HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

INVESTIGATION ON THE POTENTIAL APPLICATION IN ENVIRONMENTAL TECHNOLOGIES OF THE MICROALGA *CHLORELLA VULGARIS*

THESES OF DOCTORAL (PhD) DISSERTATION

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Background and Objectives

The atmospheric carbon dioxide (CO_2) concentration has increased from 280 ppm in the 1700s to 427 ppm today, leading to severe consequences such as rising sea levels, increasingly frequent and extreme weather events, and the adverse effects of global climate change. The European Union's climate neutrality objectives include increasing the use of renewable energy sources and developing innovative technologies such as various CO_2 capture and utilization solutions. Microalgae-based CO_2 capture presents a promising method for reducing emissions. Due to their rapid growth rate and high photosynthetic efficiency, algae can absorb significantly more CO_2 per unit of time and area than terrestrial plants. The application of algae-based methods is not only environmentally friendly but also economically advantageous, as it can yield valuable by-products. Therefore, the use of microalgae offers a solution not only for CO_2 capture but also for the sustainable production of biofuels, fertilizers, and other industrial products. Despite the numerous advantages of microalgae-based CO_2 capture systems, their implementation faces significant technological and economic challenges.

In my dissertation, I present the results of two model experiments, both aimed at promoting the use of microalgae in various environmental technologies, with a particular focus on CO_2 reduction techniques. The primary objective of the first study was to develop a simple, rapid, and low-resource-intensive method for estimating algal biomass. During the experiments, I examined and compared the applicability of widely researched and commonly used methods for quantifying the biomass of the *Chlorella vulgaris* algal species, including magnesium content, chlorophyll-a content, and algal cell count. In the first model experiment, I investigated the correlation of six different laboratory methods with an UV-VIS spectrophotometric procedure, based on parallel measurements performed on dilution series prepared from a concentrated *C. vulgaris* stock solution. The goal of the experiment was to determine the optimal wavelength at which optical density shows the strongest correlation with other parameters characterizing algal biomass. Since the recommended wavelengths for measuring chlorophyll pigments in the literature cover a wide range, I applied correlation scanning to identify the exact wavelength. This method involved performing linear regression on the optical density data measured at different wavelengths against additional laboratory parameters measured in the dilution series (e.g., cell count, dry matter content, chlorophyll-a concentration).

The second model experiment aimed to investigate the carbon uptake capacity of *C. vulgaris*. To model a separated-system microalgae-based CO_2 capture process, I used an aqueous extract of ash derived from the combustion of wood biomass as a washing medium. The selected raw material is globally widespread and sustainable, contributing to the fundamental objectives of the circular economy model. In the first step, I examined the flue gas purification capability of the ash extract using a model flue gas. CO_2 gas from a cylinder was introduced in the form of fine bubbles into the aqueous extract through a diffuser while monitoring the pH change. A secondary objective of the experiment was to develop a new method for measuring dissolved inorganic carbon content using the OxiTop respirometer.

Materials and Methods

Maintenance of *Chlorella vulgaris* strain culture

During my modeling experiments, I used a *C. vulgaris* strain culture as a test organism. The applied unicellular green alga (*Chlorella vulgaris* Beijerinck, CCAP 211/11b) originated from the Algal Collection of Charles University in Prague, Czech Republic. The cultures were maintained in a nutrient salt solution named Scharlau Algae Broth (Art. No. 02-007) provided by the Scharlab Group, Barcelona, Spain. The selected algal species is easy to maintain under laboratory conditions and tolerates environmental changes well, including elevated CO_2 concentrations during cultivation. There is extensive scientific literature available on *C. vulgaris*, and the strain is in high market demand, factors that played a crucial role in selecting this model organism. To prepare the nutrient solution, I added 1.87 g of nutrient salt per liter of algal suspension following the manufacturer's instructions to achieve a dark green color within 7–15 days. The cultures were maintained in Erlenmeyer flasks at a controlled temperature (25 ± 1°C) under continuous illumination with full-spectrum daylight fluorescent tubes (Osram Biolux, T8 L30W/965 Daylight 6500K, 26 mm x 895 mm).

For culture maintenance, I used titration flasks based on the methodology described by Vince Ördög, placing them on a glass shelf. Fluorescent tubes were positioned beneath the glass shelf to ensure uniform illumination of the flasks. Aeration was provided using an air pump (Hailea Co., Chaozhou, China), and the incoming air was dispersed into microbubbles using a pumice stone, ensuring efficient oxygenation of the medium. Before entering the flasks, the air was filtered through a 0.22 μm pore-sized (d = 13 mm) polyvinylidene fluoride syringe filter to maintain sterile and controlled experimental and cultivation conditions.

Methods for determining algal biomass

To measure algal biomass, I prepared a dilution series from the strain culture with an initial cell concentration of $1.08 * 10^{11}$ cells/L, using dilution factors of 2, 5, 10, 20, 50, 100, 200, 500, and 1000. For comparative evaluation, I analyzed six different methods against the widely used UV-VIS spectrophotometric method.

The comparative study included the following methods:

1. Biomass concentration by filtration (BMf)
2. Biomass concentration by loss of evaporation (BMe)
3. Magnesium concentration determination (Mg)
4. Chlorophyll-a extraction (Chl-a)
5. Cell number counting using a microscope (CN)
6. Dichroic fluorometer system (DFS)
7. Optical density determination via spectrophotometry (OD)

Cell counting was performed in quadruplicate for each dilution to ensure representativeness. Due to minimal deviations in UV-VIS spectra ($R^2 > 0.999$), only two parallel samples were analyzed. For the other measurement methods, three replicates were conducted per dilution step. Except for magnesium content, all parameters were measured within 24 hours to avoid potential temporal changes due to the biological nature of the samples.

Determination of algal biomass by filtration

During the determination of algal biomass by filtration, the algal suspensions were filtered through sterile membrane filters (Whatman 10401170, Maidstone, UK, cellulose nitrate membrane, pore size = 0.45 μm, d = 47 mm) that had been pre-washed with distilled water. After filtration, the filter papers were dried and then weighed. The filtered algal cells were

dried at 105°C for 24 hours along with the filter paper, after which their combined weight was measured using an analytical balance.

Determination of algal biomass by loss of evaporation

Algal suspensions of different dilutions were homogenized by shaking in volumetric flasks and then dispensed into ignition crucibles in three replicates (3 × 40 ml). The crucibles were evaporated to constant weight in a drying oven at 90°C, and the residual mass was then measured. For each dilution, the dry matter content of the algal-free solution was also measured in three replicates. To determine the dry matter content, the samples were centrifuged at 5000 rpm for 20 minutes (Ohaus Corporation, Parsippany, NJ, USA), and the supernatant was filtered through filter paper (Whatman 10401170, pore size = 0.45 μm, d = 47 mm) to remove residual algal cells. The filtered samples were then transferred to ignition crucibles and dried to constant weight in an oven at 90°C. The average residual mass per unit volume of the algal-free supernatant was determined in three replicates and subtracted from the concentrations of non-centrifuged samples, also determined by evaporation. The dissolved salt content, which could potentially originate from the nutrient solution, was deducted from the total residual dry weight in all cases, yielding the net algal biomass weight.

Determination of algal biomass based on magnesium content

To determine magnesium concentrations, the biomass samples remaining after evaporation were placed in a muffle furnace and ignited at 550°C for 3 hours. The magnesium content of the samples was extracted from the ash using acid hydrolysis and then measured using a microwave plasma atomic emission spectrometer (4210 MP-AES, Agilent Technologies, Santa Clara, CA, USA).

