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Assessing the feasibility of marine animal tissue culture for lab-grown seafood in maritime systems

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Abstract

Objective

The current study attempts to explore the viability of using marine animal TC to generate seafood in laboratories. The current work focuses on exploring the biological and technical constraints associated with the large-scale culturing of marine organisms, especially in generating CBS. The current research is expected to shed light on the potential of CBS as a sustainable alternative in the seafood sector.

Materials and Methods

Tissue cultures of marine organisms, which include fish and crustaceans, have been utilized to assess the viability of producing CBS on a large scale. This includes the use of cell line generation, the creation of serum-free media, and the design of bioreactors. The integration of genetic engineering with conventional AC techniques was used to establish an ideal growth environment for the cells. Several culture conditions were assessed, including resistance to hypoxia, regulation of pH, and adaptation to low temperatures.

Results

This experiment indicated that marine animal cells, especially those from fish and crustaceans' muscles, were better adapted to hypoxia and low temperatures than terrestrial cells. This is beneficial for CBS fabrication since it facilitates effective growth and differentiation of cells in the bioreactor. Furthermore, cell cultures derived from fish tissues were capable of forming tissue-like constructs on biodegradable scaffolds, which could pave the way for growing seafood in the laboratory. However, there were challenges associated with the creation of serum-free medium and bioreactor design.

Conclusion

In conclusion, marine animal tissue culture can potentially be used as a viable technology for growing seafood sustainably. While there are a myriad of physiological benefits associated with using marine cells in the manufacture of cultured seafood, much remains to be accomplished in

addressing some technical challenges in this area, including the development of serum-free medium, culturing of cell lines, and bioreactor design. Solving these problems could usher in a paradigm shift in the industry, offering a sustainable solution to the burgeoning global demand for seafood.

Keywords: marine, maritime, seafood, tissue culture

Paper Type: Research Paper.

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Introduction

The depletion of the earth's resources and climate change are a major concern, as the world's population keeps growing, and safe and quality food is crucial. (Maja & Ayano, 2021). The amount of food produced will need to be significantly higher to meet the growing demand in an efficient and sustainable manner, supplying the projected 12 billion people by 2060 (Hajam et al., 2023). As the world's population grows, demand for meat is expected to increase by 75% from the current level but the supply of meat on the planet by 2060 will not be enough to meet this demand. In this overall global issue, the aquatic resources offer protein-rich diet with omega-3 fatty acids and available minerals (Sen et al., 2025). The low productivity of fish harvested from the offshore fisheries and the threats of sustainability of Aquaculture (AC) activities are great concern (Mohammadabadi et al., 2021; Samarajeewa, 2023; Kwon et al., 2024). To sufficiently nourish the expanding world population by 2060, a 100 percent rise in lab-grown seafood output is anticipated as a need (Morro et al., 2022). Future estimates of capture AC at 2060 are 97.4 million tons and fishing output 150 million tons. Sustainable practices will be limited by the challenges of AC, as future improvements in fish output are largely reliant on this. Therefore, substantial efforts are needed to boost feed production for the AC industry, build up environmental quality, relax pressure on wild aquatic resources used for feed, and provide quality aquatic products to consumers (Zigui et al., 2024). These issues need the advancement of new aquatic food supplies using cell-cultivated methods.

Cell-Based Seafood (CBS)

CBS has captured attention for its potential as a sustainable and eco-friendly method of food production, with the ability to culture cells of animals in vitro using Tissue Culture (TC)

