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The Potential of Biostimulants to Enhance the Growth of *Kappaphycus alvarezii* (Rhodophyta) Propagules

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Abstract

Kappaphycus alvarezii is a globally significant tropical red seaweed renowned for its carrageenan content. While tissue culture is a valuable technique for enhancing seedling quality and stress resilience in vegetative propagation, it is time-consuming and costly. Biostimulants have demonstrated the potential to enhance plant growth. This study aimed to evaluate the potential of biostimulants in enhancing the growth of *K. alvarezii* propagules. Three different biostimulant products were tested: biostimulant A (amino acid-based), biostimulant B (bacteria-based), and biostimulant C (seaweed extract-based). Each biostimulant was applied at two different concentrations and compared to a negative control and a positive control. Artificial seawater enriched with PES and supplemented with biostimulants according to the treatment was used as the growth medium. Growth of *K. alvarezii* propagules was monitored weekly, with the primary parameters being propagule weight and specific growth rate. Growth medium quality was assessed by in situ pH measurements and ex situ nitrate and phosphate analyses. Fermented biostimulants negatively impacted growth by reducing the pH of the culture medium, while biostimulant B (photosynthetic bacteria) maintained a near-neutral pH and showed the most promising results, with treatment B2 displaying stable growth and treatment B1 achieving the highest specific growth rate at week 6. Though treatments A1 and A2 showed higher nitrate and phosphate concentrations, these did not correlate with improved growth, likely due to the low pH. While these findings suggest the potential of photosynthetic bacteria for *K. alvarezii* growth, further research is necessary to fully understand the underlying mechanisms and to develop strategies to overcome the limitations associated with the acidic nature of fermented biostimulants.

Keywords: biostimulant, *Kappaphycus alvarezii*, photosynthetic bacteria, propagule, seaweed

INTRODUCTION

Kappaphycus alvarezii (Kotoni) is a tropical red seaweed of significant economic importance. Its cell walls contain carrageenan, making it one of the world's most important sources of carrageenan. The majority of carrageenan production is derived from seaweed farming, with the species *Eucheuma* and *Kappaphycus* contributing over 90% to global production (Rupert et al., 2022). *K. alvarezii* is particularly valuable due to

its high yield and rapid growth, both of which are essential for sustaining the global demand for carrageenan and supporting the livelihoods of seaweed farmers.

Conventional seaweed farming relies on vegetative propagation from germplasm. However, this conventional propagation approach has limitations, as seaweed becomes susceptible to diseases and environmental issues (Hurtado et al., 2015). The development of effective propagation techniques, such as tissue culture, is crucial for sustainable seaweed cultivation. This method has been identified as a potential solution to improve the quality of seaweed seedlings (Sulistiani & Yani, 2014), making it particularly valuable in seaweed breeding.

During the early life stages of seaweed (including propagules and planlets), seedlings are highly vulnerable to environmental stress (Navarro et al., 2016). Both biotic and abiotic stresses, such as disease, nutrient deficiency, and extreme environmental conditions, can negatively affect the growth rates of seaweed, leading to reduced yields or even complete crop failure. Abiotic stress can be mitigated by providing optimal growth conditions through adequate nutrition and plant growth regulators (Yakhin et al., 2017). The growth of propagules produced through tissue culture is supported by nutrient supply, such as Provasoli's Enriched Seawater (PES), which is the most commonly used medium for seaweed seedling production in laboratories (Mukti & Ujang, 2019; Sulistiani & Yani, 2014; Suryati et al., 2015). PES is an artificial nutrient medium composed of various chemicals and is relatively expensive. In addition, plant growth regulators (exogenous hormones) have also been utilized to stimulate seaweed seedling growth (Majda & Robert, 2018; Mo et al., 2020; Taya et al., 2022). However, a major limitation of tissue culture is the lengthy production process, making it a costly method (Luhan & Mateo, 2017). The production of *K. alvarezii* planlets typically takes around one year (Sulistiani & Yani, 2014). Therefore, more efficient methods to shorten production time are still needed.

