

## Article

# Utilisation of CO<sub>2</sub> from Sodium Bicarbonate to Produce *Chlorella vulgaris* Biomass in Tubular Photobioreactors for Biofuel Purposes

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**Abstract:** Microalgae are one of the most promising sources of renewable substrates used for energy purposes. Biomass and components accumulated in their cells can be used to produce a wide range of biofuels, but the profitability of their production is still not at a sufficient level. Significant costs are generated, i.a., during the cultivation of microalgae, and are connected with providing suitable culture conditions. This study aims to evaluate the possibility of using sodium bicarbonate as an inexpensive alternative CO<sub>2</sub> source in the culture of *Chlorella vulgaris*, promoting not only the increase of microalgae biomass production but also lipid accumulation. The study was carried out at technical scale using 100 L photobioreactors. Gravimetric and spectrophotometric methods were used to evaluate biomass growth. Lipid content was determined using a mixture of chloroform and methanol according to the Blight and Dyer method, while the carbon content and CO<sub>2</sub> fixation rate were measured according to the Walkley and Black method. In batch culture, even a small addition of bicarbonate resulted in a significant ( $p \leq 0.05$ ) increase in the amount of biomass, productivity and optical density compared to non-bicarbonate cultures. At 2.0 g·L<sup>-1</sup>, biomass content was  $572 \pm 4$  mg·L<sup>-1</sup>, the maximum productivity was  $7.0 \pm 1.0$  mg·L<sup>-1</sup>·d<sup>-1</sup>, and the optical density was  $0.181 \pm 0.00$ . There was also an increase in the lipid content ( $26 \pm 4\%$ ) and the carbon content in the biomass ( $1322 \pm 0.062$  g·dw<sup>-1</sup>), as well as a higher rate of carbon dioxide fixation ( $0.925 \pm 0.073$  g·L<sup>-1</sup>·d<sup>-1</sup>). The cultivation of microalgae in enlarged scale photobioreactors provides a significant technological challenge. The obtained results can be useful to evaluate the efficiency of biomass and valuable cellular components production in closed systems realized at industrial scale.



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**Keywords:** sodium bicarbonate; carbon dioxide; biofixation; microalgal biomass; lipids content

## 1. Introduction

The main source of energy in the world, also used for fuel production, is still crude oil [1]. Limited fossil fuel resources and adverse environmental impact due to greenhouse gas emissions increased interest in advanced fuel production technologies [2]. The primary feedstocks used for their production are obtained from energy crops or lignocellulosic wastes. Less conventional sources include the biomass of macroalgae and microalgae [3,4].

Microalgae are unicellular or multicellular simple organisms that are metabolically diverse, but most of them are photoautotrophs [5]. A valuable property of theirs is that their fast biomass growth, which per hectare is several times higher compared to terrestrial plants [6], but just like plants, microalgae require nutrients, light and carbon dioxide to grow [7]. Under appropriate conditions, microalgae convert solar energy into chemical energy stored as starch or lipids [5,8,9], which are precursors for bioethanol and biodiesel production [10]. Given the higher photosynthetic efficiency, higher biomass production per unit area and faster growth rate compared to energy crops, microalgae are good alternative as feedstock for biofuel production [8]. An additional advantage of microalgae is the lack of competition for nutrients with food crops [11]. Furthermore, biomass production can

be located on marginal lands [6]. The negative environmental impact associated with the cultivation of microalgae for energy purposes is described as potentially negligible [12,13].

The main problems of algal biofuel production are related to cultivation costs and biomass dehydration processes [14,15]. To increase the cost-effectiveness of biomass production, nutrients contained in municipal wastewater [16,17] or in aquaculture wastewater [18], are used in cultivation. Microalgae have a high ability to remove nitrogen and phosphorus compounds, thus biomass production can be more sustainable and can be used in the bioremediation of the aquatic environment [19].

