Effects of Sodium Bicarbonate Supplements on Growth Performance and Quality of Marine Microalgal Inoculum Production

Wasana Arkronrat*, Chonlada Leearam, Rungtiwa Konsantad and Vutthichai Oniam

ABSTRACT

The effects of sodium bicarbonate ($NaHCO_3$) supplements (0.05, 0.25, 0.50, 1.0 $g \cdot L^{-1}$) in the culture medium were examined on six marine microalgae inocula cultured under laboratory conditions (25±1 °C, 28 psu, 12L:12D, 3,000 Lux light intensity, Conway medium) for 10 days and compared to a control without $NaHCO_3$. The parameters assessed included cell density (CD), specific growth rate (μ), division rate (K), and doubling time (D). The results indicated a positive effect on CD, μ, K, and D for the production of *Chaetoceros calcitrans, Thalassiosira weissflogii, Chlorella* spp., and *Nannochloropsis oculata* strains with $NaHCO_3$ additions of 0.25, 1.0, 1.0, and 0.50 $g \cdot L^{-1}$, respectively. Conversely, $NaHCO_3$ concentrations of 0.25–1.0 $g \cdot L^{-1}$ negatively impacted the CD, μ, K, and D for *Tetraselmis suecica* and *Isochrysis galbana* culture. Regarding inoculum quality after refrigerated storage at 4±1 °C, $NaHCO_3$ supplements did not sustain the growth rates of *C. calcitrans, T. weissflogii,* and *Chlorella* spp. inocula cultured after storage for 7, 15, and 30 days. However, 0.5 $g \cdot L^{-1}$ $NaHCO_3$ maintained the growth rate of *N. oculata* cultured after storage for 7 days. This study provides comprehensive insights into the optimal $NaHCO_3$ supplementation for maintaining each microalgal strain in a stable state for inoculum production applications.

Keywords: Microalgal inoculum, Sodium bicarbonate, Growth and quality of microalgae

INTRODUCTION

Microalgal cultivation is a modern biotechnology with successful applications in human food supplements, animal feed, cosmetics, medicines, health products, and biodiesel fuel (Arkronrat and Oniam, 2012; Khan *et al*., 2020; Foo *et al*., 2023). Marine microalgae, such as diatoms, green microalgae, and flagellate strains, are commonly used as important live food for many aquatic animal larvae and are produced commercially to support aquatic animal hatchery farming in Thailand (Arkronrat *et al*., 2016; Arkronrat and Oniam, 2019). The success of microalgal production depends on various factors, primarily those influencing microalgal growth, such as temperature, salinity, light, nutrients, and pH (Ramlee *et al*., 2021). The carbon source is also crucial, as it affects growth and fatty acid composition in the cultivation of numerous microalgal species by providing energy and the structural backbone for cell growth (Xiaoning *et al*., 2020; Ratomski *et al*., 2021).

Generally, the carbon source for microalgae in culture systems is carbon dioxide (CO_2). While a mixture of CO_2 gas and air can enhance microalgal growth, CO_2 supplementation also increases production costs (Pimolrat *et al*., 2010). An alternative inorganic carbon source for microalgal cultivation is sodium bicarbonate ($NaHCO_3$), which has been actively researched as a supplement, particularly for green microalgal strains (Sampathkumar and Gothandam, 2019; Caturwati and Setyati, 2020).

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The concentration of $NaHCO_3$ can be a significant factor limiting the growth of some microalgal species, and its effects vary among species. For instance, the addition of 0.086 $g \cdot L^{-1}$ $NaHCO_3$ did not affect the growth rate of *Spirulina* sp. (Caturwati and Setyati, 2020), whereas 2.0 $g \cdot L^{-1}$ $NaHCO_3$ produced the highest biomass and productivity in *Chlorella vulgaris* (Ratomski *et al*., 2021). Conversely, adding 1.0, 2.0, and 3.0 $g \cdot L^{-1}$ $NaHCO_3$ had no effect on the growth of *Chlorella sorokiniana* compared to the control without $NaHCO_3$ (Salbitani *et al*., 2020). Some researchers have reported that increasing $NaHCO_3$ concentrations in the culture medium can decrease the growth rate of certain microalgae (Ishika *et al*., 2017; Ramlee *et al*., 2021).

