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Original Research

The Possibility of Deploying CO_2 from Biogas Combustion to Improve the Productivity of a Periodical *Chlorella vulgaris* Culture

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Abstract

Background: Carbon dioxide (CO_2) is the major contributor to the global emissions of greenhouse gases, which necessitates the search for its fixation and utilization methods. Engaging photosynthesizing microorganisms for its biosequestration is one of the prospective technologies applied to this end. Considering the paucity of literature works on the possibilities of deploying CO_2 from biogas combustion to intensify microalgae production, this research aimed to identify the feasibility of using this type of CO_2 in *Chlorella vulgaris* culture by evaluating biomass production yield and CO_2 biosequestration effectiveness. **Methods**: The experiment was performed in glass PBR, in which the culture medium occupied the volume of 1.0 dm^3, and the gaseous phase occupied 0.3 dm^3. The reactors were continuously illuminated by fluorescent lamps. The temperature of flue gases and air fed to reactors, and culture temperature was 20 °C ± 2 °C. **Results**: The use of flue gases promoted a more rapid biomass growth, reaching 77.8 ± 3.1 mgVS/dm^3·d, and produced a higher microalgae concentration, i.e., 780 ± 58 mgVS/dm^3. Nevertheless, the flue gas-fed culture turned out to be highly sensitive, which was manifested in a decreased culture medium pH and relatively quickly achieved decay phase of the *C. vulgaris* population. The microalgae effectively assimilated CO_2, reducing its concentration from 13 ± 1% to 1 ± 0.5% in the effluent from the photobioreactor. **Conclusions**: The flue gases were found not to affect the qualitative composition of the microalgal biomass. However, strict control and monitoring of microalgae biomass production is necessary, as well as rapid responses in flue gas-fed systems. This is an important hint for potential operators of such technological systems on the large scale. Regardless of the possibility of deploying microalgae to fix and utilize CO_2, a justified avenue of research is to look for cheap sources of CO_2-rich gases.

Keywords: biogas; flue gases; carbon dioxide; microalgae; biomass; *Chlorella vulgaris*; photobioreactor

1. Introduction

Climate changes triggered by excessive emissions of greenhouse gases (GHG) pose the major environmental hazard today [1]. The unceasing and successive increase in concentrations of atmospheric carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), observed for many years, has significantly accelerated adverse climate changes [2]. The phenomena induced by the greenhouse effect directly affect the ecological homeostasis, destroy natural ecosystems, and adversely affect both the health status of populations and the economy [3].

CO_2 is estimated to account for almost 65% of the total global emissions of greenhouse gases (GHG) [4]. The concentration of atmospheric CO_2 has increased from approximately 310 ppm in the 1960s to over 410 ppm today [5]. This increase is mainly due to anthropogenic activities; with the exploitation of fossil fuels being the greatest contributor in this respect [6]. Therefore, there is a justified need to search for possibilities to minimize CO_2 emissions and methods to reduce its concentration in the atmosphere. This effect can be achieved by harnessing primary methods based on renewable energy sources or other low-emission or zero-emission technologies for the production and use of fuels [7]. Another way is to develop effective CO_2 fixation methods, involving all activities that lead to its capture and subsequent long-term storage and deposition [8]. The most frequently described methods of CO_2 sequestration include mineral carbonation, CO_2 deposition in geological structures, and biological methods [9].

Considering economic and ecological concerns, the CO_2 fixation methods engaging photosynthesizing microorganisms seem to be a viable approach compared to the physical and chemical techniques [10]. Due to the phytoplankton inhabiting natural marine ecosystems, this process plays a key role in maintaining CO_2 balance in the atmosphere [11]. Marine phytoplankton accounts for half of the global primary productivity, fixing approximately 50 gigatons CO_2 annually [12]. Ample studies have proved the higher efficiency of CO_2 fixation and biomass productivity by microalgae compared to vascular plants [13].

Thus far investigations have provided evidence for the feasibility of using controlled systems from microalgae biomass proliferation in the processes of wastewater and effluent treatment, waste and sewage sludge management,

Table 1. Composition of the 3N-BBM medium.

Specification	R-r initial, g/dm^3	Dose, cm^3	Microelements	R-r initial, mg/dm^3
$NaNO_3$	75.0	10	$FeCl_3 \cdot 3H_2O$	97
$CaCl_2 \cdot 2H_2O$	2.5	10	$MnCl_2 \cdot 4H_2O$	41
$MgSO_4 \cdot 7H_2O$	7.5	10	$ZnCl_2$	5
$K_2HPO_4 \cdot 3H_2O$	7.5	10	$CoCl_2 \cdot 3H_2O$	2
KH_2PO_4	17.5	10	$Na_2MoO_4 \cdot 2H_2O$	4
NaCl	2.5	10		
Microelements		6		

CO_2 bio-sequestration, bio-gas enrichment or exhaust gas purification [14,15]. The produced microalgae biomass is deemed to be a valuable raw material for producing energy carriers and a source of many economically valuable compounds and chemicals, which makes this technology economically and environmentally viable [16,17].