Commercial cultivation of microalgae requires the supply of significant amounts of inorganic carbon for photosynthesis [20]. This is mainly provided by carbon dioxide from the air and eventually from industrial emissions [21]. This may be a method for its biological sequestration [22], especially considering that some microalgae are capable of assimilating up to 1.83 Mg of CO<sub>2</sub> during the production of 1 Mg of their biomass [23]. Higher CO<sub>2</sub> concentration promotes lipid accumulation in the cells [24]. Atmospheric carbon dioxide concentration depends on anthropogenic activities [25] and industrial development stage [26] and may increase above 400 PPM [27]. Concentrations of CO<sub>2</sub> may be an important factor to limit growth and development of some microalgal species [28], however, minimum and maximum concentrations CO<sub>2</sub> dissolved in the culture medium for microalgae cultivation vary from one species to another [29]. Some microalgae can grow under atmospheric air [30], others in extremely high CO<sub>2</sub> concentrations ranging from 40 to 100 vol% [31]. Compressed CO<sub>2</sub> can also be used in cultivation [32], but this increases the costs, which are related to the capture, compression, transport or storage of this gas [33]. The cost of using compressed carbon dioxide in microalgae culture can account for up to half of the total cost of biomass production [34]. An alternative option may be using of bicarbonate salts [35]. These compounds have much higher solubility in water than CO<sub>2</sub> and higher efficiency in biomass production compared to compressed carbon dioxide [28]. A review of the recent progress in bicarbonate-based microalgae cultivation suggested potential to significantly reduce production cost. The use of sodium bicarbonate reduces the cost of carbon supply, increases the accumulation of valuable components, and is energetically efficient [36]. New technologies make it possible to produce sodium bicarbonate from carbon dioxide, including from industrial CO<sub>2</sub> emissions, which are responsible for the negative effects of climate change [37].

The aim of this study was to evaluate the ability of *Chlorella vulgaris* microalga to utilize bicarbonate as a carbon source and find out the sodium bicarbonate concentration to produce higher biomass and higher lipid contents in microalgae. Its effectiveness was assessed by the amount of biomass, optical density of the culture, lipid accumulation rate, carbon content in the biomass and CO<sub>2</sub> fixation rate. Most of the available literature presents the results of studies carried out at small-scale, so it is important to know the mechanism of microalgae growth at the industrial scale, which changes as the capacity of the photobioreactor increases. The potential benefits of using NaHCO<sub>3</sub> were assessed on a technical not laboratory scale. It is crucial for industrial application of microalgae, especially for biofuel production, and to the best of our knowledge, this is the first such study.

## 2. Materials and Methods

### 2.1. Microalgae

The strain of *C. vulgaris* (BA 002) green microalga was obtained from the Culture Collection of Baltic Algae. The material was stored in F/2 liquid medium [38] with the following composition [g·L<sup>-1</sup>]: NaNO<sub>3</sub>—0.075 g; NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O—0.00565 g; stock solution of trace elements: 1 mL·L<sup>-1</sup> (Na<sub>2</sub>EDTA 4.16 g, FeCl<sub>3</sub> 6H<sub>2</sub>O 3.15 g, CuSO<sub>4</sub> 5H<sub>2</sub>O 0.01 g, ZnSO<sub>4</sub> 7H<sub>2</sub>O 0.022 g, CoCl<sub>2</sub> 6H<sub>2</sub>O 0.01 g, MnCl<sub>2</sub> 4H<sub>2</sub>O 0.18 g and NaMoO<sub>4</sub> 2H<sub>2</sub>O 0.18 g) and stock solution of vitamin mix: 1 mL·L<sup>-1</sup> (cyanocobalamin (vitamin B12) 0.0005 g, thiamine HCl (vitamin B1) 0.1 g, biotin 0.0005 g). Microalga were stored at 4 °C, with a photoperiod of 12 h under light-emitting diode (LED).

## 2.2. Experimental Setup

*C. vulgaris* strain was grown in synthetic medium F/2. In the study, vertical tubular photobioreactors with a total volume of 100 L were used, which were supplemented with 80 L of culture medium F/2 and an appropriate dose of sodium bicarbonate ( $\text{NaHCO}_3$ ): 0.025, 0.5, 1.0, 1.5 i 2.0  $\text{g}\cdot\text{L}^{-1}$ . After sterilization of the medium with UV-C light, 8 L of microalgae inoculate were introduced into the photobioreactors. The control object in the experiment was a commercial culture medium without bicarbonate –0 (F/2). The pH of the medium was set at 7 using 1 N NaOH.