Determination of algal biomass based on chlorophyll-a concentration

The chlorophyll-a concentration of each dilution series was determined using the spectrophotometric method described by Felföldy. First, the samples were filtered through filter paper (Whatman 10401170, pore size = 0.45 μm, d = 47 mm), then the filter paper was removed from the filter funnel, folded, and cut into thin strips with scissors. The filter paper strips were placed into centrifuge tubes, and 10-10 mL of methanol was added. The centrifuge tubes were placed in a water bath, and the mixture was heated to the first boiling point (74°C). After heating in the water bath, an additional 10-10 mL of methanol was added to the samples. Following the extraction process, the chlorophyll-a content was separated from the sample matrix by centrifugation (4700 rpm, 20 minutes). The optical density of the supernatant was measured at different wavelengths (750, 666, and 653 nm) using a Jenway 6405 UV-VIS spectrophotometer (Cole-Parmer Instrument Co., Neots, UK). The chlorophyll-a content of the samples was calculated using the Felföldy formula.

Determination of algal biomass based on cell count in samples

Algal samples from different dilution series were dispensed into test tubes in two replicates. Two images were taken of each examined sample using a BTC BIM 105M microscope (Castell Nova Kft., Sopron, Hungary) equipped with a MicroQS 2-megapixel SPCMOS02000KPA microscope camera (Micro Q Inc., San Diego, CA, USA) at 100x magnification. The results were obtained by averaging image processing from four replicates to ensure the accuracy and reliability of the method. The cell count was determined using a Bürker counting chamber (Marienfeld-Superior, Lauda-Königshofen, Germany). From each microscopic image, a 0.64 mm^2 area was digitally cropped before performing detailed image analysis and cell counting. The cropping was pre-adjusted to match the known dimensions

of the counting chamber's edges, and after determining that the unit area consisted of 1110 x 1110 pixels, this size was randomly cropped from the digital microscopic images. Knowing the 0.1 mm depth of the Bürker chamber, the examined unit area allowed for the cell count of a 0.064 mm^3 suspension volume.

The number of cells in the unit image section was determined using the ImageJ image processing software (software version: 1.50i) after performing image processing steps on the microscopic images. First, a background correction process was applied using a bright background, then the images were converted to 8-bit and thresholding was performed to enhance contrast and convert the images to black and white, using a threshold range between 0 and 200. The first three steps aimed to remove unwanted background noise and improve the visibility of algal cells on the glass surface. The particles in the images were counted using the "Analyze Particles" command, ensuring that only spots between 6-150 pixels in size were considered by the software. The applied size limit was determined based on the pixel count of dozens of randomly selected algal cells, ensuring that only cells within the desired size range were counted while ignoring potential contaminants of varying sizes.

Determination of algal biomass based on excited chlorophyll-a fluorescence measurement

The biomass of algal suspensions was also determined based on the excited chlorophyll-a fluorescence measurement method with the assistance of researchers from the Agricultural Environmental Science Research Center of the MATE Institute of Environmental Sciences. Fluorescence measurements were carried out using the prototype of a dichroic fluorometer system (DFS) developed within the Aquafluosense project (NVKP_16-1-2016-0049, consortium leader: Prof. Dr. András Székács). The developed instrument is suitable for estimating algal density and analyzing its composition based on excited chlorophyll-a fluorescence measurement. The measurements were performed according to a method described and optimized by the research team involved in the instrument development, and the detected fluorescence intensity was expressed in relative fluorescence units (RFU). The DFS is equipped with a stepper motor that enables the movement of detector heads over the wells of 96-well microplates, allowing the RFU to be determined quickly and efficiently, as well as individually in the sample wells of microplates. During fluorescence measurements, the RFU values were determined based on the results measured by DFS channel 1. The applied excitation wavelength was 630 nm, while the detection wavelength was 716 nm. During the excited chlorophyll-a fluorescence measurements, an LED lamp served as the light source.

Determination of algal biomass based on optical density measurement

The optical density of the examined algal suspensions was determined using full-spectrum scanning in the 300–1100 nm wavelength range with a Jenway 6405 UV-VIS spectrophotometer (Cole-Parmer Instrument Co., Neots, UK). Due to the extremely low variance of the measured spectral data (based on linear regression analysis: $R^2 > 0.999$), measurements were performed in only two replicates for each member of the dilution series. During the measurements, all recorded spectra were corrected using the spectrum of distilled water, which was used as a blank, by subtracting the values measured for the blank from all recorded spectra.

Statistical evaluation of methods suitable for determining algal biomass

The results of the analytical measurements were visualized and analyzed using Microsoft Excel 2018 (Microsoft Corporation, Redmond, WA, USA). The correlation coefficients (R^2 values) between the algal density values measured by different methods and

the UV–VIS signal differences measured at specific wavelengths were determined using a newly developed method called correlation scanning. Correlation scanning was performed across the entire measured wavelength spectrum using a program developed in Delphi by Dr. Imre Czinkota (Borterres Delphi® 6.0, for Microsoft® Windows 98® and higher operating systems). The essence of the algorithm's calculations is that the program performs linear regression analysis for the function of concentration measured in each member of the dilution series and optical density at specific wavelengths separately and then provides the obtained R^2 values for the respective wavelengths. The maximum values of the obtained functions indicate the strongest correlation and the wavelength at which the given parameter should be measured using a spectrophotometer.

The obtained maximum values of the correlations between optical density and concentrations were subjected to further statistical analysis using Microcal Origin 6.0 (MicroCal Software, Inc., Northampton, MA, USA). Unlike in scanning, where only linear correlations could be established, the built-in functions of the program allowed for a more refined mathematical background of the correlation.

Presentation of the model experiment examining microalgal carbon uptake

Preparation of ash extract from biomass combustion

In the model experiment investigating carbon uptake in the selected *C. vulgaris* microalgal species, the wood ash sample originated from the fly ash of the Szakoly Biomass Power Plant. In the laboratory, an extract was prepared from the wood biomass ash (WBA) sample using distilled water at a 1:10 ratio, followed by a 24-hour shaking process, after which the solution was filtered through filter paper (Whatman No. 42, pore size = 0.25 μm).

Experimental setup

Using the extraction method described in the previous section, the effects of treatments with different WBA (wood biomass ash) contents (0%, 10%, 25%, and 50% WBA) on the carbon uptake of *C. vulgaris* were investigated. The effects of the treatments were examined in triplicate at room temperature (25 ± 1 °C), with three parallel control samples included in the study. The precise composition of the treated and untreated control experimental units is summarized in Table 1. To ensure results comparable to optimal growth conditions, all samples were initiated simultaneously under identical laboratory conditions, using a standard nutrient solution (Scharlau Algae Broth, Scharlab Group, Barcelona, Spain). During the experiments, CO_2 gas was introduced into the samples from a gas cylinder using a diffuser (ISTA Ceramic UFO CO_2 Diffuser, 25 mm disc) in the form of microbubbles. After the CO_2 fixation process, the pH of each sample was adjusted from its original pH to 6.00±0.05. If the samples were left in the laboratory for several hours, distilled water could absorb atmospheric CO_2, resulting in slight carbonic acid formation. This additional carbon content could influence the results of the model experiment; therefore, to prevent this, the distilled water was boiled immediately before the experiment. Consequently, boiled (then cooled to room temperature) distilled water (pH = 7.00) was used in the experiments. During the growth test, the samples were placed in 500 mL Erlenmeyer flasks sealed with stoppers and stored on an illuminated glass shelf.

Table 1: Experimental setup of the control and different CO_2 and WBA content treatments used in the model experiment

Treatment	WBA extract (mL/L)	Nutrient salt (g/L)	Algae stock (mL/L)	Boiled, distilled water (mL/L)	CO_2
vontrol	-	1.87	5	995	-
0%	-	1.87	5	995	yes
10%	100	1.87	5	895	yes
25%	250	1.87	5	745	yes
50%	500	1.87	5	495	yes

pH value monitoring

In the model experiment conducted, I determined the pH values in the control and treated groups every 12 hours using a digital pH/mV meter (OP-211/2, Radelkis Ltd., Budapest, Hungary). The pH values were measured in all three replicates for both the control and treated samples.