In Thailand, there have been limited studies on the production of marine microalgal inoculum with $NaHCO_3$ supplementation for use in aquatic animal hatcheries (Pimolrat *et al*., 2010; Chaichalerm *et al*., 2012; Arkronrat and Oniam, 2019). The supplementation of sodium bicarbonate has been studied for its effects on various marine microalgae species. Notable species with existing reports include *Nannochloropsis* spp., *Chlorella* spp., and *Dunaliella* spp. (White *et al*., 2012; Ismail *et al*., 2018; Srinivasan *et al*., 2018). Research on *Nannochloropsis* spp. and *Chlorella vulgaris* has shown significant improvements in growth and lipid accumulation (Ismail *et al*., 2018), which are essential for biodiesel production. Studies on *Dunaliella salina* revealed that sodium bicarbonate could enhance both biomass and carotenoid production (Srinivasan *et al*., 2018). However, the effects of sodium bicarbonate on many marine microalgae species, particularly regarding inoculum quality, have not been thoroughly investigated. These species include *Chaetoceros calcitrans, Thalassiosira weissflogii, Isochrysis galbana*, and *Tetraselmis suecica*. The lack of data on these species offers a significant opportunity for further research to determine if sodium bicarbonate could similarly enhance their growth and productivity. Exploring these species could provide valuable insights and expand the application of sodium bicarbonate in marine microalgae cultivation.

Repeating studies on previously reported species is essential for several reasons. Firstly, it serves to verify and confirm the consistency of earlier findings under varying experimental conditions. Secondly, it allows for the improvement of methodologies, such as adjusting the concentrations of sodium bicarbonate, to optimize results. Thirdly, testing under different environmental conditions, such as varying light intensities or temperatures, can provide a more comprehensive understanding of the effects. Lastly, expanding the knowledge base through repeated studies helps in developing more practical applications and ensures the robustness of the findings. In addition, it is well known that storage duration affects the quality of microalgal inoculum, influenced by factors such as nutrient availability, environmental conditions, and contamination risk. Proper storage practices, including nutrient supplementation, are essential for preserving the viability and quality of microalgal cultures over extended periods (Foo *et al*., 2023). Therefore, determining the ideal amount of $NaHCO_3$ supplement for microalgal production requires an alternative design to enhance the growth and quality of the inoculum. To meet these goals, we conducted experiments to cultivate microalgal inoculum with different amounts of $NaHCO_3$ supplement and evaluated the effects on the growth performance and quality of six commercial marine microalgal inocula (*Chaetoceros calcitrans, Thalassiosira weissflogii, Chlorella* spp., *Nannochloropsis oculata, Tetraselmis suecica,* and *Isochrysis galbana*), as an alternative for commercial microalgal inoculum production in Thailand.

MATERIALS AND METHODS

Microalgal preparation

The experiment was conducted in the Phytoplankton Laboratory of the Klongwan Fisheries Research Station (PL-KFRS) in Prachuap Khiri Khan province, Thailand. Original inoculum samples of six marine microalgal species (the diatoms *Chaetoceros calcitrans* and *Thalassiosira weissflogii*; the green microalgae *Chlorella* spp. and *Nannochloropsis oculata*; and the flagellates *Tetraselmis suecica* and *Isochrysis galbana*) were obtained from the Prachuap Khiri Khan Coastal Fisheries Research and Development Center, Department of Fisheries, Thailand.

Microalgal cultures were raised in 250 mL Erlenmeyer flasks containing sterilized seawater adjusted to a salinity of 28 psu and enriched with Conway medium (Wongrat, 2000). Silicate was added only for the diatoms. The cultures were inoculated at 10% (v/v) and maintained in a temperature-controlled room at PL-KFRS at 25±1 °C under a 12-h light to 12-h darkness photoperiod (12L:12D) using cool white fluorescent lamps at a light intensity of approximately 1,000 Lux. The microalgae were cultured in the Erlenmeyer flasks until the cell density reached approximately 10^6 cells·mL^{-1}. Each culture was then scaled up to 1 L glass bottles and used in the experiment (Arkronrat and Oniam, 2019).

