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Growth of Sea Grapes (*Caulerpa lentillifera*) in Laboratory-Scale Cultivation With Urea As A Single Nitrogen Nutrient

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ABSTRACT

Sea grape (*Caulerpa lentillifera*) is a strategic green macroalgae with significant potential in aquaculture and functional food industries. However, its cultivation remains constrained by limited production techniques. This study investigated the influence of nitrogen concentration on *C. lentillifera* growth in a laboratory-scale cultivation system. A completely randomized experimental design was implemented with four treatments: a control group (0 ppm) and nitrogen supplementation levels of 5, 10, and 15 ppm, each replicated thrice over 21 days. Results demonstrated a significant positive correlation between nitrogen concentration and growth parameters. 15 ppm nitrogen treatment exhibited the highest absolute growth (3.37 g), relative growth rate (13.50%), and specific growth rate (1.82% per day). Interestingly, no statistically significant difference was observed between 10 and 15 ppm treatments, suggesting a potential nitrogen uptake saturation point. Water quality parameters remained stable throughout the experiment, with temperature ranging from 28-30°C, salinity at 33-34 ppt, dissolved oxygen between 5.9-6.7 ppm, and pH maintained at 7.5-7.8. The study provides crucial insights into nitrogen's role in *C. lentillifera* cultivation, offering a scientific foundation for developing more efficient and sustainable macroalgae production strategies in aquaculture and functional food industries.

INTRODUCTION

Sea Grape (*Caulerpa lentillifera*) is one of the green macroalgae species with strategic potential in aquaculture and functional food industry development. Its distribution is widespread in tropical and subtropical waters, characterized by relatively rapid growth and adaptability to diverse aquatic environmental conditions (Renal *et al*., 2021). Nevertheless, optimal utilization of sea grapes remains constrained by limited production and sustainable cultivation techniques.

Nutritionally, *C. lentillifera* possesses critical components that elevate its value as a high-value commodity. Its primary nutritional constituents include protein, minerals, lipids, and carbohydrates, with a notable advantage in polyunsaturated fatty acids (PUFAs). The amino acid composition is predominantly aspartic and glutamic acids, while its mineral profile is rich in essential elements and photosynthetic pigments such as chlorophyll-a, chlorophyll-b, β-carotene, and caulerpin (Darmawan *et al*., 2020).

Optimal *C. lentillifera* growth critically depends on nutrient availability, particularly nitrogen. Nitrogen serves as an essential component in protein synthesis and chlorophyll formation, playing a crucial role in photosynthetic processes. Appropriate nitrogen availability can significantly influence growth rates, biomass productivity, and macroalgae nutritional quality (Ginting *et al*., 2015). Previous research has demonstrated growth response variations with different nitrogen concentrations, with growth rates ranging between 1.36-3.29% per day.

The primary challenge in *C. lentillifera* cultivation lies in its dependence on natural conditions and limited environmental factor control. Consequently, developing laboratory-scale cultivation techniques with nitrogen as a single nutrient represents a strategic approach to optimizing biomass production and quality. This study aims to investigate the influence of nitrogen concentration variations on *C. lentillifera* growth within a controlled cultivation system.

By comprehensively understanding growth responses to controlled nitrogen supplementation, this research is expected to provide significant scientific contributions toward developing more efficient and sustainable macroalgae cultivation strategies. The study's findings are projected to serve as a reference for advancing macroalgae-based aquaculture and functional food industry applications.

METHODS

Study Design and Experimental Setup

This experimental laboratory study investigated the influence of nitrogen concentration on the growth of sea grapes (*C. lentillifera*). The research was conducted over 21 days, from June 22 to July 12, 2023, at the Fish Production and Reproduction Laboratory, Aquaculture Study Program, Faculty of Agriculture, University of Mataram, Indonesia.

Experimental Design

A completely randomized experimental design was implemented to investigate the growth response of sea grapes to varying nitrogen concentrations, comprising four distinct treatments: a control group (0 ppm) and three nitrogen supplementation levels at 5, 10, and 15 ppm. The experimental setup consisted of 12 total experimental units, with each treatment replicated thrice to ensure statistical robustness and minimize potential variability, thereby providing a comprehensive assessment of nitrogen's impact on macroalgae biomass production and physiological characteristics.