Monitoring of algal biomass concentration

To monitor the changes in algal biomass concentration, I applied a method based on optical density determination, based on the relationship established in the first model experiment. Sampling was done every 12 hours, similar to the pH measurement. I examined the samples using a spectrophotometer (Jenway 6405 UV/VIS, Cole-Parmer Instrument Co., Neots, UK) at a wavelength of 440 nm. After the measurement, the samples were returned to the experimental titration flask. The biomass concentration was calculated from the optical density values determined by the instrument using equation **[2]**.

Determination of dissolved inorganic carbon content using a self-developed method

The determination of total dissolved inorganic carbon (DIC) is typically performed by acidifying the water sample, during which HCO_3- and CO_3^{2}- transform into CO_2 molecules. After extraction and titration, the DIC content of the sample can be determined. In my studies, I used the OxiTop device to measure DIC based on pressure measurement, a method I could not find any previous examples of in the literature. The OxiTop device (OxiTop-IDS B6M-2.5 WTW, Weilheim, Germany) is a closed, static respirometer based on pressure measurement, originally developed to evaluate the biological activity of microorganisms in solid and liquid samples.

During the measurements, I sealed the CO_2-enriched algal suspensions in the OxiTop device containers, placing a 50 mL flask containing 1 M HCl in each container alongside the sample. The baseline pressure was measured for 10 minutes, after which I shook the containers to overturn the flasks, causing the acid to mix with the suspension, releasing CO_2 dissolved in the sample. The final pressure stabilized after 20 minutes, and I recorded the measured values. Before the model experiment, I split the CO_2-enriched solutions containing algal cells and ash extract into two parts. One part was placed in the OxiTop devices to determine the DIC content before the algal growth experiment, with 3 replicates for each. The other part of the algal suspension was first used in the algal growth model experiment, and after completing the 4-day experiment, I also placed these samples in the OxiTop devices, enabling the determination of the initial and final DIC values of the solutions. The measurements were performed at a constant temperature (20 °C), and based on the measured pressure values, the amount of CO_2 was determined using the combined gas law.

Statistical evaluation of the results of the model experiment on the carbon uptake of the studied microalga

In my model experiment on the carbon uptake of *C. vulgaris*, I examined the effects of different treatments in triplicates and performed statistical analyses using R Statistical Program version 4.2.1 (R Development Core Team, Vienna, Austria). Prior to the statistical analysis, I checked the normality of the data and the homogeneity of variance using the Shapiro-Wilk test and Levene or Bartlett tests at a 5% significance level. During the model experiment, I evaluated the effects of treatments using one-way analysis of variance (one-way ANOVA). For comparisons between groups, I applied the Tukey test as a post-hoc test. If the assumptions for ANOVA were not met, I evaluated the data using the Kruskal-Wallis test and applied the Dunn test to compare differences between groups at a 5% significance level. If an examined parameter (e.g., DIC value) had both initial and final values, the differences between the values were evaluated using a two-sample t-test.

Results and Discussion

Comparison of different biomass determination methods

The average results of the various methods suitable for determining algal concentration are summarized in Table 2. For algal biomass measured by evaporation, I excluded results from the 200x dilution value from the correlation analyses, while for biomass values determined by filtration, I only disregarded the results from the 1000x dilution, as these fell below the detection limit (LOD). In the case of magnesium (Mg) content measurement, it is likely that for values below the 20x dilution, after ignition, the remaining material was so minimal that these results fell below the LOD. However, I did not exclude these results because they did not significantly affect the observed trend.

Table 2: Averages of measured parameters characterizing algal density based on selected biomass estimation methods

Dilution	Ratio	BMe (mg d.m./L)	BMf (mg d.m./L)	Chl-a (µg/L)	Mg (µg/L)	CN (10^6 cells/L)	RFS
1	1	960.0	770.0	7515.6	204.5	108222.7	1689562
2	0.5	436.7	420.0	4163.1	109.6	61578.1	1200521
5	0.2	150.0	177.5	1567.0	27.6	34113.3	853994
10	0.1	66.7	79.3	892.4	10.6	13136.7	610195
20	0.05	35.8	31.4	382.1	28.4	10664.1	369087
50	0.02	14.2	15.0	182.3	13.8	3757.8	189411
100	0.01	10.0	9.3	90.9	15.4	1699.2	112247
200	0.005	<LOD	2.5	54.6	17.5	472.7	63474
500	0.002	<LOD	1.4	20.1	11.0	371.1	32556
1000	0.001	<LOD	<LOD	12.3	6.9	105.5	26938

Correlations found between different biomass determination methods

When comparing the results obtained from determining algal concentration parameters, I correlated the data sets (Table 2) against each other to uncover the strength of the relationships between the individual parameters (Table 3). The numbers represent the highest R^2 values achieved by the best-fit trendlines, which were obtained after performing linear and exponential regression. With the exception of one case, the relationships can be considered statistically strong ($R^2 > 0.94$). The only weak correlation occurred between the fluorescence measurement and the magnesium content determination results ($R^2 = 0.816$). I highlighted the exceptionally high correlation values ($R^2 > 0.990$) in bold. The table does not include the spectroscopic analysis, as the correlations obtained with this parameter were examined across the entire spectrum at each wavelength, and the results are discussed in a separate section.

Table 3. Established correlations between the measured parameters

	Dilution	BMf	BMe	Mg	CN	Chl-a	RFS
Dilution	-	**0.998** (L)	**0.997** (L)	0.975 (L)	0.986 (L)	**0.998** (L)	0.989 (Lg)
BMf	-	-	0.989 (L)	0.966 (L)	**0.993** (L)	**0.999** (L)	0.987 (Lg)
BMe	-	-	-	0.982 (L)	0.974 (L)	**0.990** (L)	0.974 (Ep)
Mg	-	-	-	-	0.945 (L)	0.971 (L)	0.816 (Ep)
CN	-	-	-	-	-	0.989 (L)	0.984 (Lg)
Chl-a	-	-	-	-	-	-	0.986 (Lg)
RFS	-	-	-	-	-	-	-

linear (L), logarithmic (Lg), or exponential (Ep) functions

Results of the correlation scanning and determination of coefficients across the entire spectrum

In addition to the correlations presented above, which may be important for understanding the interconnections of laboratory-measurable parameters, one of the main focuses of my research was to establish the correlation between these parameters and optical density. As shown in Chapter 2.6, there is no consensus in the literature regarding the wavelength used for the spectrophotometric measurement of *Chlorella vulgaris*, nor the equation used for converting the measurements to biomass mass. Therefore, it was essential in my research to not follow individual authors and select a particular wavelength, but rather to first prove through mathematical methods which wavelength would provide the most accurate measurement of *C. vulgaris* cell density.

The dilution series I prepared provides spectral data at each wavelength with a series of 10 data points. These data points can then be compared with the laboratory-measured algal concentration results for the same 10 samples from the dilution series. To determine the strongest correlation between the examined parameters across the entire UV–VIS spectrum, a function and trendline must be fitted for each parameter at every data point. This would require fitting and plotting a total of 800 functions for each parameter. To automate this task, I used a Delphi-based program, which continuously scanned the development of R^2 values that characterize the strength of linear regression relationships (figure 1).

Based on the results of the correlation scanning performed across the entire measured spectrum, the stronger correlations and higher R^2 values corresponding to local maximums appeared where the original spectrum had peak values. The only exception was observed for fluorescence values, where the opposite trend was observed. The biomass values, determined by filtration and originally marked in black, are fully covered in the graph because this dataset showed an extremely strong correlation with the chlorophyll-a concentration data marked in green. The maximum correlation values determined between optical density and other algal biomass-estimating parameters in the visible wavelength range (400–800 nm) are summarized in Table 4.