Experimental design and set-up

Experiment 1: Effect of $NaHCO_3$ supplementation on growth of microalgae

This experiment was designed to investigate the impact of $NaHCO_3$ supplementation at different concentrations on the growth performance of microalgae. Five treatments were examined: a control without $NaHCO_3$ and additions of 0.05 g·L^{-1} $NaHCO_3$ (T1), 0.25 g·L^{-1} $NaHCO_3$ (T2), 0.50 g·L^{-1} $NaHCO_3$ (T3), and 1.0 g·L^{-1} $NaHCO_3$ (T4). Batch cultivation was conducted in enriched seawater (28 psu) in 1 L glass bottles with 1 mL of Conway medium (with silicate added only for the diatoms). The cultures were grown in a temperature-controlled room at PL-KFRS (25±1 °C) under a 12-h light/12-h dark photoperiod (12L:12D) at a light intensity of approximately 3,000 Lux for 10 days. All cultures were started with equal amounts of inoculum (approximately 1–2×10^5 cells·mL^{-1}), and $NaHCO_3$ was added on the initial day of the culture period. The experiment was performed with 10 replicates (n =10) using a completely randomized design (CRD).

During the experiment, algal cell samples were collected daily to estimate cell density (CD) and maximum cell density (max.CD) in cells·mL^{-1}. Cells were fixed with 5% formalin and counted using a hemacytometer under a compound microscope at 40× magnification. The specific growth rate (μ), division rate (K), and doubling time (D) of each culture were calculated using equations 1, 2, and 3, respectively (Tahiri *et al*., 2023).

$$\mu = \ln N_2 - \ln N_1 / t_2 - t_1 \quad (1)$$

$$K = \mu / \ln(2) \quad (2)$$

$$D = 1 / K \quad (3)$$

where μ = the specific growth rate (day^{-1}), N_1 = the cell count at time t_1 (cells·mL^{-1}), N_2 = the cell count at time t_2 (cells·mL^{-1}), t_1 = the first sampling time (day), t_2 = the second sampling time (day), and ln indicates the Napierian logarithm.

Experiment 2: Effect of $NaHCO_3$ supplementation on quality of microalgae after storage at low temperatures (4 °C)

This experiment was designed to investigate the impact of $NaHCO_3$ supplementation on the quality of microalgae in terms of growth patterns and cell density (CD) after storage for different periods. Samples of each microalgal inoculum in the exponential phase (D), with the optimum concentration of $NaHCO_3$ supplement (based on the results of Experiment 1), were stored in a refrigerator (4±1 °C) for 7 (T1), 15 (T2), and 30 (T3) days. After storage, each sample was used for batch cultivation under set conditions at PL-KFRS (25±1 °C, 28 psu, 12L:12D, 3,000 Lux light intensity, Conway medium, for 10 days) and evaluated for growth patterns and CD, comparing stored samples with non-stored controls. The experiment was conducted with 10 replicates (n = 10) and followed a completely randomized design (CRD).

Statistical analysis

The experimental data were represented as means±standard deviations. Variances and comparisons of growth performance (max.CD, μ, K, D) of inoculum samples between the treatments were analyzed using one-way ANOVA. Differences between means were tested using Duncan's multiple range test at the 95% confidence level, using IBM SPSS Statistics for Windows.

RESULTS

Effect of $NaHCO_3$ supplements on microalgal growth

The growth performance results of the inoculum samples were present in Table 1. There was a positive effect from using $NaHCO_3$ as a supplement in the production of the *Chaetoceros calcitrans, Thalassiosira weissflogii, Chlorella* spp., and *Nannochloropsis oculata* strains with 0.25, 1.0, 1.0, and 0.50 g·L^{-1} $NaHCO_3$ supplement, respectively. In the diatom strains, the initial CD values for *Chaetoceros calcitrans* and *Thalassiosira weissflogii* were 1.27±0.12 and 1.85±0.46×10^5 cells·mL^{-1}, respectively. The values for the growth performance parameters (max.CD, μ, K, D) of *Chaetoceros calcitrans* in the T2 experiment were significantly ($p<0.05$) higher than for the other treatments. The growth performance in terms of μ, K, and D of *Thalassiosira weissflogii* in T4 was significantly higher than for the other treatments; however,

Table 1. Maximum cell density (max.CD;×10^5 cells·mL^{-1}), specific growth rate (μ; day^{-1}), division rate (K; day), and doubling time (D; day) of marine microalgae inoculum cultured with $NaHCO_3$ supplement at different concentrations under laboratory conditions (n = 10).