LED lighting with red and blue LEDs (5:1 ratio), with a photoperiod of 18/6 h (light/dark cycle) was used. The microalgae cells were kept in suspension by mixing with gas using a membrane pump (HAILEA ACO-300A, Guangdong, China) with a power of 160 W and a capacity of  $240 \text{ L}\cdot\text{min}^{-1}$ . The experiment was carried out as batch cultures and ran for 20 d.

Biomass growth was estimated by gravimetric method [39], using a moisture analyzer (AXIS ATS60, Gdańsk, Poland). The optical density of the microalgae suspension was determined by spectrophotometry at wavelength  $\lambda = 680$  using a spectrophotometer (SPEKOL 11, Jena, Germany). Measurements were made at the beginning and on the 5th, 10th, 15th and 20th day of the experiment.

The lipid content of the biomass was determined using a solvent mixture (chloroform-methanol) according to the method of Blight and Dyer [40]. The lipid content was calculated using following equation:

$$LC = \left( \frac{mL}{mDAB} \right) \cdot 100, \quad (1)$$

where  $LC$  is the lipid content,  $mL$  is the mass of lipids (g) and  $mDAB$  is the mass of dry microalgal biomass (g).

The carbon content and  $\text{CO}_2$  fixation rate of microalgae cells were determined according to the method of Walkley and Black [41], with some modification [42]. The carbon content was calculated using following equation:

$$a = \frac{3.951}{g} \left( 1 - \frac{T}{S} \right), \quad (2)$$

where  $a$  is the carbon content,  $g$  is the mass of the microalgae sample (g) and  $T$  and  $S$  are the blank and test sample iron-ammonium sulfate, respectively (mL).

The rate of  $\text{CO}_2$  fixation was calculated from the following equation:

$$R_{\text{CO}_2} = C_C \cdot P_{\text{max}} \left( \frac{M_{\text{CO}_2}}{M_C} \right), \quad (3)$$

where  $R_{\text{CO}_2}$  is the rate of  $\text{CO}_2$  fixation ( $\text{g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ ),  $C_C$  is the carbon content of microalgae cells (%),  $P_{\text{max}}$  is the maximum biomass productivity ( $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ ) and  $M_{\text{CO}_2}$  and  $M_C$  are the molecular weight of  $\text{CO}_2$  and C, respectively.

During cultivation, the pH was controlled by a pH-meter CI-316 (Conrad Electronic SE, Hirschau, Germany).

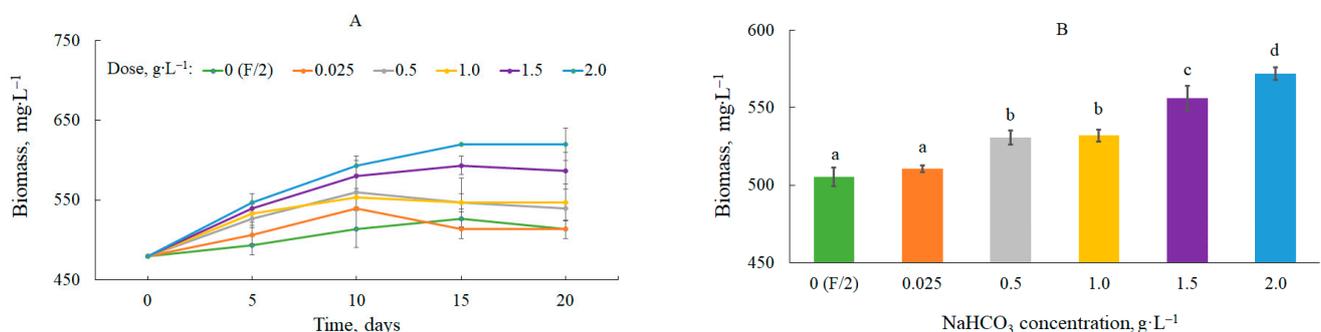
## 2.3. Statistical Analysis

All analyses were carried out in triplicate. Results were statistically analyzed using Statistica software (version 13.3, 2016; Dell Inc., Tulsa, OK, USA). Two-factor analysis of variance was used. The significance of differences between means was assessed using Tukey's test at  $p \leq 0.05$ . Pearson's linear correlation coefficient ( $r$ ) and standard deviations (SD) were also determined.

