

Developing hybrid tissue culture methods for disease-resistant marine algae in food industry applications

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Abstract

Objective

This study aimed to develop and evaluate a hybrid tissue culture method, called Somatic Embryogenesis-Micropropagation-Controlled Bioreactor Culture (SE-Mp-CBc), for producing disease-resistant marine algae. And enhance algae regeneration efficiency and marine microbial resistance for food industry-orient applications.

Materials and methods

To develop the SE-Mp-CBc system, marine algal explants were collected and cultured in nutrient-optimized media containing selective plant growth regulators and elicitors. The explants were sterilized and subjected to somatic embryogenesis and micropropagation processes under a sterile environment. To maintain the optimization, the pH, temperature, aeration, and nutrient flow were controlled and managed by the bioreactor system. The experiment was designed to improve and assess the regeneration process and relative resistance score (RS) to marine pathogens. The physical and molecular changes, like morphological changes, biomass yield, and disease resistance in the marine algae, were measured and analysed by the ANOVA statistical tool.

Results

In the algae regeneration, the SE-Mp-CBc showed a major improvement compared to the traditional methods. The regeneration process was successful by enhancing callus induction, shoot development, and tissue survival with the hybrid system. Regeneration efficiency improved across multiple strains, showing a clear advantage of the controlled bioreactor environment. Pathogen resistance also increased notably, with SE-Mp-CBc-grown algae showing a 35% decrease in infection severity and a 25% increase in biomass yield over conventionally grown samples. Disease resistance analysis confirmed more stable and elevated immune responses in bioreactor-cultured strains.

Conclusions

This hybrid tissue culture method gives a flexible, and efficient solution for marine algae cultivation. By merging somatic embryogenesis and micropropagation with bioreactor technology. The challenges like low generation rates, contamination risks and the limited disease resistance were faced by the conventional methods were overcome by the The SE-Mp-CBc system. This method gives the uniform tissue development and low stress in the algae cells, it supporting sustainable large-scale algae production. It was weather independent, yearly round operation makes it unique for food processing, nutraceuticals, and biodegradable packaging industries. Furthermore, research and development was needed for diver's algal species and economical improvement, but the approach provides a strong foundation for transforming marine biotechnology and securing algae as a reliable industrial crop.

Keywords: bioreactor, disease resistance, food industry, somatic embryogenesis, tissue culture

Paper type: Research paper.

Citation: Ramanathan, C. R., & Muthukumar (2026). Developing hybrid tissue culture methods for disease-resistant marine algae in food industry applications. *Agricultural Biotechnology Journal*, 18(3), 571-584.

Agricultural Biotechnology Journal, 18(3), 571-584.

DOI: 10.22103/jab.2026.25260.1712

Received: March 30, 2026.

Received in revised form: May 21, 2026.

Accepted: May 22, 2026.

Published online: June 30, 2026.

Publisher: Shahid Bahonar University of Kerman & Iranian
Biotechnology Society.



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Introduction

In recent years, marine algae have emerged as a focus of biotechnology research to support global food security and reduce the demand for the increasing world population (Pangestuti & Kim, 2011; Nakamura & O'Donnell, 2025). The ability, like rapid growth, high nutritional value, and growing without farmland or freshwater, makes them significantly scalable for eco-friendly food production (Sarvi & Pourmozaffar, 2024). However, the major problem in large-scale algae cultivation is microbial disease, which can efficiently affect the yield and reduce the production, which will affect the investor economically (García-Poza et al., 2020; Ayswaria et al., 2023). Conventional cultivation methods lack precision and control, limiting their ability to consistently develop disease-resistant strains (Boopathy et al., 2024; Kolandhasamy et al., 2025). Tissue culture techniques offer a sterile and controlled environment for algae propagation (Hwang & Park, 2020). However, contaminated tissue culture methods often result in poor regeneration efficiency (Sengupta & Deshmukh, 2024). These limitations underscore the need for stronger, more efficient propagation methods (Jiksing et al., 2022). A hybrid bioreactor system combining somatic embryogenesis and micropropagation, referred to as SE-Mp-CBc, is suggested as a