Item	Treatment ($NaHCO_3$ supplement: g·L^{-1})				
	Control (0)	T1 (0.05)	T2 (0.25)	T3 (0.50)	T4 (1.0)
Chaetoceros calcitrans					
max.CD	25.60±5.87^b	24.72±6.58^b	39.00±5.66^a	29.22±2.92^b	30.93±9.28^b
μ	0.34±0.02^c	0.38±0.02^{bc}	0.46±0.02^a	0.36±0.00^c	0.41±0.05^b
K	0.48±0.03^c	0.55±0.03^{bc}	0.66±0.03^a	0.52±0.00^c	0.59±0.07^b
D	2.06±0.17^c	1.81±0.25^{bc}	1.52±0.08^a	1.93±0.09^c	1.70±0.28^b
Thalassiosira weissflogii					
max.CD	33.48±15.46^a	36.81±11.60^a	42.70±6.36^a	32.73±5.38^a	37.12±13.48^a
μ	0.43±0.17^b	0.41±0.02^b	0.41±0.01^b	0.38±0.05^b	0.58±0.26^a
K	0.62±0.24^b	0.59±0.03^b	0.59±0.03^b	0.55±0.07^b	0.83±0.38^a
D	1.60±0.36^b	1.70±0.18^b	1.70±0.15^b	1.81±0.23^b	1.20±0.22^a
Chlorella spp.					
max.CD	32.50±6.85^c	44.98±9.84^b	60.81±9.40^a	45.17±10.40^b	56.65±4.87^a
μ	0.34±0.00^c	0.41±0.02^{ab}	0.41±0.00^b	0.38±0.00^b	0.43±0.02^a
K	0.48±0.00^c	0.59±0.03^{ab}	0.59±0.00^b	0.55±0.00^b	0.62±0.03^a
D	2.06±0.13^c	1.70±0.16^b	1.70±0.06^b	1.81±0.06^b	1.60±0.08^a
Nannochloropsis oculata					
max.CD	52.27±18.91^b	53.79±11.53^b	50.03±15.93^b	69.95±10.61^a	78.36±16.26^a
μ	0.43±0.05^{bc}	0.38±0.00^c	0.41±0.02^c	0.46±0.02^a	0.43±0.00^{ab}
K	0.62±0.07^{bc}	0.55±0.00^c	0.59±0.03^c	0.66±0.03^a	0.62±0.00^{ab}
D	1.60±0.20^{bc}	1.81±0.08^c	1.70±0.19^c	1.52±0.10^a	1.60±0.07^{ab}
Tetraselmis suecica					
max.CD	11.45±2.86^a	8.46±2.52^b	7.16±2.11^{bc}	5.72±1.78^c	6.66±2.33^{bc}
μ	0.26±0.02^a	0.19±0.02^{bc}	0.22±0.02^b	0.19±0.02^c	0.22±0.01^b
K	0.38±0.03^a	0.28±0.03^{bc}	0.31±0.03^b	0.28±0.04^c	0.31±0.02^b
D	2.63±0.38^a	3.61±0.44^{bc}	3.21±0.62^b	3.61±0.64^c	3.21±0.44^b
Isochrysis galbana					
max.CD	40.21±8.95^a	22.01±8.22^{bc}	14.70±1.56^{cd}	25.51±10.78^b	13.61±9.92^d
μ	0.36±0.02^a	0.31±0.00^{ab}	0.29±0.02^b	0.34±0.05^{ab}	0.34±0.07^a
K	0.52±0.03^a	0.45±0.00^{ab}	0.42±0.03^b	0.48±0.07^{ab}	0.48±0.10^a
D	1.93±0.13^a	2.22±0.14^{ab}	2.41±0.25^b	2.06±0.31^{ab}	2.06±0.51^a

