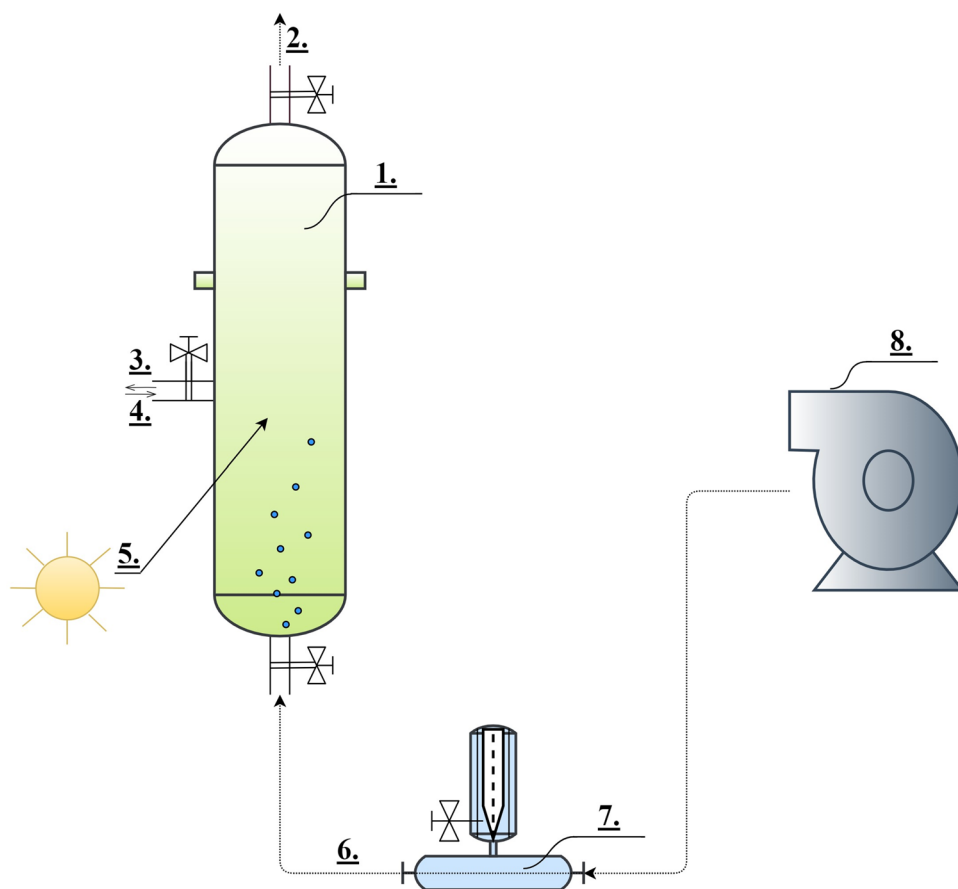
NitriVer 3 (no. 8507), and modified Nessler (no. 8038) were used to determine orthophosphates, nitrates, nitrites, and ammonium nitrogen, respectively. All nitrogen forms measured during the experiment were expressed as mgN/L allowing to determine the nitrogen removal rate. Volatile fatty acids and COD were determined with HACH-Lange tests LCK 365 and LCK 214, respectively. Turbidity, total suspended solids, and color were measured according to EN ISO 7027, the photometric method (8006 method), and the Platinum-Cobalt standard method (8025 method), respectively. TSS was then used to calculate the overall algae-bacteria biomass productivity in accordance with Min et al. [23]. Total solids, volatile solids, and pH were analyzed with the standard methods [24]. To determine the concentrations of selected metals, the following HACH-Lange methods were applied: FerroVer no. 8008 for iron, Zincon no. 8009 for zinc, AluVer no. 8012 for aluminum, and CuVer 1 no. 8506 for copper. The concentrations of sulfates, sulfides, and chlorides were determined with the respective HACH-Lange tests: SulfaVer 4 method no. 8051, a methylene blue method no. 8131, thiocyanate method no. 8113. Light intensity was measured directly on external walls of the bioreactor using the LXP-10B illuminance meter (Sonel, Poland). Surveys were taken three times a day—in the morning, at noon, and in the evening. The illumination rate inside the bioreactor (internal illumination) was not measured.

Microalgal Biomass Analysis

Microalgal biomass was determined by measuring the level of chlorophyll a (chl-a), the optical density, and the algal cell density.

The concentration of chl-a is one of the basic indicators of algal biomass growth. As all taxonomical algal groups produce this photosynthetic pigment, chl-a concentration can reflect changes and/or disturbances in algal biomass. The chl-a concentration was determined according to the methodology described previously in Sobolewska et al. [20] using 90% methanol during extraction. The final chl-a concentration was calculated with formula 1.

$$Chl - a\,[mg/L] = (16.5 \times A_{665}) - (8.3 \times A_{650}) \qquad (1)$$

where A_{665}—absorbance measured at 665 nm; A_{650}—absorbance measured at 650 nm.

The biomass growth was also evaluated by optical density measured at 680 nm (OD680), a wavelength corresponding

to the second chlorophyll absorption peak and allowing to indirectly estimate biomass levels in suspensions [21].

Cell density of each algal taxa was determined by applying cell counting on a microscopic slide using a droplet method. Together with quantitative analysis, taxonomic identification was performed based on morphological characteristics. The number of cells per 1 mL of a sample was calculated using formula 2 enabling the further evaluation of algal taxa percentage share.

$$Nx[cells/mL] = (xi \times Ap)/(Si \times V) \qquad (2)$$

where Nx is the algal cell density per 1 mL of sample; xi is the cell number of individual taxa; Ap is the total microscopic slide surface area $= 400$ mm^2; Si is the surface area used to count taxa (mm^2), and V is the drop volume (cm^2).

Determination of Bacterial Communities Using Metagenomic Analysis

At the end of the experiment, V3-V4 16S rRNA metagenomic analysis was performed on the biomass collected from the photobioreactor. Analysis was executed in accordance with the procedure described in Sobolewska et al. [21]. Acquired data was deposited in the NCBI database. Using the Sequence Read Archive tool, a bioproject and biosample with respective access numbers of PRJNA922144 and SAMN32643824 were prepared. To determine the phylogenetic bacterial biodiversity, 16S rRNA fragments were matched against their corresponding taxonomical levels from phylum to species. The presence of bacteria, confirmed with metagenomic analysis ("Bacterial Community in Biorector" section) resulted from the lack of digestate sterilization and use on nonaxenic algal cultures.