Despite their known benefits in agriculture, the use of biostimulants in seaweed farming, especially for *K. alvarezii*, remains underexplored. While biostimulants such as seaweed extracts, amino acids, and beneficial bacteria have demonstrated significant potential in optimizing nutrient use and reducing abiotic stress, their comparative effectiveness in enhancing seaweed growth has not been adequately studied in the context of seaweed farming. This gap in research is critical to address, as biostimulants could play an important role in improving both the speed and sustainability of seaweed cultivation.

Biostimulants have shown promise in enhancing plant growth by improving stress resilience, boosting nutrient uptake, and enhancing overall plant health (du Jardin, 2015; Yakhin et al., 2017). These products are widely used in agriculture and increasingly explored for their potential in seaweed cultivation, where they can help to mitigate environmental stresses, reduce growth time, and increase yield. This study aims to evaluate different types of biostimulants for their potential to enhance the growth and development of *K. alvarezii* propagules, thereby contributing to more efficient and sustainable seaweed farming practices.

MATERIALS AND METHODS

Research Location and Time

The experiment was performed at the Biology Laboratory, Fisheries High School, Tegal, Central Java. The research was carried out from June to October 2024.

Experimental Design

The research environment was designed using a Completely Randomized Design, with a factorial treatment. The treatments consisted of three different biostimulant types and two control conditions. Each biostimulant treatment was applied at two distinct concentration levels, resulting in a total of eight experimental units. Each treatment was replicated five times.

Sample Collection and Biostimulant Application

The *K. alvarezii* propagules were obtained from the Seaweed Tissue Culture Laboratory at the Brackish Water Cultivation Fisheries Center, Jepara. The growth medium used artificial seawater with a salinity of approximately 30 g/L, supplemented with Provasoli's Enriched Seawater (PES) at 1 mL/L as a positive control, without any additives as a negative control, and with biostimulants as the experimental treatments. The biostimulants applied in this study were commercial products A, B, and C. Biostimulant A is an amino acid (AA)-based product with concentrations of 1.2 mL/L and 2.5 mL/L, while biostimulant B is a bacterial (PSB)-based product with concentrations of 2.5 mL/L and 5 mL/L. Biostimulant C is a seaweed extract (SE)-based product with concentrations of 0.5 mL/L and 1 mL/L. The experimental units in this study were as follows:

- Negative Control: artificial seawater
- Positive Control: artificial seawater + PES
- Biostimulant A1: artificial seawater + PES + AA 1.2 mL/L
- Biostimulant A2: artificial seawater + PES + AA 2.5 mL/L

- Biostimulant B1: artificial seawater + PES + PSB 2.5 mL/L
- Biostimulant B2: artificial seawater + PES + PSB 5 mL/L
- Biostimulant C1: artificial seawater + PES + SE 0.5 mL/L
- Biostimulant C2: artificial seawater + PES + SE 1 mL/L

The growth medium was added to 500 mL in transparent jars. Three propagules were weighed and placed in each jar, which was then sealed. All experimental units were randomly placed on racks and aerated to facilitate nutrient mixing and enhance seaweed absorption. The temperature was maintained between 23–25°C, and the light intensity was set to approximately 1500 lux with a photoperiod of 12 hours of light and 12 hours of darkness.

Growth Monitoring

Growth observations were conducted daily to assess the condition of the growth medium and propagules. Sampling occurred weekly, during which the propagules were weighed, and the growth medium was renewed. The parameters used to evaluate propagule growth included propagule weight and specific growth rate (SGR). Propagule weight was measured using a digital analytical balance with precision 0.01g. This experiment was conducted over a period of 3 months.

$$SGR = \frac{\ln(Wt/W0)}{t} \times 100\%$$

SGR = Specific Growth Rate (% per day)

Wt = Final weight of propagule (g)

W0 = Initial weight of propagule (g)

t = Time interval between measurements (days)

Water quality in the growth medium was monitored by measuring pH (in situ) and collecting samples for nitrate and phosphate analysis (ex situ). Water sample analysis was performed at the Water Quality Laboratory of the Brackish Water Cultivation Fisheries Center, Jepara. Nitrate content was analyzed using the method outlined in SNI 19-6964.7-2003, while phosphate was analyzed according to SNI 8567:2018.