The dynamic development of bioenergy systems based on the use of methane fermentation processes often poses difficulties with post-fermentation sludge management [18]. After dehydration, the solid phase is either used as a fertilizer or dried and used in co-incineration processes. On the other hand, the liquid phase is difficult to neutralize due to its large volume and high concentration of pollutants [19]. Many studies have described the possibility of using post-fermentation leachate in microalgae biomass proliferation as a source of biogenic compounds and microelements in the culture medium [20,21]. The high concentration of CO_2 in the leachate has been proved to intensify the growth rate of microalgae, which has a direct impact on the efficiency of pollutant degradation [22]. So far, there have been few reports only describing the possibility of using exhaust gases from biogas combustion in the production of microalgae [23]. To date, this source of CO_2 has been seen as a promising element of photobioreactors, but these assumptions have not been supported by the results of experimental works [24]. Therefore, there is a justified need to assess the possibility of using waste gases generated during biogas combustion for intensive production of microalgae biomass, and to simultaneously verify the effectiveness of biological CO_2 fixation. An important step of this assessment is the selection of microalgae species that can be cultivated in a medium containing leachate and used for the assimilation of CO_2 from biogas combustion. It is necessary to take into account their growth rate, resistance to specific pollutants present in the leachate and waste gases, eurybiontic nature, high adaptability to changing environmental conditions, and pollutant removal efficiency [24]. Studies have shown that multicellular algae with a low growth rate cannot be used for this purpose due to the difficulties in maintaining their constant growth and efficiency of the purification process [25]. Instead, microalgae are preferred, including mainly the fast-growing strains of *Chlorella* sp., *Scenedesmus* sp., and *Chlamydomonas* sp. [26].

The aim of this study was to determine the possibility of using flue gases from biogas combustion in the production of *Chlorella vulgaris* biomass by assessing the impact of this technological treatment on the efficiency of CO_2 removal, growth efficiency and composition of microalgal biomass, as well as changes in chlorophyll *a* concentration and the effectiveness of nutrient removal from the culture medium.

2. Materials and Methods

2.1 Experimental Design

The experiment was conducted under laboratory conditions, in two series differing in the source of CO_2 fed to photobioreactors (PBR). In series 1 (SA), it was atmospheric air, whereas in series 2 (SE), these were exhaust emissions (flue gases) from biogas combustion. In both experimental series, microalgae were cultured for 19 days.

2.2 Microalgal Biomass and Culture Medium

The experiment was carried out with the *Chlorella vulgaris* UTEX 2714 culture obtained from the Culture Collection of Algae (University of Texas, Austin, USA). This taxon features a huge potential for utilizing pollutants, including waste gases. The advantages of this species include its eurybiontic nature, high adaptability to varying environmental conditions, resistance to pollution, and a fast growth rate.

The cultivation bold balsam medium 3N-BBM was used in microalgae culture (Table 1). At the beginning of culture, the 3N-BBM medium and microalgae were fed to PBR in the amount ensuring the initial concentration of *Chlorella vulgaris* biomass at approximately 40 mg/dm^3.

2.3 Sources of CO_2

Flue gases were derived from biogas combustion in a Bunsen burner (Sigma-Aldrich, Darmstadt, Germany), with atmospheric air as the source of oxygen. Biogas was obtained from a fermentation tank operating under mesophilic conditions [27]. The flue gases were accumulated in a metal dome fixed above the burner. Then, they were discharged through a snorkel to a 2.5-meter aluminum pipe (20 cm in diameter) to get cooled, and finally were stored in tedlar bags. The mean CO_2 concentration in the flue gases was 13% $\pm$ 0.5%. In both experimental se-

ries, the mass flux of CO_2 to PBR was ensured at 0.054 $mgCO_2$/min. In series 1 (SA), atmospheric air was fed to PBR with the yield of 100 cm^3/min (Mistral 200, Aqua Medic). In series 2 (SE), exhaust gases were fed to the PBR using a peristaltic pump (FASTLoad Programmable control peristaltic pump, VWR Germany) with the yield of 0.3 cm^3/min. In SA series, their air which had flown through the culture medium was discharged from the PBR. In SE series, the exhaust gases were recirculated with the peristaltic pump (VWR Germany) with the yield of 100 cm^3/min, owing to which the gas volume flux was analogous in both reactors. In SE series, the peristaltic pump was also used to discharge the gas outside the reactor, with the yield of 0.3 cm^3/min.

2.4 Experimental Station

The experiment was performed in glass PBR, in which the culture medium occupied the volume of 1.0 dm^3, and the gaseous phase occupied 0.3 dm^3. The inlet of gases with CO_2 to PBR was in the culture medium directly above the bottom, whereas the gases were discharged in the upper section of PBR (Fig. 1). Track of peristaltic pumps provided protection against gas backflow. The tube for collection of medium and microalgal biomass samples was equipped with a valve. The reactors had probes for pH measurements (pH meter 340/ION-Set WTW, Oberbayern, Germany). pH was measured continuously, once a day, and the results were sorted, averaged and recorded in a pH-meter memory. The reactors were continuously illuminated by fluorescent lamps (T8 Luxine Plus 15W Sylvania United Kingdom, color temperature 6500K), with the illuminance on reactor's surface from the light side at 2 klux. The temperature of flue gases and air fed to reactors, and culture temperature was 20 °C ± 2 °C.

