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From green to blue economy: Marine biorefineries for a sustainable ocean-based economy

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Despite being a vital asset for global sustenance and economy, ocean aquatic ecosystems are in danger due to the effects of incorrect management of their resources, pollution, and climate change. Considering the seafood industry discards half of its fish-product mass in the ocean, a proper valorization of its residues would decrease not only the ocean contamination but also improve the management of marine resources and increase the sector competitiveness. With these goals in mind, ocean-based industries are adopting new sustainable production models, similar to biorefineries, which are effective for waste valorization, namely, converting low-value biomass into commercially relevant by-products. Based on a deeper knowledge of aquatic feedstocks, the development and implementation of a marine biorefinery can be fundamental to consolidate a “greener” socioeconomic development, similar to that observed in green chemistry. However, biorefineries are sophisticated multi-step systems with numerous feedstocks and commodities. Therefore, their implementation requires expertise in all stages of manufacturing, in addition to a clear vision of all raw materials, residues, and products. In this sense, with this perspective, we provide an initial overview of the current state-of-the-art on marine biorefineries and the sources and applications of their by-products. Afterward, we suggest how to integrate green chemistry and blue economy principles into ocean-based industries, aiming to support a more sustainable, profitable, and conscious ocean economy.

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Introduction

The ocean is essential for our survival: it covers 70% of our planet, is the source of half of our oxygen, and is responsible for 17% of animal protein production.¹ However, the degradation of aquatic ecosystems is happening at an alarming rate, and there are not enough actions to change this grim outlook. For example, about 35 million tons of fish caught (35% of the total) are discarded into the ocean every year.² This is not only an irrational wastage of marine resources, but it also pollutes and endangers our aquatic environments. Hence, it is crucial to design smart and sustainable production systems to protect

our oceans while preserving the capacity of marine industries to generate jobs and supply food, especially by protecting these vital assets for future generations. In this sense, green and blue economies can provide the tools required for this paradigm shift in ocean-based industrial operations. One model that follows green chemistry precepts and could reduce seafood wastage and pollution is the “biorefinery”, considering it can convert low-value biomass into beneficial and valuable by-(bio)products.³

By-products are any secondary substances in a manufacturing process that are not its main commodity, which can be considered marketable compounds or simply waste.⁴ An industrial residue with the same composition can be discarded as waste or used as a source of commercially relevant by-products by different sectors. Hence, no waste or residue is truly “worthless”, and it is an ecological and financial loss to discard by-products without harnessing their complete potential as a secondary source of raw material.

Nowadays, each industry’s processing capacities, values, and sector regulations dictate how their residues are managed. For example, the energy field already has many successful

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examples of biorefineries for producing biofuels from residues.⁵ Ideally, more companies should apply a biorefinery approach to harness the most out of their raw materials, reducing wastage and environmental degradation.

The development of cost-effective, environmentally friendly, and circular technologies for marine-based biomass processing into value-added products are decisive for successfully implementing sustainable ocean-based industries, focusing on strategies that simultaneously contribute to decreasing waste and energetic demand.^{6,7} By employing a biorefinery concept for marine industries, it is possible to save natural resources and avoid the production of seafood waste while creating jobs and increasing profits. This model embodies the blue economy principles of preserving marine environments while improving the economy and public welfare. Having these principles in mind, in this perspective, we will introduce concepts of the ocean economy along with those of the blue and green economies, while presenting the potential of marine biorefineries for seafood waste valorization. We will also pinpoint sources and applications of seafood waste by-products and discuss the state-of-the-art on marine biorefineries. Finally, we will examine the potential and trends in this field and offer our perspective on the next steps required to spread blue economy practices.

