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Research Article

Evaluating Sodium Carbonate as an Alternative Carbon Source for *Euglena gracilis* Growth

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Abstract

Microalgae utilize dissolved inorganic carbon species such as dissolved carbon dioxide (CO_2), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) for photosynthesis, depending on medium pH. Carbonate salts have been investigated as an alternative inorganic carbon carrier in microalgal cultivation systems. This study evaluated the effects of different sodium carbonate (Na_2CO_3) concentrations on the growth behavior of *Euglena gracilis* under non-aerated cultivation conditions. Cultivation was conducted in F1 medium supplemented with Na_2CO_3 concentrations of 0, 20, 40, 60, 80, and 100 mg/L. Biomass was monitored using UV–Vis spectrophotometry at 680 nm and converted to dry weight (g/L). Rather than promoting growth, Na_2CO_3 supplementation generally accelerated biomass decline, which was analyzed using a first-order decay model. Higher Na_2CO_3 concentrations were associated with greater biomass decline, reaching up to approximately 49% reduction after 15 days. The decay rate constants were 2.2×10^{-3}, 3.9×10^{-3}, 2.5×10^{-2}, 3.79×10^{-2}, 4.26×10^{-2}, and 3.08×10^{-2} day^{-1} for 0, 20, 40, 60, 80, and 100 mg/L, respectively. The results indicate that Na_2CO_3 supplementation under acidic and non-aerated conditions did not improve *E. gracilis* growth within the tested concentration range. The observed biomass decline was likely influenced by multiple interacting factors, including altered ionic conditions and limited gas transfer. These findings provide an initial evaluation of Na_2CO_3 supplementation as an alternative inorganic carbon source for *E. gracilis* cultivation.

Keywords: Biomass decay, carbonate chemistry, *Euglena gracilis,* inorganic carbon, sodium carbonate (Na_2CO_3)

1. INTRODUCTION

Euglena gracilis is a unicellular photosynthetic microalga known for its metabolic flexibility and ability to produce valuable biocompounds such as paramylon, wax esters, and tocopherols. Due to these characteristics, *E. gracilis* has attracted considerable attention for applications in biotechnology, nutraceuticals, and biofuel-related research [1–3]. Similar to other photosynthetic microalgae, the growth of *E. gracilis* strongly depends on the availability of inorganic carbon for photosynthesis and biomass formation [4]. Previous studies have shown that carbon availability and cultivation conditions significantly influence microalgal growth performance and physiological responses.

In aqueous cultivation systems, inorganic carbon exists mainly as dissolved carbon dioxide ($CO_2(aq)$), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}). The distribution of these species is strongly influenced by pH [5]. Under acidic conditions, dissolved $CO_2(aq)$ and carbonic acid (H_2CO_3) predominate, whereas bicarbonate becomes dominant at near-neutral pH and carbonate species become increasingly important under alkaline conditions [6]. Many microalgae are capable of utilizing dissolved CO_2 and bicarbonate through carbon concentrating mechanisms (CCMs) [7], although their responses to carbonate-rich conditions vary among species. Consequently, changes in carbonate equilibrium and pH may strongly affect inorganic carbon availability,

physiological stress, and biomass productivity in microalgal cultivation systems.

Conventionally, gaseous CO_2 supplementation is widely used as the primary inorganic carbon source in microalgal cultivation. However, direct CO_2 aeration may suffer from low absorption efficiency and carbon loss during gas transfer processes [8,9]. Therefore, alternative inorganic carbon sources such as sodium bicarbonate ($NaHCO_3$) have been investigated in bicarbonate-based integrated carbon capture and algae production systems (BICCAPS) [10–13]. Previous studies demonstrated that bicarbonate supplementation can support microalgal growth and carbon fixation under controlled cultivation conditions. In contrast, sodium carbonate (Na_2CO_3) introduces carbonate species together with stronger alkalinity [14], potentially altering inorganic carbon equilibrium, medium chemistry, and ionic conditions differently from bicarbonate-based systems. Consequently, Na_2CO_3 supplementation may influence microalgal growth not only through inorganic carbon availability but also through indirect effects related to pH alteration and ionic stress.