### 3. Results and Discussion

#### 3.1. Biomass Production with CO<sub>2</sub> from Sodium Bicarbonate

The effect of the NaHCO<sub>3</sub> dose on the growth dynamics of *C. vulgaris* is shown in Figure 1A. The initial amount of biomass was, on average, 480 ± 6 mg·L<sup>-1</sup>. High bicarbonate doses influenced the cell growth and biomass production and prolonged the logarithmic growth phase. After 20 days, the highest biomass (620 ± 16 mg·L<sup>-1</sup>) was determined in the photobioreactor with a dose of 2.0 g·L<sup>-1</sup> NaHCO<sub>3</sub> (over 20% more than in control 0 (F/2) object). Similar results were presented by Yeh et al. [43], who, in the culture of *C. vulgaris*, used NaHCO<sub>3</sub> at a dose ranging from 100 to 1600 mg·L<sup>-1</sup> and obtained the maximum biomass at the highest dose. The same results (0.769 g·L<sup>-1</sup>) were obtained by Mokashi et al. [44], who applied NaHCO<sub>3</sub> in the culture of *C. vulgaris* in a range from 0.025 to 1.0 g·L<sup>-1</sup>. Molazadeh et al. In addition, [45] cultivated *C. vulgaris* in wastewater with compressed CO<sub>2</sub> at 16% and obtained 0.790 g·L<sup>-1</sup>. An equally high biomass content (0.740 g·L<sup>-1</sup>) was obtained by Rodas-Gaitán et al. [46], who cultured *C. vulgaris* in 15-L photobioreactors and used sodium bicarbonate at 8 g·L<sup>-1</sup> as a carbon source. The high solubility of bicarbonate in the culture medium [47] promotes the absorption of inorganic carbon and the production of biomass. In the present study, the average biomass concentration ranged from 505 ± 6 mg·L<sup>-1</sup> in the culture medium without bicarbonate – 0 (F/2) object, to 572 ± 4 mg·L<sup>-1</sup> in a medium enriched with NaHCO<sub>3</sub> at a dose of 2.0 g·L<sup>-1</sup> (Figure 1B). An increase in biomass was also observed at lower doses of bicarbonate, which may be due to the beneficial effect of NaHCO<sub>3</sub> on photosynthesis and cellular component accumulation. A study by Salbitani et al. [48] confirmed the positive relationship between bicarbonate, the chlorophyll a content and the photosynthetic activity of *Chlorella sorokiniana*. The use of NaHCO<sub>3</sub> may be an alternative to CO<sub>2</sub>, which decreases the pH of culture medium and may reduce the availability of carbon for photosynthesis [49].



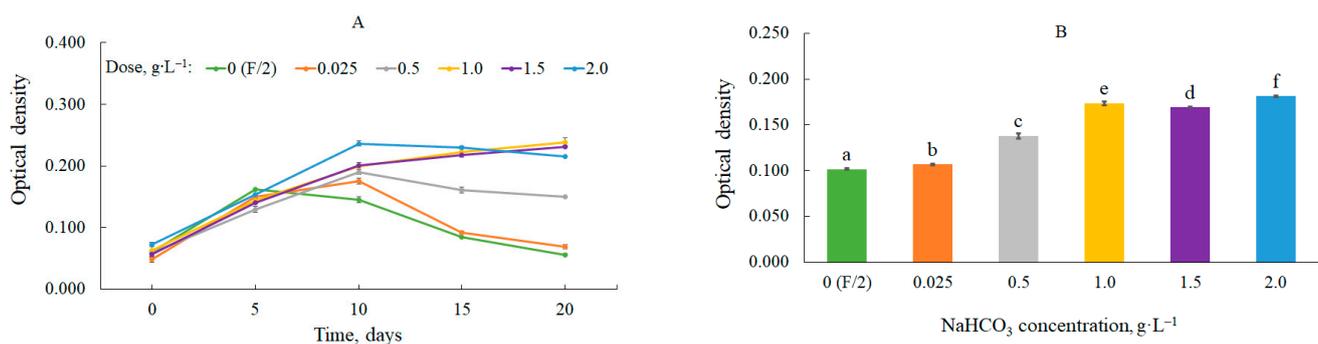
**Figure 1.** Dynamics of changes in biomass content (A) and average biomass content in the culture (B). Mean over each column not marked with the same letter is significantly different at  $p \leq 0.05$ .