As expected, it is best to measure where the algae absorb light more efficiently, so higher correlation coefficients are observed at the characteristic peaks of *C. vulgaris* photosynthetic pigments. However, it was surprising that the peak around 680 nm did not yield the maximum determination coefficient for any parameter, despite this being a popular wavelength often suggested in the literature, as it has a clear and easily detectable local maximum in the spectrum. Based on the biomass measurement methods, the 439 nm and 437 nm wavelengths showed the highest correlation, so – considering that many spectrophotometers cannot handle scales smaller than 5 nm – I selected the 440 nm

wavelength for fine-tuning the correlation and establishing the relationship for biomass estimation.

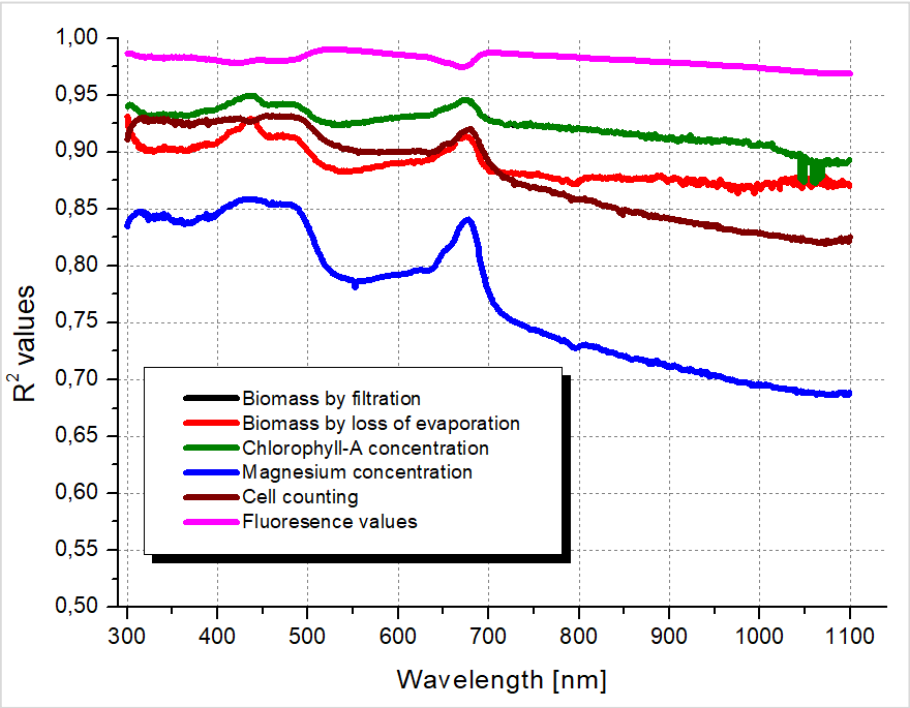

Figure 1: Strength of correlation coefficients (R^2) between optical density measurements and additional parameters estimating algal biomass across the entire spectrum

Table 4: Wavelengths corresponding to the strongest correlation values (highest R^2) determined for each algal biomass estimation parameter based on the full spectra

dilution	BMf	BMe	Mg	CN	Chl-a	DFS
R^2 max	0.94961	0.92922	0.85855	0.93257	0.94961	0.99000
R^2 max wavelength	**439**	**437**	**442**	461	**439**	525

Recommended formula for estimating algal biomass

After selecting the appropriate wavelength, I further refined the correlation by testing various built-in functions of the Origin software (Figure 2). When correlating biomass concentration measured along the dilution series with optical density values, the data fit most accurately to an exponential (saturation) curve. This is because, above a certain number of algal cells, the likelihood of cells partially overlapping increases statistically, meaning that the appearance of an additional algal cell in a more concentrated suspension does not cause as significant an increase in light intensity as in the more diluted samples. Based on the well-known saturation phenomenon, it was logical to select the saturation curve, so from the built-in functions of the program, I applied the Box–Lucas function **[1]**:

$$y = a\left(1 - e^{-bx}\right) \qquad \textbf{[1]}$$

where *a* and *b* are constants, and *x* and *y* are the variables of the function.

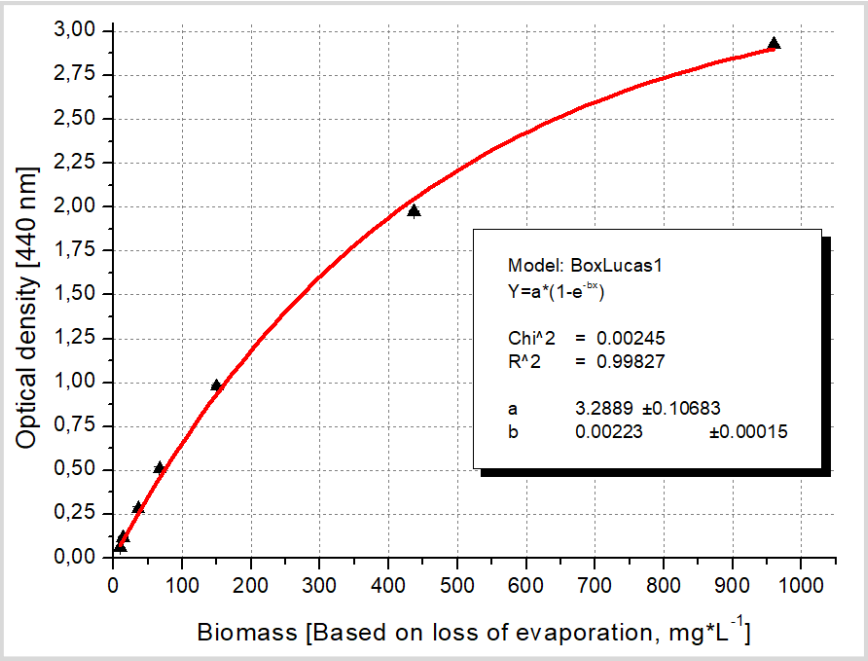

Figure 2: Optical density values correlation to dry algal biomass data determined by loss of evaporation method

By selecting the 440 nm wavelength, I established an exponential relationship **[2]** between the dry algal biomass concentration (c), measured using the evaporation residue method, and the signal difference measured by optical density (OD):

$$c_{\text{algal biomass}}\ \text{mg / L (d.m.)} = \ln\left(1 - OD_{440\ \text{nm}} / 3.29\right) / (-0.0022) \qquad \textbf{[2]}$$

Results of a model experiment investigating carbon uptake by the microalga *C. vulgaris*

The results obtained during the model experiment are summarized in Table 5. Statistical analysis of the results of WBA treatments showed higher final algal concentrations, biomass change (Figure 3), and specific growth rates compared to the control group ($p \leq 0.038$). The highest final algal concentrations, biomass changes, and specific growth rates were observed in the 10% and 25% WBA treatments compared to the control group ($p < 0.001$). Significant differences were found between the initial and final algal concentrations for all treatments ($p \leq 0.006$).