Despite increasing interest in carbonate-based cultivation systems, studies investigating the effects of Na_2CO_3 supplementation on *E. gracilis* growth remain limited. In particular, the concentration-dependent response of *E. gracilis* to Na_2CO_3 under non-aerated cultivation conditions is not yet fully understood. Furthermore, it remains unclear whether Na_2CO_3 primarily functions as an effective inorganic carbon source or indirectly influences growth through changes in carbonate equilibrium and cultivation conditions. Therefore, this study aimed to evaluate the effects of different Na_2CO_3 concentrations on the growth behavior of *E. gracilis* under non-aerated cultivation conditions and to assess the possible influence of carbonate supplementation on biomass decay behavior and medium chemistry.

2. RESEARCH METHODS

Microalgae and Culture Medium Preparation

The microalga used in this study was *E. gracilis*, obtained from the Center for Energy Studies, Universitas Gadjah Mada (UGM), Indonesia. The cultivation medium used was F1 medium consisting of ammonium sulfate ($(NH_4)_2SO_4$, 2 g/L), potassium chloride (KCl, 0.04 g/L), magnesium sulfate ($MgSO_4$, 0.4 g/L), triple superphosphate (TSP, 1.98 g/L), sodium molybdate (Na_2MoO_4, 4×10^{-5} g/L), vitamin B1 (4×10^{-9} g/L), and vitamin B12 (2.5×10^{-11} g/L), dissolved in 600 mL distilled water. Prior to use, the water was exposed to UV irradiation for approximately 15 min.

For each experimental unit, 100 mL of F1 medium was transferred into a 250 mL Erlenmeyer flask, followed by the addition of 100 mL of *E. gracilis* stock culture. Different concentrations of Na_2CO_3 were added into each flask, namely 0, 20, 40, 60, 80, and 100 mg/L. All chemicals were purchased from local chemical suppliers in Yogyakarta, Indonesia.

Cultivation Conditions and Na_2CO_3 Treatment

Cultivation was carried out for 15 days at a constant temperature of 30 °C. Each Erlenmeyer flask was submerged in a water bath to maintain a stable cultivation temperature. Continuous illumination was provided using white light-emitting diode (LED) lamps with a light intensity of 2000 lux throughout the cultivation period [15,16]. Manual mixing was performed during each sampling event.

The cultivation was not conducted under axenic conditions, and no specialized microbiological sterilization procedures were applied during cultivation preparation. Each Na_2CO_3 treatment was conducted using a single cultivation flask; therefore, the results were analyzed descriptively and should be interpreted as preliminary observations.

Growth Monitoring and Biomass Measurement

Microalgal growth was monitored by measuring optical density (OD) using a UV–Vis spectrophotometer at a wavelength of 680 nm, following previous studies on *E. gracilis* cultivation [17]. Sampling was conducted on cultivation days 0, 1, 4, 6, 8, 11, 13, and 15 throughout the cultivation period. Approximately 7 mL of culture was withdrawn during each sampling event, and no volume replacement was performed afterward.

The absorbance values were converted into dry weight biomass concentration (DW, g/L) using the following empirical relationship:

$$DW\ (g/L) = 1.6425 \times OD_{680} \quad (1)$$

The calibration curve showed moderate correlation ($R^2 = 0.6906$), indicating an approximate biomass estimation.

Correlation Between Optical Density and Biomass Concentration

The OD_{680}–dry weight correlation was established using nine independent samples collected during different cultivation periods. For each calibration sample, 10 mL of culture was centrifuged at 3000 rpm for 5 min to obtain biomass pellets. The pellets were subsequently dried in an oven at 80 °C for 1 h prior to weighing. The measured dry biomass values were then correlated with OD_{680} measurements to obtain the biomass conversion equation.

