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Designing integrated bioreactors for marine plant tissue culture and coastal habitat restoration

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Abstract

Objective

The coastal ecosystem plays a major role in maintaining the environment and economic assets for humans. But the ecosystem was severely polluted by human activity and climate change. This study investigates the integration of a bioreactor with marine symbiotic microbes that restore the coastal habitat through innovative marine plant tissue culture. The objective of the study is to design the integrated bioreactors for Marine plant tissue culture and restore the coastal habitat. The restoration was done by the marine symbiotic micro-organisms, like Mycorrhizae, Nitrogen-fixers, Endophytes, Rhizosphere microbes, and Pathogens. The review was taken by focusing on the plant-microbe interaction across diverse coastal ecosystems, such as dunes, mangroves, swamps, and forests.

Materials and methods

On the other side, the bioreactors were designed by non-agitated systems, rotating wall vessels, tubular membrane bioreactors, and single-use reactors were evaluated for their ability and flexibility to use in the plant tissue and cell cultivation. Key design features such as aeration, mixing efficiency, nutrient uptake, and shear stress mitigation were thoroughly examined.

Results

As a result, the coastal symbiotic microbes can efficiently influence the coastal ecosystem in coastal plant community structure and function, promoting resilience against salinity, nutrient limitation, and biotic stress. The advanced bioreactor engineering has improved mass propagation potential, reduced operational cost, and facilitated large-scale micropropagation of marine plant culture. The integration of microbiome-based inoculation within a bioreactor system provides a solution to naturally restore degraded habitats, improve plant growth, and monitor the ecosystem health by indicator microbes.

Conclusions
The combination of microbial ecology with plant tissue culture with bioreactor technology can give a valuable and efficient scalable restoration of the marine ecosystem. Further research is needed on the inoculation of the culture and bioreactor optimization is arranged for species-specific application. This approach holds the substantial promise for reinforcing the ecological resilience of vulnerable coastal regions amid global environmental change.

Keywords: Bioreactors, costal ecosystem, plant-microbes interaction, plant tissue culture, symbiotic microbes

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Introduction

Coastal regions provide essential benefits to humanity. They make up 21% of the global land surface, housing 39% of the human population and 55% of the world's major cities (Kerfouf et al., 2023). These areas contain unique environments that serve as reservoirs of biodiversity while delivering crucial environmental services such as food production, storm surge mitigation, pollution control, carbon sequestration, recreational opportunities, and preservation of cultural heritage (Heckwolf et al., 2021). Despite their importance to humanity, many coastal systems are declining due to various stresses that threaten their integrity and the provision of ecological services (Negi et al., 2024). Prior evaluations of marine ecosystems have examined the present condition of these systems, the effects of climate change, their resistance to such changes, and management strategies (Khanam et al., 2022; Myoa et al., 2023). These evaluations have thoroughly established that human global change significantly affects coastal ecosystems. Climate change results in rising sea levels, leading to flooding and deterioration of coastal habitats, including marshlands and dune ecosystems (Shukla et al., 2021; Myoa et al., 2023). Warming correlates with heightened strength and frequency of cyclones, which impact coastal regions. Industrial, agricultural, and oil spill pollution has destroyed and eroded marshes and mangroves (Roy et al., 2023; Süren & Angin, 2019). Prior evaluations have emphasized the challenges and unique characteristics of the repair and reconstruction of coastal systems, highlighting the predominance of partial recuperation and feedback mechanisms that sustain coastal structures despite deteriorated conditions (Veera et al., 2024; Tan et al., 2022). Previous research underscores the significance of biodiversity and interconnectivity in maintaining the adaptability of coastal ecosystems. Classical in vitro development, including direct and indirect paths, has been established for several plant species, enabling organism generation from explants through organogenesis or embryogenesis. Plant micropropagation via Somatic Embryogenesis (SE) offers numerous advantages over organogenesis, such as the bipolar structure of the embryo, the ability for single cell regrowth without vascular connection to maternal callus cells or a cultured explant, and the need for only one hormonal introduction (Quinn et al., 1985: Barredo-Damas et al, 2010; Khan & Taha, 2023). Despite these benefits, the automation of large-scale embryo production is only achievable through Bioreactor (BR) technologies. BRs also provide additional advantages, including continuous adjustment of operational parameters, enhanced nutrient absorption, and streamlined management of large culture volumes. Given these characteristics, BRs have become suitable systems for modern industrial Plant Tissue and Cell Cultivation (PTCC). BRs have evolved since their initial use in cultivating *Begonia hiemalis* (Gantait & Mukeherjee, 2021; Sethupathi et al., 2024). Therefore, investigating advancements in BR technology over time sheds light on the current state and future innovations. This research outlines trends and significant advancements in BR technologies within PTCC, including associated challenges and future potential (Quinn et al., 1985; Khan & Taha, 2023; Ravshanova et al., 2024).