The ocean economy

The ocean economy includes all commercial ocean-based activities, with a mix of well-established and emerging

industries.^{1,8} Established ocean industries operate on different sectors, such as food (e.g., industrial capture of fish and seafood processing), transport (e.g., maritime transport and shipping, port activities), construction (e.g., shipbuilding and marine manufacture and construction), marine education and research, business, tourism (maritime and coastal), energy (shallow-water offshore oil and gas drilling) and dredging.¹ As for emerging ocean industries, they apply new technologies to solve current problems and reduce environmental risks. For example, to boost seafood supply and face an increase in demand due to exponential population growth, the fishing industry designed an intensive production model known as aquaculture (controlled cultivation of aquatic organisms).⁹ The development of modern technologies to extract deep and ultra-deep oil and gas as well as for marine and seabed mining are also helping to prevent shortages of these non-renewable compounds. For example, offshore crude oil and natural gas already represent 30% of this market.¹⁰ In addition, exploiting the potential of the sea for the production of renewable energy from waves and wind could help with the growing demand for sustainable energy sources.¹¹ There is also great interest in exploring marine biomolecules using biotechnology for medical and industrial applications due to the vast biodiversity present in the ocean. Furthermore, considering that 80% of the volume of products is transported by sea, maritime safety and surveillance and maritime technology are gaining importance in our globalized commerce.¹² To elucidate key areas of the ocean economy, Fig. 1 includes the established and emerging sectors of ocean-based industries defined by the

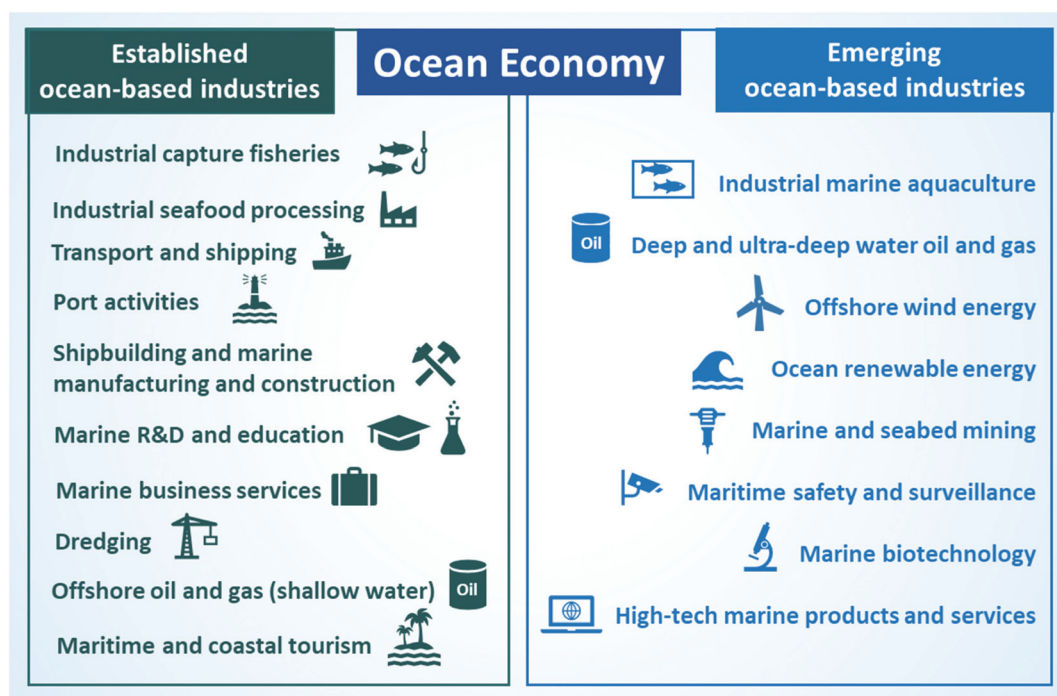


Fig. 1 Established and emerging ocean-based industries. Image produced by the authors with Information from OECD.¹³

Organization for Economic Cooperation and Development (OECD).¹²

As illustrated in Fig. 1, the ocean economy encompasses and impacts a wide range of industrial and commercial areas. In addition to these traditional fields recognized by OECD, the sea is also used for certain irregular activities, such as piracy and illicit trafficking of people and illegal products. These irregular actions mainly stem from the difficulty of prosecuting and defining jurisdictions for offshore crimes, which facilitate their expansion and turn them into a global concern.¹⁴ Interestingly, along with the relevance of traditional sectors, emerging ocean-based industries already have an extensive impact on the economy, supply of food, and extraction of natural resources. The best example is aquaculture, which already accounts for half of seafood production and is the fastest-growing food sector today.⁹