potential solution. It aims to enhance the regeneration rates and disease resistance by optimizing media and maintaining a sterile culture environment (Sharma & Nair, 2025; Sundarraj et al., 2025). It is also used to enhance tissue regeneration and disease resistance (Yuorieva et al., 2023). Strong algal development is promoted in the approach by the use of selective elicitors and better nutritional media (Topalova et al., 2024). Maintaining a sterile environment by bioreactor management helps lower the microbial infiltration risk (Sugumaran et al., 2022). The ultimate effect is consistent mass-producing disease-resistant algae using the great grade technique. This approach has greatly raised disease resistance and biomass yield (Wang et al., 2024). Using the hybrid technique in marine algae biotechnology guarantees the long-term survival of the food sector (Mahdy et al., 2022; Gürlek & Atay, 2021). This work investigates spore-based propagation methods (S-PMs) to improve development practices, increase genetic diversity, and strengthen selection of seaweed germplasm by Sundarraj et al., (2025). Stressing controlled reproduction methods for large-scale agriculture promotes sustainability and strain reduction for commercial goals (Vakhguel & Jianzhong, 2023). This method preserves the best features of seaweed and improves its longevity and breeding capacity (Chen et al., 2024). According to the studies, microalgae may provide a fresh approach to obtaining antimicrobial peptides (AMPs) that fight against resistance to antibiotics, bacteria, and viruses by Ayswaria et al., (2025) (Wang et al., 2024). It explores their operations, present limitations, and future possible therapeutic applications (Hu et al., 2021). According to bioplastics and bioenergy to food and agriculture (BBFA), this overview addresses the growing industrial and technical applications of seaweed by Kolandhasamy et al., (2025) (Sharma et al., 2025; Vivek et al., 2012). It clarifies how processing and technological developments have benefited the environment, increased people's resistance to climate change, and diversified the bioeconomy in many other ways, making seaweed a super crop (Parthasarathy et al., 2024). The SE-Mp-CBc system, proposed as a fresh approach to cultivating marine algae, is examined in this paper. It explains how the system operates, its benefits over conventional methods, and the components that cooperate. Furthermore, the paper explores the wider effects of implementing this hybrid culture technology on a large scale in the company. It emphasizes its use in generating sustainable, highly productive, disease-resistant algae. In addition, climate change has widespread impacts on biodiversity and has posed survival challenges to many plant and animal species (Farahvashi et al., 2026a). Increasing temperatures, changing rainfall patterns, rising sea levels, and extreme weather events have led to habitat changes, species population declines, and even extinctions (Pakgohar et al., 2026). Climate change also affects ecological interactions such as food chains, species distribution, and the timing of biological activities (Mohammadabadi et al., 2021). Climate change is one of the major global challenges that has widespread impacts on ecosystems and biodiversity. Increasing temperatures, changing rainfall patterns, rising sea levels, and extreme weather events have led to changes in habitat conditions, species population declines, and even the extinction of some of them. This phenomenon not only disrupts the ecological balance, but also affects the relationships between species, such as food chains and competition for resources (Farahvashi et al., 2026b). Therefore, this study aims to develop and evaluate a hybrid tissue culture method (SE-Mp-Cc) that can improve both regeneration efficiency and disease resistance in marine algae. This technique could

enable mass production of algae biomass for the food industry while reducing losses due to disease, contributing to sustainable marine agriculture.

Materials and methods

This part describes the experimental techniques for developing and running the SE-Mp-CBc system for marine algae propagation. It addresses subjects including bioreactor configurations, micropropagation techniques, somatic embryogenesis procedures, culture media formulation, and explant selection. These techniques were created to maximize regeneration effectiveness under regulated, repeatable conditions, assuring disease resistance; hence, they are perfect for use on a commercial basis.