Design principles of bioreactors for plant tissue culture cultivation

The BR concept for PTCC involved transitioning from solid (agar) medium and liquid media to enhance the efficacy and productivity of plant growth (Quinn et al., 1985; Khan & Taha, 2023). An optimal BR design must fundamentally consider various factors, such as the generation of a substantial quantity of plantlets in a single batch, scalability, a minimal number of culture vessels, straightforward vaccination and harvesting processes, continuous contact between the growing surfaces and the substrate to facilitate nutrient absorption and promote growth acceleration, and airflow capacity to ensure an adequate supply of oxygen for improved growth rates and final biomass output (Lee et al., 2010). Establishing an effective free-flow circulating method for PTCC aims to reduce apical dominance and boost shoot bud formation (Rincon et al., 2024; Nair & Rao, 2023; Assegid & Ketema, 2023). Based on the features, substance, and steel-based BR equipped with turbines for plant micropropagation, they are mainly classified into: (a) mechanically agitated structures, for example aeration-agitated BR, turning drum BR, and spin filtering BR, (b) manually agitated structures, and (c) non-agitated structures, for example gas-phase BR, air-transparent membrane aerator BR, and overlaid airflow BR. Contemporary BR models seek to address foaming, strain, and pressure issues (Lee et al., 2022).

Coastal plant-microbial symbioses

The research concentrates on five main categories of microbial symbionts: mycorrhizae, nitrogen-fixing organisms (diazotrophs), endophytes, rhizosphere microbes, and pathogens. Certain groups intersect (nitrogen-fixing organisms are rhizosphere microorganisms), although these are prevalent classifications of symbionts addressed in microbiology and conservation.

Mycorrhizae: Mycorrhizal fungi establish mutualistic relationships with plant roots in many environments, especially coastal areas like marshes, waterways, mangroves, sandy beaches, and heaths. The symbiotic relationship between fungi and plants is particularly crucial for plants in demanding settings, such as initial succession in dune fields, since mycorrhizae enhance nutrient intake and improve resistance to salt and drought damage. Arbuscular mycorrhizal fungi are associated with 72% of plant species, such as grasses, shrubs, and trees that inhabit coastal regions. Several plant families or species prevalent along coastlines are non-mycorrhizal, for example Chenopodiaceae, which encompasses *Salicornia* and pickleweed or exhibit poor or inconsistent mycorrhizal colonization, for example Cyperaceae, which contains Carex and sedges. There is ongoing discourse over how elevated saline stages, such as those in oceans and soils, impede arbuscular mycorrhizae. The role of arbuscular fungi as either facilitators or inhibitors of plant development in salty settings varies depending on context and the host species.

Nitrogen-fixers: Initial production and breakdown in mangroves, swamps, and dunes are constrained by nitrogen, and coastal ecosystems rely significantly on nitrogen supplies from plant-associated algae. In mangrove ecosystems, Nitrogen-fixers are estimated to provide 45% to 65% of the ammonium necessary for plant growth. Nitrogen-fixation is constrained by carbon availability, leading to heightened activity of Nitrogen-fixers in the soil's shape, roots, and litter strata, with N-fixing rates closely linked to plant metabolism and breakdown processes. N-fixation occurs in anaerobic conditions; hence, saturated anoxic environments such as wetlands and swamps are optimal homes for this process. Multiple adaptations facilitate N-fixation in variable or elevated oxygen settings, including the rhizosphere and arid soils.