The ocean economy is so relevant that, if it were a country, it would be the 8th largest economy in 2021, with an annual gross domestic product (GDP) of USD 2.5 trillion.^{1,15} Moreover, the World-Wide Fund for Nature (WWF) has estimated the value of major ocean assets to be at least USD 24 trillion.¹ The impressive value of the oceans is not only economic, but also social, as the fishing industry employs around 200 million people in the capture, harvesting, and processing of fish products, and provides more than 17% of animal protein worldwide.¹⁶ From 1990 to 2018, there was a 122% rise in total fish products consumption, propelling a 14% and 527% increase in fish capture and aquaculture, respectively, producing 200 million tons of seafood per year.² Most of the seafood production is still employed for direct human consumption. In 2018, from 178.6 million tons (live weight) of seafood, 156.4 million tons were used for food products (87.6%), while 22.2 million tons (12.4%) were applied for non-food purposes (*i.e.*, not used for human consumption).^{2,17} Of the food segment, 44% comprised fresh or chilled fish.² About non-food products, 80% was reduced to fish meal and oil, while the rest was applied in different sectors, from pharmaceutical and nutraceutical formulations to ornamental fish.^{2,18} Since FAO members adopted the Code of Conduct for Responsible Fisheries in 1995, the seafood industry is progressing towards more sustainable practices.^{2,19} For example, 25 to 35% of fish meal and oil are already produced from fish by-products.² However, the seafood industry still discards countless residues rich in high-value biomolecules that could be valorized and commercialized with efficient processing.

Above all, it is crucial to emphasize the environmental relevance of the seas, which play a leading role in our survival. For example, aquatic ecosystems generate 50% of the oxygen and absorb 30% of the carbon dioxide (CO₂) emissions on Earth.¹ Therefore, the ocean is a vital asset for global livelihoods and economy, providing reliable sources of food, energy, chemicals, medicines, transport, tourism, and jobs. However, while the ocean's vastness makes it seem unwavering, growing and uncontrolled human activity at sea, ocean waste disposal, and climate change are increasingly putting pressure on "our

oceans", quickly showing the true fragility of "our aquatic ecosystems".

The excessive extraction of ocean resources and sea pollution is rapidly degrading marine environments, especially destroying these natural biodiversity hotspots. For example, in 1990, 90% of fish stocks were within sustainable biological levels.² However, these levels declined to an alarming 65.8% in 2017.² This figure is even more worrying because, according to the Food and Agriculture Organization (FAO), 35% of global fish catches are discarded dead or dying in the ocean.² There is also the problem of disposal of fish waste in marine environments, which can reduce oxygen levels in seawater, bury or smother sea life, and introduce diseases and invasive or non-native species to the aquatic ecosystems.²⁰ Considering that nearly 70% of the production of marine products depends on a healthy ocean, if no action is taken soon, ocean-based industries and related activities will face a severe crisis in the recent future.¹

To counter both of these issues, we believe that creating marine biorefineries for the sustainable valorization of seafood waste/residues can save ocean resources and maximize revenues, as well as, at least partially, reduce marine pollution and minimize the negative environmental impact of human activity. Considering that ocean-based industries discard more than half of seafood mass during processing, there is a wide variety of waste materials that can be used to generate value-added products, namely: algae biomass residues, fish frames, heads, fins, scales, viscera and bones, and marine invertebrates shells, viscera, and skin.²¹ Therefore, by applying green economy principles and strategies to reuse fishery waste, it would be possible to reduce the environmental problems associated with fishing and aquaculture while increasing revenue and creating jobs.