Data Analysis

Data were tabulated using Microsoft Excel, and analysis of variance (ANOVA) was performed using SPSS statistical software. If significant differences were detected, Tukey's post-hoc test was conducted with a 95% confidence level.

RESULTS AND DISCUSSION

The control groups (both negative and positive) showed a general decline in growth over the course of the experiment (Figure 1). Specifically, the negative control group experienced a decrease of approximately 19% from the initial measurement, while the positive control group saw a reduction of about 13%.

In the treatment groups, some initial improvements in growth were observed (such as in A1, B1, and C1), but growth tended to decline over time. Treatment A1 exhibited an initial increase of around 16%, but this decreased by approximately 5% by the end of the observation period. Similarly, treatments B1 and C1 showed initial growth increases, but more significant declines occurred later, with reductions of 20% by the end of the study. The decline in growth for treatments A1, B1, and C1 began around week 4, with a steady decrease observed through the remaining observation period.

However, treatment B2 demonstrated a more stable growth pattern compared to the other treatments. Treatment B2 showed an initial increase of about 2%, and despite some decrease, the final growth value remained relatively stable, showing an 18% change compared to the initial measurement. Treatment C2, which exhibited the lowest growth among all treatments, experienced a significant decline. The observed decrease of 25% from the initial value suggests that this treatment may be ineffective or even detrimental to the growth of the propagules.

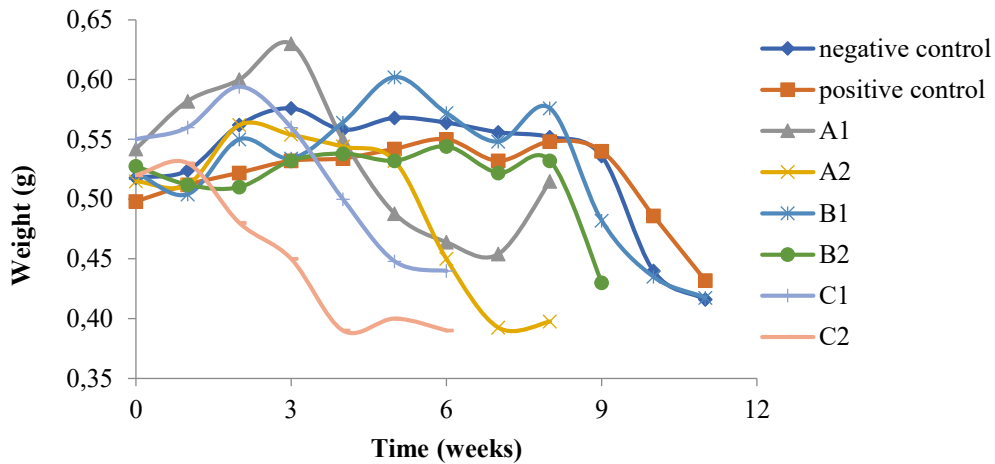


Figure 1. Growth of *Kappaphycus alvarezii* propagules in different growth media

Several treatments initially had a positive impact on growth, but this effect did not persist. The significant decrease observed at the end of the experiment indicates that these treatments did not provide the expected long-term benefits. Specifically, the decline in growth for treatments A1, B1, and C1 began around week 4 of the experiment, with a consistent downward trend observed in subsequent weeks. This suggests that while the initial application of biostimulants may stimulate growth, without sustained support, the results tend to diminish. The decline could be attributed to external factors affecting all treatments or limitations in the treatments themselves, causing their effectiveness to decrease over time.