3. RESULTS AND DISCUSSION

Effect of Na_2CO_3 Concentration on Biomass Profile

The growth patterns of *E. gracilis* under various Na_2CO_3 concentrations are shown in **Figure 1**. The results indicate that Na_2CO_3 concentration influenced the slope of the biomass profile. A first-order exponential decay model was used to approximate the declining biomass trend:

$$dX/dt = - kX \quad (2)$$

whose analytical solution is:

$$X(t) = X_0 \exp(-kt) \quad (3)$$

where X is biomass concentration (g/L), X0 is the initial biomass concentration (g/L), t is cultivation time (days), and k is the apparent biomass decay constant (day^{-1}). Model parameters were estimated using nonlinear least-squares regression.

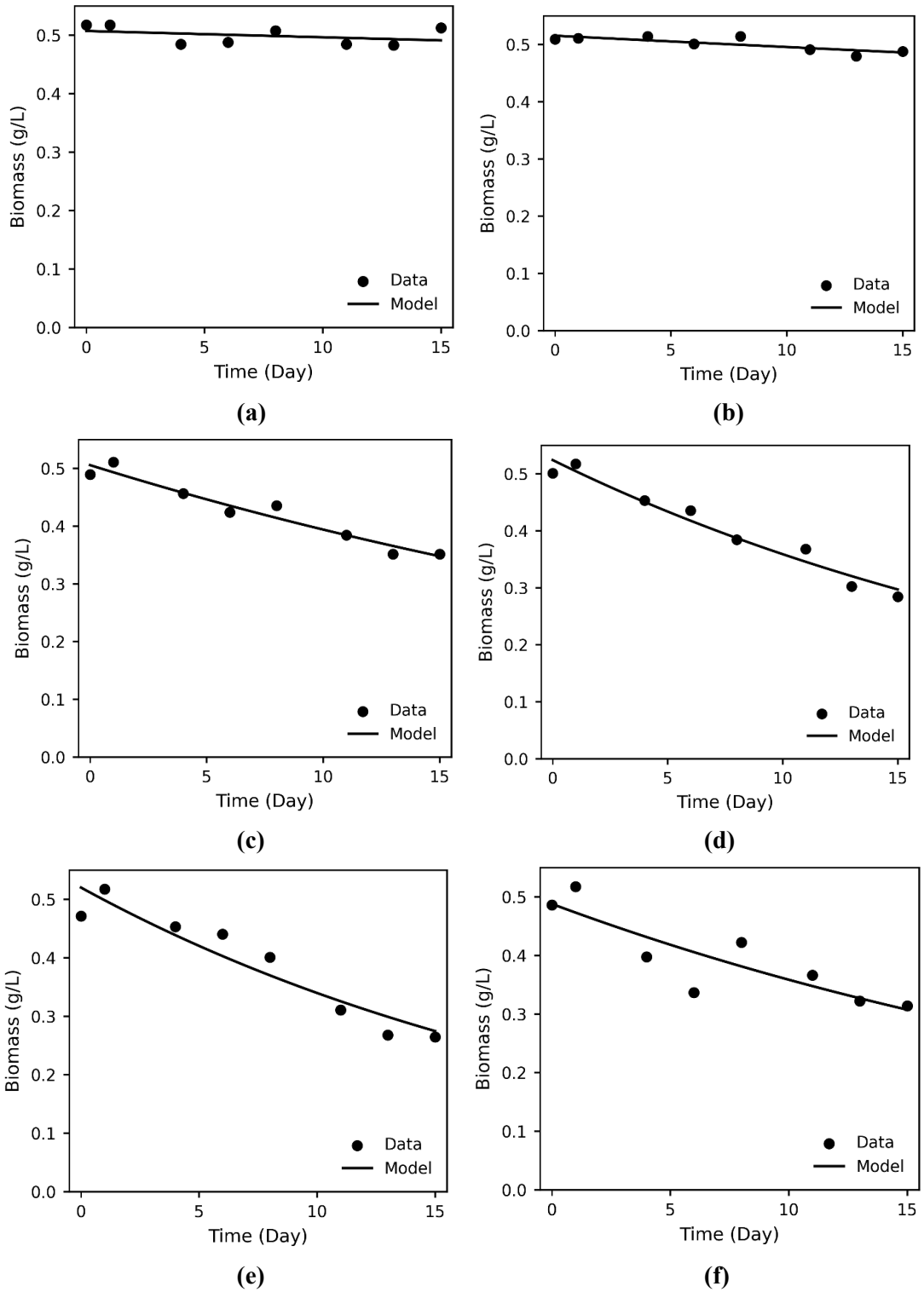

Figure 1. Biomass concentration versus cultivation time at various Na_2CO_3 concentrations: (a) 0 mg/L; (b) 20 mg/L; (c) 40 mg/L; (d) 60 mg/L; (e) 80 mg/L; and (f) 100 mg/L

Table 1. Exponential decay model fitting parameters for different Na_2CO_3 concentrations

Na_2CO_3 Concentration, mg/L	k (day^{-1})[a]	X_0 (g/L)[a]	R^2	RMSE
0	0.0022 ± 0.0022	0.5071 ± 0.0097	0.1383	0.0137
20	0.0039 ± 0.0012	0.5152 ± 0.0052	0.6572	0.0073
40	0.0250 ± 0.0025	0.5056 ± 0.0098	0.9456	0.0131
60	0.0379 ± 0.0032	0.5242 ± 0.0122	0.9612	0.0159
80	0.0426 ± 0.0061	0.5201 ± 0.0223	0.9008	0.0287
100	0.0308 ± 0.0073	0.4880 ± 0.0267	0.7531	0.0352

Footnote: [a]Data presented as value ± standard error (SE)

Overall, biomass reduction after 15 days of cultivation was approximately 6%, 4%, 30%, 44%, 49%, and 37% for Na_2CO_3 concentrations of 0, 20, 40, 60, 80, and 100 mg/L, respectively. The largest biomass decline occurred at 80 mg/L, although the inhibitory trend was not strictly monotonic at higher concentrations.