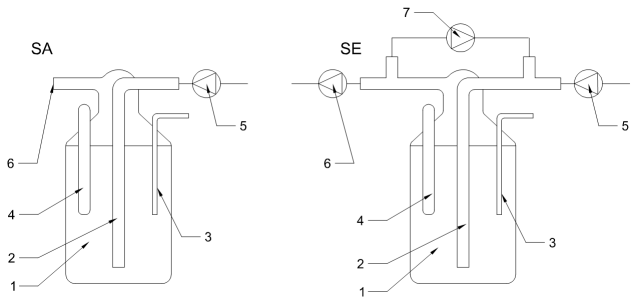

Fig. 1. Experimental station scheme. SA: (1) glass photobioreactor; (2) valve supplying compressed air to the culture medium; (3) collection of microalgal biomass samples; (4) pH measurement; (5) air feeding pump; (6) air discharge. SE: (1) glass photobioreactor; (2) valve supplying flue gases to the culture medium; (3) collection of microalgal biomass samples; (4) pH measurement; (5) flue gas feeding pump; (6) flue gas discharging pump; (7) gas recirculating pump.

2.5 Analytical Methods

The culture medium (20 cm^3) was collected from the PBR once a day and determined for organic dry matter content with the gravimetric method. The filtrated samples were analyzed for total nitrogen content (LCK Hach-Lange USA). Chlorophyll *a* content was determined with the fluorescent method using an algae online analyzer (AlgaeOnlineAnalyser – bbe Moldaenke GmbH, Germany). Algae of a given taxonomic class possess a similar composition of photosynthetic pigments and thus have a typical *in vivo* fluorescence-excitation spectrum, whereby the emission wavelengths of the measured fluorescent light are between 680 and 700 nm. It is thus possible to allocate an algal species to a spectral algal class based on its fluorescence spectrum. In order to obtain a meaningful fluorescence excitation spectrum, six LEDs were used at frequencies of 370 nm, 430 nm, 470 nm, 525 nm, 590 nm, and 610 nm, respectively. The excitation wavelengths of the LEDs were adapted to the absorption wavelengths of the light-harvesting pigments of different algal classes: phycocyanin, phycoerythrin, fucoxanthin, peridinin, and chlorophyll *a*. The excitation of the algal pigments was performed after dark adaptation by switching on the LEDs one after the other at a high frequency. The fluorescence emission of the chlorophyll *a* resulting from the excitation was measured in the phases between these pulses. Spectra of different algal classes of an algal sample consisting of cyanobacteria, chlorophytes, diatoms, dinoflagellates, and cryptophytes were recorded. A mean excitation spectrum normalized by chlorophyll *a* content (fingerprint) of an algal class was determined. Using these "fingerprints" and a mathematical operation (best-fit procedure) enabled calculating the chlorophyll *a* concentration from a complex mixture and the distribution of up to 4 different algal classes in a water sample. The fifth pre-installed class was reserved for the detection of fluorescent yellow substances (humic substances) and used for chlorophyll *a* correction. The chlorophyll determination (calibration) was quantitatively based on an established HPLC separation method of algal pigments [28].

Once a day, samples of gases (flue gases and air) were collected at the inlet to and the outlet from PBR (CO_2, CO, NO_x, SO_x, O_2, N_2), whereas samples of crude biogas were collected before combustion (CH_4, H_2S, H_2, N_2, O_2). The quality of gases was measured using an Agilent Technologies gas chromatograph (GC) with a TC detector (Model 7890A with columns 6Ft 1/8 2 mm MolSieve 5A 60/80 Ultimetal, 9Ft 1/8 2 mm Porapak Q 80/100 Ultimetal) under the following conditions: detector temperature 250 °C; oven temperature 40 °C; carriers: He 10 mL/min and N_2 10 mL/min; and a portable flue gas analyzer Testo 340 (Testo Ltd., Poland) certified for compliance with the EN 50379 standard. Inside the reactors, pH electrodes were tightly installed for on-line measurements (WTW 340/ION-Set WTW, Oberbayern, Germany). pH was measured con-

Table 2. Composition of dried gases supplied to and discharged from PBR in particular experimental series.

Component	Unit	Series			
		SE		SA	
		Inflow to PBR	Outflow from PBR	Inflow to PBR	Outflow from PBR
CO_2	%	13.0 ± 1.0	1 ± 0.5	0.039 ± 0.001	0.031 ± 0.001
N_2	%	76.2 ± 0.4	77.9 ± 0.2	78.1 ± 0.1	78.1 ± 0.1
O_2	%	9.2 ± 0.3	21.4 ± 0.1	20.9 ± 0.1	21.2 ± 0.1
CO	ppm	1.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NO_x	ppm	150 ± 20	0.0 ± 0.0	42.0 ± 3	0.0 ± 0.0
SO_x	ppm	1200 ± 70	0.0 ± 0.0	19.0 ± 2	0.0 ± 0.0

tinuously, once a day, and the results were averaged and saved in analyzer memory.