Overview of proposed system SE-Mp-CBc: A big advantage of the SE-Mp-CBc system is its improvement of scalability, disease resistance, and regeneration in algal tissue culture. Using bioreactor-controlled conditions and improved processes addresses significant issues with traditional methods. In marine biotechnology, its economic feasibility makes it a significant advancement since it could result in more dependable and sustainable algae cultivation for various industrial applications. Parameters such as temperature, aeration, pH, and nutrient delivery are precisely regulated under bioreactor control as shown in Figure 1. Without sacrificing genetic integrity, the platform permits real-time monitoring and mass production. Its modular approach allows it to be easily altered to suit many types of algae. By optimizing biological output in a controlled and predictable environment and decreasing inputs, this integrated technology not only boosts propagation efficiency but also promotes sustainable aquaculture goals.



Figure 1. Schematic representation of the SE-Mp-CBc system and its impact on algal tissue culture efficiency

Quantitative evaluation formulations: Unlike traditional approaches, to quantitatively assess the effectiveness of the SE-Mp-CBc system, two key metrics were used. Regeneration success percentage and relative resistance score (RS). It will help to measure both tissue

regeneration efficiency and resistance to marine pathogens under bioreactor-controlled conditions.

$$\text{Regeneration success (\%)} = \left(\frac{\text{Number of regenerated tissue (PT)}}{\text{Total explants (sm)}} \right) \times 100 \quad (1)$$

This formula was used to evaluate the efficiency of the SE-Mp-CBc system in promoting tissue regeneration from algal explants. A higher regeneration percentage indicates a more effective tissue culture protocol. In this study, explants were cultured under controlled bioreactor conditions with optimized media and selective elicitors. The total number of explants inoculated (sm) and the number of successfully regenerated tissues (PT) were recorded after a fixed culture period. Regeneration success was calculated for each experimental group, and comparisons were made with control cultures maintained under traditional conditions. Results were statistically validated using ANOVA to determine the significance of observed differences ($p < 0.05$).

$$RS(st) = \sum_{t=1}^n \left(\frac{PT_{dt} \cdot ef_i}{if} \right) \quad (2)$$

Calculation of Relative Resistance Score (RS) for each algal strain based on accumulated pathogen response data over multiple time intervals was performed using Equation 2. The equation adjusts observed pathogen tolerance PT_{dt} by the system's resistance-enhancing effectiveness ef_i and normalizes it against the initial infection factor if .

Data collection and study design justification: To support the development of the SE-Mp-CBc system, a thorough literature review was conducted leveraging scholarly sources including PubMed, ScienceDirect, and Google Scholar. Scholarly publications, technical papers, and case studies provided research sources on bioreactor technologies, micropropagation, tissue culture from marine algae, and somatic embryogenesis. The main areas of attention were bioactive chemical interactions, microbial contamination management, and regeneration challenges. This paper obtained and assessed information on disease resistance indices, environmental factors, and nutritional media composition. This data from the literature helped to guide the choice of protocols and materials, ensuring that the proposed hybrid framework was based on tested-and-true processes and in accordance with existing research trends.

Methodological framework and statistical analysis: The methodological framework for the SE-Mp-CBc system integrates somatic embryogenesis and micropropagation within a bioreactor-controlled environment. Selected marine algae explants were sterilized and cultured in nutrient-optimized media containing specific plant growth regulators and elicitors. Somatic embryos were induced and multiplied through subculturing, followed by acclimatization in bioreactors under sterile, monitored conditions. Analytical methods included morphological assessment, biomass yield measurement, and pathogen resistance assays. Comparative data were collected from conventional culture methods as controls. Statistical analysis using ANOVA validated the significance of observed differences in growth rate and resistance, confirming the efficacy and reproducibility of the proposed hybrid cultivation approach.

Results

Analysis of regeneration efficiency: The SE-Mp-CBc system demonstrated significantly higher regeneration efficiency than conventional tissue culture methods (S-PMs, AMPs, and

BBFA) as shown in Figure 2. Regeneration rates increased with the number of samples in the range (10-100), and statistical analysis confirmed the differences were significant ($p < 0.01$ to < 0.0001) compared to other methods. The consequences of the optimal nutritional media and controlled bioreactor environment, which are accountable for these advantages, were reduced stress factors and homogenous tissue development across numerous strains of algae. The SE-Mp-CBc system led to improved callus induction, accelerated shoot development, and higher overall survival rates in algal samples. These factors collectively contributed to the consistently high regeneration ratios observed in SE-Mp-CBc-treated cultures.