techniques to generate edible, lab-grown seafood products without killing the animal (Ganesan et al., 2024 and Badr-Eldin et al., 2022). Cellular farming is a major revolutionary food production technology that was developed to address the above challenges, and which started with the development of goldfish. The production of large numbers of lab-grown units of cells and organs relevant to seafood production requires a large TC in the case of CBS. With traditional food processing techniques these tissues or cells are used to make unstructured products such as surimi or fish bones (Wang et al., 2021). They can be grown on 3D biomaterial scaffolds to generate structured products, such as fish fillets. The advantages of growing CBS over local fish species are better freshness, reduction of inedible wastes like bones, skin, shells, and scales which negatively impact the environment (Setthawong et al., 2026). CBS can shorten time in food manufacturing process and allow the production of food continuously; TCs require several weeks to make functional food and can produce it continuously. Compared to terrestrial animals, fisheries and AC are relatively environmentally friendly farming methods; the challenges of overfishing, pressure on wild stocks, emergence of new diseases and bacterium resistant to antibiotics, climate change, and ocean acidification have a negative impact on organism biology, biodiversity, and migration of species (Zhang et al., 2023). Additionally, byproducts of manufacturing, microplastics, chemical pollutants in aquatic environments, and insufficient clean water necessitate the lab-grown seafood industry to adopt alternatives and creative farming structures to address these pressing issues (Yan et al., 2024). AC, an ancient technique shown by visual engravings indicating its usage by Egyptians for fish about 2600 BC, gained significance beyond survival and small-scale industry only in the 1980s. AC has transitioned from extensive systems reliant on Mother Nature with limited human involvement to semi-intensive and demanding systems, where producers meticulously manage conditions for development through nutrition, reproduction, disease management, and waste elimination, leading to significantly increased production rates (Yogamadhavan & Mannayee, 2024). The spread of AC also poses problems, analogous to those of farming on land, such as the production of phosphate and nitrogenous wastes due to the uneaten feed and metabolic wastes. This makes it more likely that environmental conditions will be impacted, such as streams being degraded and the development of algae blooms. The higher the intensity, the greater will be the risk of transmission of disease transmission energy (Xu et al., 2024) from the cultivated fish to indigenous species. Research on the welfare of many species that are cultivated for AC has been lacking, which has led to no definitive welfare guidelines for cultured aquatic organisms and thus remains unclear the ethical implications of AC (Goswami et al., 2024). Marine catch no longer surpasses the amount of seafood that people consume through AC. The landings from marine fishery have reached a plateau of 92 million tons per year on average throughout the world (Chandrasekharan & Radhakrishnan, 2025). But at the global level, the mariculture of marine life and shellfishes has increased almost four times in the same period, which means that agriculture has not reduced the dependence on the wild caught fisheries. AC is viewed as a remedy for overpopulation; however, a number of farmed fish use feed made from marine catch. AC actually reduces the number of fish in the world, as a predatory farmed fish will eat multiple marine species that are caught (Myoa et al., 2023). While fully-controlled AC can be more efficient for farming than wild-growing ones,

there is no research into fully controlled lab-grown seafood (cell cultivation) vs marine livestock husbandry. Genetic manipulation has significantly enhanced production levels, feed conversion efficiencies, and shortened growth periods for fish in controlled environments. Genetically engineered rohu grow nearly four times more rapidly than their conventionally produced equivalents, and engineered mud loaches grow 38 times quicker and too much greater sizes. Genetically-modified tilapia, which include a salmon development hormone linked to an oceanic pout coolant promoter gene, achieve growth exceeding 340% compared to non-transgenic fish raised under the same conditions. The gene, encoding regulatory components from ocean pout and growth hormones, constitutes the "all fish" chimeric sequence of genes that AquaBounty fish utilizes. AquaBounty salmon, authorized for production and distribution in Canada, grow at double the pace of their wild counterparts and have a feed conversion rate 12% superior to that of farmed fish. The species are important sources of food and are of environmental interest, so they are only raised as triplets, sterile females, in a closed, inland aquaculture system. Despite these security measures, significant regulatory barriers and legislative pressures have blocked the fish route to the US market. These fish are a shining example of how bioengineering can help increase the production of lab-grown seafood in closed systems. These genomic improvements in closed AC facilities are an essential stepping-stone to future improvements in CBS production.