Statistical Analysis

During the experimental run, physicochemical indicators and microbiological parameters were measured in triplicates. The average values, standard deviation (SD), error bars, and correlation coefficients (where 0.0–0.2 very weak correlation, 0.2–0.4 weak correlation, 0.4–0.7 moderate correlation, 0.7–0.9 strong correlation, 0.9–1.0 very strong correlation) were calculated in Microsoft Excel 365 version 2211.

Results and Discussion

Treatment Efficiency

The photobioreactor was exclusively operated under sunlight conditions with no additional light sources. The variations of physical–chemical indicators in the course of the run are plotted in Fig. 2. The post-digestion effluent contains vast amounts of nitrogen, phosphorous, and organic carbon (Table 1), which can be utilized by microalgae for their growth and other metabolic processes [1]. Considering the above, the treatment process was mainly evaluated based on the removal of orthophosphates, nitrogen forms, and COD with simultaneous control of biomass productivity as well as microalgal biomass parameters.

During the experimental run, the concentration of nitrates increased until the 31st day of the process. This can be related to the overall biomass growth as reflected by the increase of optical density, turbidity, and TSS. Due to no changes observed in pH and no decrease in ammonium nitrogen, the participation of nitrification bacteria (confirmed in small share with *Nitrosomonas* sp. and *Nitrospira* sp. by metagenomic analysis) in a cultivation period is unlikely. After this period, the concentration of N-NO$_3$ significantly dropped to stabilize at a relatively low level since the 38th day of the run. In contrast, the ammonium nitrogen content in the treated digestate showed much lower fluctuations throughout the experiment. Importantly, the concentration of nitrites was close to zero, which could indicate oxygen-sufficient conditions in the reactor (data not included in Fig. 2c). There is an indication that the presence of *Microglena* sp. could slightly affect changes in ammonium nitrogen concentration. After the 49th day of the experiment, the trend for the slow and gradual rise in ammonium nitrogen concentration was observed, corresponding to the decrease in *Microglena* sp. cell abundance. This trend was offset for the period of 84th and 105th day, correlating to the small increase in cell density. Moreover, after the complete disappearance of *Microglena* sp. registered at the end of the experimental run, the ammonium nitrogen increased relatively sharply again. Nitrogen plays a significant role in the growth of living organisms. Eukaryotic algae can directly absorb inorganic nitrogen only in the form of ammonium nitrogen, nitrates, and nitrites. Due to lower energy requirements, ammonium nitrogen is usually assimilated faster than nitrates by algae. On the other hand, an excessive ammonium concentration might have a repressive growth effect. Besides, nitrogen in the form of nitrates is thermodynamically more stable in aqueous, oxidized environments and therefore might be dominant in the substrate [25]. However, it should be noted that the assimilation capabilities as well as a tolerated nutrient concentration are usually species- and strain-dependent [26]. Nitrogen transformations did not considerably affect pH. On one hand, ammonium nitrogen increases the pH of the environment, whereas the appearance of nitrates through nitrification has a reverse effect. However, as depicted in Fig. 2a, the pH value showed greater variations only in the first part of the experiment, and since the 38th day of the run, it stabilized at 9.5. The high pH level could be attributed to the significant activity of microalgae, which consume
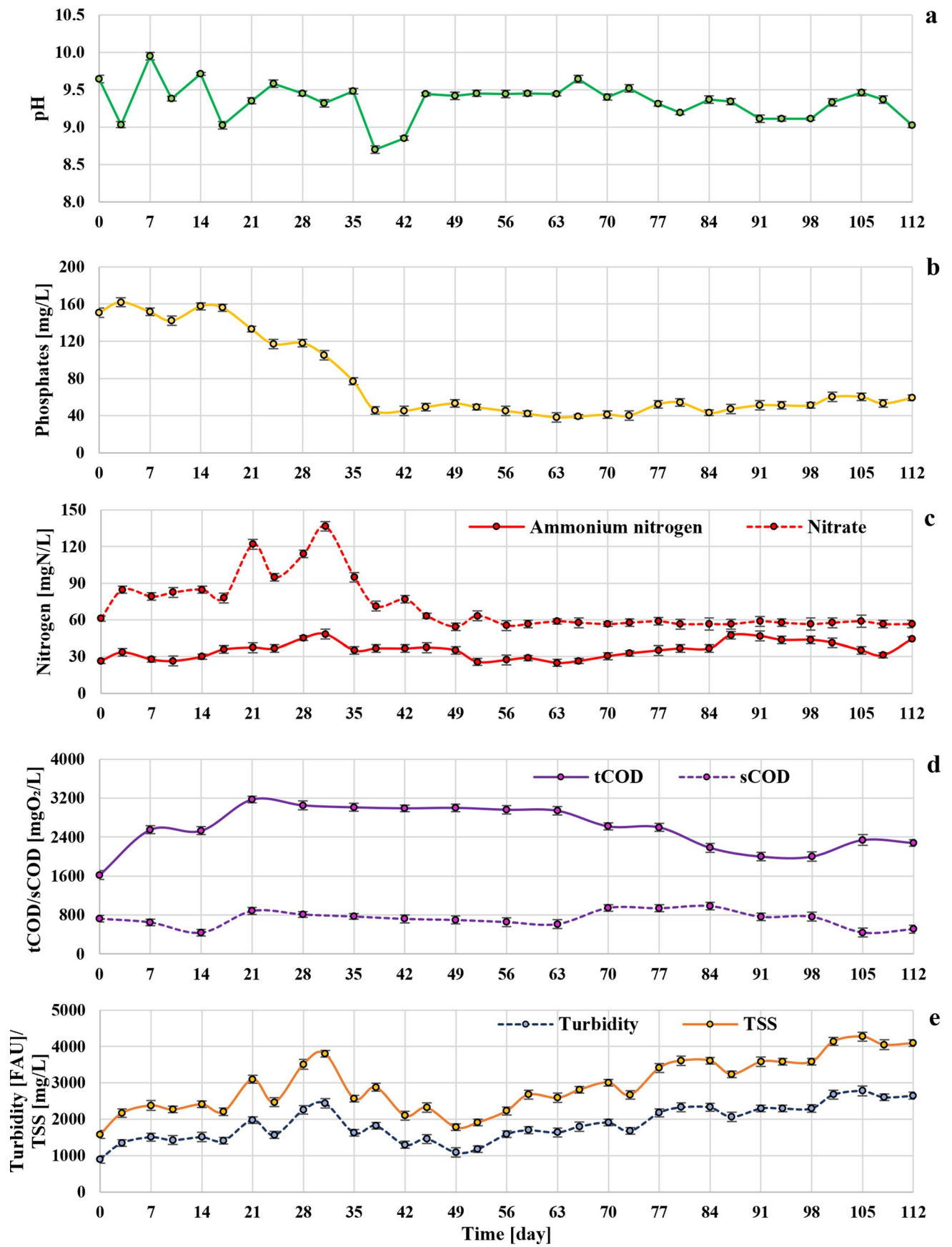
◀**Fig. 2** Changes in physicochemical indicators during semi-continuous experimental period (**a** pH; **b** phosphates; **c** nitrogen; **d** chemical oxygen demand; **e** total suspended solids/ turbidity); the figure does not include 4 initial weeks of microalgae multiplication