At the end of the culture, microalgal biomass was subjected to quality analysis. Contents of organic dry matter and mineral dry matter in the biomass were determined with the gravimetric method. Biomass samples dried at 105 °C were determined for contents of total carbon (TC), total organic carbon (TOC), and total nitrogen (N_{tot}). The above analyses were performed using a Flesh 2000 Organic Elementary Analyzer (Thermo Scientifics, USA). The content of total phosphorus (P_{tot}) was determined with the colorimetric method with ammonium metavanadate (V) and ammonium molybdate after prior mineralization of the sample in a mixture of sulfuric (VI) and chloric (VII) acids at a wavelength of 390 nm using a DR 2800 spectrophotometer (HACH Lange). The content of total protein was determined with the Kjeldahl method. The samples were mineralized in sulfuric acid (VI) in the presence of catalysts. Protein nitrogen is converted under these conditions to the ammonium ion which, after alkalization, is distilled as ammonia. The ammonia content was determined by acid-base titration. The conversion factor of 6.25 was used to convert nitrogen to protein. The content of reducing sugars was determined with the colorimetric method with an anthrone reagent, at the wavelength of 600 nm, using a DR 2800 spectrophotometer (HACH Lange). Lipid concentration was determined with the Soxhlet method using an extraction apparatus (Buchi).

2.6 Statistical Analysis

Analyses were conducted in five replications for both experimental series. The results were subjected to one-way analysis of variance at the assumed significance level ($p < 0.05$). Differences between mean values were determined with the Tukey test for honestly significant differences (HSD).

3. Results and Discussion

3.1 Changes in the Composition of Gases

The qualitative composition of crude biogas produced in the fermentation tank and used for combustion was as follows: CH_4 – 64.2 ± 1.9%; CO_2 – 35.4 ± 2.4%; H_2O – 3.1 ± 0.4%; N_2 – 1.4 ± 0.2%; O_2 – 0.3 ± 0.1%; H_2 – 9800 ± 1300 ppm; H_3 – 700 ± 140 ppm, and H_2S – 1500 ± 560 ppm. Table 2 presents the composition of flue gases supplied to the PBR in series SE. It needs to be emphasized that CO_2 concentration in the flue gases outflowing from the photobioreactor in series SE was stable throughout the 19-day culture period and reached 1.0% ± 0.5%. The gas assimilation rate was not correlated with the observed growth phases of *C. vulgaris* biomass in PBR. Considering that CO_2 concentration in the exhaust gases was 13% ± 1.0%, the efficiency of CO_2 fixation in the technological system reached 0.05 mgCO_2/min. This technological effect was influenced by two factors, namely: CO_2 assimilation by the growing microalgal biomass and its dissolution in the culture medium solution [29]. Given the high hardness of water used to prepare the culture medium, i.e., 490 ± 20 mg $CaCO_3/dm^3$, it had significant buffering properties and a significant capability for CO_2 fixation by calcium or magnesium ions. This phenomenon has been earlier described by Liu *et al.* (2022) [30], who optimized the growth rate of *Prymnesium parvum*. In addition, PBR supply with exhaust gases in SE series enabled the complete removal of NO_x and SO_x, whose concentrations in waste gases were at 150 ± 20 ppm and 1200 ± 70 ppm, respectively, and allowed increasing oxygen content to 21.4 ± 0.1%. Analyses conducted in SA series demonstrated a decrease in CO_2 concentration from 390 ± 10 ppm to 310 ± 10 ppm, an increase in oxygen content to 21.2 ± 0.1%, and the removal of nitrogen and sulfur oxides from the air. The effectiveness of CO_2 removal from exhaust emissions was also described by Jiang *et al.* (2013) [31] who demonstrated that the effectiveness of CO_2 utilization by *Scenedesmus dimorphus* might reach even up to 75.61%. In addition, they proved that *S. dimorphus* may tolerate high concentrations of CO_2 and NO, and that $CaCO_3$ addition mitigated the inhibiting effect of flue gases on microalgae [31].

Other researchers [32] suggested flue gases to be a fine source of CO_2 and concluded that their use allowed reducing costs of microalgal culture supplementation with other CO_2 sources. This technological treatment may reduce both this gas emissions to the atmosphere and the costs of chemical and physical purification of flue gases [33,34]. Limitations in deploying crude flue gases are driven by their high

temperature and potentially toxic pollutants they contain [35]. The studies conducted so far have proved that only a few species of microalgae tolerate high concentrations of SO_x and NO_x. For this reason, the choice of species is essential to ensure high effectiveness of CO_2 fixation from flue gases [31,36]. An eurybiontic and resistant to harsh environmental conditions genus *Chlorella* sp. is claimed to be promising in this respect as it ensures CO_2 fixation rates from 0.73 to 1.79 g/dm^3/d [37].

A complete assessment of the effectiveness of net CO_2 fixation by microalgae can be made taking into account the amount of energy introduced into the cultivation system (lighting, mixing, gas injection, separation and drainage, nutrient dosing, etc.). This can only be reliably done in installations operated on a technical or pilot scale, where the operating conditions are similar to full-scale systems. This is an important aspect that determines the application potential of each technology. Research of this kind in the novel photobioreactor with a total volume of 30 m^3 which required merely 100 m^3 of land footprint was carried out by Chen *et al*. (2012) [38]. These researchers determined the potential of CO_2 fixation in the culture of *Spirulina platensis* and proved that the total capture of CO_2 in a photoautotrophic culture was 2234 kg CO_2 year. However, after taking into account the annual energy consumption of 1494 kg CO_2, the found that the net amount of fixed CO_2 in the biomass was only 740 kg CO_2/year. Ultimatelty, upon deducting the energy consumption of bioreactor unit operation, the estimated amount of CO_2 to be fixed by a scaled-up reactor would be 74 tons/ha·year [38].