the max.CD values among treatments were not significantly different. In the green microalgal strains, the initial CD values for *Chlorella* spp. and *Nannochloropsis oculata* were 1.91±0.25 and 2.05±0.26×10^5 cells·mL^{-1}, respectively. The max.CD, μ, K, and D values of *Chlorella* spp. in the T1–T4 treatments were significantly higher than the control, while the values for *μ* and *K* of *Chlorella* spp. in T4 were significantly higher than in T2 and T3. D in T4 had a significant rapid increase compared to T1, T2, and T3. For the *Nannochloropsis oculata* culture, the max.CD, μ, K, and D values in T3 and T4 were significantly better than in T1 and T2, and its growth performance in T3 was significantly higher than the growth in the control.

In contrast, the addition of concentrations of $NaHCO_3$ in the range 0.25–1.0 g·L^{-1} had a negative effect on both the flagellate strains in this study. In *Tetraselmis suecica*, at an initial cell density of 1.34±0.11×10^5 cells·mL^{-1}, the growth performance in T1–T4 in terms of max.CD, μ, and K was significantly lower than for the control, while D in T1–T4 significantly decreased compared to the control. For *Isochrysis galbana*, the initial CD value was 1.79±0.27×10^5 cells·mL^{-1}, with its max.CD values in T1–T4 being significantly lower than for the control. There were no significant differences among the values for μ, K, and D of *Isochrysis galbana* in the control, T1, T3, and T4, while T2 had significantly lower growth performance than the control.

Effect of $NaHCO_3$ supplement on quality of microalgae

Based on the results of Experiment 1, we used the *Chaetoceros calcitrans, Thalassiosira weissflogii, Chlorella* spp. and *Nannochloropsis oculata* inoculum strains with 0.25, 1.0, 1.0, and 0.50 g·L^{-1} $NaHCO_3$ supplements, respectively. The growth patterns of each inoculum cultured under laboratory-scale conditions after refrigerated storage (4±1 °C) at 7 (T1), 15 (T2), and 30 (T3) days compared with and without storage (control) are shown in Figure 1.

In this experiment, the initial CD values of *Chaetoceros calcitrans, Thalassiosira weissflogii, Chlorella* spp. and *Nannochloropsis oculata* were 1.66±0.73, 1.65±0.44, 2.64±0.74, and 3.21±1.00×10^5 cells·mL^{-1}, respectively. The results showed