Materials

The experimental apparatus comprised a comprehensive set of specialized laboratory equipment designed to facilitate precise scientific measurements and controlled cultivation conditions. Glass containers with a 3-liter capacity served as primary culture units, complemented by fluorescent lamps for regulated lighting, aerators to ensure optimal oxygen distribution, and calibrated measurement instruments including pH meters, dissolved oxygen (DO) meters, refractometers, and analytical balances. These instruments were carefully selected to provide accurate monitoring and control of critical environmental parameters throughout the research process, enabling rigorous documentation and analysis of *C. lentillifera* growth dynamics.

Seawater for the experiment was sourced from Batu Layar Beach in West Lombok, characterized by a stable salinity of 34 practical salinity units (ppt). Nitrogen supplementation was achieved using urea, a nitrogen-rich fertilizer with a 46% nitrogen content, from which a concentrated stock solution was meticulously prepared. The stock solution, containing 10,000 mg Nitrogen/L, was generated by precisely dissolving 4.348 g of urea in 200 mL of sterile seawater. This methodical preparation ensured a precise and controlled approach to nitrogen concentration variations, allowing for systematic investigation of nitrogen's impact on sea grape macroalgae growth and development.

Cultivation Procedure

- ***Seawater Preparation and Handling***

Seawater collection and preparation were conducted with meticulous attention to quality and environmental consistency. High-quality seawater was sourced from Batu Layar Beach, carefully filtered through cotton filtration to remove potential particulate contaminants and unwanted microorganisms. The filtered seawater was systematically stored in a 120-liter storage container, ensuring a sufficient volume for the entire experimental duration. Each experimental unit received a precisely measured 2.5 liters of meticulously prepared seawater, maintaining uniform environmental conditions across all treatment groups.

- ***Macroalgae Selection and Adaptation***

The *Caulerpa lentillifera* specimens were selectively collected from Batu Layar Beach, with a rigorous screening process prioritizing young, robust, and visually healthy samples. Specimen selection focused on individuals exhibiting optimal morphological characteristics and minimal physical damage. Prior to experimental initiation, the collected sea grape specimens underwent a critical two-day acclimatization period. This controlled adaptation phase was strategically designed to minimize physiological stress, reduce potential mortality rates, and prepare the macroalgae for the experimental environment, thereby enhancing the reliability and reproducibility of research outcomes.

- ***Experimental Unit Configuration***

Each experimental unit was precisely configured to provide an optimal cultivation environment for *Caulerpa lentillifera*. The units were equipped with a comprehensive set of standardized components to ensure consistent and controlled growth conditions. Fluorescent lighting, calibrated at 18 watts and maintained at 3,000 lux, provided a uniform light intensity crucial for photosynthetic processes. A continuous aeration system was implemented to maintain adequate oxygen levels and promote gentle water circulation. Nitrogen concentrations were precisely adjusted according to experimental treatments, with each unit initially stocked with a standardized macroalgae biomass of approximately 25 grams. This systematic approach enabled a comprehensive investigation of nitrogen's impact on sea grape growth under meticulously controlled laboratory conditions.

Cultivation Protocol

The experimental protocol involved three critical maintenance procedures: precise nitrogen supplementation, where calculated volumes of nitrogen stock solution were meticulously added to each experimental unit using a graduated pipette to ensure homogeneous nutrient distribution; consistent illumination and aeration, with fluorescent lamps maintained at a constant 3,000 lux while aerators provided continuous oxygen supply; and periodic water quality management through partial water exchange, wherein every three days, 50% of the culture medium was replaced with filtered seawater, with nitrogen supplementation proportional to the volume of new water added, to sustain optimal nutrient balance and maintain appropriate water chemistry for *Caulerpa lentillifera* cultivation.