Endophytes: Endophytic fungi and bacteria inhabit coastal sedges' roots, stems, leaves, and flowers, grazing forbs, and forests. Despite little research, coastal flora has been seen to harbor significant differences in endophytes, such as bacteria and fungi, previously documented in marine, terrestrial, and freshwater environments. Endophytes reside unnoticed inside plant tissues, serving purposes such as antagonizing or facilitating plant diseases and enhancing the stress resistance of host plants. Extensive studies on endophyte roles in plants have focused on agricultural species, with less investigation into coastal species. Research on dune systems indicates that a fungus leaf lichen significantly enhances the survival, belowground material, vegetative development, and sand build-up of *Ammophila breviligulata*, with serious consequences for dune history and stability. A notable instance is the use of endophytes, isolated from mangrove organisms, to inoculate and enhance the development of a kind of tree utilized for rehabilitation in Brazil. The pressures of living in a coastal region have prompted changes in the host species and their internal microbes, making endophyte research in coastal environments promising for comprehending stress tolerance and symbiotic relationships.

Microorganisms in the rhizosphere: Like other terrestrial ecosystems, the rhizosphere microbiological populations in coastal environments exhibit considerable differences from bulk soil, characterized by increased populations of taxa recognized as endophytes, mycorrhizae, and crop growth-promoting microbes. In dunes, plant roots serve as vital carbon sources for microbial communities within a matrix marked by resource scarcity and significant physical stress. In flooded coastal ecosystems, roots serve as a substantial supply of carbon, facilitating microbial activities essential for N-fixation and plant breakdown. Sulfate-reducing microbes account for over fifty percent of the overall breakdown of biological material in salt marsh environments and have shown tolerance to settings such as the rhizosphere, characterized by rapidly fluctuating redox circumstances. Indeed, the processes of fixation of nitrogen and sulfate reduction, at times conducted by the same creature, are significantly elevated in the rhizosphere compared to bulk soil.

Microorganisms that cause disease: Pathogens have a crucial role in influencing the dynamics of natural communities in coastal ecosystems. In coastal deserts, soil infectious agents, including pathogenic fungi and parasitic worms, induce plant species substitution and support succession in the ecosystem. Fungal infections are inherent in coastal forests, resulting in winter seedbank loss within coastal sage scrub ecosystems. In coastline sandy dune fields, the invasive *Ammophila arenaria* facilitates its invasion by collecting local diseases that adversely impact native flora. The extent of invasion of *Ammophila arenaria* in coastline dune ecosystems is determined by its escape from nematode parasites and the biotic barrier provided by local soil pathogens that impede its invasion. Likewise, indigenous diseases restrict the spread of *Bromus diandrus* into the California coastline sage scrub. Organisms greatly affect the ecological makeup of coastal areas. Future research aimed at comprehending and regulating pathogens linked to die-off incidents, managing those associated with species assaults, and elucidating the impact of shifting circumstances on the evolution of diseases will be crucial for preserving the vitality of coastlines.

Principal types of bioreactors for plant tissue culture cultivation

BRs with helical ribbon impellers: By the early 1990s, spiral and dual Helix Ribbon Turbines (HRT) were integrated into BRs to accommodate shear-sensitive suspensions in suspension. Notwithstanding the presence of surface barriers that influence power input, HRT biological reactors mitigated shear damage and enhanced power dissolution, mixing rates, and

Oxygen Transfer Rate (OTR), particularly in viscosity plant cell cancellations, by producing, re-dispersing, and encircling bubbles from the top gas contact. Helical protesters proved to be effective for the suspended culture of high-density crops. A further attempt at effectively extracting California poppies was undertaken in BRs.

Rotating wall vessels (RWV) BRs: RWV biological reactors were first developed for mammal cultures in gravity and continue to be utilized in human cell research. The cell types of the Japanese yew tree were uniquely treated in an RWV-BR. The findings indicated that an additional metabolite generated by the plant, known as Taxol, is adjusted in response to shear stress. On day 15, 5 mg L-' of Taxol was generated in the culture at a shear stress of 2.4×10^{-3} Nm^{-2}. Moreover, the generation of the element was inhibited under high shear circumstances, during which cells have sustained significant damage in the latter stages of the culture phase. Under zero shear conditions, Taxol production was comparable to that seen under medium shear strain levels. The process by which plant cells perceive acute shear pressures and translate them into electrophysiological and biological responses remains ambiguous. Tubular Membrane BR (TMBR) using various barrier arrangements, including hollow fibers or ceramic veins, has been engineered to possess elevated surface-to-volume percentages, facilitating dense plant cell growth beneath minimal stress conditions. The disadvantages of these BRs include inadequate oxygen transfer rates, intricate operations, and restricted scale. The TMBR idea emulates tissue-like circumstances akin to the vessels, arteries, and lymphatic vessels seen in live tissue. TMBRs are seamlessly coupled with cell immobility structures, since the outer layer functions as an immobilizer, retaining the cell culture inside the reactor chamber. It serves as a substrate for adhering cell types. TMBR has been effectively used to improve the recovery of betanin, which is from carrot, and ajmalicine, a substance from Madagascar snail cells.

