



## Sustainable biotechnology applications of marine macroalgae Example model: Sea cucumber nutrition

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### Abstract:

Brown seaweeds (Phaeophyceae) represent a vast and underutilized marine biomass, thriving in coastal ecosystems all over the world, including the coasts of the Oman Sea and Sistan and Baluchestan Province. Their unique biochemical composition, rich in polysaccharides (alginate, fucoidan, laminarin), polyphenols (phlorotannins), and minerals, positions them as a highly promising feedstock for the burgeoning field of marine biotechnology. This review synthesizes recent advancements in the biotechnology of brown algae, moving beyond traditional extraction methods to explore cutting-edge applications. We detail the development and optimization of enzymatic and microbial-based processes for the efficient saccharification and fermentation of algal biomass into high-value products, including biofuels (bioethanol, biobutanol), biochemicals (organic acids, pigments), and nutraceuticals. Furthermore, we highlight the innovative use of genetic and metabolic engineering tools to enhance seaweed cultivation, tailor composition, and create sustainable bioprocessing pipelines. The integration of brown seaweed into a circular bioeconomy model, addressing challenges and future perspectives, is also discussed. The following is an example model that justifies the use of brown algae extract in feeding sea cucumber larval stages.

### Review History:

Received: 7/27/2025

Revised: 8/25/2025

Accepted: 9/29/ 2025

Available Online: 10/1/2025

### Keywords:

Bioactive compounds  
refinery  
Enzymatic hydrolysis  
Bioethanol  
Prebiotics  
Metabolic engineering  
Circular bioeconomy  
Sea cucumber nutrition

### How To Cite This Article:

Ajdari A., Hafezieh M., & Raei, M. (2025). Sustainable biotechnology applications of marine macroalgae Example model: Sea cucumber nutrition. *Int. J. Biotech. Adv. Res.*, 1(1) 19-25

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## Introduction

The global demand for sustainable resources is driving the exploration of alternatives to terrestrial biomass. Brown seaweeds, or macroalgae, have emerged as a compelling candidate due to their rapid growth rate, high biomass yield, and ability to be cultivated without arable land, freshwater, or fertilizer. Unlike lignocellulosic biomass, brown algal cell walls lack lignin, theoretically simplifying deconstruction. The core components of their biomass—the structural polysaccharides alginate, fucoidan, and laminarin—form the basis for a wide array of biotechnological valorization pathways. This article reviews the scientific and technological progress in harnessing brown seaweed through biotechnological approaches for the production of energy, food, materials, and health products.

## The biochemical foundation: Key compounds of interest

The valorization of brown seaweed is predicated on its distinct chemistry:

- Alginate: A linear copolymer of  $\alpha$ -L-guluronic acid (G) and  $\beta$ -D-mannuronic acid (M). It is the primary structural component, responsible for flexibility and strength. It is widely used as a viscosifier, gelling agent, and stabilizer in food, textile, and pharmaceutical industries.

- Fucoidan: A sulfated, fucose-rich heteropolysaccharide with renowned bioactivities, including anticoagulant, antiviral, anti-inflammatory, and anticancer properties.

- Laminarin: A storage  $\beta$ -glucan (1,3- with 1,6-branching) that serves as an energy reserve. It is easily hydrolyzable into glucose and has prebiotic and immunostimulatory effects.

- Phlorotannins: Polyphenolic compounds exclusive to brown algae, exhibiting potent antioxidant, UV-protective, and antidiabetic activities.

- Mannitol: A sugar alcohol derived from photosynthesis, acting as a carbon storage compound and compatible solute. It is a valuable platform chemical.

## Biotechnological processing: From biomass to products

The traditional model of extracting a single compound is being superseded by the biorefinery concept, which aims to utilize the entire biomass efficiently.

1. Enzymatic Saccharification A critical bottleneck in brown seaweed biotechnology is the hydrolysis of complex polysaccharides into fermentable sugars. This is overcoming the limitations of harsh acid hydrolysis, which can degrade sugars and generate inhibitors.

- Alginate Lyases: These enzymes cleave alginate via a  $\beta$ -elimination mechanism, producing unsaturated oligomers that can be further processed. Microbial screening and protein engineering are producing novel lyases with improved thermostability, specific activity, and pH tolerance.