Parameter Measurements

- **Biomass Parameters**

Wet biomass of *Caulerpa lentillifera* was quantified at predetermined intervals (0, 7, 14, and 21 days) employing a standardized measurement protocol: specimens were meticulously extracted from their culture units, subsequently drained of excess moisture by gently blotting with tissue paper for 25-30 seconds to ensure consistent moisture removal, precisely weighed using an analytical balance with 0.01 g precision to capture minute biomass variations, and systematically documented through photographic records to maintain a comprehensive visual and quantitative research archive.

- **Water Quality Parameters**

Water quality parameters were systematically monitored throughout the experimental period to ensure optimal growth conditions for *Caulerpa lentillifera*. Daily measurements were conducted using calibrated scientific instruments to track critical environmental variables: water temperature was measured using a precise thermometer, maintaining a consistent record of thermal conditions; pH was assessed with a digital pH meter to monitor potential hydrogen ion concentration; salinity was quantified using a refractometer to ensure marine environmental stability; and dissolved oxygen (DO) levels were determined using a dissolved oxygen meter. These comprehensive daily measurements provided a robust dataset for evaluating the physiological response of sea grapes to varying environmental conditions, with each parameter serving as a crucial indicator of potential stress or optimal growth potential in the marine culture system.

Tested Parameters

In this study, four critical aspects were evaluated to comprehensively assess the growth and environmental conditions of *Caulerpa lentillifera*: absolute growth, relative growth, specific growth rate (SGR), and water quality parameters.

- Absolute Growth

  The determination of absolute growth was calculated using the formula proposed by Effendie (1997):

  $M = Wt - Wo$ .... (1)

  Where: M: Absolute growth (g/observation period);
  Wt: Final biomass weight (g);
  $W0$: Initial biomass weight (g).

- Relative Growth

  Relative growth was also determined using Effendie's (1997) established formula:

  $R = (Wt - Wo) / Wo$ .... (2)

  Where: R: Relative growth (%);
  Wt: Final biomass weight (g);
  $W0$: Initial biomass weight (g)

- Specific Growth Rate (SGR)

  The specific growth rate was derived from the formula introduced by Huisman (1976):

  $S = [(Wt / Wo^*)^{(1/t)} - 1] * 100\%$ .... (3)

  Where: S: Daily specific growth rate (% per day);

Wt: Final biomass weight (g);
$W0$: Initial biomass weight (g);
t: Observation time interval (days)

- Water Quality Parameters

Water quality parameter measurement represents a critical aspect of environmental management in *Caulerpa lentillifera* research. Six primary parameters were investigated, each playing a crucial role in the species' life and productivity: temperature, pH, salinity, dissolved oxygen, and light intensity.

Statistical Analysis

Data growth was input into the CoStat application for analysis of variance (ANOVA), with an analytical significance level of $\alpha = 0.05$ (95% confidence interval). The statistical analysis followed a systematic approach to determine the potential significant differences among the experimental treatments. When the ANOVA results indicated a statistically significant effect ($p < 0.05$), the analysis was subsequently proceeded with the Least Significant Difference (LSD) test at a 95% confidence level. This post-hoc test allowed for a more detailed comparison between individual treatment means, providing a comprehensive understanding of the variations observed in the dataset.

Water quality parameters were analyzed through descriptive statistical methods by comparing the measured parameter values against established standards for sea grape (*Caulerpa* sp.) habitat suitability. This comparative approach enabled a comprehensive evaluation of the environmental conditions and their potential implications for the marine organism's growth and survival. The descriptive analysis provided insights into the ecological context of the study, ensuring a holistic interpretation of the water quality data in relation to the research objectives.

RESULTS

Wet Weight Dynamics

The wet weight of *C. lentillifera* exhibited a complex growth pattern across different nitrogen concentrations during the 21-day cultivation period (Figure 1). Initial observations revealed a significant increase in biomass during the first week, with the 15 ppm nitrogen treatment demonstrating the most pronounced growth performance. This initial phase highlighted the crucial role of nitrogen availability in supporting metabolic processes and protein synthesis, with the highest response observed at the maximum concentration.