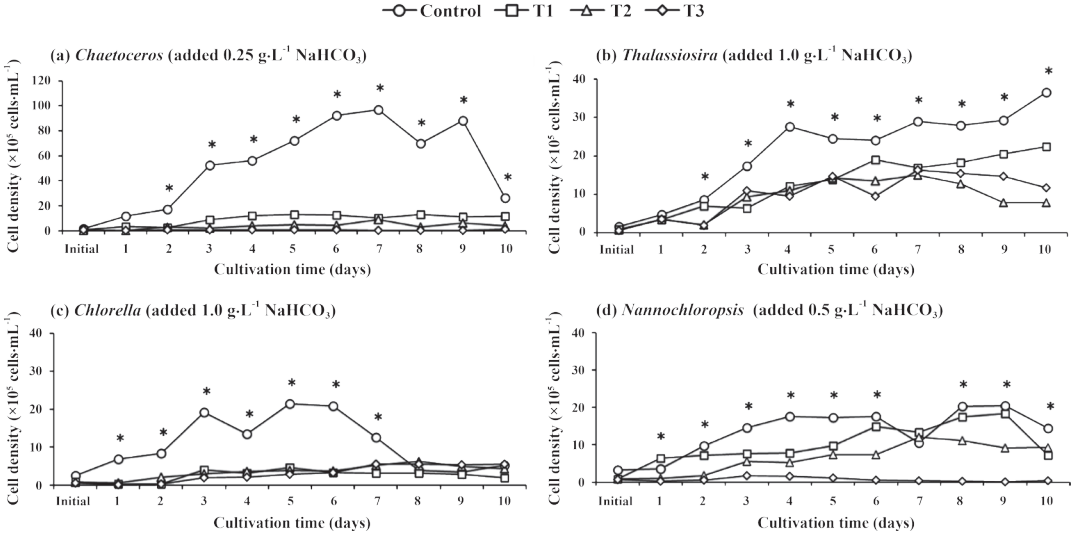

Figure 1. Growth patterns of inocula of *Chaetoceros calcitrans* (a), *Thalassiosira weissflogii* (b), *Chlorella* spp. (c) and *Nannochloropsis oculata* (d) with added $NaHCO_3$ and cultured under laboratory-scale conditions after refrigerated storage at 4±1 °C for different times: without storage (control) and after storage for 7 days (T1), 15 days (T2), and 30 days (T3). An asterisk denotes statistical significance ($p<0.05$).

that the CD of *Chaetoceros calcitrans* in the control increased rapidly from 17.11±4.17 to 52.69±11.42×10^5 cells·mL^{-1} at days 2–3 of cultivation (D) after which it reached the stationary phase where the max.CD was 97.22±16.63×10^5 cells·mL^{-1}, then the CD decreased until the end of experimental period. In contrast, there were slow growth patterns for *Chaetoceros calcitrans* in T1, T2, and T3 as well as the max.CD values in T1, T2, and T3 being significantly lower (13.20±2.36, 8.91±1.40, and 1.28±0.51×10^5 cells·mL^{-1}, respectively) than in the control (Figure 1a). The values for μ of *Chaetoceros calcitrans* in T1 (0.43±0.12 day^{-1}), T2 (0.25±0.08 day^{-1}), and T3 (0.15±0.11 day^{-1}) were significantly lower than for the control (0.59±0.09 day^{-1}) too (Figure 2). The CD of *Thalassiosira weissflogii* in control and T1 continued to increase until the end of the experimental period without any apparent exponential phase of growth. In T2 and T3 there was a gradual decrease in CD at days 6–7 of cultivation onward. The values for the max.CD (36.61±7.07×10^5 cells·mL^{-1}) and μ (0.31±0.02 day^{-1}) of *Thalassiosira weissflogii* in the control were significantly higher than in T1–T3 (14.67±2.98–22.49±4.67×10^5 cells·mL^{-1}; 0.19±0.03–0.24±0.04 day^{-1}, respectively), as shown in Figures 1b and 2.

For the *Chlorella* spp. culture, there were slower growth patterns in T1, T2, and T3 compared to the control. The CD value of *Chlorella* spp. in the control increased rapidly from 8.48±1.70 to 19.17±2.63×10^5 cells·mL^{-1} at days 2–3 of cultivation (D); then, the CD decreased considerably from day 7 of cultivation onward. The max.CD and μ values of *Chlorella* spp. in the control (21.50±3.22×10^5 cells·mL^{-1}, and 0.44±0.13 day^{-1}, respectively) were significantly higher than in T1–T3 (4.65±1.05–6.32±1.56×10^5 cells·mL^{-1}; 0.11±0.04–0.13±0.05 day^{-1}), as shown in Figures 1c and 2. For the *Nannochloropsis oculata* culture, the CD values in the control, T1, and T2 continued to increase, with rapid increases from 3.53±0.21 to 9.81±2.37, 9.73±4.38 to 14.82±5.35, and 1.68±0.16 to 5.48±1.04×10^5 cells·mL^{-1} at days 2–3, 5–6, and 2–3 of cultivation (D), respectively. Then, the CD of *Nannochloropsis oculata* in the control, T1, and T2 decreased slowly