The biomass productivity changed as a function of the bicarbonate dose and time (Table 1). The highest values were observed at the beginning of the study at a dose of 2.0 g·L<sup>-1</sup> (13.3 ± 2.3 mg·L<sup>-1</sup>·d<sup>-1</sup>). The productivity decreased with time. The changes could be due to the time-limited availability of nutrients as observed in batch cultures [50] or to the level of carbon dioxide utilisation.

**Table 1.** Biomass productivity in relation to bicarbonate dose.

| Level of Sodium Bicarbonate (g·L <sup>-1</sup> ) | Biomass Productivity (mg·L <sup>-1</sup> ·d <sup>-1</sup> ) |            |           |           |
|--|---|------------|-----------|-----------|
|  | Day 5   | Day 10     | Day 15    | Day 20    |
| 0 (F/2)  | 2.7 ± 2.3   | 3.3 ± 2.3  | 3.1 ± 0.8 | 1.7 ± 0.6 |
| 0.025  | 5.3 ± 2.3   | 6.0 ± 0.0  | 2.2 ± 0.8 | 1.7 ± 0.6 |
| 0.5  | 9.3 ± 2.3   | 8.0 ± 2.0  | 4.4 ± 0.8 | 3.0 ± 0.0 |
| 1.0  | 10.7 ± 2.3  | 7.3 ± 1.2  | 4.4 ± 2.0 | 3.3 ± 1.2 |
| 1.5  | 12.0 ± 0.0  | 10.0 ± 2.0 | 7.6 ± 0.8 | 5.3 ± 1.2 |
| 2.0  | 13.3 ± 2.3  | 11.3 ± 1.2 | 9.3 ± 0.0 | 7.0 ± 1.0 |

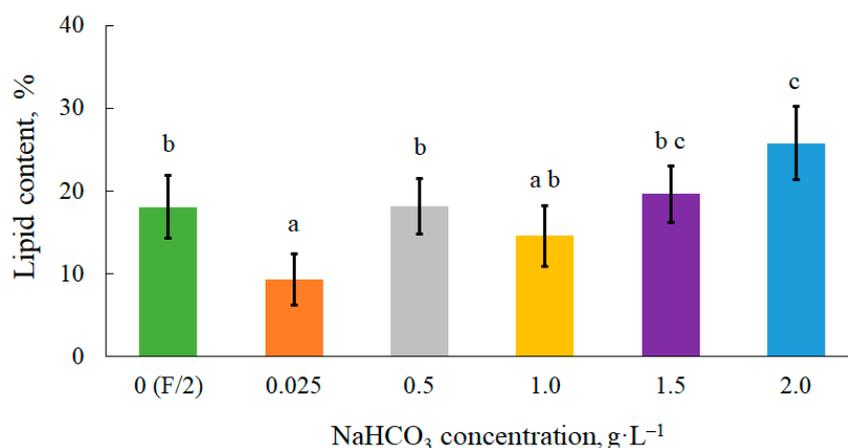
Optical density in culture, as well as biomass content, changed with bicarbonate dose. Higher values were observed at higher doses of  $\text{NaHCO}_3$ . After 20 days,  $\text{OD}_{680}$  in the culture medium ranged from  $0.215 \pm 0.00$  at a dose  $2.0 \text{ g}\cdot\text{L}^{-1}$  to  $0.239 \pm 0.01$  at a dose  $1.0 \text{ g}\cdot\text{L}^{-1}$ , more than 300% in relation to  $0.056 \pm 0.00$  in control 0 (F/2) object (Figure 2A). The amount and the availability of nutrients affects the growth of microalgae [51]. The mean optical density ranged from  $0.101 \pm 0.0$  in control 0 (F/2) object to  $0.181 \pm 0.0$  at a dose  $2.0 \text{ g}\cdot\text{L}^{-1}$  (Figure 2B). Similar results were presented by Jegan et al. [52], who obtained the highest optical density (0.477) in *C. vulgaris* cultures at the highest dose of sodium bicarbonate (2 M). Different results were presented by Salbitani et al. [48], who analysed the effect of three doses of  $\text{NaHCO}_3$  (1, 2 and  $3 \text{ g}\cdot\text{L}^{-1}$ ) on the growth of the microalgae *Chlorella sorokiniana* and found no significant differences between the values obtained at the highest dose and in the control object. The experiment was conducted over 72 h; it represents the short-term effect of bicarbonate addition on algae cultures. According to Chi et al. [53], a high  $\text{NaHCO}_3$  content in the culture medium can affect the growth and development of some microalgae, especially freshwater species. The authors studied the effect of  $\text{NaHCO}_3$  concentration (from 0.01 to 0.60 M) on the growth of *C. sorokiniana* and observed a significant decrease in optical density with increasing  $\text{NaHCO}_3$  concentration (from about 1.3 to 0.1). This may be associated with  $\text{Na}^+$  ions, increase with increasing  $\text{NaHCO}_3$  dose [54]. This ion can support the growth of some halotolerant algal strains [55], which can also include the *Chlorella* strain tested in the present study.