Proposed marine animal TC for lab-grown seafood

Just as with cell-based meat production, CBS relies on complicated scientific progress for maximizing cell line efficiency, media composition, and bioreactor design. A CBS manufacturing system, like any closed TC system, is made up of the following components integrated into the process: appropriate cell type(s) from the desired tissue type; a growth medium providing the nutrients for cell proliferation and differentiation; and a fermentation chamber providing the closed environment required for cell growth. A biodegradable scaffold is a key component to provide structural support for cell development and maturation in 3D organs. During this period, a Muscle Protein Manufacturing System (MPPS) was developed for zebrafish, funded by the National Aeronautics and Space Administration (NASA) and no other cases of CBS production were reported. Bioreactors are highly complex closed system environments which require many parameters to be monitored, maintained and modified through feedback loops in order to control the biomass production process. The characteristics of the native fish muscle cells make the ocean cell lines more tolerant of the temperature, pH and oxygen levels than mammalian cell lines. This difference significantly affects energy use and economy of the mass production of CBS (Figure 1). These disparities provide a novel array of research prospects for cellular farming and large-scale tissue design; most medically driven tissue science concentrates on producing smaller cells, often not exceeding the size of organs.

Oxygen necessities

Mammalian TC: Suboptimal oxygen levels adversely affect mammalian cell proliferation and recombinant protein synthesis; thus, monitoring and maintaining dissolved oxygen content and air humidity in bioreactor devices are critical parameters. Air saturation values of 45-65% are

typically necessary for mammalian cell growth. In three-dimensional, highly-vascularized cells, the saturation rates and levels are more important, because the oxygen transport limits the tissue growth to approximately 1.7mm.