Single-use BRs: Disposal BRs (DBRs), or single-use BRs, are constructed from single-use plastic bags and designed for plant cell suspension culture. DBRs are efficient regarding time and expenses, preventing cross-contamination and minimizing changeover time among runs. Given the significant influence of neuroproteomics-assisted methodologies in medicinal cultivation and molecular agriculture, DBRs provide innovative characteristics for producing pharmaceuticals and translational protein derivatives. DBRs are integrated with diverse BR technologies; scalability presents a significant hurdle to their advancement.

Super-spinning bioreactors (SSBR): SSBRs, equipped with an internal cell stirrer operating at low velocity, are appropriate for plant cell suspension cultivation due to their ability to minimize shear stresses. The efficacy of SSBR varies across various species of plants. The investigation yielded outstanding outcomes with the cell cultivation of Maire's yew. However, the identical BR proved ineffective for the somatic embryogenesis of *Lilium*.

Microbiomes as nature-based strategies for the rehabilitation of marine ecosystems

Nature-based solutions, including those using microorganisms, are increasingly recognized worldwide as a means to tackle ecological and social concerns. The research proposes integrating microbiome research with nature-based solutions to enhance restoration efforts for various habitat-forming marine organisms. Characterising the microbiome and detecting advantageous microbial species or their possible metabolites might facilitate the screening and choice of the "meta-community and meta-ecosystem", for example vegetation and its associated sediment microbiota and minerals for transplantation. Historically, screening and selection were conducted empirically, for example through visuals); however, contemporary methodologies utilize multi-omics and computerized methods to find living things, colonies, or sand clods for translocation to reconstruction sites and to determine the optimal substrate for egg or propagule agreement. Alterations in the structure of the microbiome are regarded as an early indicator of holobiont wellness; microbiome analysis is especially beneficial in deep-sea environments, for example cold-water reefs for choosing and monitoring fit living things post-translocation or transplantation. Biomonitoring microbiota in habitat-forming animals is now feasible due to established methodology and readily available tools (Mohammadabadi et al., 2021). It does not serve as a means to rectify unsuccessful restoration efforts, but rather as a tool for the early identification of possible pathogens and for facilitating prompt decision-making, such as halting disease transmission. Probiotics and bio-promoter nutrients, upon demonstration of their efficiency, might enhance the holobiont's resilience, for example to algae blooms and other stressors before transplantation, as well as preserve or enhance its physiological conditions post-transplantation. Alternative microbiome-based options for restoration interventions involve separating microbiome constituents using culturomics methodologies, enabling the selection of bacteria that generate viable probiotics or bio-promoters for inoculation into transplanted species. Alternative approaches using culturomics involve natural microbiome-assisted development, which is employed to select microbiological taxa exhibiting optimal tolerance to stress, hence enhancing the fitness of transferred organisms. These methodologies are marked by limitations, for example challenges in culturing marine microbes and probiotic production, rendering their implementation particularly challenging for large-scale treatments. Due to the pressing need for marine ecosystem reconstruction, it is essential to enhance culturomics-based methodologies.

Conclusion: Mycorrhizae, N-fixing organisms, endophytes, rhizosphere microorganisms, and pathogens are essential to properly functioning coastal environments. They significantly influence the health of the plants that are the basis of coastal towns, upon which a vast network of animals, fisheries, and people depends. These tiny symbionts influence plant community, development, competitive capacity, and stress resilience while regulating biogeochemical turnover in coastal ecosystems. The use of microbial communities to enhance the restoration of stressed and degraded coastal ecosystems is still nascent; yet, it has significant potential for improving the effectiveness of restoration along the coast. An extensive study is essential to evaluate the role of microbes in various coastal environments, which will be crucial for creating localized, efficient inoculation for application in restoration areas worldwide. This presents a unique chance for cooperation between restoration professionals and microbial environmentalists to further the shared objective of enhancing the durability of the coastal environments. These interactions are significant since these structures face heightened vulnerability to several stressors, while their ecological functions gain significance due to global warming.