Table 5: Summary results of the model experiment investigating carbon uptake by *Chlorella vulgaris*

Parameter	Dimension	Blank	0% WBA	10% WBA	25% WBA	50% WBA
initial algae conc.	mg L^{-1} (d.m.)	5.6 ± 0.5 a	5.5 ± 0.6 a	5.5 ± 0.8 a	6.5 ± 0.7 a	5.7 ± 0.8 a
final algae conc.	mg L^{-1} (d.m.)	*17.1 ± 0.5 a	* 49.8 ± 3.6 b	* 160.2 ± 17.2 c	* 176.0 ± 5.6 cd	* 133.3 ± 18.0 d
change in biomass	mg L^{-1} (d.m.)	11.5 ± 1.6 a	44.3 ± 3.8 b	154.7 ± 17.1 c	169.5 ± 5.5 cd	125.8 ± 18.3 d
mass multiplication	-	3.10 ± 0.4 a	9.24 ± 1.3 a	29.32 ± 3.5 b	27.21 ± 1.8 b	17.83 ± 3.2 c
specific growth rate (μ)	day^{-1}	0.28 ± 0.03 a	0.55 ± 0.04 b	0.84 ± 0.03 c	0.83 ± 0.02 c	0.72 ± 0.04 d
doubling time	days	1.97 ± 0.11 a	1.29 ± 0.07 b	0.86 ± 0.04 c	0.89 ± 0.02 c	1.02 ± 0.06 c
pH without CO_2 addition	-	6.94 ± 0.01 a	6.79 ± 0.01 a	9.67 ± 0.20 b	11.18 ± 0.06 c	12.15 ± 0.04 d
change in pH	-	0.05 ± 0.01 a	0.87 ± 0.04 b	3.95 ± 0.17 c	2.98 ± 0.09 d	1.83 ± 0.06 e
initial DIC	mg C L^{-1}	LOD	10.4 ± 0.00 a	48.6 ± 7.96 b	156.3 ± 9.03 c	309.2 ± 7.96 d
final DIC	mg C L^{-1}	LOD	3.5 ± 3.01 a	* 3.5 ± 3.01 a	* 90.3 ± 3.01 b	* 218.9 ± 10.42 c
DIC removal	%	-	66.7 ± 28.9 ab	92.8 ± 6.5 a	42.2 ± 1.4 b	29.1 ± 5.15 b

Note: BM biomass, Doubling time (td) specific growth (μ) WBA: wood biomass ash extract; DIC: dissolved inorganic carbon; <LoD: result below the Level of Detection; letters a-b-c-d: indicate a statistically significant difference between the control and the treated group ($p \leq 0.05$); * indicates a significant difference between the initial and final values ($p \leq 0.05$)

Compared to the control group, significantly higher pH values were observed in the 10%, 25%, and 50% WBA treatments ($p < 0.001$), while significantly different pH changes were observed in all groups ($p < 0.050$). The initial dissolved DIC content in the treated groups was significantly different, with the highest final DIC values measured in the 50% WBA treatment ($p < 0.001$). Significant differences were found between the initial and final DIC values in the 10%, 25%, and 50% WBA treatments ($p \leq 0.003$). However, no significant difference was observed between the initial and final DIC values in the 0% WBA treatment ($p = 0.057$). The greatest decrease in DIC content was observed in the 10% WBA treatment ($p \leq 0.014$), although the difference was not significant compared to the 0% WBA treatment ($p = 0.223$). Based on the parameters related to algal growth, the 10% and 25% WBA treatments proved to be the most suitable, while the greatest decrease in DIC content was observed in the 0% and 10% WBA treatments (Table 5).

In summary, the results of the examined parameters suggest that the 10% WBA treatment is considered optimal for algal growth, carbon uptake, and the regeneration capacity of the ash extract as an alkaline CO_2 absorbing medium.

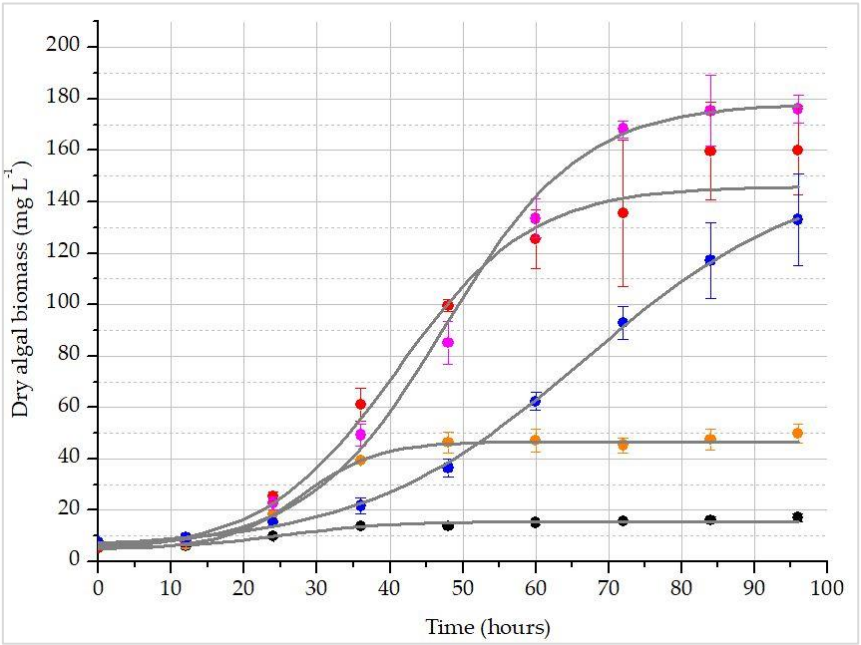

Figure 3: The growth of algal biomass in the differently carbonized treatments: blank treatment without CO_2 diffusion (black); 0% WBA addition with CO_2 scrubbing (orange); 10% WBA addition with CO_2 scrubbing (red); 25% WBA addition with CO_2 scrubbing (purple); 50% WBA addition with CO_2 scrubbing (blue).

Conclusions and Recommendations

Conclusions drawn from the results of different biomass determination methods

Magnesium and chlorophyll-a content in *Chlorella vulgaris* algal cells

The average magnesium (Mg) content in *C. vulgaris* algae can be determined based on the measured biomass mass and the Mg content of the same dilution group. The average single-cell mass of the algae species can be calculated from the biomass mass and cell count values. For the planned calculations, I determined the average values of the data from each dilution sample. Due to the assumed inaccuracies in laboratory measurements at higher dilution levels, samples with dilutions greater than 20 times were not included in the calculations (Table 6).

Table 6: Specific values derived from measured algae concentration data.

Dilution	BMe (mg d.m./L)	Mg (µg/L)	Mg (mg/100 g d.m.)	Chl-a from Mg (µg/L)	Chl-a (µg/L)	CN (10^6 cells/L)	AW (µg /10^6 cells)
1x	960	205	21.31	7521	7516	108223	8.871
2x	437	110	25.09	4029	4163	61578	7.091
5x	150	28	18.40	1015	1567	34113	4.397
10x	67	11	15.83	388	892	13137	5.075
Average			**20.16 ± 3.98**				**6.36 ± 2.03**

Based on the performed calculations, the dry mass of 1 million *C. vulgaris* algal cells is 6.36 ± 2.03 µg, or $6.36 \pm 2.03 \times 10^{-12}$ g/cell. It is surprisingly difficult to find relevant data in the literature with which to compare this result. The only available data suggests that the estimated mass of 1 million cells is 22.4 µg ($2.24 \pm 0.16 \times 10^{-11}$ g/cell), which comes from a thesis and is not considered an official scientific publication. Nevertheless, this result is approximately in the same order of magnitude as the value I measured. In terms of the magnesium content in *C. vulgaris*, according to my calculation, it is 20.156 ± 3.98 mg/100 g (on a dry weight basis). When comparing this result with literature values, an interesting scientific situation arises because the values determined by authors can roughly be categorized into two groups (Table 6).

Potential applications of the study's results

The wavelengths determined through the correlation scan and further correlation analyses using the saturation (exponential) curve open up the possibility of rapid biomass determination methods suitable for industrial algae production, without damaging the sampled biomass. However, it remains a question whether fluorescence detection of excited chlorophyll-a or UV–VIS spectrophotometric optical density measurements should be applied. The ISO 7027-1:2016 water testing standard recommends the use of two types of measurement instruments, depending on the number of contaminant particles. In the case of less contaminated samples (e.g., water treatment), nephelometric measurements can be applied. In the case of heavily contaminated, turbid samples (e.g., wastewater treatment), turbidimetric methods are recommended.