The growth trajectory post-first week revealed intriguing variations among treatments. The 15 ppm nitrogen treatment maintained more stable biomass until day 21, while 5 ppm and 10 ppm treatments showed a tendency for weight reduction after 14 days of cultivation. The control treatment (without nitrogen supplementation) exhibited the earliest weight decline, confirming the significance of nitrogen supplementation in supporting *C. lentillifera* growth.

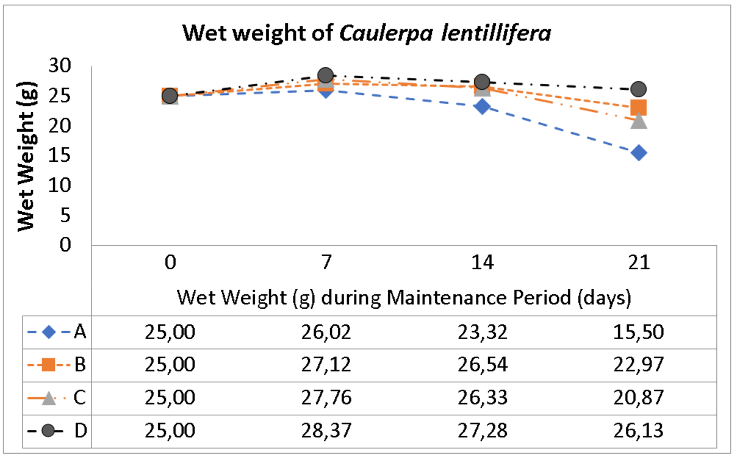

Figure 1. Wet weight of *Caulerpa lentillifera* observed every 7 (seven) days in media with various nitrogen concentrations. Notes: A = 0 ppm (control); B = 5 ppm; C = 10 ppm; and D = 15 ppm.

- **Absolute Growth Analysis**

Statistical analysis of absolute growth demonstrated a positive correlation between nitrogen concentration and algal development (Table 1). The 15 ppm nitrogen treatment yielded the highest absolute growth of 3.37 grams within one week, statistically significant (p<0.05) compared to the control and 5 ppm treatments. This performance suggests *C. lentillifera's* capacity to optimize nitrogen utilization at this concentration, potentially attributed to intensified photosynthetic activities and cellular metabolism.

Table 1. Effect of Nitrogen Concentration on Growth Parameters of *Caulerpa lentillifera*

Parameters	A (Control)	B (5 ppm)	C (10 ppm)	D (15 ppm)
Abs (g)	1.02 ± 0.68 ^{a}	2.12 ± 0.53 ^{ab}	2.76 ± 0.56 ^{bc}	3.37 ± 0.70 ^{c}
RGR (%)	4.09 ± 2.71 ^{a}	8.50 ± 2.13 ^{ab}	11.04 ± 2.22 ^{bc}	13.50 ± 2.78 ^{c}
SGR (% per day)	0.57 ± 0.37 ^{a}	1.17 ± 0.28 ^{ab}	1.51 ± 0.29 ^{bc}	1.82 ± 0.36 ^{c}

Note : *Abs* = Absolute Growth (g); *RGR* = Relative Growth Rate (%); *SGR* = Specific Growth Rate (% per day)

The 10 ppm nitrogen treatment produced an absolute growth of 2.76 grams, occupying the second position in treatment hierarchy. Interestingly, the growth at 10 ppm was not significantly different from the 15 ppm treatment, indicating an optimal nitrogen range between 10-15 ppm for *C. lentillifera* cultivation.

- **Relative Growth**

The relative growth analysis revealed a similar pattern, with the 15 ppm treatment achieving the highest relative growth rate of 13.5% (Table 1). The 10 ppm treatment showed comparable performance at 11.04%, suggesting potential nutrient-use efficiency at lower concentrations. The control treatment recorded the lowest relative growth at 4.09%.

- **Specific Growth Rate**

The specific growth rate ranged from 0.57% to 1.82% per day (Table 1), with the 15 ppm treatment producing the highest rate of 1.82% daily. Notably, no significant difference was observed between 15 ppm and 10 ppm treatments, further supporting the potential of 10 ppm as an optimal, cost-effective nitrogen concentration.