The exponential decay model reasonably represented the overall declining biomass trend at Na_2CO_3 concentrations of 40–100 mg/L, as indicated by relatively high R^2 values (**Table 1**). At 0 and 20 mg/L, biomass remained relatively constant, indicating that the exponential decay assumption was not suitable for describing biomass behavior under these conditions. The consistent decrease in biomass at 40 mg/L and above (**Figure 1 c-f**) suggests noticeable biomass decline in *E. gracilis* cultures.

Relationship Between Na_2CO_3 Concentration and Biomass Decay Rate

The relationship between Na_2CO_3 concentration and the estimated apparent biomass decay constant (k) is shown in **Figure 2**. An increasing trend was generally observed up to 80 mg/L, although the decay constant decreased slightly at 100 mg/L. Linear regression across all concentrations yielded R^2 = 0.7226, indicating a moderate positive association between Na_2CO_3 concentration and biomass decay rate. Error bars represent the standard error obtained from nonlinear regression fitting. The results suggest that increasing Na_2CO_3 concentration generally accelerated biomass decline.

Overall, the increase in the estimated decay constant (k) aligned with the reduction in biomass observed in **Figure 1**, suggesting that higher Na_2CO_3 concentrations created less favorable cultivation conditions for E. gracilis. Relatively high decay rates were observed at 60–80 mg/L, beyond which further increases in concentration produced only minor changes in k values. This apparent plateau may indicate that the inhibitory effect became less sensitive to further increases in Na_2CO_3 concentration within the tested range. However, because the differences among these higher concentrations were relatively small and no statistical analysis was performed, this trend should be interpreted with caution.