Following the recommendations, I divided the 10 diluted samples into 5 more dilute and 5 more concentrated sample groups. Then, I compared the strength of the obtained correlations for each group based on the separated data sets. For the more diluted samples, the data obtained through fluorescence detection resulted in a higher determination coefficient, which can be explained by the principle of operation of the measuring instrument. In this device, the arrangement of the light source, sample, and detector forms a 90-degree angle, so the instrument measures light scattered by the particles at this angle, which is especially effective when there are few algae cells present in the sample. Unsurprisingly, for the less diluted, more concentrated samples, UV–VIS spectroscopy provided more accurate results ($R^2 = 0.99993$) compared to the fluorescence detection results ($R^2 = 0.98533$). In the applied device, the light source, sample, and detector are aligned in a straight line, forming a 180-degree angle, and the device measures the light absorbed by the particles. This configuration allows for greater accuracy when the particles are more concentrated in the suspension.

Given that the selected measurement wavelength has been mathematically validated, it directly follows that future developments of measuring instruments should focus on the 440 nm wavelength.

Conclusions drawn from the model experiment investigating microalgal carbon uptake

The measurement performed with the OxiTop device is suitable for determining the DIC (dissolved inorganic carbon) content (Figure 4).

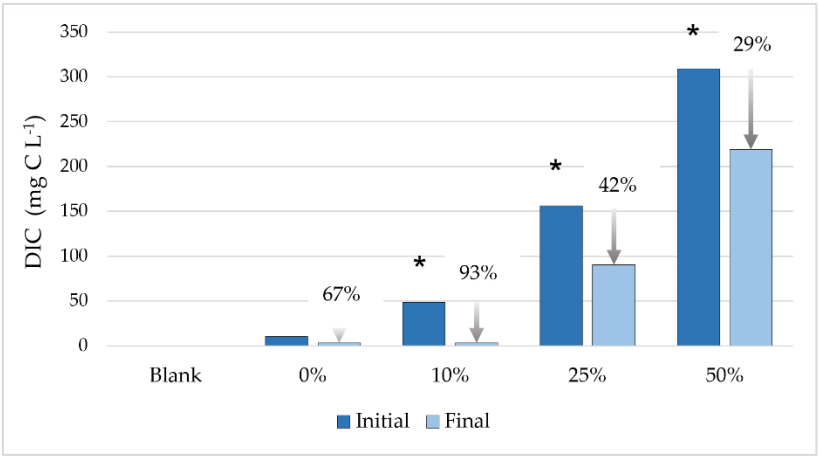

Figure 4: The measured total dissolved inorganic carbon (DIC) contents at the beginning and the end of the algae cultivating model experiment (* indicates significant difference between the initial and final DIC values at the significance level of 0.050).

The aqueous extract of wood biomass ash (WBA) formed as a byproduct of biomass combustion is capable of absorbing CO_2 gas, and *C. vulgaris* microalga is able to uptake this carbon during its growth. During the carbon uptake process, the alga was able to regenerate the CO_2-absorbing medium.

In my model experiment, the initial medium was alkaline due to the addition of the WBA (wood biomass ash) extract. By increasing the proportion of WBA extract, the medium became increasingly alkaline, which also allowed for more CO_2 binding. At the start of the experiment, I set all treatments to a uniform pH (pH = 6) using CO_2 diffusion treatment. During the growth of the algal biomass, the alkalinization of the medium is a phenomenon documented in numerous studies, and it was clearly observable during my model experiment as well. The highest final pH value was measured during the 10% WBA treatment, reaching the pre-treatment level of CO_2 diffusion, which indicates that in these samples, the algae growth fully regenerated the pH of the washing medium.

I demonstrated that CO_2 diffusion and ash extract treatment did not show toxic effects on the growth of *Chlorella vulgaris* microalgae. Based on my results, the WBA extract effectively bound CO_2 without having a toxic effect on algae growth. However, at higher concentrations, it inhibited growth, resulting in an extended lag phase. The algal cells were able to utilize CO_2 from the WBA extract

as a carbon source, which led to CO_2 consumption, biomass growth, and an increase in pH. According to the literature, *Chlorella* species generally grow optimally in the pH range of 6–6.5, while *C. vulgaris* can grow effectively under alkaline conditions as well (pH = 10.5). *C. vulgaris* is also capable of reproducing at very low pH, a feature that is cited in several sources as the basis for zooplankton control methods in laboratory-scale cultures.

Based on the results of the model experiment, I was able to select the 10% WBA treatment, which can be applied in further research and industrial applications. During the optimization of the WBA extract, the 10% and 25% WBA treatments were found to be the most suitable for algae growth, producing 154.7 ± 17.1 mg and 169.5 ± 5.5 mg of excess biomass over 4 days, respectively. When calculating the growth per unit of initial biomass, the specific growth rates were 0.84 ± 0.03 and 0.83 ± 0.02 1/day, while the doubling times were 0.86 ± 0.04 and 0.89 ± 0.02 days. The biomass increase was 29.32 ± 3.5 and 27.21 ± 1.8 times for the two treatments over the 4-day growth period (Table 5). Although these values are not statistically significantly different from one another. When considering the efficiency of CO_2 removal, I observed the highest efficiency for the 10% WBA treatment (92.8 ± 6.5%). The 25% WBA treatment showed much lower efficiency (42.2 ± 1.4%), and thus, the algae were not able to raise the pH to the original level prior to CO_2 diffusion. In the case of the 10% WBA treatment, the washing solution used for cultivation was fully regenerated, and the pH reached a value of 9.95 at the end of the treatment.

Critical review of algal yield data in the literature

In this section, I attempt a brief critical review to analyze the realism of the algal yield data found in the literature. Using my own measurement results, I will demonstrate, employing multiple approaches, that the data often rely on overly optimistic estimates for algal biomass growth, leading to industrial sectors achieving results that fall significantly short of the promises made in the literature.

Argument 1: The initial element of my dilution series

The initial element of my dilution series was a highly concentrated suspension containing 1.095 x 10^{11} cells per liter. This sample had a very dark green, almost black appearance. I determined its biomass concentration using two different methods, neither of which reached 1 g/L (filtration: 0.77 g/L, evaporation: 0.96 g/L). The spectrophotometer also measured values near its detection limit (OD > 2.990) for the individual peaks, despite the fact that the light only needs to pass through a narrow cuvette (d = 5 mm). Considering these facts, I find values above 30 g/L, as reported in some literature, highly unlikely.

Argument 2: Pixel coverage

For the densest sample with the algal concentration described above, image processing in ImageJ resulted in an average of 13% pixel coverage. Based on this, I determined a theoretical maximum value for when the surface is completely covered by algae, which gives a value of 7.38 g/L. It is worth noting that the actual maximum could be slightly higher since the Bürker chamber also has depth, and we are not reading only from a flat projection.

Argument 3: The volume occupied by the cells

Knowing that the geometry of *C. vulgaris* is a regular sphere with a known diameter between 2–10 μm in the literature, the cell volume can be calculated. Multiplying this by the number of cells per liter in the concentrated sample results in values between 0.45 cm^3 and 56.67 cm^3. This corresponds to 0.05% and 5.67% V/V per liter. From this, I also calculated a theoretical maximum where 100% of the volume is occupied by algae. Based on the two cell diameters, the calculations give values of 1920 g/L and 16.9 g/L. This suggests that with 10 μm cells, at biomass concentrations above 17 g/L, the cells would begin to touch each other. This is, of course, impossible to achieve, especially when considering the light requirement for growth, which would be inefficient at such high densities, with very little light reaching individual cells.