3.2 Changes in Microalgal Biomass Concentration and Characteristics

The experimental series differed significantly in the rate and amount of microalgal biomass produced. In SE series, within the first 9 days of culture, in the logarithmic growth phase, the biomass growth rate was 77.8 ± 3.1 mgVS/dm^3·d, and biomass concentration reached 745 ± 42 mgVS/dm^3. In the subsequent 4 days of *C. vulgaris* population development, no significant changes were observed in biomass concentration, and the culture entered into the stationary phase of growth. On day 13 of the culture, biomass concentration peaked to 754 ± 45 mgVS/dm^3 and then successively decreased in the consecutive days of culture (the decay phase). At the end of culture in SE series, i.e., after 19 days, the concentration of *C. vulgaris* reached 365 ± 62 mgVS/dm^3 (Fig. 2). The above profile of *C. vulgaris* population development is consistent with observations made by Lee *et al*. (2000) [39], who proved that the growth rate of microalgae can be affected by tolerance of their species to the concentrations of major inhibitory compounds (NO_x and SO_x) in flue gas. Other authors [40] have emphasized that microalgae cannot be used for direct CO_2 fixation from exhaust emissions because industrial flue gases contain approximately 100–300 ppm SO_x [40]. In the present study, the SO_x concentration determined in SE series was substantially higher and reached 1200 ± 70 ppm. In turn, other authors [41] have concluded that the NO_x compounds have no direct impact on microalgae growth at concentrations below 300 ppm NO_x. The growth of microalgae may be inhibited by the excess of acidic gases, part of which cannot be effectively consumed by algae nor dissolved in water, producing multiple ionized H^+. This may lead to culture medium acidification and, consequently, to the inhibition of microalgal population development. Huang *et al*. (2016) [42] have emphasized that certain methods are effective in mitigating the toxic effects of SO_x and NO_x on microalgal biomass. Lee *et al*. (2000) [39] have reached this goal in the case of *Chlorella* sp. KR-1 strain by maintaining optimal pH values and using high concentrations of inoculating cells.

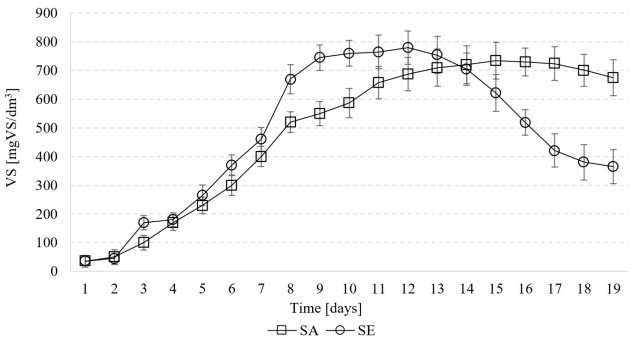

Fig. 2. Changes in the concentration of *C. vulgaris* biomass in particular experimental series.

In SA series, the rate of microalgal biomass growth determined within the first 9 days was significantly lower and reached 56.1 ± 2.7 mgVS/dm^3·d. In contrast to SE series, the concentration of *C. vulgaris* biomass in PBR was also observed to increase within the 6 subsequent days, peaking to 735 ± 64 mgVS/dm^3. But still, this value was lower than the value determined in SE series, where it reached 780 ± 58 mgVS/dm^3 on day 12 of culture. In SA series, the lag phase and stable biomass concentration in the culture medium, approximating 700 mgVS/dm^3, were observed till the end of culture (Fig. 2). Sydney *et al*. (2010) [43] achieved similar results in their study, where the *C. vulgaris* LEB-104 biomass concentration showed exponential growth from the 96th to the 168th hour of the experiment. The maximal cell concentration, reaching 1.94 g/dm^3, was achieved in the last day of culture (15th day), whereas the maximal microalgal biomass productivity reached 0.31 g/dm^3·d [43].

The analysis of the present study results allows concluding that the *C. vulgaris* population grew faster and achieved a higher final biomass concentration in SE series. In addition, it earlier entered into the lag phase, followed by decay phase as early as on day 14 of culture. Although

the rate of microalgal biomass production turned out to be lower in SA series, this culture featured greater stability and significantly lesser fluctuations in biomass concentration in PBR.