CO_2 and carbonates thus increasing buffering capacity of the environment. On the other hand, increased pH results in conversion of ammonium nitrogen into free ammonia, which, in turn, could inhibit the growth of microalgae [27], but the effect of ammonia reduction occurring with volatilization during aeration of culture media was not measured. It can be therefore assumed that the pH increase could be the reason for the microalgae density decrease observed after 42 days of the process (Fig. 3). Additionally, during the experiment, high intensity of sunlight (the average of 6000 Lux) and high the temperature (30 ± 2 °C) could inhibit nitrification process with photooxidation of bacterial cytochrome as described in Lavrentyev and Gardner [28]. The calculated total nitrogen concentration (as the sum of nitrogen from ammonium nitrogen, nitrates, and nitrites) in the reactor effluent in the steady-state period was equal to 95 mgN/L. This gave the average nitrogen removal rate of 86% (compared to raw liquid digestate and observed for this period). Hence, a complete nitrogen removal was not achieved, suggesting the presence of remaining organic compounds not assimilable by microalgae and their native, accompanying bacteria. Generally, the recorded removal rate was greater than the values reported in the literature. In a raceway pond treatment system operated with food waste digestate, over 80% reduction in total nitrogen was reached [10]. In the cited study, unfiltered digestate was used as a substrate, and nitrogen was removed not only through microbial transformation but also by ammonia oxidation. In another study, a consortium of *Scenedesmus* spp. and *Chlorella* spp. was applied for the treatment of agricultural digestate [12]. The authors documented only 20–29% of nitrogen removal rate with a significant impact of nitrification in the overall biological process. In turn, Tan et al. [29] reported total nitrogen reduction at approximately 84%, which was achieved for initially filtered starch wastewater treated in an outdoor photobioreactor using *Chlorella pyrenoidosa*.

Phosphorus is another key macronutrient for living organisms, and algae are believed to possess a great capacity to store and use phosphorus. Inorganic phosphates (PO_4-P) are the dominant phosphorus forms in the digestate (up to 90% contribution), and they play a significant role in the overall microalgal growth [1, 29]. As illustrated in Fig. 2b, initially, the phosphate concentration in the treated digestate showed a downward trend until day 38 of the experiment, after which it stabilized at 49 mg/L. It can be assumed that phosphates could be removed from the digestate not only as a result of microalgal activity but also through precipitation, which is intensified at a high pH of 8–10 [30]. This gave the average phosphate removal rate of 86%, which was comparable to the findings of Xu et al. [13] where *Scenedesmus obliquus* (now also known as *Tetradesmus obliquus*) was applied for the treatment of sterilized piggery anaerobic effluent. A similar efficiency was also documented in a study of Tan et al. [29], where filtered starch wastewater was treated by *Chlorella pyrenoidosa*.

Algae are also known to be able to utilize both inorganic and organic carbon [25]. The soluble COD indicator reflects the presence of organic compounds in the liquid digestate, whereas total COD also includes the concentration of organic compounds in biomass. Changes in total and soluble COD are plotted in Fig. 2d. The value of total COD increased twice in the initial experimental stage, which could be linked to the biomass growth in this period, as this indicator moderately correlates to TSS (Table 2). In contrast, soluble COD remained at a relatively constant level throughout the experiment and did not correlate with tCOD. The calculated removal rate of soluble COD in a steady-state period reached 91%. For comparison, Abu Hajar et al. [11] reached a 78–82% COD removal rate using *Scenedesmus dimorphus* to treat unsterilized animal manure and food waste. Likewise, the algal treatment of piggery anaerobic liquid digestate allowed to reduce 62–75% sCOD [13]. In another study, the sCOD removal efficiency oscillated between 40 and 75%, which was dependent on the digestate dilution [9]. In our study, the reduction rate of total COD (tCOD) was lower (70%), probably due to the biomass growth. In algae-bacteria systems, both microbial groups can be involved in the removal of organic compounds via anabolic and catabolic reactions [31]. However, a complete removal of COD is very unlikely, because hardly degradable compounds and the products of cell lysis remain in the effluent [29, 32]. As mentioned earlier, the growth of biomass was indicated by both tCOD and TSS increase as well as turbidity, and a strong correlation between these indicators was observed. Total suspended solids also correlated with optical density; however, the strongest correlation of over 0.99 was noted between TSS and turbidity (Table 2). In a steady-state period, the mean levels of TSS and turbidity were 3793 mg/L for TSS and 2462 FAU, respectively.