- Fucoidanases and Sulfatases: These enzymes specifically break down fucoidan into oligofucoidans and fucose, which are higher-value products than crude extracts. The development of tailored enzyme cocktails is enabling the simultaneous saccharification of alginate, laminarin, and cellulose, maximizing sugar release for downstream fermentation.

2. Microbial Fermentation and Metabolic Engineering The released monosaccharides (e.g., glucose from laminarin, mannuronic/guluronic acids from alginate, fucose from fucoidan) serve as substrates for engineered microorganisms.

- Biofuel Production: Engineered *Saccharomyces cerevisiae* and *Escherichia coli* strains can co-ferment glucose, mannitol, and alginate-derived sugars to produce bioethanol and biobutanol. A key breakthrough was engineering microbes to express the

pathway for importing and metabolizing uronic acids (a major product of alginate degradation).

- Production of Biochemicals: Beyond fuels, fermentation can be directed towards sustainable production of lactic acid, succinic acid, and polyhydroxyalkanoates (PHAs, bioplastics) from seaweed hydrolysates.

- Prebiotics and Nutraceuticals: Controlled enzymatic hydrolysis is used to produce specific oligosaccharides (alginate oligosaccharides - AOS, laminari-oligosaccharides - LOS) that act as prebiotics, stimulating the growth of beneficial gut bacteria.

### **Emerging frontiers and genetic engineering**

1-Strain Improvement and Cultivation Biology Research is focusing on selecting and breeding brown algal strains (e.g., *Saccharina latissima*, *Sargassum* spp., *Ascophyllum nodosum*) with desirable traits: high carbohydrate content, fast growth, and disease resistance. Understanding the molecular biology of development and stress responses is key to improving cultivation yields.

2- Genetic and Metabolic Engineering of Seaweeds While still in its infancy compared to terrestrial crops, genetic transformation protocols for brown algae are being developed. The long-term goal is to create designed cultivars with:

- Reduced recalcitrance (e.g., modified alginate structure for easier processing).

- Enhanced production of specific high-value compounds (e.g., elevated phlorotannin or fucoidan content).

- Increased abiotic stress tolerance for wider cultivation range.

### **Challenges and future perspectives**

Despite the promise, several challenges remain:

- Economic Viability: The cost of enzyme production, cultivation, and harvesting must be reduced to compete

with established terrestrial and fossil-based routes.

- Process Integration: Developing efficient, continuous, and scalable biorefinery processes that minimize waste and energy input is crucial.

- Ecological Impact: Sustainable cultivation and harvesting practices must be ensured to avoid disrupting marine ecosystems.

Future progress will rely on interdisciplinary research combining marine biology, enzymology, fermentation technology, and process engineering. The integration of artificial intelligence for bioprocess optimization and the exploration of novel extremophile enzymes for hydrolysis are particularly promising avenues.

### **Sea cucumber nutrition, an example model**

The global demand for sea cucumbers, a high-value delicacy in Asian markets, has led to severe overfishing of wild populations. In response, aquaculture has emerged as the only sustainable way to meet market needs and allow wild stocks to recover. However, a major bottleneck has stymied the industry: how to reliably feed the delicate, microscopic larvae. Traditional methods rely on cultured microalgae, which are expensive, time-consuming to produce, and prone to crashing. But a promising, natural solution is emerging from the depths: extracts from brown seaweed.

### **The Larval Challenge: A Picky Eater in a Microscopic World**

Sea cucumber larvae, particularly in the auricularia stage, are suspension feeders. They require a diet of tiny, nutrient-dense particles that are easy to capture and digest. In the wild, they consume a complex soup of plankton and organic detritus. Replicating this diet in a hatchery is complex. The inconsistent nutritional quality and availability of live microalgae can lead to poor larval survival, slow

growth, and ultimately, failed production cycles.