Argument 4: Industrial statistics

The variation in growth values for *Chlorella* species is extremely high. This is partly explained by the expression of specific values discussed in the previous section, where there are numerous potential sources of error, such as the selection of surface/volume/time intervals for measurement. Even within a single article, the variation between values is significant, as demonstrated by the work of Je and Yamaoka (2022), where growth rates ranged between 3 and 5370 mg/L/day. Comparing these values to the EU's annual microalgae production volume of 182 tons, we can estimate that the two capacity values would correspond to growth volumes of 170,000 m^3 and 93 m^3 of cultivation medium, respectively. The latter corresponds to three standard shipping containers, which seems highly unlikely, as otherwise, the microalgae technology would be far more widespread globally.

Suggestions

Based on my results, the growth of *C. vulgaris* in monoculture and axenic conditions should be examined at the 440 nm wavelength, for which LED lighting paired with a photodiode, or a laser light source and laser diode pair, should be used. To eliminate noise and light absorption that is not from the algae, a similar pair of devices at the 750 nm wavelength, as recommended by the ISO and Felföldy methods, can be applied for this purpose. The absorption of blue light by algae should also be further investigated in cultivation experiments where the light source simultaneously produces peaks around 680 nm and 440 nm, for example, by using white LED lights. The model for estimating algal biomass should be further validated with unknown samples and the results compared with other types of spectrophotometric analyses.

Furthermore, additional comparisons and validation are needed for the digital cell counting method and the determination of dissolved inorganic carbon using the OxiTop device. The biomass ash extract should be examined in samples from the power plant during different seasons and compared with quality variations due to seasonality. Additionally, it should be compared with ash extracts/solutions from other plant combustions. It would also be worthwhile to examine the ash extract after the algal treatments to determine how elements potentially taken up by the algae (e.g., potassium) affect the future usability of the washing solution.

In the future, instead of using CO_2 gas in the model, it would be advisable to use flue gases and biogas from industry to absorb into the washing solution, and then examine the algae growth to filter out any growth-inhibiting effects and identify potentially usable trace gases that could be beneficial for the algae. This could potentially reduce the costs associated with gas purification in practice. It would also be highly beneficial to examine the nutritional content of the produced algal biomass, which would reveal how the treatment alters the composition of the biomass compared to the control, as well as which industries the resulting biomass could be used in, based on the relevant quality parameters.

New Scientific Results

1. A New Method for Determining the Most Reliable Measurement Wavelength for *Chlorella vulgaris* Growth Using a new technique called *correlation scanning*, I mathematically proved that the most reliable measurement wavelength for *Chlorella vulgaris* during its exponential growth phase, using UV-VIS spectrophotometry, is at the peak of 440 ± 3 nm. This mathematical confirmation addresses a long-debated issue in the scientific literature. By selecting the appropriate wavelength and choosing an exponential function to describe the phenomenon more accurately, I established the following relationship between optical density (OD) and the dry algal biomass concentration (c) in suspension:

$$c_{\text{algal biomass}} \text{ (mg/L d.m.)} = \ln(1 - OD_{440\text{ nm}} / 3.29) / (-0.0022)$$

2. Average Dry Cell Weight and Magnesium Content of *Chlorella vulgaris* Based on my experimental results, I determined the average dry cell weight of *Chlorella vulgaris* to be $6.36 \pm 2.03 \times 10^{-12}$ g, and the average magnesium content of the dry biomass to be 20.156 ± 3.98 mg/100 g. This result also demonstrated that the values above 300 mg/100 g found in the literature are not achievable under the cultivation conditions presented in this study.

3. Use of the OxiTop Device for Measuring Dissolved Inorganic Carbon (DIC) I demonstrated that the OxiTop (IDS B6M-2.5 WTW) device is suitable for measuring dissolved inorganic carbon (DIC) in both carbonate and bicarbonate forms. The device's pressure measurement capability detects the additional pressure caused by the CO_2 gas appearing in the gas phase during the process. The procedure involves placing a sav solution alongside the suspension in a boiling flask. After sealing the container and establishing the baseline pressure, the flask is tipped to mix the acid solution with the suspension, releasing CO_2. The measured pressure change is directly proportional to the DIC concentration according to the combined gas law. The smallest detectable DIC concentration, with a 200 mL sample, is 5.21 mg C/L.

4. CO_2 Absorption by Ash Extracts from Biomass Combustion I showed that the aqueous extract of ash produced during biomass combustion is capable of absorbing CO_2. This is supported by the measured DIC concentrations, where treated samples exhibited higher DIC values compared to untreated control samples. *Chlorella vulgaris* can absorb and utilize this carbon during growth, as evidenced by a decrease in DIC and a significant

increase in biomass in treated samples compared to controls. The algae were also able to regenerate the CO_2-absorbing medium, as indicated by the pH measurements in the 10% ash extract treatment. Initially, the pH of the ash extract was 9.67 ± 0.20, which decreased to 5.99 ± 0.02 due to CO_2 diffusion. After observing algal biomass growth over 4 days, the pH increased back to 9.95 ± 0.18, returning to its original alkalinity.

5. Non-Toxicity of Ash Extracts and CO_2 Diffusion to *Chlorella vulgaris* Growth I demonstrated that treatments with 10%, 20%, and 50% ash extract, combined with CO_2 diffusion, did not have a toxic effect on the growth of *Chlorella vulgaris*. This was supported by significant increases in biomass compared to the control treatment. Based on the model experiment conducted at room temperature (25 ± 1 °C), the 10% ash extract treatment with CO_2 diffusion was the most effective. The biomass concentration increased by 29.32 ± 3.5 times from the initial 5.5 ± 0.8 mg/L to 160.2 ± 17.2 mg/L. During the treatment, the algae completely regenerated the medium's pH (9.95 ± 0.18) and reduced the initial DIC concentration by 92.8 ± 6.5%.

The author's publications related to the topic of the dissertation

Foreign language scientific journal articles published in foreign journals:

Fekete Gy., Klátyik Sz., Sebők A., Dálnoki A.B., Takács A., Gulyás M., Czinkota I., Székács A., Gyuricza Cs., Aleksza L. (2024): Optimization of a *Chlorella vulgaris*-based carbon sequestration technique using an alkaline medium of wood biomass ash extract. *Water* **16**: 3696.

Fekete Gy., Sebők A., Klátyik Sz., Varga Zs.I., Grósz J., Czinkota I., Székács A., Aleksza L. (2024): Comparative analysis of laboratory-based and spectroscopic methods used to estimate the algal density of *Chlorella vulgaris*. *Microorganisms* **12**: 1050.

Summaries published in a foreign language conference publication:

Fekete Gy., Varga Zs., Aleksza L., Gulyás M., Czinkota I. (2020): An investigation on replacement alternatives of the nutrient requirements of microalgae with agricultural byproducts. *Abstract book of the 19th Alps-Adria Scientific Workshop* (Wisła, Lengyelország) pp. 31-31.

Fekete Gy., Aleksza L., Köles P., Grósz J., Dálnoki B.A. (2018): Investigation on the growth of microalgae affected by biomass ash extract treatment. *17th Alps-Adria Scientific Workshop: Abstract book* (Hnanice, Csehország) pp. 150-151.

Fekete Gy., Gulyás M., Longhurst P., Jiang Y. (2014): Impact of biogas injection on growth of algal biomass (Biogáz-áramoltatás hatása alga biomassza növekedésére). *II. Sustainable development in the Carpathian Basin" international conference – book of abstracts* (Gödöllő) pp. 79-81.

Summaries published in the Hungarian conference publication:

Fekete Gy., Aleksza L., Köles P., Czinkota I., Dálnoki A.B., Grósz J. (2018): Faalapú biomassza hamu vizes kivonatában megkötött szén-dioxid felhasználása mikroalga termesztésben. *XIV. Kárpát-medencei Környezettudományi Konferencia kiadványa*. MAG Mezőgazdaságért Alapítvány (Gödöllő), pp. 87-92.