The correlations noted in biomass growth were confirmed by the observed changes in chlorophyll *a* concentration in PBR. The course of these changes in time was alike, though not identical compared to the changes in *C. vulgaris* biomass concentration. No statistically significant differences were observed in chlorophyll *a* concentration until day 13 of culture. The rate of its increase and its final concentration were analogous in both experimental series, i.e., 7056 $\pm$ 785 μg/dm^3 in SE and 6790 $\pm$ 258 μg/dm^3 in SA (Fig. 3). In the subsequent days of culture, a significant and rapid decrease in chlorophyll *a* concentration was observed in SE, reaching 4650 $\pm$ 521 μg/dm^3 at the end of the culture (Fig. 3). In SA series, its concentration remained stable, ranging from 6820 $\pm$ 478 μg/dm^3 on day 14 to 6180 $\pm$ 480 μg/dm^3 on day 19 of culture (Fig. 3). The analysis of chlorophyll *a* concentration in the culture medium confirmed that the short-time supply of flue gases was a viable technological solution, while the long-term feeding of this CO_2 source had an adverse effect on *C. vulgaris* population and contributed to a rapid decrease in microalgae count after 14 days of culture. Also Yang and Gao (2003) [44] investigated the impact of supplying microalgal cultures with flue gases and their effect on changes in chlorophyll *a* concentration. They observed that high concentrations of bisulfites, reaching 2 mmol/dm^3, caused damage to chlorophyll *a* in *B. braunii* and ascribed this toxic effect to the generation of active oxygen radicals contributing to chlorophyll *a* whitening and peroxidation of membrane lipids [44]. In turn, Vuppaladadiyam *et al.* (2018) [45] have emphasized that acidic conditions may enhance the toxic effects of bisulfites, which is related to the tolerance to SO_x.

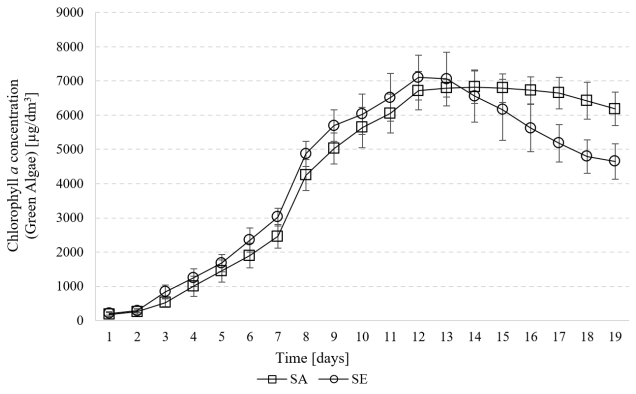

Fig. 3. Changes in the concentration of *C. vulgaris* biomass in particular experimental series.

The source of CO_2 had no significant effect on the composition and characteristics of *C. vulgaris* microalgae biomass. The contents of basic parameters characterizing the biomass were similar in both experimental series. The content of volatile solids oscillated around 91%, that of protein approximated 30%, whereas contents of lipids and sugars were at 19% and 37%, respectively. Table 3 presents detailed biomass characteristics. A similar lipid concentration in *C. vulgaris* culture, reaching 17.23%, was achieved by Álvarez-Díaz *et al.* (2017) [46]. In turn, Yeh *et al.* (2010) [47], who used a dissolved inorganic carbon source (sodium bicarbonate) and a fluorescent light source (TL5), produced the biomass of *C. vulgaris* microalgae with the following composition: 25–30% of proteins, 6–10% of carbohydrates, and 30–40% of lipids.

CO_2 capture and utilization (CCU) is defined as the conversion of this gas into valuable products with lower or no emissions such as fuels, chemicals, carbon fibers, biomass, and building materials [48]. CCU should contribute even to negative net emissions [49]. CCU with the use of microalgae is a biological process in which CO_2 is assimilated in the photosynthesis process [50], and the produced biomass replaces non-renewable resources in the production of chemicals, fuels, plastics, building materials, dyes, dietary supplements, cosmetics, pharmaceuticals, feed, and fertilizers [51]. An example is their use in the production of cement [52] or biochar, which, when introduced into the soil, allows for long-term storage of CO_2 and promotes sustainable agriculture [53]. Another direction of deploying microalgae biomass is the production of bioplastics [54]. These types of plastics are environmentally friendly because they do not increase the CO_2 pool and are more easily biodegradable [55]. Microalgae biomass can be an alternative to other bioplastics and replace traditional plastics or biodegradable plastics such as polylactic acid and polyhydroxyalkanoates [56].

3.3 Changes in Nitrogen Concentration and pH Value

The more rapid development of the microalgal culture observed at the initial period of culture in SE series was also confirmed by the analysis of changes in the concentration of nitrogen compounds in the culture medium. A significantly more effective consumption of this medium component was observed between day 4 and day 6 of culture. The nitrogen concentration reached 26 $\pm$ 4 mgN/dm^3 in SE and 38 $\pm$ 4 mgN/dm^3 in SA (Fig. 4). An interesting phenomenon was observed in SE series, namely the increase in nitrogen concentration from 6.8 $\pm$ 2.6 mgN/dm^3 on day 15 to 22.2 $\pm$ 4.1 mgN/dm^3 on day 19 (Fig. 4). This increase was correlated with the decay process of microalgal biomass, mineralization of organic matter, and nitrogen release to the dissolved phase. For comparison, PBR supplied with atmospheric air allowed reaching a stable nitrogen concentration in the culture medium, fitting within a narrow range from 5.5 $\pm$ 2.6 mgN/dm^3 to 5.7 $\pm$ 3.2 mgN/dm^3 in the analogous days of culture (Fig. 4).

In SA series, the pH value increased significantly since the onset till day 10 of culture (Fig. 5). This increase

Table 3. Composition of *C. vulgaris* biomass in particular experimental series.