Analysis of disease resistance: Algal strains grown with the SE-Mp-CBc system showed improved resistance to marine pathogens. Indices of tissue immune response were raised in controlled environments when elicitors were utilized, as shown in Figure 3. Disease resistance analysis (measured by the Analysis Ratio %) increased with sample size, with SE-Mp-CBc-treated samples displaying a more stable and elevated resistance pattern.

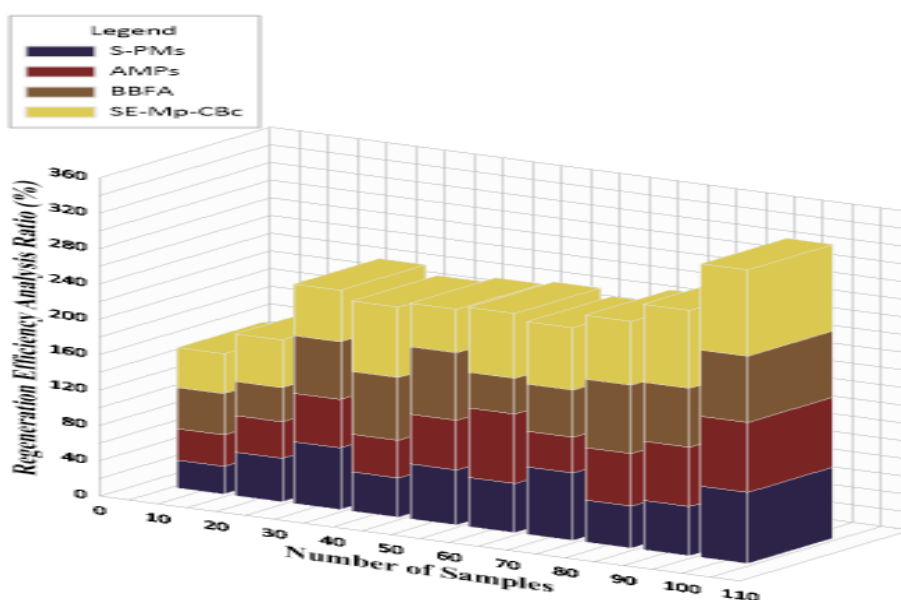


Figure 2. Comparative analysis of regeneration efficiency across different bioengineering approaches in algal tissue culture

When SE-Mp-CBc-grown algae showed a 35% decrease in infection severity over conventionally produced strains, the method's promise for producing disease-resistant algae for industrial usage was proven. Integrating the SE-Mp-CBc method to achieve high-efficiency algae growth. Standardized processes, clean surroundings, and ideal medium compositions guaranteed consistent results. The techniques guaranteed that the system could spread disease-resistant strains on a large scale without contamination, opening the path for industrial algae production and future biotechnological applications.

Common use of marine algae in various sectors, including agriculture: Particularly for the food industry, there is high anticipation that the SE-Mp-CBc system can transform industrial-

scale marine algae production. High-yielding, disease-resistant strains help remove a major barrier in traditional seaweed farming: crop loss brought on by diseases. The SE-Mp-CBc system enables a continuous, weather-independent production of processed algal biomass using bioreactor technology. Unlike manual processing and natural cultivation methods, this approach ensures year-round scalability as shown in Figure 4.