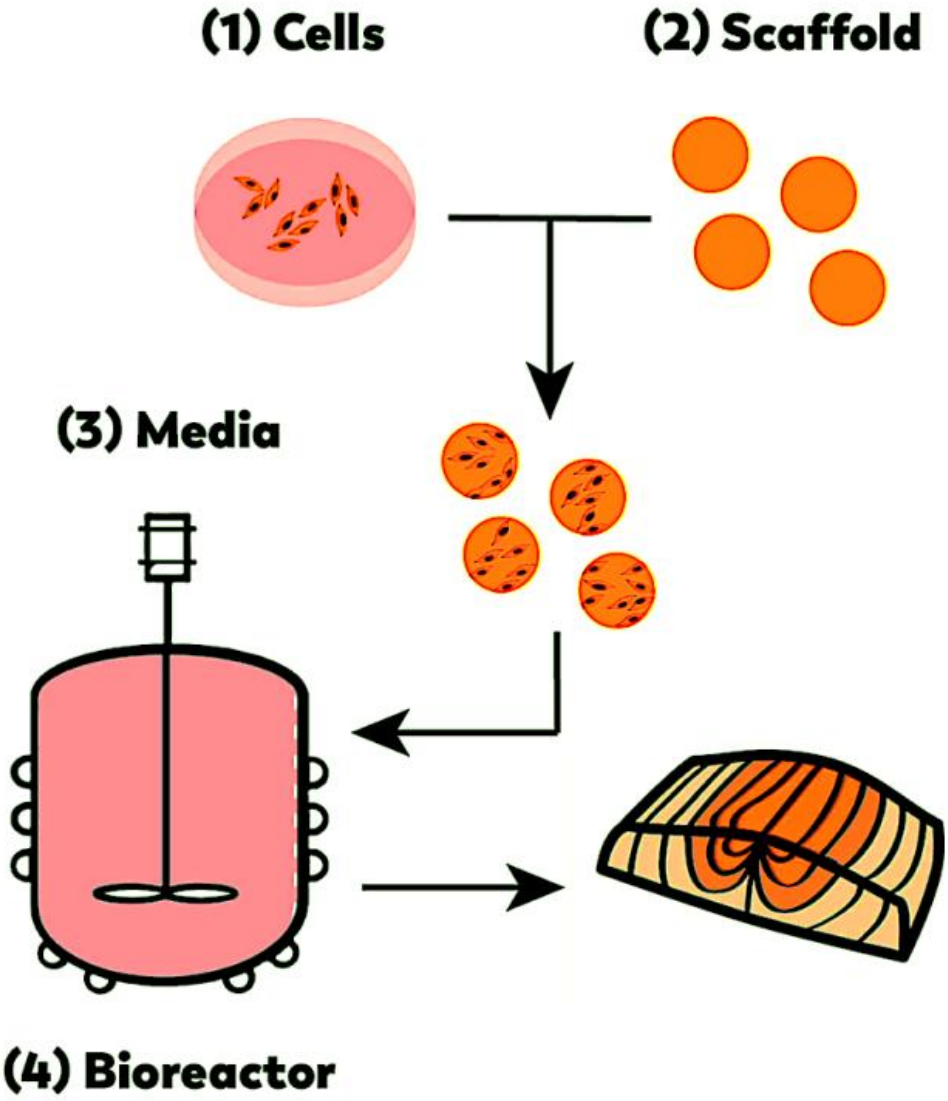

Figure 1. Marine animal TC for lab-grown seafood

Ichthyic TC: As aquatic organisms, fish are highly capable of coping in low-oxygen environments where oxygen is reduced. The researchers noted that the oxygen demand of fish cells cultured in bioreactor structures has not been studied and that genetic susceptibility to hypoxia found in fish makes fish cells ideal for the oxygen-restricted environments in TC and those limited by the bioreactor structure. Homologous DNA sequences of hypoxia response proteins were found in the mammalian genome, which may serve as candidates for making the cell membrane hypoxia-tolerant.

pH considerations

Mammalian cell cultivation: The pH in the external medium is correlated to the pH inside the cell in the case of human TC, and lactic acid generated by the cells under anaerobic conditions, accumulates for longer periods of time in bioreactor configurations. This buildup leads to the acidity of the bioreactor atmosphere. The well-defined link between cellular acidification during cell death and the decrease in external pH from 6.7 to 6.2, which induces quiescence in cells, makes it important to carefully control the pH in bioreactor devices that aim to increase cell proliferation. pH modification is an optimisation approach, because pH variation affects the synthesis and glycosylation of proteins; increasing the pH to increase the protein yield and improve the process efficiency inevitably reduces the metabolic activity and cell growth. The pH of the bioreactor system in mammalian cell cultivation should be carefully managed to prevent the buildup of acidic substances (e.g. lactic acid) depending on the desired purpose of cell culture: cell growth or protein production.

Ichthyic TC: A basic physiological characteristic of in vivo muscle across species is its internal buffering ability, that is, the ability of a cell to maintain a neutral pH after it has added to it waste products of metabolism, such as lactic acid. In the cell itself the buffering capacity is expressed in Slykes, which are small amounts of base required to change the pH of the homogenate cell by one unit per gram of wet mass. Terrestrial animals like pigs have 45.2 Slykes and cows have 50.3 Slykes. By comparison, aquatic species like the fur seal have 75.4 Slykes and striped whales have 83.6 Slykes. The buffering capacity of the white muscle in the warm-bodied fishes is very high (105 Slykes for black skipjack, and 109 Slykes for albacore), double that of several terrestrial animals. Muscle Lactate Dehydrogenase (LDH), the enzyme that degrades lactic acid in muscle cells, shows up to a twofold increase in activity among warm-bodied fish like the albacore as opposed to terrestrial animals such as cattle.

Thermal specifications

Mammalian TC: The temperature of 38°C (the human body temperature) is typically used for mammalian cells cultured in TC, and is an important factor in designing mammal bioreactor processes. Alternate culture conditions of 38 and 36°C increased the vitality of the mutated CHO cells. On the other hand, cultures grown at 32°C showed a higher longevity and protein yield than the cultures grown at 38°C.

Ichthyic TC: There is a high degree of variation in the adaptation strategies of fish species between the poles and the equator, with rates of speciation being higher closer to the poles than closer to the equator. Antifreeze adaptation mechanisms have been evolved, depending on the temperature range (0° to 12°C) and marine vitamin E, a potent antioxidant, has been synthesized and occurs in greater amounts in salmon from colder waters than those from equatorial areas. Other changes involve unique oxygen carrying and metabolic activities.