The TSS-based overall biomass productivity was equal to 22 mg/L/d and corresponded to the lower limit values obtained in analogous digestate treatment studies. It should be noted that biomass productivity can be influenced by *inter alia* a type of substrate, its characteristics and pretreatment methods, environmental conditions such as lightning and temperature [10], and strain-dependent microbial capabilities. The strains used in this study were environmental isolates, acquired from substrates other than liquid digestate, and therefore the natural ability of algal cells to develop in such substrates may have been lower. The applied process conditions and specific substrate (liquid digestate) could

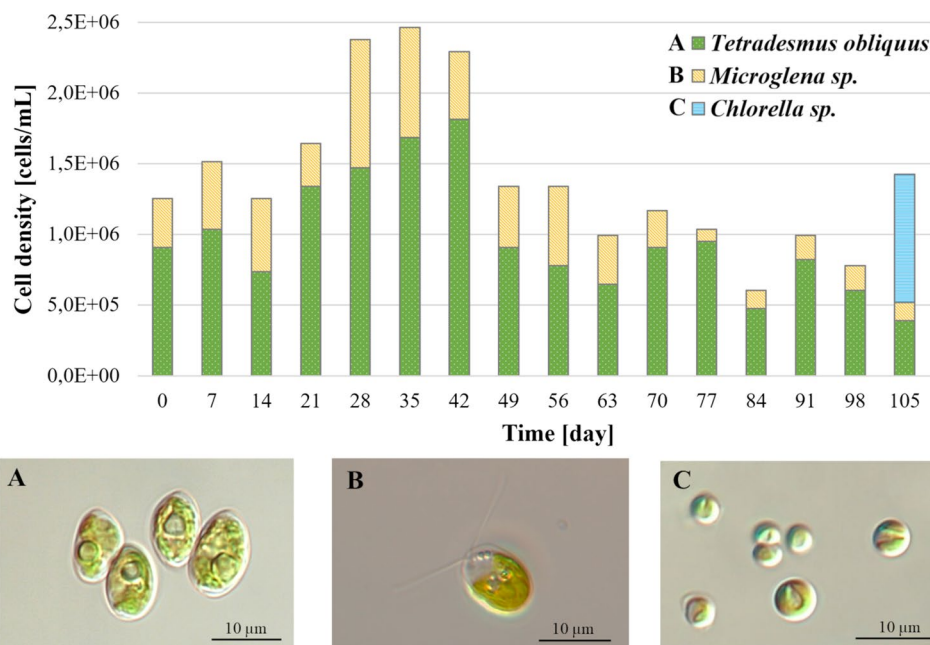**Fig. 3** Changes in cell density and percentage share of algal taxa

Table 2 Correlation coefficients between physicochemical indicators

	N-NH$_4^+$	N-NO$_3^-$	PO$_4^{3-}$	Turbidity	TSS	tCOD	sCOD
pH	−0.48231	0.061255	0.238633	−0.2256	−0.2332	−0.03963	−0.50907
N-NH$_4^+$		0.242812	−0.12156	0.591365	0.602802	0.311713	0.203013
N-NO$_3^-$			0.614033	−0.05062	−0.04119	0.6822	0.006229
PO$_4^{3-}$				−0.34733	−0.3414	0.460198	−0.13249
Turbidity					0.997686	0.17569	−0.06417
TSS						0.6285	−0.01967
tCOD							0.072974

0.0–0.2 very weak correlation; 0.2–0.4 weak correlation; 0.4–0.7 moderate correlation; 0.7–0.9 strong correlation; 0.9–1.0 very strong correlation

have the greatest impact on the results obtained, which differ from those reported in the literature. For instance, Xu et al. [13] achieved 6–14 times higher biomass productivity, whereas the treatment efficiencies were 62–75% for COD, 58–75% for TN, and 70–89% for TP. Similar values (78–82% of COD removal, 65–72% of TN removal, and 63–100% of TP reduction) were reported by Abu Hajar et al. [11], with biomass productivity higher when the substrate dilution rate was increased. On the other hand, the research of Barzee et al. [10] shows that with the equally low biomass productivity (17.7 ± 1.8 mg VS/L/d), high treatment efficiencies can still be achieved (total nitrogen removal over 80%). It should also be noted that, despite the relatively low biomass productivity, the other parameters characterizing cell growth, i.e., the chlorophyll *a* concentration and optical density suggested the development of the algal culture allowing to achieve satisfactory treatment results. The analogous biomass values (chl-*a* = 19.04 mg/L) were also reported by Ansari et al. [33], who applied *Scenedesmus* sp.

and achieved high removal rates of COD (95%), phosphate (over 80%), and nitrates (over 99%).

Changes in the Structure of Algal Community During Treatment

From the beginning of the experiment until the 98th day, the only detected algae were *Tetradesmus obliquus* and *Microglena sp*. Within the first month, the algal cell number increased after which a decrease in cell density and its further stabilization was observed (Fig. 3). The initial rapid growth of algae could be induced by phosphates that remained in the environment from the 3N-BBM medium used before the digestate was introduced to the bioreactor. Moreover, in the first weeks, the growth of algae was unaffected by the biomass density, which was initially low. Conversely, determined cell density began to decrease after the 42nd day of the process, despite high, increasing chl-*a* and OD680 values suggesting a potential mutual shading

effect (see more in the "Mutual Shading Effect and Influence of Light Intensity" section). The determination of cell density indicates the presence of a significant number of *Microglena* sp. cells, mainly in the first half of the experiment, after which the share of this taxon decreased significantly till complete disappearance occurring between the 105th and 112th day. Starting from the 56th day, a drop in average light intensity was noted suggesting the influence on *Microglena* sp. regression. This effect was enhanced by the occurrence of mutual shading evidenced *inter alia* by progressive turbidity and TSS increase. Although this phenomenon most likely affected both microalgal strains, obtained results indicate that *Microglena* cells were less resistant. It should also be noted that the adaptive capabilities of *Microglena* sp. to substrates such as digestate have not yet been described in scientific literature. The growth of algae could be affected by pH and vice versa. It is commonly known that pH strongly impacts algal cell division and metabolic processes, and the maximal growth of these organisms can be observed in neutral environments [22]. As noted in the "Treatment Efficiency" section, alkaline pH favors result in a release of free ammonia, which is much more inhibitory to microalgae cells than ammonium ions. The growth of algae in the first month of the run corresponded to the increase in both optical density and chl-*a* concentration. Interestingly, after this period, the algae cell density significantly dropped as discussed above, but the concentration of chl-*a* remained at a constant level of 23.0 mg/L until the end of the experimental run (Fig. 4). The correlation factor between these indicators suggested weak correlation ($R > 0.36$) in contrast to the literature findings [34]. Additionally, the chl-*a* concentration can be affected by multiple factors such as phosphorous and nitrogen concentrations or temperature [35]. With the slow increase of phosphates (registered from 77 to 112 days of the process), a small increase in nitrates concentration, and highly probable daily temperature rise (occurring naturally for the summer period, especially in August), maintaining high chlorophyll *a* concentration despite cell