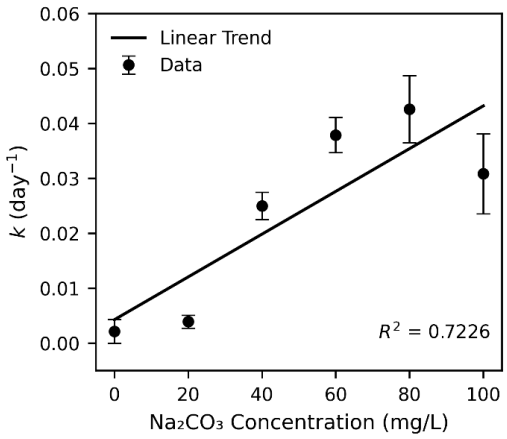

Figure 2. Relationship between Na_2CO_3 concentration and apparent biomass decay constant (k). Error bars represent standard error (SE).

Possible Factors Contributing to Biomass Decline

Although the exact inhibitory mechanism was not experimentally investigated in this study, the biomass decline was likely influenced by multiple interacting factors rather than a single toxicological pathway. Na_2CO_3 supplementation altered the ionic composition and alkalinity of the medium. In addition, the non-aerated cultivation system may have affected gas transfer and dissolved oxygen dynamics.

Specifically, Na_2CO_3 supplementation simultaneously increased sodium ion concentration and inorganic carbon input to the culture medium. However, under the acidic conditions observed in this study (pH ~4), carbonate species were likely converted predominantly into dissolved $CO_2(aq)/H_2CO_3$. Consequently, the net increase in bioavailable inorganic carbon may have been relatively limited compared with the accompanying changes in ionic conditions. This imbalance may have contributed to the unfavorable growth response observed at higher Na_2CO_3 concentrations.

Previous studies have shown that changes in salinity and ionic strength can negatively affect microalgal growth. Amelia et al. [18] reported growth inhibition in several microalgae species under saline conditions, while Timotius et al. [19] observed that increased salinity induced physiological adaptation and reduced biomass accumulation in *Euglena sp*. Similarly, Suyono et al. [20] demonstrated that the

addition of inorganic salts altered biomass production and metabolite composition in *Euglena* cultures. However, these studies mainly investigated chloride-based salinity systems, which differ chemically from Na_2CO_3 supplementation due to differences in alkalinity, carbonate equilibrium, and buffering behavior. Therefore, the comparison should be interpreted cautiously.

Another possible contributing factor was the absence of continuous aeration and agitation during cultivation. In photosynthetic systems, limited mixing may reduce CO_2 replenishment while allowing dissolved oxygen accumulation in the culture medium [21]. Elevated O_2/CO_2 ratios may increase photorespiratory activity and oxidative stress in microalgal cells [22]. Although dissolved oxygen concentration and reactive oxygen species (ROS) were not measured in this study, the non-aerated conditions may have intensified cellular stress responses even at relatively low Na_2CO_3 concentrations.

This concentration-dependent inhibitory response is further supported by preliminary range-finding observations conducted at substantially higher Na_2CO_3 concentrations (up to 10 g/L). At concentrations above 100 mg/L, *E. gracilis* cultures rapidly lost their green coloration and appeared pale or whitish. Microscopic observation also showed little to no cellular motility, indicating severe growth inhibition or possible cell death. Because only the 100 mg/L culture still exhibited observable cell movement, the concentration range in this study was limited to 0–100 mg/L. These baseline observations confirm that excessive Na_2CO_3 concentrations create highly unfavorable cultivation conditions for *E. gracilis* under the tested cultivation setup.

Carbonate Chemistry and Experimental Limitations

To better understand the carbonate chemistry behavior in the culture medium, pH was monitored continuously until cultivation day 11 (**Table 2**). Despite Na_2CO_3 supplementation, the culture remained acidic, with pH values ranging from 3.7 to 4.3 during the monitored period. Higher Na_2CO_3 concentrations slightly increased the initial pH; however, the medium did not reach alkaline conditions typically associated with carbonate-rich systems.