- **Water Quality Characteristics**

Water quality parameters remained stable throughout the cultivation period, with temperature ranging from 28-30°C, salinity at 33-34 ppt, dissolved oxygen between 5.9-6.7 ppm, and pH maintained at 7.5-7.8. These conditions aligned with recommended standards for marine macroalgae cultivation, ensuring an optimal environment for *C. lentillifera* growth. The research provides comprehensive insights into the nitrogen-growth relationship of *C. lentillifera*, offering valuable implications for sustainable seaweed aquaculture strategies.

DISCUSSION

Physiological Response to Nitrogen Concentration

Nitrogen plays a critical role in the metabolic processes of marine macroalgae, serving as a fundamental element in protein synthesis, nucleic acid formation, and other essential molecular functions (Hanisak, 1983). In this study, the growth response of *Caulerpa lentillifera* to varying nitrogen concentrations revealed complex physiological dynamics that provide insights into the species' adaptive capabilities.

The research demonstrated a significant positive correlation between nitrogen concentration and growth parameters, with the highest absolute growth, relative growth rate, and specific growth rate observed at 15 ppm nitrogen treatment. These findings align with previous studies by Chung *et al*. (2011), who reported that increased nitrogen concentrations substantially enhance macroalgal growth rates. The positive correlation reflects *C. lentillifera's* response to nitrogen availability, consistent with Raikar *et al*. (2001) who documented biomass increases with elevated nitrogen levels in culture media.

Comparative analysis between treatments revealed statistically significant differences, particularly between the control (0 ppm) and nitrogen-supplemented treatments. Interestingly, the 10 ppm and 15 ppm treatments showed no significant statistical difference, suggesting a potential nitrogen uptake saturation point. This phenomenon corresponds to the "luxury consumption" concept proposed by Cade-Menun and Paytan (2010), wherein organisms can absorb nutrients beyond their normal metabolic requirements, storing excess nitrogen as proteins or nucleic acids.

The specific daily growth rate of *C. lentillifera* ranged from 0.57% to 1.82% per day, with the highest value at 15 ppm nitrogen concentration. This range falls within the normal growth parameters for marine macroalgae, as reported by Dawes (1998). The increasing growth rate correlating with nitrogen concentration indicates that nitrogen is a primary limiting factor in *C. lentillifera* growth under the experimental conditions.

The nitrogen's influence on growth can be attributed to its role in enhancing photosynthetic efficiency and protein synthesis. Nitrogen is a crucial component in chlorophyll formation and enzymes involved in photosynthesis (Hanisak, 1983). While the 15 ppm concentration yielded the best results, the statistically non-significant difference with the 10 ppm treatment suggests potential optimization of nitrogen use in *C. lentillifera* cultivation.

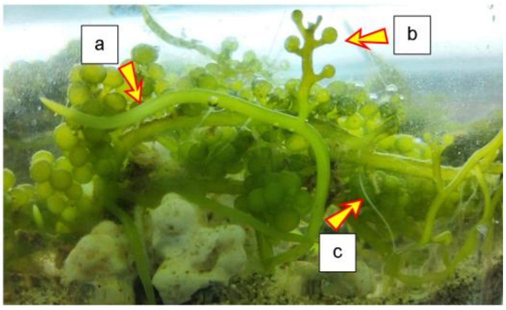

Figure 2. Stolon elongation (a), Ramuli growth (b), and Rhizoid development (c) of *Caulerpa lentillifera* in controlled containers.

Visual observations (Figure 2) revealed distinctive morphological changes, including Stolon elongation (a), Ramuli growth (b), and Rhizoid development (c) of *Caulerpa lentillifera* in controlled cultivation conditions. These morphological adaptations further illustrate the significant impact of nitrogen concentration on *C. lentillifera's* structural development and growth dynamics.