density reduction was possible. This observation may also be explained by the presence of other phototrophic microorganisms in the nonaxenic community of the photobioreactor as discussed below. Another interesting observation is the appearance of the *Chlorella* sp. algae determined metagenomically as *Chlorella sorokiniana* at the end of the experimental run. Their growth could be induced by the decrease in sunlight intensity, occurring naturally at the beginning of September (corresponding to the end of the experiment). It is highly probable that singular *C. sorokiniana* cells were present in the substrate or reactor since the beginning of the experimental run but were not detected analytically, and the high light intensity could have inhibited their photosystem II activity resulting in overall photoinhibition. Gao et al. [36] documented that *C. sorokiniana* growth is being inhibited when light intensity exceeds 150 μmol/m²/s, while Li et al. [37] described that *Chlorella kessleri* biomass growth stops at 200 μmol/m²/s. Hence, the decrease in light intensity to the levels tolerated by *Chlorella* cells could lead to their multiplication and taxonomical domination at the end of the run. Moreover, between the 105th and 112th day of the experiment, a rising trend in ammonium nitrogen concentration (correlating with a pH decrease) was observed, which might be the cause of a rapid development of *Chlorella* sp. [38].

Bacterial Community in Bioreactor

The bacteria presence, determined at the end of the experiment using metagenomic analysis, resulted from the lack of digestate sterilization and the use of nonaxenic algal cultures. It was found that the most dominant bacteria at the phylum level were Firmicutes (26.31%) and Patescibacteria (17.38%) as well as Actinobacteriota (14.86%). This may suggest that bacteria were mainly responsible for organic contaminants oxidation, phosphorus removal, and nitrogen transformation in the digestate [39]. Furthermore, a significant abundance (around 8%) of Chloroflexi, Cyanobacteria,

Fig. 4 Changes in optical density and chlorophyll *a* concentration
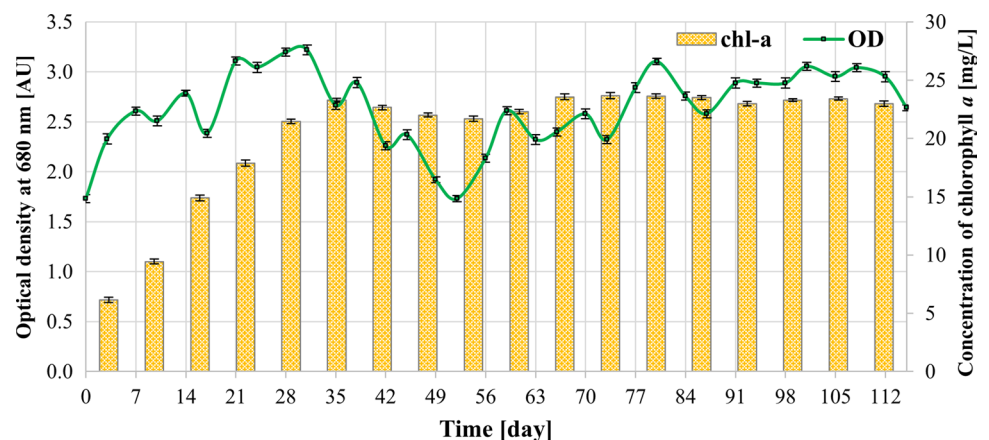
and Proteobacteria was also detected. Bacteria from the Proteobacteria phylum could be responsible for the degradation of organic matter and detoxification of inorganic pollutants [40]. Moreover, bacteria from the Chloroflexi phylum could contribute to phosphorous removal [39]. At the order level, Bacillales, Dojkabacteria, Microtrichales, and Chloroplast record dominated, whereas a high relative abundance of Rhizobiales, Oligoflexales, Caldilineales, and Deinococcales was also noted (Fig. S1 -Supplementary material). Metagenomic analysis revealed that *Tetradesmus obliquus* and *Chlorella sorokiniana* observed at the end of the experiment (see the "Changes in the structure of algal community during treatment" section) were assigned to the Chloroplast record (Cyanobacteria phylum). In turn, members of Bacillales are known to be important denitrifying and hydrocarbon-degrading bacteria [41], while Microtrichales represented mostly by bacteria *Iamia* sp. (relative abundance higher than 8%) are heterotrophs responsible for the mineralization of organic carbon [42]. Likewise, the activity of

Trupera sp. from the order Deinococcales could be linked to the removal of COD from the digestate [43]. In contrast, the family *Rhizobiaceae* includes bacteria responsible for nitrogen fixation [44]. Additionally, an important role could be exhibited by *Chloroflexaceae* autotrophic denitrifiers [45] and chemoorganotrophic *Blastocatellaceae* showing an ability to decompose complex carbon compounds [46] (Fig. 5).

Mutual Shading Effect and Influence of Light Intensity

The average light intensity over the experimental period is shown in Fig. 6. It should be noted that illumination was measured on the external walls of the bioreactor and therefore mutual shading effect was not directly evaluated. Throughout the first 63 days of the experiment, the mean light intensity was similar. The semi-continuous experimental period started in spring (May) in which the number of sunny days is relatively high [47]. A significant decrease of