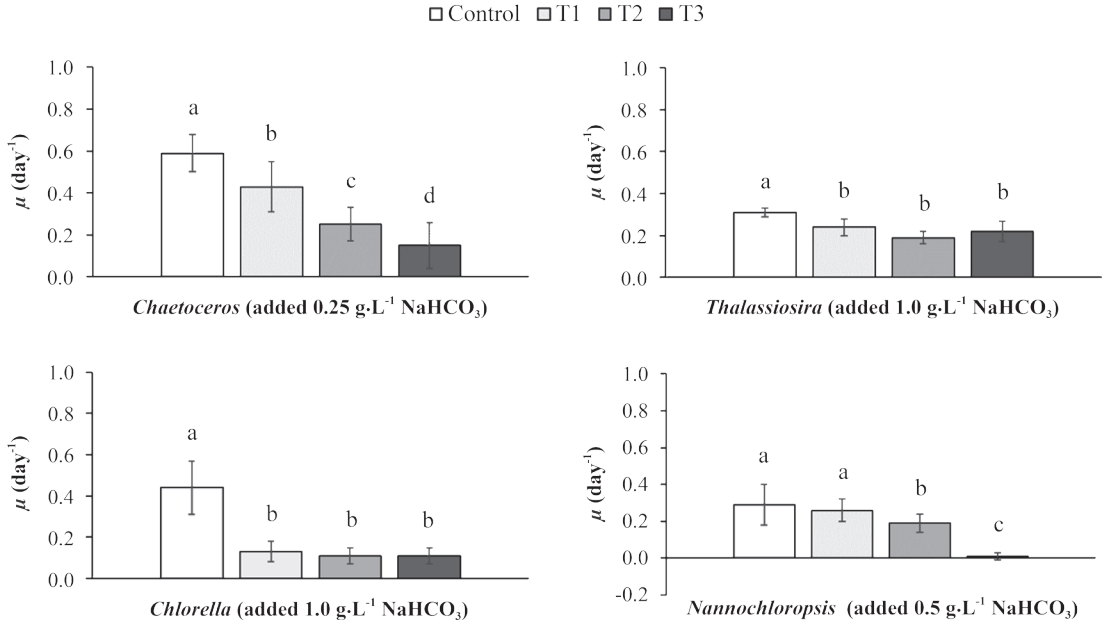

Figure 2. Specific growth rate (μ) of four inocula with added $NaHCO_3$ cultured under laboratory-scale conditions after refrigerated storage at 4±1 °C for different times: without storage (control) and after storage for 7 days (T1), 15 days (T2), and 30 days (T3). Error bars indicate mean±standard deviation and different lowercase letters above bars indicate a significant ($p<0.05$) difference.

from days 7–8 of cultivation onward. The max.CD and μ values of *Nannochloropsis oculata* among the control ($20.31\pm6.51\times10^5$ cells·mL^{-1} and 0.29 ± 0.11 day^{-1}, respectively) and T1 ($18.41\pm5.59\times10^5$ cells·mL^{-1} and 0.26 ± 0.06 day^{-1}, respectively) were not significantly different, with both being significantly higher than in T2 ($12.03\pm3.14\times10^5$ cells·mL^{-1}; 0.19 ± 0.05 day^{-1}). In addition, the growth pattern of *Nannochloropsis oculata* in T3 was very low, with the values for the max.CD ($1.70\pm0.25\times10^5$ cells·mL^{-1}) and μ (0.01 ± 0.02 day^{-1}) being significantly lower than in the control, T1, and T2. In addition, the CD of *Nannochloropsis oculata* in T3 decreased from day 4 of cultivation onward (Figures 1d and 2).

DISCUSSION

The growth pattern and rate of microalgae are crucial indicators of culture efficacy, influenced by factors like temperature, salinity, light, nutrients, and pH (Ishika *et al*., 2017; Arkronrat and Oniam, 2019; Xiaoning *et al*., 2020; Ramlee *et al*., 2021). While microalgae can thrive on environmental CO_2 alone, their growth can be enhanced by bicarbonate supplementation in culture systems (de Farias Silva *et al*., 2016; Sampathkumar and Gothandam, 2019; Caturwati and Setyati, 2020; Tahiri *et al*., 2023). Our study provides comprehensive insights into $NaHCO_3$ supplementation for microalgal strains, crucial for stable inoculum production in laboratory conditions. We observed positive effects on growth performance (CD, μ, K, and D) in *Chaetoceros calcitrans, Thalassiosira weissflogii, Chlorella* spp., and *Nannochloropsis oculata* inocula with $NaHCO_3$ additions of 0.25, 1.0, 1.0, and 0.50 g·L^{-1}, respectively. Conversely, 0.25–1.0 g·L^{-1} $NaHCO_3$ negatively impacted *Tetraselmis suecica* and *Isochrysis galbana* inocula. Singh *et al*. (2022) noted inhibition of microalgal growth with higher $NaHCO_3$ supplementation, leading to elevated pH levels detrimental to growth. However, optimal $NaHCO_3$ concentrations vary among species and cultivation conditions (Caturwati and Setyati, 2020; Salbitani *et al*., 2020; Ratomski *et al*., 2021).