Parameter	Unit	Series	
		SE	SA
Volatile solids	% dry matter	91.1 ± 1.2	90.6 ± 2.0
Mineral solids	% dry matter	8.9 ± 1.2	9.4 ± 0.6
N_{tot}	mg/g dry matter	49.4 ± 3.1	50.7 ± 3.5
P_{tot}	mg/g dry matter	18.7 ± 1.7	19.9 ± 2.3
TC	mg/g dry matter	511.2 ± 39.2	504.8 ± 52.4
TOC	mg/g dry matter	469.5 ± 11.9	455.2 ± 19.6
Proteins	% dry matter	30.6 ± 1.4	29.1 ± 1.0
Lipids	% dry matter	19.4 ± 1.7	18.9 ± 0.9
Sugars	% dry matter	37.2 ± 2.6	36.6 ± 3.3

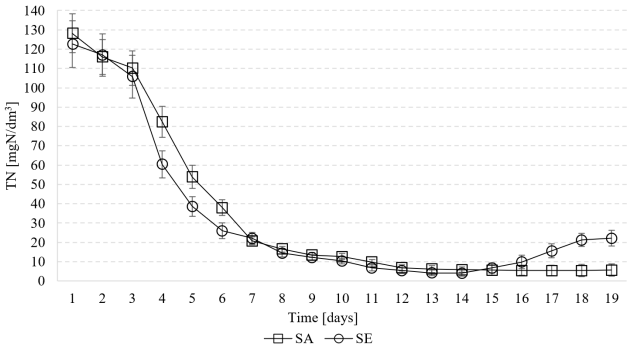

Fig. 4. Changes in total nitrogen concentration in the culture medium in particular experimental series.

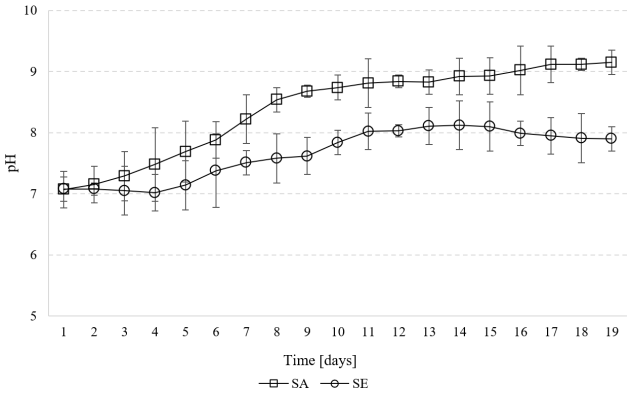

Fig. 5. Changes in culture medium pH in particular experimental series.

was strongly correlated with the growth dynamics of the *C. vulgaris* population and an increased microalgal biomass concentration in PBR. This phenomenon is typical of periodical cultures, where the increasing concentration of exometabolites produced during photosynthesis leads to culture medium pH increase. Once the threshold concentrations are achieved and pH value increases substantially, the growth of the microalgal population is firstly diminished and then ultimately inhibited. In SA series, the intensive production of the microalgal biomass was inhibited around day 11 of culture, as manifested by the recorded concentrations of biomass and chlorophyll *a*. Afterward, the pH value reached 8.81 ± 0.4 (Fig. 5). In the subsequent days of culture, the concentration of *C. vulgaris* biomass remained stable, thereby limiting dynamic pH changes. At the end of SA culture (day 19), the pH value reached 9.15 ± 0.2 (Fig. 5).

The pH values recorded in SE series were lower in the entire culture period. Analogous pH changes to SA series were observed at the microalgal population development and growth phases. The pH value increased from 7.08 ± 0.2 at the beginning of the experiment to 8.12 ± 0.4 after 14 days of PBR operation (Fig. 5). In the subsequent days, the SE series culture was observed to decay, which was reflected in reduced production of exometabolites by microalgae and pH decrease to 7.9 ± 0.2 (Fig. 5). The lower pH values recorded in SE series were also affected by CO_2 source. Even though the total CO_2 concentration was the same in both experimental series, the concentration of carbon dioxide was higher in exhaust emissions. A greater difference of concentrations contributes to faster diffusion, penetration, and dissolution of CO_2 in the culture medium, which caused pH to decrease when the buffering capability had been depleted. The pH of the culture is an important effector of the microalgal CO_2-concentrating mechanism [57]. Valdes *et al.* (2012) [58] have demonstrated that the pH profile provides information about the behavior of the microalgae/photobioreactor system in terms of CO_2 consumption effectiveness.

3.4 Algal CO_2 Fixation—Limitations and Challenges

Effective CO_2 biosequestration in the systems for intensive microalgae biomass production raises multiple controversies due to the lack of carbon neutrality and, in many cases, even a positive carbon footprint of this type of technology, as earlier proven [59]. As autotrophic organisms, microalgae fix CO_2 through photosynthesis, which should directly affect the low or none carbon footprint and high handprints. It takes place in natural ecosystems, where the growth of phytoplankton population occurs without additional elements intensifying the rate of biomass produc-

tion. However, many technological solutions implemented for the industrial processing of microalgae are based on specialized installations, like photobioreactors; heating and lighting systems; devices for mixing and for dosing a carbon dioxide source and nutrients; as well as solutions for biomass thickening and dehydration [60]. These solutions require energy consumption both for the production of components and for the operation and service of the technology. What is more, their carbon footprint can be high. This is especially important in climatic zones with low temperatures and poor insolation. Therefore, it is necessary to strive for implementing simple, energy-saving installations, and for identifying and optimizing solutions operating similarly to natural ecosystems.