Fig. 5 Bacterial community and bacteria cell abundance at the level of family

- ⊠ *Hyphomicrobiaceae; =1.25E+03 cells/ mL*
- ⊟ *Ilumatobacteraceae; =1.27E+03 cells/ mL*
- ☐ *Lachnospiraceae; =1.36E+03 cells/ mL*
- ▦ *Rubritaleaceae; =1.47E+03 cells/ mL*
- ▨ *Cellulomonadaceae; =1.55E+03 cells/ mL*
- ◩ *Blastocatellaceae; =2.05E+03 cells/ mL*
- ▥ *WPS-2; =2.09E+03 cells/ mL*
- ▥ *Solirubrobacteraceae; =2.23E+03 cells/ mL*
- ▨ *Chloroflexaceae; =3.61E+03 cells/ mL*
- ▨ *Rhizobiaceae; =4.00E+03 cells/ mL*
- ▨ *Trueperaceae; =4.23E+03 cells/ mL*
- ◪ *Caldilineaceae; =5.98E+03 cells/ mL*
- ▨ *Oligoflexales; =6.25E+03 cells/ mL*
- ▬ *Iamiaceae; =1.07E+04 cells/ mL*
- ▨ *Chloroplast; =1.09E+04 cells/ mL*
- ▨ *Dojkabacteria; =2.12E+04 cells/ mL*
- ▤ *Planococcaceae; =3.06E+04 cells/ mL*
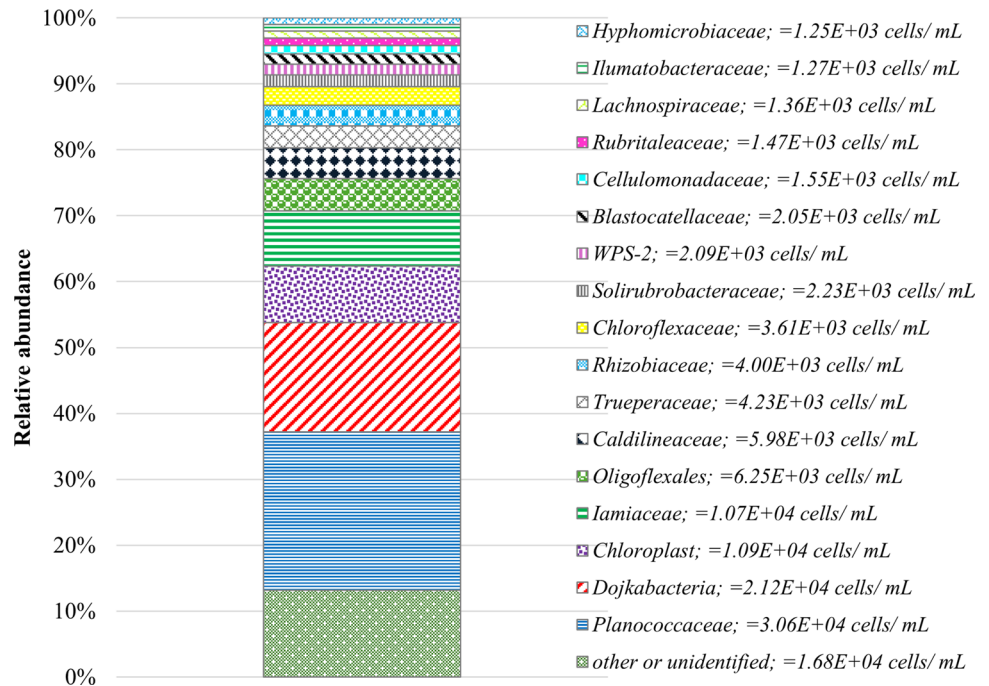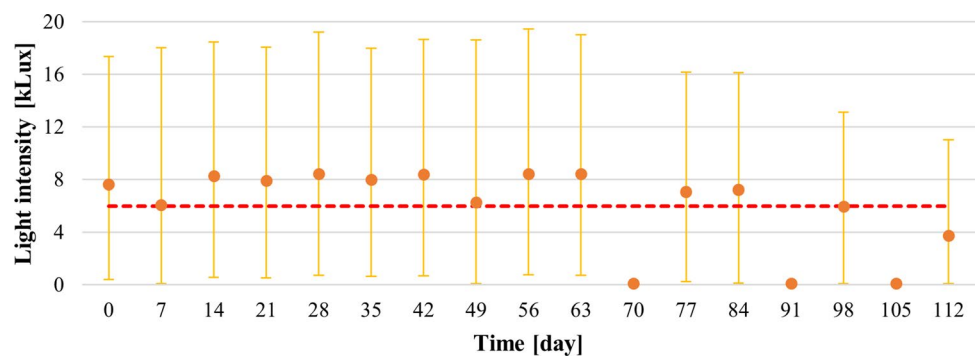- ▨ *other or unidentified; =1.68E+04 cells/ mL*

Fig. 6 Changes in light intensity occurring during the experimental run (orange dots represent mean values; orange bars show maximal and minimal values; red dotted line represents mean light intensity calculated for all measurements); for the 70th, 91st, and 105th day, registered values were lower than 0.5 kLux

illumination was registered in the 70th, 91st, and 105th days (with mean values of 0.103–0.106 kLux) suggesting cloudy weather conditions. A decrease in illumination was noted since the beginning of August as a result of lower insolation, more cloudy weather, and more abundant in rainy days [47]. However, no correlation between lower illumination and biological parameters such as cell density, optical density, and chlorophyll *a* was found proving that the decrease in sunlight was most likely the result of single cloudy days, which did not significantly affect the deterioration of the quantitative parameters of the algae (see more in the "Changes in the structure of algal community during treatment" section). Light parameters inside the bioreactor and the ability of light to penetrate and reach microalgal cells also depend on the mutual shading effect. Its occurrence may be indicated by a decrease in cell density after the 42nd day of the experimental run ("Changes in the structure of algal community during treatment" section) and confirmed by visual observations. The phenomenon of biofilm overgrowing the internal walls and the biomass gathered in the bottom of the bioreactor (despite mixing) became visible at the beginning of August (about the 70th day of the process) and lasted till the end of the experiment (105th day). A significant impact of mutual shading on the development and stability of microalgal cultures as well as treatment efficiency has already been described in the literature [48–50]. Guieysse et al. [48] reported a negative correlation between cell density, photosynthetic activity, and increased O_2 consumption. The shading effect might be indirectly evidenced by a significant increase in the TSS concentrations from the 49th day of the experiment with the highest value (4270 mg/L) reported on the 105th day. The relationship between TSS and the mutual shading effect was described by Foladori et al. [49] and Uggetti et al. [50]. The increased TSS concentrations (and thus the shading effect) could have been the reason for the upward trend in phosphates concentration starting from the 77th day, as shown in Fig. 2b. However, it should be noted that liquid digestate had dark color, which might have affected penetration and spread of light intensifying mutual shading effect. The decrease in sunlight intensity enhanced by the mutual shading effect as well as TSS and turbidity rise observed from the 49th day forward were probably the main factors causing the decrease and disappearance of *Microglena* sp. Overall, this taxa showed less resistance to changes registered for light conditions than *Tetradesmus obliquus*.

Conclusions

The study showed that natural sunlight can be successfully used for efficient liquid digestate treatment with algae-bacteria systems even in temperate climate zones. As high as 91% sCOD and 86% nutrients were removed from non-sterile and unfiltered liquid digestate proving that initial substrate pre-treatment is not required to achieve high treatment efficiency. *Microglena* sp. not used previously for digestate treatment showed low resistance to light intensity reduction and effects of mutual shading. The results also indicated that *Microglena* sp. can potentially promote ammonium nitrogen removal.