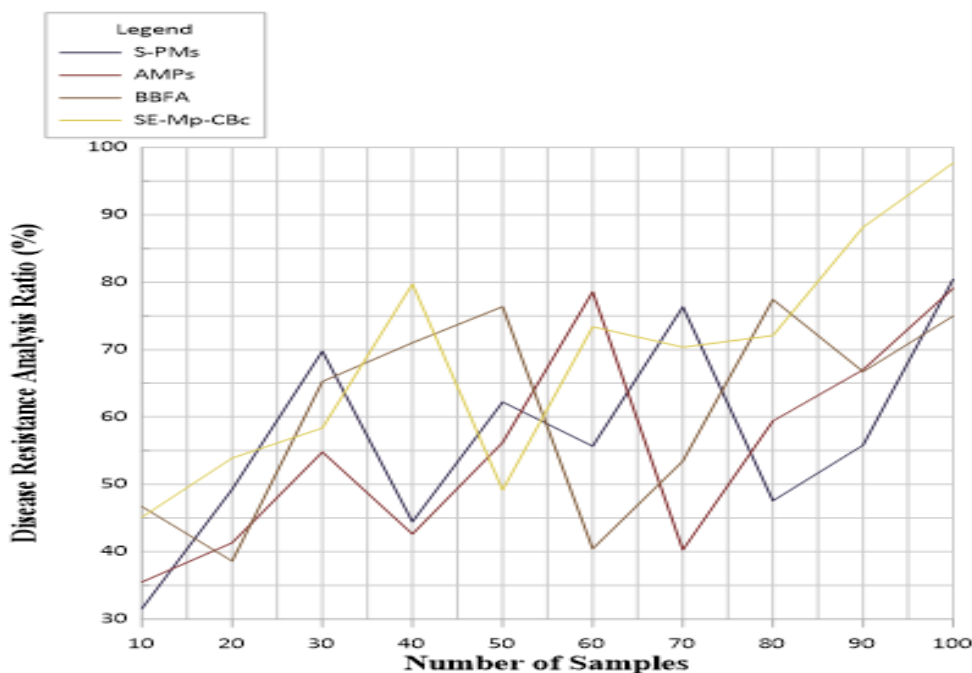


Figure 3. Disease resistance analysis across algal samples under different bioengineering treatments

Manufacturing can run nonstop all year round, independent of the weather, since bioreactor platforms allow one to scale up, as shown in Figure 2. Among other industrial uses where a consistent biomass supply is vital, this homogeneity is helpful in food processing, nutraceuticals, and sustainable packaging. A good thing is the declining need for chemical treatments and antibiotics in line with clean-label movements and legislative requirements for environmentally conscious manufacturing.

Discussion

A new method called the SE-Mp-CBc system was discussed in this study, which combines three techniques to grow the marine algae more efficiently, such as somatic embryogenesis, micropropagation, and bioreactor culture. The aim of this study is to develop marine algae with efficient, rapid growth and multiple disease resistance for the large-scale food-based industries and their application. Traditional tissue culture methods often face problems like slow growth and contamination. But with the SE-Mp-CBc system, these issues were greatly reduced. The controlled environment inside the bioreactor helped the algae grow more uniformly and avoided

contamination from harmful microbes. Using some chemicals and nutrients known as elicitors helped to improve the efficient disease resistance against the marine pathogens.