The specific growth rate (SGR) measured in the sixth week indicated that treatment B1 was the most effective in stimulating the growth of *K. alvarezii* propagules (Figure 2). Treatment B1 recorded a growth rate of 0.22% per day ($p < 0.05$), which was significantly higher than the controls and all other treatments, making it the most prominent treatment among all tested. In contrast, treatments A1, A2, C1, and C2 all showed a reduction in growth rates, indicating that these treatments had a negative effect on growth. Treatment A1 had a decrease in growth rate of 0.39% per day, while A2 showed a decline of 0.34% per day. Treatments C1 and C2 also showed decreases of 0.54% per day and 0.68% per day, respectively. The significant reductions in A1, A2, C1, and C2 may be due to factors such as inappropriate composition or dosing, which could cause stress or imbalance in the propagules, ultimately affecting their growth.

Both the negative and positive controls exhibited relatively stable results, with a slight increase in the positive control group. The positive control exhibited a slightly higher growth rate (0.22% per day) than the negative control. This indicates that although no additional treatments were applied, the positive control maintained or slightly improved its growth rate.

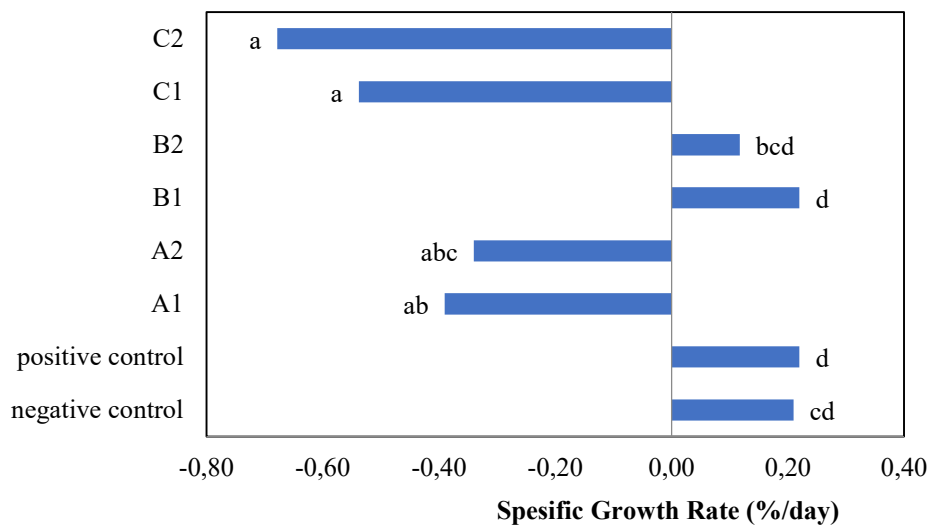


Figure 2. Specific growth rate of *Kappaphycus alvarezii* propagules in different growth media after 6 weeks of cultivation. Different letters at the end of the bars indicate significantly different at $p < 0.05$.

Based on these findings, it can be concluded that the treatments applied to the experimental groups were not as effective as anticipated, except for B1. The results for A1, A2, C1, and C2 may have been influenced by mismatched treatment composition or dosage. Environmental changes or imbalances in growth conditions could also have contributed to these declines. These results highlight the importance of sustainability and balance in treatment application to maintain optimal growth rates in *K. alvarezii* propagules.

The limited growth observed in several treatments is suspected to be caused by a decrease in pH of the medium. Treatment A1 showed a low pH, ranging from 6.59 to 7.11 (Figure 3), while treatment A2 exhibited even lower pH values, between 6.13 and 6.55. Both A1 and A2 led to a significant decrease in pH, indicating increased acidity in the medium. This acidity likely caused stress on the propagules, impairing their ability to grow optimally.

Treatment C1 had a lower pH range of 6.14 to 6.44, while C2 exhibited even lower pH values, ranging from 5.96 to 6.34. This indicates that treatment C significantly lowered the pH of the medium, making it more acidic than other treatments. Excessive acidity in the medium could hinder the metabolic processes in the *K. alvarezii* propagules, which may explain why treatment C was ineffective in promoting growth.