Nitrogen Absorption Dynamics of Caulerpa lentillifera in Marine Aquaculture Systems

Sea Grape, *C. lentillifera*, exhibits remarkable nitrogen absorption capabilities across multiple nitrogen forms in marine environments. As a significant macroalgal species, it demonstrates complex nitrogen uptake mechanisms crucial for ecological and aquaculture applications. Fish waste generates substantial nutrient loads that potentially contaminate cultivation waters (Yang *et al*., 2015). Integrating marine cultivation systems with seaweeds like C. lentillifera significantly mitigates dissolved nutrient discharge, offering an innovative bioremediation strategy (He *et al*., 2008).

Nitrogen Source Utilization

Sea grape, *C. lentillifera*, demonstrates extraordinary nitrogen absorption capabilities across various nitrogen forms in marine environments, with primary nitrogen sources including nitrate (NO_3^-) and ammonium (NH_4^+) (Liu *et al*., 2016). Nitrate represents the most common inorganic nitrogen form in marine environments (Hanisak, 1983). *C. lentillifera* can effectively absorb nitrate and ammonium, showing a preference for ammonium. This preference aligns with most macroalgal species, as ammonium absorption requires less energy for assimilation compared to nitrate (Raven *et al*., 1992).

Beyond the primary inorganic forms, this species can also utilize urea and dissolved organic nitrogen (DON), including amino acids, providing metabolic flexibility in marine environments with nutrient variations (Tyler *et al*., 2005; Morand & Briand, 1996). This characteristic offers a competitive advantage in environments with limited inorganic nitrogen availability.

Environmental Influence on Nitrogen Absorption

The intricate relationship between environmental conditions and nitrogen absorption in marine macroalgae represents a critical area of scientific inquiry, with *C. lentillifera* emerging as a particularly compelling model organism. Environmental parameters demonstrate a profound and nuanced influence on nitrogen absorption efficiency.

- **Temperature Dynamics and Nitrogen Metabolism**

Environmental parameters play a crucial role in modulating nitrogen absorption efficiency in macroalgae, with variables such as temperature having a highly significant influence on nutrient absorption dynamics. The temperature range of 28-30°C supports robust growth, consistent with findings by Guo *et al.* (2015b) who identified 28°C as the peak growth temperature. This temperature range enhances photosynthetic rates and metabolic processes, critically influencing *C. lentillifera* development. At these temperatures, cellular metabolism accelerates dramatically, directly enhancing enzymatic capacity for nitrogen absorption and assimilation. The study by Gao *et al.* (2017) demonstrated that temperature increases not only enhance nitrogen uptake rates but also modify molecular transport mechanisms in algal cell membranes. Within specific temperature ranges, nitrogen transport protein activity experiences increased efficiency, enabling *C. lentillifera* to extract more nitrogen from its environment with relatively lower energy expenditure. The implications of these findings are critically important in the context of global climate change. With ongoing global warming, macroalgae like *C. lentillifera* have the potential to undergo physiological adaptations that allow them to survive and even leverage changing environmental parameters. This impacts not only marine ecosystems but also potentially influences future seaweed cultivation strategies.

- **Salinity Tolerance and Nutrient Dynamics**

Salinity emerges as a critical environmental variable influencing nutrient uptake mechanisms, with *C. lentillifera* demonstrating remarkable physiological adaptability across diverse ionic conditions. Empirical investigations by Paul *et al.* (2014) revealed *C. lentillifera's* exceptional salinity tolerance, characterized by robust metabolic performance across a broad salinity spectrum ranging from 20 to 50 practical salinity units (ppt). Notably, the species exhibits optimal growth characteristics within the 30-40 ppt range, suggesting a sophisticated osmoregulatory capacity that enables efficient nutrient assimilation under variable marine environmental conditions.

The observed salinity range of 33-34 ppt in this study represents a physiologically optimal zone, facilitating enhanced nutrient uptake efficiency and cellular metabolic processes. This narrow yet critical salinity window demonstrates the species' finely tuned adaptation mechanisms, which are essential for maintaining nutrient absorption capabilities in dynamic marine ecosystems.