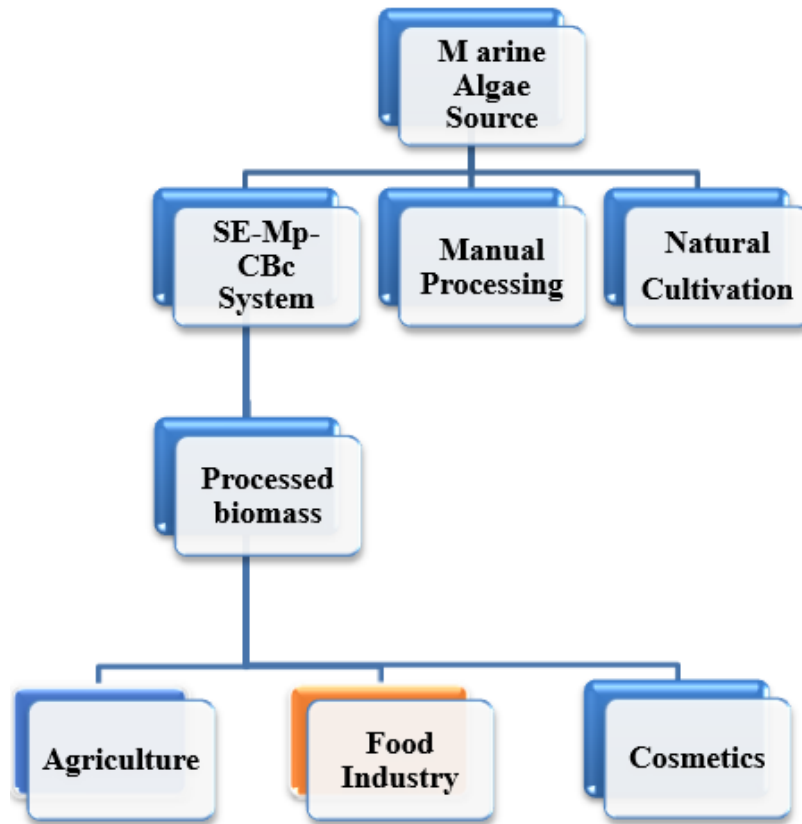


Figure 4. Process Flow from Marine Algae Source to Industrial Applications via SE-Mp-CBc System

The results showed that the algae grown by the hybrid method have 35% better disease resistance to the marine algae and gave 25% more biomass compared to the traditional method. It shows that the SE-Mp-CBc method is more efficient and promising for commercial applications. The next huge advantage is that the process can be done once a year, and it is weather-independent for the bioreactor. This allows for steady production, which is useful not just in food but also in making eco-friendly products like biodegradable packaging and health supplements. In short, the SE-Mp-CBc system provides a clean, controlled, and efficient way to grow strong, high-yielding algae. It helps overcome many of the problems found in traditional methods and supports the growing need for sustainable solutions in agriculture and the food industry.

Conclusions: With the SE-Mp-CBc system incorporating somatic embryogenesis with micropropagation and bioreactor management, marine algae production can be more tolerant to illnesses, more readily scaled up, and more regenerative. Combining the best aspects of conventional tissue culture techniques and our hybrid approach will help us achieve more success with less microorganism contamination, stronger strains for industrial usage, and extensive algae farming with an eye toward its use in food manufacture. Though promising, further development,

including changing bioreactor settings for different algae species and looking at affordable scaling techniques, will be required for the system's general acceptance. Two areas that will be the focus of the next research are the long-term stability of disease-resistant traits across generations and increasing diversity of algae species that can profit from the SE-Mp-CBc system. Practically speaking, one should also look at the extensive implementation's financial feasibility and environmental effects.

Author contributions

All authors contributed equally to all stages of the research. Both authors were involved in conceptualizing the study, methodology, data collection, analysis, and interpretation.

Acknowledgments

The authors would like to thank the Department of GMDSS (Global Maritime Distress and Safety System) and Marine Engineering, AMET University, Kanathur, Tamil Nadu, for providing access to academic resources and research facilities for the research.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The work was supported by the authors' respective institutions through internal resources and academic collaboration

Data availability statement

The data that support the findings of this study are currently not publicly available as the manuscript is under review and the study has not yet been published. However, the data may be made available from the corresponding author upon reasonable request.

Ethical considerations

This research did not involve human participants, animal testing, or the use of sensitive personal data. Therefore, ethical approval was not required. All methods and procedures used in this study comply with institutional, national, and international guidelines and regulations applicable to the field of research. The authors affirm that there are no ethical conflicts or issues associated with this work.

Conflict of interest

The authors declare no conflict of interest.

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توسعه روش‌های هیبریدی کشت بافت برای جلبک‌های دریایی مقاوم به بیماری در کاربردهای صنایع غذایی

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تاریخ دریافت: ۱۴۰۵/۰۱/۰۹ تاریخ دریافت فایل اصلاح شده نهایی: ۱۴۰۵/۰۲/۳۱ تاریخ پذیرش: ۱۴۰۵/۰۳/۰۱

چکیده

هدف: هدف این مطالعه توسعه و ارزیابی یک روش هیبریدی کشت بافت با عنوان «جنین‌زایی سوماتیکی-ریزازدیادی-کشت کنترل‌شده در بیوراکتور (SE-Mp-CBC)» برای تولید جلبک‌های دریایی مقاوم به بیماری بود. همچنین این پژوهش به دنبال افزایش کارایی باززایی جلبک‌ها و مقاومت آن‌ها در برابر میکروارگانیسم‌های بیماری‌زای دریایی برای کاربردهای مرتبط با صنایع غذایی بود.