**Figure 2.** Dynamics of changes in optical density (A) and mean  $\text{OD}_{680}$  values in the culture (B). Mean over each column not marked with the same letter is significantly different at  $p \leq 0.05$ .

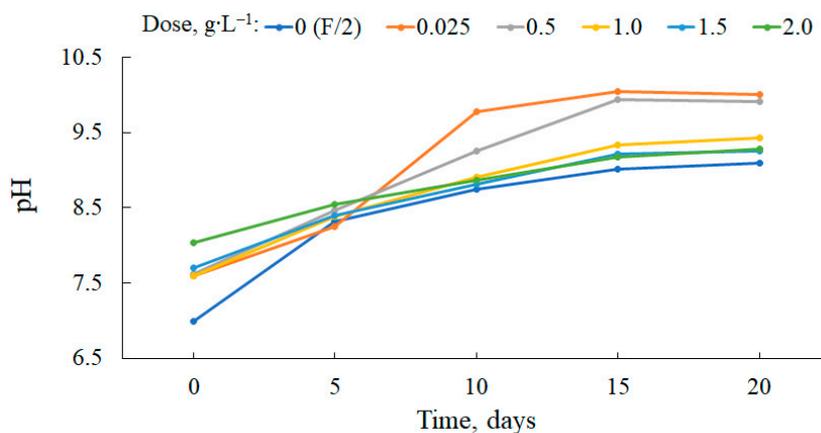
### 3.2. Effect of Sodium Bicarbonate on Lipid Accumulation in Microalgal Biomass

The presence of bicarbonate in the culture medium, in excess of carbon storage in algal cells, can promote lipid accumulation [56]. According to Figure 3, adding low concentrations of bicarbonate from  $0.5 \text{ g}\cdot\text{L}^{-1}$  to  $1.5 \text{ g}\cdot\text{L}^{-1}$  had no distinct effect on the lipid production of *C. vulgaris*. From own research indicate that lipid synthesis in cells requires a higher dose of inorganic carbon in the medium. In the present study, a significant increase in lipids in the presence of  $\text{NaHCO}_3$ , compared to the control 0 (F/2) object, was observed in the culture at a highest dose of  $2.0 \text{ g}\cdot\text{L}^{-1}$ . The lipid content was  $26 \pm 4\%$  and this was 8% higher than the values obtained without bicarbonate. Li et al. [57] observed an increase in lipid content in *C. vulgaris* cells with increasing  $\text{NaHCO}_3$  dose, but a decrease in the amount of biomass. At a dose of 160 mM the authors obtained approx.  $450 \text{ mg}\cdot\text{g}^{-1}$  of lipids. A linear dose-dependent increase in lipid content of algal biomass was reported by Bywaters and Fritsen [58]. A too-high concentration of  $\text{NaHCO}_3$  may adversely affect lipid accumulation in microalgae cells. Significantly reduced lipid accumulation capacity in *C. pyrenoidosa* biomass, after introduction of 200 mM  $\text{NaHCO}_3$ , was observed by Sampathkumar and Gothandam [59]. This is also confirmed by Pimolrat et al. [60], who analyzed the effect of  $\text{NaHCO}_3$  at doses ranging from 0.05 to  $5 \text{ g}\cdot\text{L}^{-1}$  on the stimulation of triacylglycerol production in *Chaetoceros gracillis* cells.