Treatments B1 and B2 exhibited pH values ranging from 7.32 to 7.90, which were closer to neutral and slightly alkaline. These pH values, which are closer to neutral, may have provided more favorable conditions for propagule growth. This result is consistent with the observed positive growth effects for these treatments, as shown in Figure 2, where treatment B1 exhibited the highest specific growth rate.

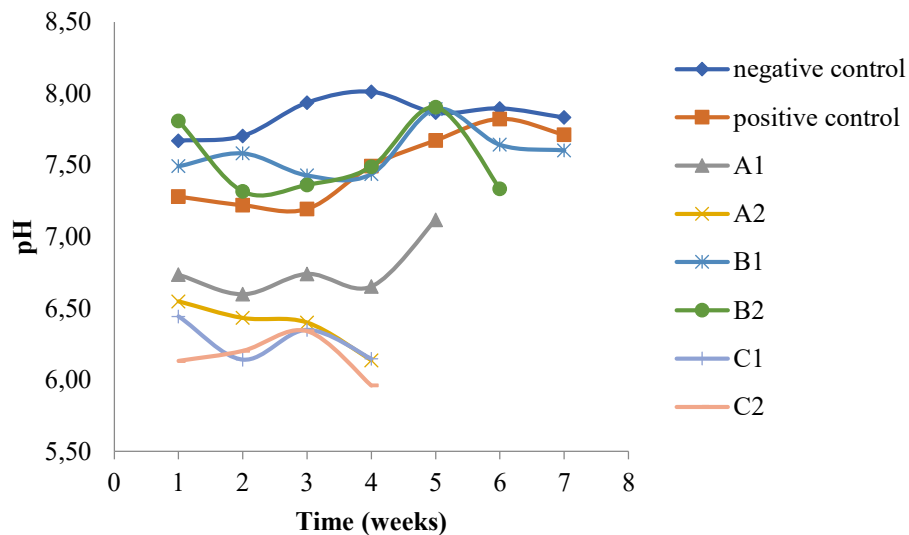


Figure 3. pH levels of the growth media with different biostimulant addition

As seen in Figure 3, as the medium became more acidic, the duration of growth observation shortened. This suggests that lower pH levels may worsen the conditions for propagule growth, whereas a pH closer to neutral, such as that found in treatments B1 and B2, supports optimal growth. A significant negative correlation was observed between pH and SGR, with a correlation coefficient (r) of -0.85 ($p < 0.01$). This strong negative correlation indicates that a decrease in pH significantly reduces SGR in *K. alvarezii* propagules. Therefore, pH appears to play a crucial role in determining the effectiveness of treatments on propagule growth, with overly acidic pH hindering growth processes.

Visual observations showed that propagules in media with low pH experienced changes in color and thallus structure (Figure 4). The thallus color changed to red or pink, and the structure became increasingly soft. In contrast, propagules in media with neutral or slightly alkaline pH remained brown, with the thallus structure staying elastic.



Figure 4. The color of *Kappaphycus alvarezii* propagules in growth media with neutral pH (A) and low pH (B).

Treatment A involved the addition of organic amino acids, while treatment C involved the addition of seaweed extract. Both products were derived from fermentation processes, which were indicated by the characteristic acidic odor when the products were opened. Fermented biostimulants, such as those used in treatments A and C, contributed to the observed reduction in pH. The fermentation process results in the production of acidic metabolites, which significantly lowered the pH of the media when these products were added. This decrease in pH is believed to have negatively impacted the growth of propagules.

Fermented organic biostimulants typically have low pH, making them acidic. This characteristic is primarily due to the fermentation process, which involves lactic acid bacteria. The low pH of these organic biostimulants helps extend their shelf life and reduces the need for special storage conditions (Atfaoui et al., 2021).