مواد و روش‌ها: برای توسعه سامانه SE-Mp-CBC، ریزنمونه‌های جلبک دریایی جمع‌آوری و در محیط‌های کشت غنی‌شده از مواد مغذی حاوی تنظیم‌کننده‌های رشد گیاهی و القاکننده‌های اختصاصی کشت داده شدند. ریزنمونه‌ها استریل شده و تحت فرایندهای جنین‌زایی سوماتیکی و ریزازدیادی در شرایط استریل قرار گرفتند. برای حفظ شرایط بهینه، pH، دما، هوادهی و جریان مواد مغذی توسط سامانه بیوراکتور کنترل و مدیریت شد. آزمایش به‌گونه‌ای طراحی شد که فرایند باززایی و امتیاز نسبی مقاومت (RS) به عوامل بیماری‌زای دریایی بهبود یافته و ارزیابی شود. تغییرات فیزیکی و مولکولی شامل تغییرات مورفولوژیک، میزان تولید زیست‌توده و مقاومت به بیماری در جلبک‌های دریایی اندازه‌گیری و با استفاده از آزمون آماری ANOVA تحلیل شدند.

نتایج: در فرایند باززایی جلبک‌ها، سامانه SE-Mp-CBC در مقایسه با روش‌های سنتی بهبود چشمگیری نشان داد. این سامانه موجب افزایش موفقیت در القای کالوس، توسعه شاخه‌ها و بقای بافت شد. کارایی باززایی در چندین سویه افزایش یافت و مزیت آشکار محیط کنترل‌شده بیوراکتور را نشان داد. مقاومت به پاتوژن‌ها نیز به‌طور قابل توجهی افزایش یافت، به‌طوری‌که جلبک‌های

پرورش یافته در سامانه SE-Mp-Cbc کاهش ۳۵ درصدی شدت عفونت و افزایش ۲۵ درصدی تولید زیست توده را نسبت به نمونه های کشت شده به روش های متداول نشان دادند. تحلیل مقاومت به بیماری نیز پاسخ های ایمنی پایدارتر و قوی تری را در سوبه های پرورش یافته در بیوراکتور تأیید کرد.

نتیجه گیری: این روش هیبریدی کشت بافت، راهکاری انعطاف پذیر و کارآمد برای کشت جلبک های دریایی فراهم می کند. ترکیب جنین زایی سوماتیکی و ریزازدیادی با فناوری بیوراکتور موجب غلبه بر چالش هایی مانند نرخ پایین باززایی، خطر آلودگی و مقاومت محدود به بیماری در روش های سنتی شده است. سامانه SE-Mp-Cbc توسعه یکنواخت بافت و کاهش تنش سلولی در جلبک ها را فراهم کرده و از تولید پایدار جلبک در مقیاس وسیع حمایت می کند. استقلال از شرایط آب و هوایی و امکان بهره برداری در تمام طول سال، این روش را برای صنایع فرآوری مواد غذایی، نوتراسیوتیک ها و بسته بندی های زیست تخریب پذیر بسیار ارزشمند ساخته است. با وجود نیاز به پژوهش های بیشتر در زمینه گونه های مختلف جلبکی و بهبود اقتصادی، این رویکرد پایه ای قوی برای تحول در زیست فناوری دریایی و تبدیل جلبک ها به یک محصول صنعتی قابل اعتماد فراهم می آورد.

کلمات کلیدی: بیوراکتور، جنین زایی سوماتیکی، صنایع غذایی، کشت بافت، مقاومت به بیماری

نوع مقاله: پژوهشی.

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Publisher: Shahid Bahonar University of Kerman & Iranian

Biotechnology Society.



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