### **The Kelp Solution: A Powerhouse in a Bottle**

Brown seaweeds, like kelps (e.g., *Saccharina japonica*, *Macrocystis pyrifera* and Iranian brown seaweed *Gracilaria illicifolium*), are renowned for their rich composition of bioactive compounds, making them an ideal candidate for an alternative larval feed. The key lies in the extraction process. By breaking down the tough cellular walls of the seaweed, valuable nutrients are released into a liquid suspension. This "seaweed soup" is a potent mix of:

- Soluble Proteins and Amino Acids: The essential building blocks for larval growth and development.
- Complex Carbohydrates: Including laminarin and mannitol, which provide a steady source of energy.
- Polysaccharides (Fucoxanthin & Alginate): These are not just nutrients; they act as prebiotics, stimulating the larval gut microbiome and boosting immune response.
- Lipids and Fatty Acids: Critical for healthy cell membrane development, especially during metamorphosis.
- Vitamins & Minerals: Rich in vitamins A, B, C, E, and K, as well as iodine, potassium, magnesium, and calcium, which are crucial for metabolic processes and skeletogenesis (formation of calcareous ossicles).
- Pigments: Fucoxanthin, a powerful antioxidant, can enhance stress resistance and survival rates.
- Sustainability & Cost: Seaweed is a rapidly renewable resource. Cultivating or harvesting it for extraction can be more sustainable and potentially cheaper than producing microalgae (e.g., *Chaetoceros*, *Isochrysis*) at the scale required for commercial hatcheries.

### **Proven Benefits: What the Research Shows**

Studies on various high-value species like the sandfish (*Holothuria scabra*) and the temperate species *Apostichopus japonicus* have demonstrated significant advantages of using brown seaweed extract over traditional diets:

1. Enhanced Survival Rates: Larvae fed with seaweed extract suspensions consistently show higher survival rates through the critical auricularia and doliolaria stages. A robust immune system, supported by prebiotics, helps them resist bacterial infections.
2. Accelerated Growth and Development: The highly bioavailable nutrients in the extract are easily absorbed, leading to faster larval growth. This often results in a shorter time to metamorphosis, getting juveniles to the settlement stage more quickly.
3. Improved Gut Health: The prebiotic nature of fucoidan promotes a healthy population of beneficial gut bacteria. A healthy gut is directly linked to improved nutrient absorption and overall larval vigor.
4. Cost-Effectiveness and Stability: Producing a standardized liquid extract from cultivated seaweed is far more scalable and stable than maintaining vast, fragile cultures of multiple microalgae species. It reduces the labor, infrastructure, and risk associated with live feed production.

### **The Future of Sustainable Hatcheries**

The integration of brown seaweed extract represents a paradigm shift in sea cucumber hatchery management. It moves away from a reliance on unpredictable live feeds towards a standardized, off-the-shelf nutritional product. This approach offers a triple win:

- Environmental Win: It promotes sustainable aquaculture, reducing pressure on wild sea cucumber populations.
- Economic Win: It provides hatcheries with a reliable, cost-effective

tool to increase production success and profitability.

- Scientific Win: It underscores the value of marine bioresources and natural solutions in solving modern aquaculture challenges.

As research continues to optimize extraction methods and species-specific formulations, brown seaweed is poised to become a cornerstone of the next generation of sea cucumber aquaculture, helping to turn a fragile process into a robust and sustainable industry.

### Key extraction methods for larval food

The goal is to break down the tough seaweed cell walls to make the nutrients bioavailable for the tiny, filter-feeding larvae.

A. Water-Based Extraction (Most Common and Relevant). This is the simplest and safest method, avoiding harsh chemicals.

1. Drying and Milling: The seaweed is first sun-dried or oven-dried at low temperature (< 60°C) to preserve heat-sensitive nutrients. It is then milled into a fine powder.

2. Hot Water Extraction: The powdered seaweed is mixed with hot water (typically 70-90°C) at a specific ratio (e.g., 1:10 to 1:20 seaweed-to-water). The mixture is stirred for 1-3 hours. Heat helps break down the cell walls and solubilize the nutrients.

3. Filtration and Concentration: The mixture is filtered through mesh or filter paper to remove large, insoluble particles. The resulting liquid is a crude extract containing soluble polysaccharides, proteins, vitamins, and minerals. This liquid can be used directly or concentrated further via evaporation (under reduced pressure to avoid nutrient degradation) or ultrafiltration.