Some microalgae, like *C. vulgaris*, may experience growth inhibition with increased $NaHCO_3$ levels (Ishika *et al*., 2017; Li *et al*., 2018; Ramlee *et al*., 2021), consistent with our findings. However, our study didn't analyze pH changes post-$NaHCO_3$ supplementation, warranting further investigation to determine optimal $NaHCO_3$ levels for microalgal cultivation. For *C. calcitrans, T. weissflogii, Chlorella* spp., and *N. oculata* inocula, $NaHCO_3$ supplementation can boost growth under laboratory conditions by providing a CO_2 source. Conversely, *T. suecica* and *I. galbana* inocula might benefit from alternative carbon sources, such as CO_2 gas and aeration, as reported in other microalgal cultivation systems (Batac *et al*., 2020; Umetani *et al*., 2021). Exploring alternative carbon sources could be a promising avenue for enhancing growth rates in *T. suecica* and *I. galbana* inoculum production in future studies.

$NaHCO_3$ supplementation did not maintain the quality of *C. calcitrans, T. weissflogii,* and *Chlorella* spp. inocula after storage at 4±1 °C for 7, 15, and 30 days. While $NaHCO_3$ is not typically recognized as a cryoprotectant agent, the addition of 0.5 g·L^{-1} $NaHCO_3$ preserved the quality of *N. oculata* inoculum after 7 days of storage at 4±1 °C compared to non-stored samples. This finding underscores another positive aspect of $NaHCO_3$ in maintaining the quality of *N. oculata* cells, particularly after low-temperature storage, consistent with observations for other microalgae. Li *et al*. (2018), for instance, noted that optimal $NaHCO_3$ concentrations-controlled protozoa and stimulated lipid accumulation in green microalgae like *Neochloris oleoabundans*, enhancing overall algae quality. Both additives and low temperatures have been shown to impact microalgae preservation (Lindberg *et al*., 2022; Foo *et al*., 2023). Storing microalgae at 4 °C offers a promising alternative to fresh samples, while cryopreservation at -20 °C has proven effective for long-term preservation, facilitating large-scale production (de Silva *et al*., 2020; Foo *et al*., 2023). The current findings highlight the importance of considering $NaHCO_3$ concentration, which can limit the growth rates of certain microalgal species. Moreover, the effect of $NaHCO_3$ on marine microalgae growth is species-specific and dependent on concentration, underscoring the need for careful consideration in culture design.

CONCLUSIONS

Sodium bicarbonate ($NaHCO_3$) can have varying effects on microalgae depending on the concentration and species. Adding 0.25, 1.0, 1.0, and 0.50 $g \cdot L^{-1}$ $NaHCO_3$ to the cultures of *Chaetoceros calcitrans, Thalassiosira weissflogii, Chlorella* spp. and *Nannochloropsis oculata*, respectively, enhances growth performance. Conversely, 0.05–1.0 $g \cdot L^{-1}$ $NaHCO_3$ reduces the growth rates of *Tetraselmis suecica* and *Isochrysis galbana.* Regarding inoculum quality after refrigerated storage (4±1 °C), $NaHCO_3$ supplements do not maintain the growth rates of *C. calcitrans, T. weissflogii*, and *Chlorella* spp. after 7, 15, and 30 days. However, adding 0.5 $g \cdot L^{-1}$ $NaHCO_3$ preserves the growth of *N. oculata* after 7 days of storage. Therefore, using $NaHCO_3$ in the culture medium can enhance the production of certain microalgal strains.

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