Author Contribution Ewelina Sobolewska: conceptualization, data curation, methodology, investigation, formal analysis, writing—original draft, writing—review and editing, and visualization. Sebastian Borowski: conceptualization, methodology, formal analysis, resources, writing—original draft, writing—review and editing, and supervision. Paulina Nowicka-Krawczyk: formal analysis, investigation, methodology, writing—original draft, writing—review and editing.

Declarations

References

1. Xia A, Murphy JD (2016) Microalgal cultivation in treating liquid digestate from biogas systems. Trends Biotechnol 34:264–275. https://doi.org/10.1016/j.tibtech.2015.12.010
2. European Biogas Association (2020) Eba Statistical Report 2020. https://www.europeanbiogas.eu/eba-statistical-report-2020/. Accessed 20 Dec 2023
3. European Parliament and Council (2019) Regulation (EU) 2019/1009 of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003. OJ L 170, 25.6.2019. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02019R1009-20230316. Accessed 6 Mar 2024
4. Franchino M, Tigini V, Varese GC et al (2016) Microalgae treatment removes nutrients and reduces ecotoxicity of diluted piggery digestate. Sci Total Environ 569–570:40–45. https://doi.org/10.1016/j.scitotenv.2016.06.100

5. Akhiar A, Battimelli A, Torrijos M, Carrere H (2017) Comprehensive characterization of the liquid fraction of digestates from full-scale anaerobic co-digestion. Waste Manag 59:118–128. https://doi.org/10.1016/j.wasman.2016.11.005

6. Song S, Lim JW, Lee JTE et al (2021) Food-waste anaerobic digestate as a fertilizer: the agronomic properties of untreated digestate and biochar-filtered digestate residue. Waste Manag 136:143–152. https://doi.org/10.1016/j.wasman.2021.10.011

7. Yang D, Chen Q, Liu R et al (2022) Ammonia recovery from anaerobic digestate: state of the art, challenges and prospects. Bioresour Technol 363:127957. https://doi.org/10.1016/j.biortech.2022.127957

8. Christenson L, Sims R (2011) Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol Adv 29:686–702. https://doi.org/10.1016/j.biotechadv.2011.05.015

9. Torres-Franco AF, Silva G, Freitas MP et al (2022) Effect of digestate loading rates on microalgae-based treatment under low LED light intensity. Environ Technol 43:3023–3036. https://doi.org/10.1080/09593330.2021.1914178

10. Barzee TJ, Yothers C, Edalati A, et al (2022) Pilot microalgae cultivation using food waste digestate with minimal resource inputs. Bioresour Technol Reports 19:. https://doi.org/10.1016/j.biteb.2022.101200

11. Abu Hajar HA, Riefler RG, Stuart BJ (2017) Cultivation of Scenedesmus dimorphus using anaerobic digestate as a nutrient medium. Bioprocess Biosyst Eng 40:1197–1207. https://doi.org/10.1007/s00449-017-1780-4

12. Pizzera A, Scaglione D, Bellucci M et al (2019) Digestate treatment with algae-bacteria consortia: a field pilot-scale experimentation in a sub-optimal climate area. Bioresour Technol 274:232–243. https://doi.org/10.1016/j.biortech.2018.11.067

13. Xu J, Zhao Y, Zhao G, Zhang H (2015) Nutrient removal and biogas upgrading by integrating freshwater algae cultivation with piggery anaerobic digestate liquid treatment. Appl Microbiol Biotechnol 99:6493–6501. https://doi.org/10.1007/s00253-015-6537-x

14. Zambrano J, Krustok I, Nehrenheim E, Carlsson B (2016) A simple model for algae-bacteria interaction in photo-bioreactors. Algal Res 19:155–161. https://doi.org/10.1016/j.algal.2016.07.022

15. Chuka-ogwude D, Ogbonna J, Moheimani NR (2020) A review on microalgal culture to treat anaerobic digestate food waste effluent. Algal Res 47:101841. https://doi.org/10.1016/j.algal.2020.101841

16. Uma VS, Usmani Z, Sharma M et al (2023) Valorisation of algal biomass to value-added metabolites: emerging trends and opportunities. Phytochem Rev 22:1015–1040. https://doi.org/10.1007/s11101-022-09805-4

17. Sayedin F, Kermanshahi-pour A, He QS et al (2020) Microalgae cultivation in thin stillage anaerobic digestate for nutrient recovery and bioproduct production. Algal Res 47:101867. https://doi.org/10.1016/j.algal.2020.101867

18. Khalid AAH, Yaakob Z, Abdullah SRS, Takriff MS (2018) Growth improvement and metabolic profiling of native and commercial Chlorella sorokiniana strains acclimatized in recycled agricultural wastewater. Bioresour Technol 247:930–939. https://doi.org/10.1016/j.biortech.2017.09.195

19. Fernandes F, Silkina A, Gayo-Peláez JI, et al (2022) Microalgae cultivation on nutrient rich digestate: the importance of strain and digestate tailoring under PH control. Appl Sci 12:. https://doi.org/10.3390/app12115429

20. Sobolewska E, Borowski S, Nowicka-Krawczyk P, Banach K (2022) Treatment of liquid digestate by green algal isolates from artificial eutrophic pond. Molecules 27:6856. https://doi.org/10.3390/molecules27206856

21. Sobolewska E, Borowski S, Nowicka-Krawczyk P (2023) Effect of solar and artificial lighting on microalgae cultivation and treatment of liquid digestate. J Environ Manage 344:118445. https://doi.org/10.1016/j.jenvman.2023.118445

22. Andersen RA (2005) Algal culturing techniques. Elsevier Science

23. Min M, Wang L, Li Y et al (2011) Cultivating Chlorella sp. in a pilot-scale photobioreactor using centrate wastewater for microalgae biomass production and wastewater nutrient removal. Appl Biochem Biotechnol 165:123–137. https://doi.org/10.1007/s12010-011-9238-7

24. American Public Health Association (2017) Standard methods for the examination of water and wastewater, 23rd edition American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington D.C., USA. American Public Health Association.