While fermented biostimulants have potential benefits in agricultural soils, their application in aquatic environments may be less favorable due to differences in biological and chemical dynamics between soil and aquatic systems. Seaweed-based biostimulants produced through Effective Microorganisms (EM) fermentation can enhance microbial abundance in agricultural soils during the vegetative stage, positively affecting soil fertility and crop yield (Prasedya et al., 2022).

Moreover, the fermentation process, which is beneficial for soil applications, may not yield similar results in aquatic environments. The pH and organic composition levels that optimize the production of volatile fatty acids (VFAs) in soil may not be suitable for aquatic systems, where microbial consortia and environmental conditions differ (Cheah et al., 2019). Considering the potential of fermented biostimulants to enhance growth, further studies may be required to develop treatments that reduce their acidity for more effective aquatic applications.

The biostimulant used in treatment B involved the addition of photosynthetic bacteria, which maintained a near-neutral pH and showed the most promising results in enhancing the growth of seaweed propagules compared to other experimental groups. The results for treatment B1 were considered most promising because the specific growth rate (SGR) of 0.22% per day was significantly higher than that of the controls and other treatments ($p < 0.05$). Photosynthetic bacteria play a significant role in promoting plant growth through various mechanisms, including nutrient acquisition, phytohormone production, and induction of systemic resistance to pathogens. One of the primary ways photosynthetic bacteria contribute to plant growth is through their ability to fix nitrogen and carbon dioxide, both of which are critical for plant development. These bacteria positively influence plant health by increasing nutrient availability and enhancing resistance to diseases (Cheng et al., 2022; Su et al., 2017).

The metabolic activities of photosynthetic bacteria not only enhance nutrient uptake but also lead to the production of various phytohormones, such as indole-3-acetic acid (IAA), which is essential for root and shoot growth in terrestrial plants (Lee et al., 2021). For instance, studies have shown that inoculating plants with photosynthetic bacteria can significantly increase chlorophyll content and overall photosynthetic efficiency, leading to improved carbohydrate synthesis (Rajpoot & Topno, 2023).

Moreover, photosynthetic bacteria can induce systemic resistance in plants, which is crucial for plant defense against pathogens. This induction occurs through the production of signaling molecules that trigger the plant's immune response, thereby enhancing its resistance to diseases (Cheng et al., 2022; Su et al., 2017). The interaction between photosynthetic bacteria and plants also involves complex microbial-microbial interactions, which can further improve plant health and productivity. This interaction can lead to changes in gene expression and physiological responses in plants, thereby boosting growth and crop yields (Harman et al., 2021).

Studies have demonstrated that the application of photosynthetic bacteria can significantly improve crop quality and yield in terrestrial plants. For example, a study on French bean plants showed that the use of photosynthetic bacteria combined with other biofertilizers resulted in improved growth and yield compared to the control group (Rajpoot & Topno, 2023). Additionally, photosynthetic bacteria have been shown to

enhance photosynthesis and carbon fixation in various plant species, contributing to better growth outcomes (Wu et al., 2020).

The growth and development of seaweeds are profoundly influenced by the availability of key nutrients, specifically nitrate and phosphate. These nutrients are essential for facilitating photosynthetic activity and supporting cellular growth within seaweed species. Furthermore, the application of biostimulants has been shown to enhance the concentrations of nitrate and phosphate in the growth medium during the initial of maintenance (Figure 5). Treatments A1 and A2 exhibited higher concentrations of nitrate and phosphate compared to the other experimental conditions. Specifically, Treatment A1 demonstrated a nitrate concentration of 0.70 mg L^{-1} and a phosphate concentration of approximately 0.76 mg L^{-1} . In contrast, treatment A2 showed elevated levels, with nitrate and phosphate concentrations of 3.04 mg L^{-1} and 1.37 mg L^{-1} , respectively. Meanwhile, Treatment C2 presented a higher nitrate concentration relative to phosphate, measuring approximately 0.60 mg L^{-1} .