Under these acidic conditions (pH ~4), dissolved inorganic carbon predominantly exists as dissolved $CO_2(aq)$ and H_2CO_3, while carbonate species (CO_3^{2-}) become negligible [6]. Therefore, although Na_2CO_3 supplementation increased total inorganic carbon input, much of the added carbonate likely shifted toward dissolved $CO_2(aq)/H_2CO_3$ species in the medium. This suggests that Na_2CO_3 functioned primarily as an indirect inorganic carbon contributor rather than as a stable carbonate reservoir under the tested conditions.

Table 2. pH profile during cultivation at different Na_2CO_3 concentrations

Na_2CO_3 Concentration, mg/L	pH Value (Days)				
	0	1	4	6	11
0	3.8	3.8	3.8	3.7	3.7
20	3.9	3.9	3.9	3.8	3.8
40	4.0	4.0	3.9	3.9	3.9
60	4.0	4.1	4.0	3.9	3.9
80	4.1	4.1	4.1	4.0	4.0
100	4.3	4.3	4.2	4.2	4.1

Another important limitation of this study was the absence of continuous aeration and controlled agitation. Manual mixing was performed only during sampling, which may have limited gas transfer, nutrient homogeneity, and biomass distribution within the culture medium. Consequently, the observed biomass decline cannot be attributed solely to Na_2CO_3 concentration because non-ideal mixing conditions may also have contributed to growth inhibition and decay behavior. Furthermore, the absence of biological replicates limited statistical evaluation of treatment effects and kinetic trends.

Implications and Future Perspectives

Although Na_2CO_3 was evaluated as a potential alternative inorganic carbon source, the present results suggest that the tested cultivation conditions were not favorable for *E. gracilis* growth at higher Na_2CO_3 concentrations. Previous studies have demonstrated that *E. gracilis* can grow effectively under CO_2 aeration systems, with reported specific growth rates substantially higher than those observed in the present study [15,17]. Nevertheless, microalgal cultivation without direct aeration has also been reported to remain feasible under sufficient agitation conditions. For example, orbital shaking at 100 rpm was shown to support biomass growth by improving gas transfer and maintaining biomass homogeneity [23].

In contrast, the manual mixing approach used in this study likely resulted in non-uniform gas distribution and insufficient turbulence, allowing dissolved oxygen to accumulate while limiting CO_2 replenishment. Because these variables were not independently controlled, the present findings should be interpreted as an initial exploratory evaluation of Na_2CO_3 supplementation under non-aerated cultivation conditions.

These findings also highlight several opportunities for future research. Further studies incorporating controlled aeration, continuous agitation, dissolved oxygen monitoring, biological replicates, and direct measurements of inorganic carbon species would help clarify the relative

contributions of ionic effects, carbonate chemistry, and gas-transfer limitations on *E. gracilis* growth.

4. CONCLUSIONS

This study evaluated the effects of Na_2CO_3 supplementation on the growth behavior of *E. gracilis* under non-aerated cultivation conditions. Increasing Na_2CO_3 concentration generally accelerated biomass decay, with biomass reductions ranging from approximately 4–49% after 15 days of cultivation. The decay model analysis showed that the biomass decay constant (k) generally increased with increasing Na_2CO_3 concentration, with relatively high values observed at 60–80 mg/L. Beyond this range, additional Na_2CO_3 supplementation produced only minor changes in k values, suggesting an apparent plateau-like inhibitory response under the tested cultivation conditions. The results indicate that Na_2CO_3 supplementation under acidic and non-aerated conditions does not improve *E. gracilis* biomass growth within the tested concentration range. However, the observed growth response was likely influenced by multiple interacting factors, including altered ionic conditions, limited gas transfer, and insufficient mixing. Because continuous aeration, dissolved oxygen monitoring, and biological replicates were not included in this study, the findings should be interpreted as an initial exploratory evaluation of Na_2CO_3 supplementation under the tested cultivation setup. Further studies incorporating controlled agitation, aeration, and inorganic carbon monitoring are needed to better understand the relationship between carbonate supplementation and *E. gracilis* growth behavior.

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