**Figure 3.** Lipid content in microalgal biomass. Mean over each column not marked with the same letter is significantly different at  $p \leq 0.05$ .

Changes in the pH of the culture medium are shown in Figure 4. Initially, the values ranged from 7.00 (at control 0 (F/2) object) to 8.04 (at 2.0 g·L<sup>-1</sup>). The study presented here was carried out as a batch culture, without adjusting the pH of the medium. An increase in pH was observed which was associated with microalgae cell growth, carbon dioxide fixation, and dissolution of bicarbonate salts in the culture [61,62], therefore pH regulation can be an important parameter during cultivation [63]. In continuous cultures with recirculation of the culture medium, it would be necessary to evaluate whether the introduction of bicarbonate would lead to a possible accumulation of Na in the medium.

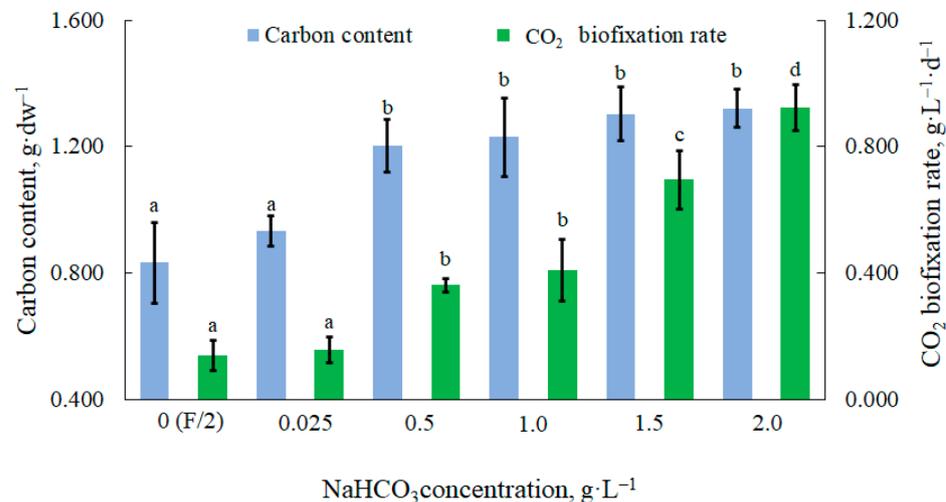


**Figure 4.** Dynamics of changes in pH during cultivation.

### 3.3. Carbon Content and CO<sub>2</sub> Fixation Rate in Microalgal Biomass

The carbon content in microalgal biomass ranged from  $0.832 \pm 0.127$  g·dw<sup>-1</sup> in control 0 (F/2) object to  $1.322 \pm 0.062$  g·dw<sup>-1</sup> at a dose 2.0 g·L<sup>-1</sup> NaHCO<sub>3</sub> (Figure 5). With increasing carbon in biomass, there was an increase in CO<sub>2</sub> fixation, which in the study ranged from  $0.139 \pm 0.047$  g·L<sup>-1</sup>·d<sup>-1</sup> to  $0.925 \pm 0.073$  g·L<sup>-1</sup>·d<sup>-1</sup>. (Figure 4B). A high carbon content and rate of fixation indicate a high potential for CO<sub>2</sub> sequestration in *C. vulgaris* biomass [64]. Similar results were reported by Prabakaran and Ravindran [65], who cultured three different algae strains (*Chlorella sp.*, *Ulothrix sp.* and *Chlorococcum sp.*) and obtained the highest carbon content and CO<sub>2</sub> fixation rate for *Chlorella sp.* at  $0.486$  g·dw<sup>-1</sup> and  $0.68$  g·mL<sup>-1</sup>·d<sup>-1</sup>, respectively. Some authors indicate a linear relationship between NaHCO<sub>3</sub> dose and carbon accumulation in algal biomass [56,57]. Mokashi et al. [44] applied bicarbonate in *C. vulgaris* cultures at dose from 0.25 to 1 g·L<sup>-1</sup> and determined the highest carbon content and CO<sub>2</sub> fixation rate of  $0.497$  g·dw<sup>-1</sup> and  $0.69$  g·mL<sup>-1</sup>·d<sup>-1</sup>, respectively, at the highest dose. The level of carbon dioxide fixation varies depending

on the microalgae strain and the carbon source (Table 2). The efficiency of the process carried out at the technical scale was higher compared to the results obtained by other authors, regardless of whether sodium bicarbonate or carbon dioxide was used in the microalgae cultivation.