4. Final Product: The concentrate can be: Liquid Extract: Added directly to the larval rearing tanks. Powdered Extract: Spray-dried (using a carrier like

maltodextrin) for longer shelf life and easier dosing.

### B. Enzymatic Hydrolysis (Advanced and Highly Effective)

This method uses specific enzymes to break down seaweed macromolecules into smaller, more easily absorbed components (e.g., peptides, simple sugars).

- Process: The seaweed powder is suspended in water. Enzymes (e.g., alginate lyase, cellulase, protease) are added under controlled temperature and pH conditions for several hours.

- Advantage: Produces hydrolysates with greatly enhanced bioavailability for larvae. It can yield a higher proportion of bioavailable nutrients compared to hot water extraction alone.

- Disadvantage: More expensive due to the cost of enzymes and requires precise process control.

C. Fermentation (Beneficial but Complex). This uses microorganisms (bacteria or yeast) to break down the seaweed.

- Process: The seaweed substrate is inoculated with specific probiotic strains. These microbes digest the polysaccharides, enriching the product with microbial biomass, enzymes, and beneficial metabolites.

- Advantage: Creates a probiotic-rich feed that can significantly improve larval gut health and disease resistance.

- Disadvantage: The most complex method to control consistently on a large scale.

### D. Application in Sea Cucumber Larval Rearing

Sea cucumber larvae (auricularia, doliolaria, pentactula stages) are filter-feeders.

- Dosage: This is not standardized and requires on-site testing. Start with very low concentrations (e.g., 1-5 ppm of the extract in tank water) and adjust based on water quality (avoiding

cloudiness) and larval density. Overfeeding will foul the water.

- Feeding Regime: The extract is typically used as part of a mixed diet:

- Primary Diet: The seaweed extract serves as the main nutritional source.

- Supplement: It can be used to enrich live microalgae or yeast before they are fed to the larvae, boosting their nutritional value.

- Co-feeding: Fed alongside live microalgae. The extract can help sustain larvae during periods when microalgae culture crashes.

- Delivery: The liquid extract or rehydrated powder is diluted in water and added to the larval tanks several times a day.

#### E. Considerations and Challenges

- Water Quality: Unlike live microalgae that can improve water quality by producing oxygen, particulate organic matter from extracts can decompose and degrade water quality. Strong filtration and water exchange systems are mandatory.

- Particle Size: Larvae can only ingest particles a few microns in size. The extraction and filtration process must result in a product with a very fine particle size distribution.

- Nutritional Balance: While nutritious, a pure seaweed extract might lack optimal levels of specific Highly Unsaturated Fatty Acids (HUFAs like EPA and DHA) crucial for larval development. Co-feeding with microalgae rich in HUFAs (e.g., *Chaetoceros*, *Nannochloropsis*) is often necessary.

- Standardization: Batch-to-batch variation in seaweed composition (due to season, species, location) can lead to variation in the nutritional quality of the extract. Sourcing and processing must be consistent.

- Hatchery Trials: Before full-scale adoption, any new extract must be rigorously tested in small-scale larval

rearing trials against the current standard diet (usually live microalgae) to assess: Larval survival rate, growth rate (time to metamorphosis), abnormal development rate and settlement success

#### Conclusion

Brown seaweed biotechnology has transitioned from a concept to a dynamic field of research with tangible outputs. By leveraging advanced enzymatic and microbial tools within an integrated biorefinery framework, brown macroalgae offer a sustainable and renewable pathway to produce a portfolio of biofuels, biochemicals, and nutraceuticals. Overcoming existing technical and economic hurdles through continued scientific innovation will be pivotal in realizing the full potential of brown seaweeds as a cornerstone of the future blue bioeconomy. As a model, using brown seaweed extracts as food for sea cucumber larvae is a highly promising and sustainable practice. Water extraction is the most accessible starting point for hatcheries, while enzymatic hydrolysis offers a superior, high-performance product. Success hinges on producing a high-quality, bioavailable extract and integrating it into a careful feeding and water management regime to avoid the primary pitfall of water fouling. This approach can reduce hatchery reliance on labor-intensive microalgae cultures and contribute to more efficient and sustainable sea cucumber aquaculture.

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