Fekete Gy., Aleksza L., Köles P., Czinkota I., Grósz J., Dálnoki A.B. (2018): Oldatban elnyeletett széndioxiddal kezelt mikroalgák vizsgálata. Alkalmazkodó Vízgazdálkodás: lehetőségek és kockázatok. Víztudományi Nemzetközi Konferencia (Szarvas). pp. 43-43.

Fekete Gy., Rétháti G., Kovács A., Dálnoki A.B., Grósz J., Tóth P.P. (2017): Mikroalgák szervesanyag-tartalmának vizsgálata. *6. Környezetkémiai Szimpózium*, Program és előadáskivonatok (Bakonybél) pp. 15.

Other scientific works in foreign and Hungarian languages:

Fekete Gy. (2017): Industrial use of microalgal biomass. *Biohulladék* **11**: 13-19.
Fekete Gy. (2016): Mikroalga tenyésztési módszerek. *Biohulladék* **10**: 35-40.

The author's publications non-related to the topic of the dissertation

Foreign language scientific journal articles published in foreign journals:

Mörtl M., Damak M., Gulyás M., Varga Zs.I., **Fekete Gy**., Kurusta T., Rácz Á., Székács A., Aleksza L. (2024): Biodegradation assessment of bioplastic carrier bags under industrial-scale composting conditions. *Polymers* **16**: 3450.

Javed N., Muhammad S., Iram S., Ramay M.W., Jaffri S.B., Damak M., **Fekete Gy**., Varga Zs., Székács A., Aleksza L. (2023): Analysis of fuel alternative products obtained by the pyrolysis of diverse types of plastic materials isolated from a dumpsite origin in Pakistan. *Polymers* **15**: 24.

Üveges Zs., Damak M., Klátyik Sz., Ramay M.W., **Fekete Gy**., Varga Zs., Gyuricza Cs., Székács A., Aleksza L. (2023): Biomethane potential in anaerobic biodegradation of commercial bioplastic materials. *Fermentation* **9**: 261.

Gulyás M., Someus E., Klátyik Sz., Fuchs M., Varga Zs.I., Dér S., **Fekete Gy**., Czinkota I., Székács A., Gyuricza Cs., Aleksza L. (2022): Effects of combined application of solid pyrolysis products and digestate on selected soil properties of arenosol and plant growth and composition in laboratory experiments. *Agronomy* (Basel) **12**: 1440.

Harsányi E., Juhász Cs., Kovács E., Huzsvai L., Pintér R., **Fekete Gy**., Varga Zs.I., Aleksza L., Gyuricza Cs. (2020): Evaluation of organic wastes as substrates for rearing *Zophobas morio, Tenebrio molitor,* and *Acheta domesticus* larvae as alternative feed supplements. *Insects* **11**: 604.

Foreign language scientific journal articles published in domestic journals:

Gulyás M., **Fekete Gy**., Varga Zs., Dér S., Sebők A., Vojtela T., Aleksza L. (2022): Biogas potential and calorific value of different agricultural main and by-product. *Hungarian Agricultural Res.: Environ. Manag. Land Use Biodiversity* **3**: 11-15.

Pintér R., **Fekete Gy**., Varga Zs.I., Gyuricza Cs., Aleksza L. (2022): Evaluation of organic wastes as substrates for rearing *Zophobas morio, Tenebrio molitor,* and *Acheta domesticus* larvae as alternative feed supplements. *J. Cent. Eur. Green Innov.* **10**: 143-156.

Pintér R., Aleksza L., **Fekete Gy**., Béres A., Gyuricza Cs. (2021): Effect of different substrate mixtures on crude fat and crude protein content of zophobas morio larvae. *Hungarian Agricultural Res.: Environ. Manag. Land Use Biodiversity* **30**: 13-16.

Hungarian-language scientific journal articles published in domestic journals:

Dálnoki A.B., Huszár Sz., Sebők A., **Fekete Gy**., Czinkota I. (2017): Talaj és növény beltartalmi paramétereinek változása trágyakazelések hatására. *Talajvédelem* **2017**: 363-370.

Járdi I., Pápay G., **Fekete Gy**., S-Falusi E. (2017): Marhalegelők vegetációjának vizsgálata az Ipoly-völgy homoki gyepeiben. *Gyepgazdálkodási Közlemények* **15**: 9-21.

Sebők A., Czinkota I., **Fekete Gy**., Dálnoki A.B., Grósz J. (2017): Humuszoldat adszorpciós és deszorpciós kinetikájának meghatározása homokos talajon oszlopkísérlet segítségével. *Talajvédelem* **2017**: 251-260.

Yadav D.V., Gulyás M., **Fekete Gy**., Béres A., Czinkota I. (2016): Effects of digestate on growth and health of soil plant system. *Növénytermelés* **65**: 111-114.

Fekete Gy., Issa I., Tolner L., Czinkota I., Tolner I.T. (2016): Invsetigation ont he indirect correlation and synergistic effects of soil pH and moisture content detected by remote sensing. *Növénytermelés* **65**: 203-206.

A book in Hungarian published by a domestic publisher, edited by:

Aleksza L., **Fekete Gy**. (2023): A körforgásos gazdaságra történő átállás előkészítési feladatai a mezőgazdasági és zöldhulladékok esetében. (Aleksza L., **Fekete Gy**. szerk.) Magyar Agrár- és Élettudományi Egyetem, Gödöllő, Magyarország, 54 p.

Summaries published in a foreign language conference publication:

Rúbia Borges Silva L., **Fekete Gy**., Varga Zs., Aleksza L., Kardos L. (2024): Sustainable remediation and valorization of pálinka spent wash: overcoming environmental challanges through composting in Hungary. *19th Carpathian Basin Environmental Science Conference, book of abstract.* HUN-REN Atommagkutató Intézet (Debrecen) pp. 39-40.

Gulyás M., Someus E., Varga Zs., **Fekete Gy**., Aleksza L. (2020): Effects of combined application of solid pyrolysis products and digestate on selected soil and plant properties in laboratory experiment. *Abstract book of the 19th Alps-Adria Scientific Workshop* (Wisła, Lengyelország) pp. 20-20.

Gulyás M., Tolner L., **Fekete Gy**., Erdősi R., Czinkota I. (2018): Rapid biotest method in precision agriculture. *17th Alps-Adria Scientific Workshop: Abstract book* (Hnanice, Csehország) pp. 162-163.

Summaries published in the Hungarian conference publication:

Teski A., Wichmann B., Penksza K., **Fekete Gy**., Kerényi-Nagy V. (2018): Kárpát-medencei rózsafajok alaktani vizsgálatai. *XII. Aktuális Flóra- és Vegetációkutatás a Kárpát-medencében - program és összefoglalók*. Debreceni Egyetem TTK Növénytani Tanszék (Debrecen). pp. 108.

Czinkota I., **Fekete Gy**., Gulyás M., Tolner L., Sebők A., Köles P. (2016): Biomassza hamu alkalmazhatóságának vizsgálata talajsavanyúság javítására. *Talajtani Vándorgyűlés: "Okszerű talajhasználat - Talajvédelem" előadások és a poszterek összefoglalója.* Magyar Agrártudományi Egyesület Talajtani Társaság (Debrecen) pp. 215-222.

Tolner L., Rétháti G., Kovács A., Dálnoki A.B., **Fekete Gy**., Czinkota I. (2016): Biodízelgyártás melléktermék (glicerin) hatása a talaj nitrogénformáira és az angolperje kezdeti fejlődésére. *5. Környezetkémiai Szimpózium*, Program és előadáskivonatok (Tihany) pp. 21-21.

Hungarian language educational material:

Aleksza L., Varga Zs., **Fekete Gy**. (2023): *Komposztmester: A közösségi komposztálás kézikönyve*. Magyar Agrár- és Élettudományi Egyetem, Gödöllő, 79 p.