Ichthyic cell lines: Until recently, fish lineages were used in limited ways, such as evaluating viral loads, water toxicity and even in the production of vaccines against AC. Few of these cells have been used to generate edible lab-grown seafood, except for research to create fish-derived proteins for travelers. The American TC Collection (ATCC) has about 4500 cell lines but lacks

fish muscle cell lines. Only a limited number of cell line repositories include two fish lineages. FICELdb is a fish cell repository with several heritage lines from 1960 to 2000. Cellosaurus is a collection of cell lines established in 2013 and released in 2019. It is under ExPASy and is a resource curated by the Swiss Institute of Bioinformatics. It comprises global cell line repositories, classifying approximately 150000 identified cell lines. Notably, only 560 have been immortalized salmon cell lines, with just nine derived from fish muscle, none of which are myoblastic, all having undergone spontaneous immortalization. Although not every preserved fish cell line is probably included in these records, nine are documented in Cellosaurus.

Techniques for cell isolation: The absence of fish cell lines necessitates that most fish TC research uses primary cells extracted from fish. Fish cell separation techniques resemble those used for mammalian cell separation. The fish is first sterilized in alcohol, then sedated, and a tissue sample is extracted by biopsy. The tissue specimen is thereafter either explanted or subjected to enzymatic digestion using collagenase or protease. Explanted cells can adhere to the culture dish, facilitating cellular migration from the organ to the culture surfaces. Culture plates are coated with proteins to improve cell adherence (e.g., gelatin, collagen, laminin). Digested cells are suspended in prepared aqueous solutions in which the cells are liberated from the tissues by degradation of the tissues by trypsin or collagenase. Impurities are removed from the tissues by washing them in buffer, and often the cells are filtered out from any remaining debris. These procedures provide a good means of isolating tissue from mature fish, but current research has investigated the extraction of cells from prenatal teleosts. It is difficult to isolate the developing cells from several naturally and deliberately mated fish that survive in incubators. A number of fish species have higher levels of telomerase, and initial embryonic cells do not senesce as quickly as human systems. Although there are no true stem cells in the embryo of fish, there are embryonic precursors that are along many differentiation pathways.

Constraints and obstacles to be conquered in the future

Accurate extraction of cells from aquatic organisms like fish, crabs, and mollusks is essential for the production of cultured lab-grown seafood. It's hard to isolate muscle neural stem cells from them because most of the human antibodies don't cross-react with cells made from fish. Identifying the cell exterior proteins that differentiate progenitor cells from muscle cells in fish using cell sorting and other techniques is challenging. One of the main challenges is finding suitable and practical methods to isolate stem cells from aquatic species. To produce cultured shellfish, marine organisms like crustaceans and crabs must be cultivated for a long period of time and in the proper manner. Very little literature has been available, and only short-term culturing has been described, which makes it difficult to determine the optimum conditions for culturing. In the cultivation of crustaceans and other organisms for human food, innovative methods need to be developed to obtain permanent or long-term cell lines of the relevant lineage of interest, including adipose tissue or muscle. Another challenge relates to the Fetal Bovine Serum (FBS). FBS is a commonly used supplement in media, supplying essential components for optimum cell growth. Due to its high cost and ethical issues, producers of CBS are looking for alternatives to FBS. As concerns regarding FBS have increased, so have the number of options that developers

are looking at, such as making seasonal adjustments to FBS in the winter and spring seasons, and considering regional differences in FBS quality because of region-specific contaminations. The problems of FBA affect the experimental results and stability of cells. FBS substitutions need to be made. Another challenge in making lab-grown seafood replacements is the need for automated extraction equipment which can remove finished lab-grown seafood products from scaffolds or small carriers. Some manufacturers also now incorporate editable scaffolds as part of the product such as changed soy protein. More suitable edible scaffolding or a computer-controlled removal technique will make manufacturing easier. Another concern is that the production of farmed lab-grown seafood could increase the cost of production initially, because of scientific and technological investments. As the activities are scaled up and skills are gained manufacturing costs are expected to come down in the future. As investments grow, in technological development, and consumer demand grows, it is probable that costs will be reduced further. There is still limited research into client demand for seafood alternatives and consumer behaviour. Even though there is no genetic manipulation in farmed seafood production, consumers will most likely identify and reject farmed seafood as being akin to genetically modified products. Educating society on the security of the technology that underlies it and the use of cultured fish and shellfish is essential, thereby alleviating public concerns and aversions that need modernization to overcome this challenge. Table 1 contrasts the characteristics of mammalian tissue culture (TC) and ichthyic TC in the context of cultured seafood, emphasizing their distinctions and benefits. Fish cells display greater tolerance to hypoxia, meaning there will be no need for extensive monitoring and management of oxygen concentration, in addition to high capacity in buffering pH. Unlike mammalian cells, fish cells are more tolerant to temperature changes, leading to energy savings in the process. However, there are few fish cell lines available in the market for muscle tissues, requiring additional research in the future. Moreover, both mammalian and ichthyic TC are faced with the same limitation in terms of the use of Fetal Bovine Serum (FBS), necessitating research on serum-free methods. Furthermore, although mammalian TC has an existing infrastructure, ichthyic TC does not have an automated extraction system yet.