- **pH-Mediated Nutrient Absorption Mechanisms**

The slightly alkaline marine environment, characterized by a pH range of 7.5-7.8, plays a pivotal role in modulating nutrient absorption dynamics. Guo *et al*. (2015a) elucidated the critical relationship between pH parameters and inorganic carbon availability, highlighting the complex interplay between cellular membrane transport mechanisms and environmental pH conditions. Within this specific pH range, *C. lentillifera* demonstrates enhanced capability for inorganic carbon utilization, directly influencing nitrogen absorption efficiency. The observed pH parameters critically mediate ion exchange processes, cellular membrane permeability, and enzymatic activity, thereby optimizing nutrient assimilation strategies.

- **Dissolved Oxygen and Metabolic Equilibrium**

Dissolved oxygen levels maintained at 5.9-6.7 ppm represent another crucial parameter supporting cellular metabolic processes. Zuldin *et al.* (2019) emphasized the significance of dissolved oxygen in facilitating fundamental physiological functions, including ATP production and maintaining cellular homeostasis. The observed oxygen levels provide substantial metabolic support, enabling *C. lentillifera* to sustain optimal energy metabolism and nutrient transport mechanisms. This oxygen range ensures efficient cellular respiration, supporting the intricate biochemical processes associated with nitrogen absorption and assimilation.

Research Implications and Aquacultural Significance

The comprehensive analysis of water quality parameters—encompassing temperature, salinity, pH, and dissolved oxygen—offers critical insights into the environmental requirements governing marine macroalgae cultivation. By demonstrating the intricate interactions between these parameters, the study provides valuable perspectives for developing sustainable seaweed aquaculture strategies.

The research illuminates the complex nitrogen-growth relationship in *C. lentillifera*, underscoring the importance of holistic environmental management in marine biological systems. These findings contribute significantly to our understanding of macroalgae physiological adaptation and provide a robust framework for optimizing cultivation practices.

Strategic Considerations

Future research must focus on developing a comprehensive, holistic, and in-depth nutritional approach to understand the growth dynamics of *C. lentillifera.* This approach needs to consider the complexity of multifactorial interactions that significantly influence the growth and productivity of macroalgae in aquaculture systems.

Key strategies that can be implemented to optimize *C. lentillifera* production include:

1) **Periodic Media Replacement**: A systematic method of regularly replacing nutrient media to maintain chemical balance and prevent the accumulation of growth-inhibiting substances. This enables optimal nutritional conditions that can support maximal seaweed growth.
2) **Population Density Management**: Regulating the number and distribution of *C. lentillifera* individuals in cultivation space to maximize nutrient access, minimize inter-individual competition, and support uniform growth. This approach requires an in-depth understanding of population dynamics and species-specific requirements.
3) **Dynamic Cultivation Systems**: Development of aquaculture systems responsive to environmental condition changes, with real-time adaptation capabilities for parameters such as temperature, light intensity, nutrient availability, and water chemical composition. These systems can include automated monitoring mechanisms and advanced environmental control technologies.

Through the implementation of these strategies, researchers can address observed growth limitations, optimize *C. lentillifera* production, and develop more efficient and sustainable cultivation models. This comprehensive approach will not only enhance productivity but also provide valuable insights into the ecology and physiology of this important macroalgal species.

CONCLUSION

This research comprehensively explored the impact of nitrogen concentration on *Caulerpa lentillifera* growth in a controlled laboratory environment. The key findings reveal critical insights into macroalgae cultivation strategies:

1) Nitrogen concentration significantly influences *C. lentillifera* growth, with the 15 ppm treatment demonstrating the most substantial growth parameters.
2) The 10 ppm and 15 ppm treatments showed statistically similar performance, suggesting an optimal nitrogen concentration range for efficient cultivation.
3) The specific daily growth rate varied from 0.57% to 1.82%, directly correlating with nitrogen supplementation levels.
4) Morphological observations confirmed nitrogen's pivotal role in supporting *C. lentillifera* structural development, including stolon elongation, ramuli growth, and rhizoid development.

The study provides a scientific foundation for optimizing *Caulerpa lentillifera* cultivation techniques, offering valuable implications for sustainable aquaculture and functional food industries. Future research should explore multi-factorial nutrient interactions, advanced cultivation systems, and broader environmental parameter influences to further enhance macroalgae production strategies.

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