Author Contributions

Both authors were contributed equally.

Data Availability Statement

No new data were generated or analysed during this study. All information presented is derived from previously published sources, which are appropriately cited.

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Ethical Considerations

This study is based solely on a review of existing literature and does not involve any experiments on humans, animals, or genetically modified organisms conducted by the authors. As such, ethical approval and informed consent were not required. All references, data, and studies have been properly cited by academic and publishing ethics.

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چکیده

هدف: اکوسیستم‌های ساحلی نقشی اساسی در پایداری محیط‌زیست و حفظ منابع اقتصادی انسان دارند، اما این زیست‌بوم‌ها به‌طور جدی تحت تأثیر آلودگی ناشی از فعالیت‌های انسانی و تغییرات اقلیمی قرار گرفته‌اند. این پژوهش به بررسی تلفیق بیورآکتور با میکروارگانیسم‌های همزیست دریایی می‌پردازد که از طریق کشت بافت نوآورانه‌ی گیاهان دریایی، به بازسازی زیستگاه‌های ساحلی کمک می‌کنند. هدف این مطالعه، طراحی بیورآکتورهای یکپارچه برای کشت بافت گیاهان دریایی و بازسازی زیستگاه‌های ساحلی است. بازسازی با بهره‌گیری از میکروارگانیسم‌های همزیست دریایی نظیر میکوریزا، تثبیت‌کننده‌های نیتروژن، اندوفیت‌ها، میکروب‌های ناحیه ریشه (ریزوسفر) و پاتوژن‌ها انجام می‌شود. این مرور با تمرکز بر تعامل گیاه و میکروب در اکوسیستم‌های گوناگون ساحلی از جمله تپه‌های شنی، جنگل‌های مانگرو، مرداب‌ها و جنگل‌های ساحلی انجام گرفته است.

مواد و روش‌ها: در مرحله طراحی، انواع بیورآکتورها شامل سیستم‌های بدون هم‌زن، راکتورهای دیواره‌چرخان، بیورآکتورهای غشایی لوله‌ای و راکتورهای یک‌بار مصرف مورد ارزیابی قرار گرفتند تا میزان قابلیت و انعطاف‌پذیری آن‌ها در کاربردهای کشت بافت و سلول‌های گیاهی تعیین شود. ویژگی‌های طراحی کلیدی مانند هوادهی، کارایی اختلاط، جذب مواد مغذی و کاهش تنش برشی به‌صورت جامع بررسی شدند.

نتایج: نتایج نشان داد که میکروارگانیسم‌های همزیست ساحلی می‌توانند به‌طور مؤثر ساختار و عملکرد اجتماعات گیاهان ساحلی را تحت تأثیر قرار داده و مقاومت آن‌ها را در برابر شوری، کمبود مواد مغذی و تنش‌های زیستی افزایش دهند. به‌کارگیری مهندسی پیشرفته بیورآکتورها موجب افزایش توان تکثیر انبوه، کاهش هزینه‌های عملیاتی و تسهیل ریزتکثیر در مقیاس وسیع کشت گیاهان

دریایی شده است. ادغام تلقیح مبتنی بر میکروبیوم در سامانه بیورآکتور، راهکاری طبیعی برای بازسازی زیستگاه‌های تخریب‌شده، بهبود رشد گیاهان و پایش سلامت اکوسیستم از طریق میکروب‌های شاخص ارائه می‌دهد.

نتیجه‌گیری: ترکیب بوم‌شناسی میکروبی با فناوری کشت بافت گیاهی و فناوری بیورآکتور، رویکردی ارزشمند، کارآمد و مقیاس‌پذیر برای بازسازی اکوسیستم‌های دریایی فراهم می‌آورد. انجام پژوهش‌های بیشتر در زمینه تلقیح کشت‌ها و بهینه‌سازی بیورآکتورها برای کاربردهای اختصاصی گونه‌ای ضروری است. این رویکرد چشم‌انداز امیدبخشی برای تقویت تاب‌آوری بوم‌شناختی مناطق ساحلی آسیب‌پذیر در مواجهه با تغییرات زیست‌محیطی جهانی دارد.

کلمات کلیدی: اکوسیستم ساحلی، بیورآکتور، تعامل گیاه و میکروب، کشت بافت گیاهان، میکروارگانیسم‌های همزیست

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