Conclusion: Marine animal tissue culture (TC) is a novel and potentially promising method for the production of in vitro seafood in a more environmentally friendly way than conventional fishing and aquaculture. The results show that fish and crustacean muscle cells have important advantages compared to terrestrial animal cells, including high-level hypoxia tolerance, strong buffering capacity, and adaptability to cold temperatures, which makes them great candidates for the production of large amounts of seafood in bioreactors. These properties improve the efficiency of the cells' growth, and reduce the energy needed to grow mammalian cells. However, challenges remain to be addressed: the need to develop efficient and sustainable fish cell lines, to develop better serum-free media and to develop bioreactors specifically for marine organisms. The study also shows the need for improvement in tissue scaffolding and automation in the harvesting of seafood products.

Table 1. Comparison of Mammalian and Ichthyic Tissue Culture for Lab-Grown Seafood Production

Aspect	Mammalian TC	Ichthyic TC (Fish Cells)	Key Differences/Advantages for Marine Animal TC
Oxygen Tolerance	Sensitive to low oxygen, requires 45-65% saturation	Superior tolerance to hypoxia, thrives in low-oxygen environments	Fish cells have better tolerance to hypoxic conditions, reducing the need for intensive oxygen regulation
pH Regulation	Sensitive to pH fluctuations (pH 6.7-6.2)	Fish cells have high buffering capacity (e.g., 105 Slykes)	Fish cells' high buffering capacity reduces the need for pH adjustments
Temperature Adaptability	Typically maintained at 38°C	Adaptable to varying temperatures, especially cold-water species	Fish cells can thrive in lower temperatures, providing energy savings compared to mammalian systems
Cell Line Development	Established cell lines for various tissues	Limited fish cell lines, especially for muscle tissue	Fish cell line development is a challenge; more research needed
FBS (Fetal Bovine Serum)	Commonly used, but expensive and ethically controversial	Similar challenges with FBS use, but alternatives are being explored	Fish TC faces the same FBS issues, prompting research into serum-free media alternatives
Technological Challenges	Robust systems in place for mammalian cell culture	Lack of automated extraction systems for seafood production	Overcoming the isolation of fish cells and developing automated harvesting systems is essential

All these challenges notwithstanding, the rise in the popularity of cellular agriculture is a positive signpost to how food security might be addressed in the future, and the effects on the environment could be mitigated. Thus, the possibilities of marine TC and the evolution of biotechnology, such as genetic engineering and bioreactor systems, are very promising to revolutionize the seafood industry. Future studies should be aimed at addressing the technical limitations revealed in this study, such as the need for long-term fish cell lines, better media formulations, and scalable production systems, to prepare for the commercialization and adoption of cultured seafood as a viable, sustainable food source.

Author contributions

A. K. P.: Conceptualized the study, designed the overall experimental framework, and was responsible for supervising the research. Led the tissue culture experiments involving marine organisms, especially focusing on cell line development, serum-free media formulations, and bioreactor setup. Analyzed experimental data and interpreted the results. Contributed to the

manuscript's drafting, revision, and final approval. D. B.: Assisted in the experimental design, focusing on the marine engineering aspects related to bioreactor configuration and optimizing conditions for cell growth. Contributed significantly to the design of culture systems, data collection, and analysis of cell culture responses under varying conditions. Reviewed and critically revised the manuscript for scientific accuracy and provided technical insights in marine engineering.