**Figure 5.** Carbon content and CO<sub>2</sub> fixation rate in *C. vulgaris* biomass. Mean over each column not marked with the same letter is significantly different at  $p \leq 0.05$ .

**Table 2.** CO<sub>2</sub> fixation of microalgae biomass.

| Strain                         | Carbon Source and Dose                     | Experimental Scale | CO <sub>2</sub> Fixation, g·L <sup>-1</sup> ·d <sup>-1</sup> | References |
|--------------------------------|--|--------------------|--|------------|
| <i>Chlorellavulgaris</i>       | NaHCO <sub>3</sub> , 2.0 g·L <sup>-1</sup> | 100 L              | 0.93   | This study |
| <i>Chlorella vulgaris</i>      | NaHCO <sub>3</sub> , 1.0 g·L <sup>-1</sup> | 100 mL             | 0.69   | [44]       |
| <i>Chlorella sp.</i>           | NaHCO <sub>3</sub> , 7.5 g·L <sup>-1</sup> | 500 mL             | 0.21   | [66]       |
| <i>Scenedesmus abliquus</i>    | CO <sub>2</sub> , 10%                      | 1 L                | 0.26   | [67]       |
| <i>Scenedesmus almeriensis</i> | CO <sub>2</sub> , 3%                       | 28.5 L             | 0.24   | [68]       |

The optical density used to determine biomass growth does not always correlate with actual biomass content [69]; however, in the presented study, there was a significant and positive correlation between these parameters ( $r = 0.863$ ) and moreover between the amount of biomass and the carbon content of microalgae cells ( $r = 0.785$ ), as well as CO<sub>2</sub> fixation rate ( $r = 0.806$ ).

#### 4. Conclusions

The present study confirmed that the addition of NaHCO<sub>3</sub> to culture medium provides effective carbon source and facilitates cell growth and *C. vulgaris* biomass production. The highest biomass content ( $572 \pm 4 \text{ mg} \cdot \text{L}^{-1}$ ) and productivity ( $7.0 \pm 1.0 \text{ mg} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ ) were obtained with bicarbonate at a dose of  $2.0 \text{ g} \cdot \text{L}^{-1}$ . Under these conditions, the average optical density in culture was also the highest ( $\text{OD}_{680} 0.181 \pm 0.00$ ). An increase in NaHCO<sub>3</sub> dose increased lipid accumulation, carbon content in microalgae cells and carbon dioxide fixation rate. The highest values were observed at the highest dose of NaHCO<sub>3</sub>. The average lipid content of the biomass was  $26 \pm 4\%$ . The carbon content of the biomass increased to  $1.322 \pm 0.062 \text{ g} \cdot \text{dw}^{-1}$ , while the rate of CO<sub>2</sub> fixation increased to  $0.925 \pm 0.073 \text{ g} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ . There was a positive correlation between the biomass amount and the optical density and between the biomass, the carbon content and the CO<sub>2</sub> fixation rate.

The study was carried out in photobioreactors used in the industrial production of microalgae biomass and therefore the results obtained showed the real values that are possible to achieve at this scale. The lipid content in the biomass increased with the

increasing dose of sodium bicarbonate. Future research should focus on determining the maximum dose of  $\text{NaHCO}_3$  for optimal microalgal growth. It is important for the economic sustainability of microalgae cultivation for fuel purposes. The commercial production of microalgae biomass is carried out as a semi-continuous or continuous culture, so the correlation between the  $\text{NaHCO}_3$  dose and the overaccumulation of  $\text{Na}^+$  ions and the possibility of limiting microalgal growth should be verified.

**Author Contributions:** Conceptualization, M.H.-P.; methodology, M.H.-P. and P.R.; formal analysis, P.R.; investigation, P.R. and M.H.-P.; data curation, P.R. and M.H.-P.; writing—original draft preparation, P.R. and M.H.-P.; writing—review and editing, P.R. and M.H.-P.; visualization, P.R. and M.H.-P.; supervision, P.R. and M.H.-P.; project administration, P.R., M.H.-P. and A.K. All authors have read and agreed to the published version of the manuscript.

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