Ekendahl *et al.* (2018) [61] have demonstrated that the year-round cultivation of microalgae under natural conditions is possible in the far north, like Borås in Sweden, thanks to the supply of heat from waste heat and carbon from the flue gases of pulp and paper mills. Due to the low photosynthesis efficiency of only 1.1%, the biomass was collected only once a year. Despite the relatively low photosynthesis efficiency, research works have documented applicable practices for carbon biosequestration in microalgae cultivation, even at higher latitudes. The trophic relationships (autotrophy, heterotrophy and mixotrophy) occurring in local microalgae consortia were determined and technological recommendations for CO_2 capture, reduction of energy consumptionm and minimization of carbon footprint were developed [61].

Other studies have proven the possibility of energy-efficient cultivation of microalgae in northern latitudes. The production system achieved a photosynthetic efficiency of 1.1% net, an energy index (NER) of 0.25, and the predicted annual energy biomass yield from the area was 5.2 times higher than the respective oilseed rape production [62]. Energy-efficient production of microalgae in a cold continental climate has been proven as well. The recovered biomass had high calorific values of 20–23 MJ kg^{-1} and contained 14–19% of oil with a predominance of C16 and then C18 fatty acids. The presented technological solution was found utile for carbon sequestration and energy storage in biomass [62]. In turn, Deprá *et al.* (2019) [63] investigated new bioreactor designs to maximize carbon mass transfer from the culture medium to the biomass or microalgae metabolites. A hybrid photobioreactor containing a bubble column and an 'illumination platform' was designed, as inspired by recent advances in biofilm culture strategies. The configuration of the model resulted in an average CO_2 conversion rate of 45.32 kg $CO_2/m^3/d$, but only 1.28% of CO_2 was incorporated into the biomass. Most of the converted CO_2, i.e., 82.75% carbon mass transfer, was consumed for the synthesis of volatile organic compounds [63].

The carbon footprint of each subsequent stage of microalgae processing (harvesting, dehydration, upgrading) needs to be balanced so that the end products are emission-negative, i.e., have a positive net carbon footprint. The Life Cycle Analysis (LCA) is the tool to balance CO_2 emissions and energy of any biologically-mediated carbon capture and utilization (bio-CCU) system. In this way, it is possible to verify the carbon footprint. The development and implementation of sustainable practices and energy-saving technologies is essential for the development of CO_2-neutral microalgae biorefineries [60]. Undoubtedly, the use of cheap sources of nutrients and CO_2 is an important element allowing to increase energy and economic efficiency. Therefore, it seems advisable to conduct research on the possibility of using waste CO_2, including that from biogas combustion installations, as a source of this microalgae biomass production-limiting chemical compound.

4. Conclusions

Exhaust emissions from biogas combustion may be deployed to intensify the culture of *C. vulgaris* species microalgae. The use of this CO_2 source (series SE) caused a higher rate of biomass growth in the acceleration and logarithmic growth phases, reaching 77.8 ± 3.1 mgVS/dm^3·d. In addition, it enabled producing a higher concentration of microalgae, i.e., 780 ± 58 mgVS/dm^3.

Nevertheless, it needs to be emphasized that the *C. vulgaris* culture supplied with flue gases turned out to be very sensitive and after a few days of the stationary phase rapidly entered into the decay phase. This phenomenon enforces strict control and monitoring of microalgal biomass production as well as rapid responses in flue gas-fed systems. This is an important hint for potential operators of such technological systems on the large scale. In turn, the culture fed with atmospheric air as a CO_2 source was far more stable and featured a long phase of stationary growth.

The course of the *C. vulgaris* culture and the observed changes in biomass concentration were correlated with changes in chlorophyll *a* concentration, culture medium pH, and effectiveness of nitrogen consumption by the microalgae. PBR feeding with flue gases had no significant effect on biomass characteristics in terms of contents of organic substances, including lipids, proteins, and sugars.

C. vulgaris biomass effectively assimilated CO_2 from the emissions, and its concentration recorded before the decay phase decreased from 13% in crude flue gases to 1% in the effluent from the photobioreactor. The concentration of CO_2 in gases fed to PBR approximated 1% throughout the culture period.

It should be understood that the complete assessment of the effectiveness of net CO_2 fixation by microalgae can be made taking into account the amount of energy introduced into the cultivation system. By converting the energy consumed into the CO_2 produced, it is possible to determine the net reduction of this gas. This can only be reliably done in installations operated on a technical or pilot scale, where the operating conditions are similar to full-scale systems.

Determining the size of the carbon footprint by means of a properly conducted LCA is a prerequisite for recognizing the technologies based on the production of microalgae biomass as those enabling the real CO_2 sequestration. The possibility of long-term storage of carbon in the microalgae biomass by developing products that can be used in products or building materials is important as well. Regardless of the possibility of deploying microalgae to fix and utilize CO_2, a justified avenue of research is to look for cheap sources of CO_2-rich gases.

Author Contributions

MZ and MD designed the research study. JK and MD performed the research. MZ provided help and advice. MZ and MD analyzed the data. MD and JK wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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