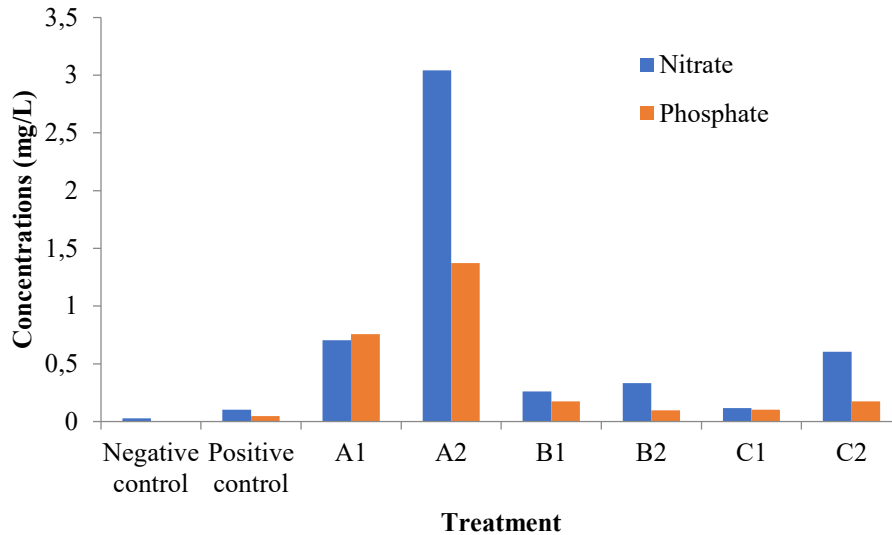


Figure 5. Nitrate and phosphate concentrations in the growth medium with different biostimulant additions

The concentrations of nitrate and phosphate were lowest in the negative control, which aligns with the expectation that, in the absence of any specific intervention or treatment, nitrate and phosphate levels would remain minimal. This suggests that in natural conditions, without additional inputs, nutrients such as nitrate and phosphate do not accumulate significantly. Conversely, the positive control group exhibited an increase in both nitrate and phosphate concentrations, indicating the presence of additional nutrient sources or inputs. The addition of PES in the positive control contributed to the increased concentrations, which were intended to support seaweed growth by providing supplemental nutrients. The optimal phosphate concentration for *K. alvarezii* is generally between 0.5 and 1.0 mg/L , while the ideal nitrate concentration ranges from 0.2 to 0.5 mg/L (Maradhy et al., 2022).

While treatments A1 and A2 exhibited higher nitrate and phosphate concentrations, this did not translate to improved growth. This suggests that nutrient availability alone is not the sole determinant of growth in *K. alvarezii* propagules. High nutrient concentrations may not be effective if other environmental factors, such as pH, are not within optimal ranges. Specifically, the low pH observed in treatments A1 and A2 likely limited nutrient uptake and utilization, even with high nutrient availability. This highlights the importance of maintaining a balanced environment, where pH and nutrient levels are both within optimal ranges.

Previous studies have shown varying relationships between nitrate/phosphate levels and *K. alvarezii* growth. For instance, some research has indicated that moderate increases in nitrate and phosphate can enhance growth, particularly in nutrient-limited environments (Suryati et al., 2015). However, other studies have found that excessive nutrient concentrations can lead to negative effects, such as increased epiphytic growth or physiological stress (Azad et al., 2017).

Conclusion

Biostimulants derived from bacteria, especially at a concentration of 2.5 mL/L (treatment B1), demonstrated significant potential in enhancing *K. alvarezii* propagule development compared to other treatments. However, while fermented biostimulants offer promising benefits, their associated acidity negatively impacted propagule health. Further research is recommended to optimize biostimulant formulations by balancing growth-promoting effects with pH stability to minimize adverse effects.

Additionally, field-scale trials should be conducted to assess the long-term viability of biostimulant applications in open-water cultivation systems.

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