Data availability statement

The dataset used in this research is available on request from the corresponding author. In situations where the data are openly accessible, the precise names of the repositories, links, and accessibility constraints should be included.

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Ethical considerations

This experiment is conducted ethically by ensuring that the marine animal cells are treated ethically through non-invasive techniques in collecting samples from the animals without causing any harm or distress to them.

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Conflict of interests

The authors declare no conflict of interest.

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ارزیابی امکان‌پذیری کشت بافت جانوران دریایی برای تولید غذای دریایی کشت‌شده در آزمایشگاه در سامانه‌های دریایی

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چکیده

هدف: مطالعه حاضر تلاش می‌کند امکان استفاده از کشت بافت (TC) جانوران دریایی برای تولید غذای دریایی در آزمایشگاه را بررسی کند. پژوهش حاضر بر بررسی محدودیت‌های زیستی و فنی مرتبط با کشت در مقیاس بزرگ جانداران دریایی، به‌ویژه در تولید غذای دریایی کشت‌شده (CBS)، تمرکز دارد. انتظار می‌رود پژوهش حاضر بتواند بر ظرفیت غذای دریایی کشت‌شده (CBS) به‌عنوان یک جایگزین پایدار در بخش تولید غذای دریایی روشنگری کند.

مواد و روش‌ها: از کشت‌های بافتی جانداران دریایی، شامل ماهی‌ها و سخت‌پوستان، برای ارزیابی امکان‌پذیری تولید غذای دریایی کشت‌شده (CBS) در مقیاس بزرگ استفاده شد. این فرایند شامل تولید رده‌های سلولی، ایجاد محیط‌های کشت بدون سرم و طراحی زیوراکتورها بود. تلفیق مهندسی ژنتیک با روش‌های متداول کشت سلولی حیوانی (AC) برای ایجاد یک محیط رشد ایده‌آل برای سلول‌ها مورد استفاده قرار گرفت. شرایط مختلف کشت، از جمله مقاومت به کمبود اکسیژن، تنظیم pH و سازگاری با دماهای پایین، مورد ارزیابی قرار گرفتند.

نتایج: این آزمایش نشان داد که سلول‌های جانوران دریایی، به‌ویژه سلول‌های عضلانی ماهی‌ها و سخت‌پوستان، در مقایسه با سلول‌های جانوران خشکی‌زی سازگاری بیشتری با کمبود اکسیژن و دماهای پایین داشتند. این ویژگی برای تولید غذای دریایی کشت‌شده (CBS) سودمند است، زیرا رشد و تمایز مؤثر سلول‌ها را در زیوراکتور تسهیل می‌کند. علاوه بر این، کشت‌های سلولی حاصل از بافت‌های ماهی قادر به تشکیل ساختارهای شبه‌بافتی بر روی داربست‌های زیست‌تخریب‌پذیر بودند که می‌تواند زمینه را

برای پرورش غذای دریایی در آزمایشگاه فراهم کند. بااین‌حال، چالش‌هایی در ارتباط با ایجاد محیط کشت بدون سرم و طراحی زیوراکتور وجود داشت.

نتیجه‌گیری: در نتیجه، کشت بافت جانوران دریایی می‌تواند به‌طور بالقوه به‌عنوان یک فناوری قابل‌اجرا برای تولید پایدار غذای دریایی مورد استفاده قرار گیرد. اگرچه استفاده از سلول‌های دریایی در تولید غذای دریایی کشت‌شده با مزایای فیزیولوژیکی متعددی همراه است، اما برای رفع برخی چالش‌های فنی موجود در این حوزه، از جمله توسعه محیط‌های کشت بدون سرم، کشت رده‌های سلولی و طراحی زیوراکتورها، هنوز اقدامات زیادی مورد نیاز است. حل این مشکلات می‌تواند موجب ایجاد یک تغییر اساسی در این صنعت شود و راهکاری پایدار برای پاسخ‌گویی به تقاضای فزاینده جهانی برای غذای دریایی ارائه دهد.

کلمات کلیدی: دریایی، دریانوردی، غذای دریایی، کشت بافت

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