25. Cai T, Park SY, Li Y (2013) Nutrient recovery from wastewater streams by microalgae: status and prospects. Renew Sustain Energy Rev 19:360–369. https://doi.org/10.1016/j.rser.2012.11.030

26. Salbitani G, Carfagna S (2021) Ammonium utilization in microalgae: a sustainable method for wastewater treatment. Sustain 13:1–17. https://doi.org/10.3390/su13020956

27. Azov Y, Goldman JC (1982) Free ammonia inhibition of algal photosynthesis in intensive cultures. Appl Environ Microbiol 43:735–739

28. Lavrentyev PJ, Gardner WS (2000) Effects of the zebra mussel on nitrogen dynamics and the microbial community at the sediment-water interface. Aquat Microb Ecol 21:187–194

29. Tan X, Chu H, Zhang Y et al (2014) Chlorella pyrenoidosa cultivation using anaerobic digested starch processing wastewater in an airlift circulation photobioreactor. Bioresour Technol 170:538–548. https://doi.org/10.1016/j.biortech.2014.07.086

30. Hao X-D, Wang C-C, Lan L, van Loosdrecht MCM (2008) Struvite formation, analytical methods and effects of pH and Ca2 +. Water Sci Technol 58:1687–1692. https://doi.org/10.2166/wst.2008.557

31. Marazzi F, Sambusiti C, Monlau F et al (2017) A novel option for reducing the optical density of liquid digestate to achieve a more productive microalgal culturing. Algal Res 24:19–28. https://doi.org/10.1016/j.algal.2017.03.014

32. Hadj-Romdhane F, Zheng X, Jaouen P et al (2013) The culture of Chlorella vulgaris in a recycled supernatant: effects on biomass production and medium quality. Bioresour Technol 132:285–292. https://doi.org/10.1016/j.biortech.2013.01.025

33. Ansari AA, Khoja AH, Nawar A et al (2017) Wastewater treatment by local microalgae strains for CO2 sequestration and biofuel production. Appl Water Sci 7:4151–4158. https://doi.org/10.1007/s13201-017-0574-9

34. Harun M, Matias Peralta HM, Gonawan NH, Shahri Z (2018) Effect of mixing on the density and chlorophyll a content on Botryococcus Sp. J Sci Technol 10:22–29. https://doi.org/10.30880/jst.2018.10.02.004

35. Huang H, Wang W, Lv J et al (2022) Relationship between chlorophyll a and environmental factors in lakes based on the random forest algorithm. Water (Switzerland) 14:1–11. https://doi.org/10.3390/w14193128

36. Gao K, Xue C, Yang M et al (2022) Optimization of light intensity and photoperiod for growing Chlorella sorokiniana on cooking cocoon wastewater in a bubble-column bioreactor. Algal Res 62:102612. https://doi.org/10.1016/j.algal.2021.102612

37. Li Y, Zhou W, Hu B et al (2012) Effect of light intensity on algal biomass accumulation and biodiesel production for mixotrophic strains Chlorella kessleri and Chlorella protothecoide cultivated in highly concentrated municipal wastewater. Biotechnol Bioeng 109:2222–2229. https://doi.org/10.1002/bit.24491

38. Ayre JM, Moheimani NR, Borowitzka MA (2017) Growth of microalgae on undiluted anaerobic digestate of piggery effluent with high ammonium concentrations. Algal Res 24:218–226. https://doi.org/10.1016/j.algal.2017.03.023

39. Luo Y, Yao J, Wang X et al (2020) Efficient municipal wastewater treatment by oxidation ditch process at low temperature: bacterial community structure in activated sludge. Sci Total Environ 703:135031. https://doi.org/10.1016/j.scitotenv.2019.135031

40. Sharma P, Tripathi S, Chandra R (2021) Metagenomic analysis for profiling of microbial communities and tolerance in metal-polluted pulp and paper industry wastewater. Bioresour Technol 324:124681. https://doi.org/10.1016/j.biortech.2021.124681

41. Liu G, Ye Z, Tong K, Zhang Y (2013) Biotreatment of heavy oil wastewater by combined upflow anaerobic sludge blanket and immobilized biological aerated filter in a pilot-scale test. Biochem Eng J 72:48–53. https://doi.org/10.1016/j.bej.2012.12.017

42. Miksch S, Meiners M, Meyerdierks A et al (2021) Bacterial communities in temperate and polar coastal sands are seasonally stable. ISME Commun 1:26–29. https://doi.org/10.1038/s43705-021-00028-w

43. Shan L, Zhang Z, Yu Y et al (2017) Performance of CSTR–EGSB–SBR system for treating sulfate-rich cellulosic ethanol wastewater and microbial community analysis. Environ Sci Pollut Res 24:14387–14395. https://doi.org/10.1007/s11356-017-9022-5

44. Mirmohamadsadeghi S, Karimi K, Azarbaijani R et al (2021) Pretreatment of lignocelluloses for enhanced biogas production: a review on influencing mechanisms and the importance of microbial diversity. Renew Sustain Energy Rev 135:110173. https://doi.org/10.1016/j.rser.2020.110173

45. Vilajeliu-Pons A, Puig S, Pous N et al (2015) Microbiome characterization of MFCs used for the treatment of swine manure. J Hazard Mater 288:60–68. https://doi.org/10.1016/j.jhazmat.2015.02.014

46. Huber KJ, Pascual J, Foesel BU, Overmann J (2017) Blastocatellia. In: Bergey's Manual of Systematics of Archaea and Bacteria. pp 1–2

47. The Institute of Meteorology and Water Management - National Research Institute Climate standards 1991–2020. https://klimat.imgw.pl/pl/climate-normals/TSR_AVE. Accessed 16 Mar 2024

48. Guieysse B, Borde X, Muñoz R et al (2002) Influence of the initial composition of algal-bacterial microcosms on the degradation of salicylate in a fed-batch culture. Biotechnol Lett 24:531–538. https://doi.org/10.1023/A:1014847616212

49. Foladori P, Petrini S, Andreottola G (2020) How suspended solids concentration affects nitrification rate in microalgal-bacterial photobioreactors without external aeration. Heliyon 6:e03088. https://doi.org/10.1016/j.heliyon.2019.e03088

50. Uggetti E, Sialve B, Latrille E, Steyer JP (2014) Anaerobic digestate as substrate for microalgae culture: the role of ammonium concentration on the microalgae productivity. Bioresour Technol 152:437–443. https://doi.org/10.1016/j.biortech.2013.11.036