The green and blue economies

The term "green chemistry" was effectively proposed by Paul Anastas in 1990, during his position as a staff chemist at the US Environmental Protection Agency's (EPA).²² However, concerns about the impacts of industries (mainly the chemical, agricultures, and arms sectors) on the environment emerged around 1950.²³ At that time, the main ecological issues were the eutrophication and pollution of potable water sources by synthetic detergents and industrial residues. The environmental impact of detergents was first demonstrated after the cleaning products manufacturer company Henkel began monitoring its surfactant concentrations in the Rhine River (Germany) to assess the biodegradability of its products.²⁴ The discovery of the harmful environmental impacts of synthetic (crude oil-based) tetrapropylenebenzene sulphonate, together with an increased environmental awareness of consumers, initiated the chemical industry's "green shift" to the development and implementation of environmentally-friendly products and processes.²³ These efforts also evolved and led to the creation of principles and guidelines to

prevent pollution and environmental risks, such as: “Twelve Principles of Green Chemistry” published in 1998 by Paul Anastas and John C. Warner; “The Responsible Care® Global Charter” from the International Council of Chemical Associations (ICCA).^{23,25,26} These green chemistry principles began to be actively applied in the chemical industry, leading to the effective establishment of the concept of “green economy”.

The United Nations (UN) Environment Program defines the green economy as a “low carbon, resource-efficient, and socially inclusive” economy.²⁷ It aims to reduce pollution and carbon emissions, improve resource and energy efficiency, as well as preserve ecosystems and biodiversity.²⁷ The pillars of green economy include:²⁸

1. Climate change – It is crucial to reduce carbon emissions and preserve our forests and oceans to slow down climate change and related effects;

2. Resource-saving and management – The efficient and resourceful use of natural resources and the reduction of wastage are imperative to prevent products and energy shortages and a global environmental crisis;

3. Circular economy – It aims to preserve natural systems by reusing and repurposing waste and residues, thus generating value while maintaining the sustainability and economic viability of the productive chain;

4. Environmental protection – Environmental degradation has negative impacts on all social and economic sectors of society, therefore, preserving the environment is essential to maintain our sustenance and health;

5. Ecosystem protection and recovery – The preservation and recovery of ecosystems and their native biodiversity will have positive social and economic impacts on the world;

6. Water conservation – Water is one of our most vital natural resources, which is primordial to protecting our drinking water sources and ensuring access to clean water for disadvantaged communities around the world;

7. Natural disaster prevention – Large-scale natural disasters are becoming more frequent due to climate change, deforestation, and rising sea levels, making it essential to avoid them as a way for saving human and animal lives, as well as natural ecosystems, urban and rural infrastructures.

Notably, these seven pillars of the green economy presented above are directly aligned with fourteen of the seventeen UN Sustainable Development Goals (SDGs), as schematized in Fig. 2.²⁹

As Fig. 2 shows, the green economy is addressing most of the pressing issues we face today. Therefore, as a direct consequence, the green economy has efforts to accomplish environmental goals, such as clean water and sanitation (SDG 6), affordable and clean energy (SDG 7), responsible consumption and production (SDG 12), climate action (SDG 13), life below water (SDG 14), and life on land (SDG 15), as well as socio-economic aims, like no poverty and zero hunger (SDGs 1 and 2, respectively), allowing decent work and economy growth and reduced inequalities (SDGs 8 and 10, respectively), good health and well-being (SDG 3), industry, innovation and infrastructure (SDG 9), and sustainable cities and communities (SDG 11), and partnership for the goals (SDG 17). As schematized in Fig. 2, there are only three SDGs, *i.e.*, quality education (SDG 4), gender equality (SDG 5), and peace, justice, and strong institutions (SDG 16), which are not directly aligned with the green economy precepts. In any case, we must stress out that if society will become “greener”, the world will be more inclusive, fair, and socially sustainable.



Fig. 2 United Nations (UN) Sustainable Development Goals (SDGs) that are directly related to green economy pillars and aims (in color). Image produced by the authors with pictograms from <http://www.un.org>.²⁹

Although the use of green economy principles has been a step in the right direction towards more sustainable production systems and economies, there are still not enough efforts to protect our aquatic ecosystems, *i.e.*, “the blue of the oceans is not yet the new green”. While 15% of terrestrial lands are conservation areas, only 1% of the ocean surface is legally protected.³⁰ This difference is quite surprising, especially considering that the initial efforts to define guidelines for protecting the environment aimed to preserve our water resources.²³ Additionally, climate change disproportionately affects the oceans, which absorb 90% of heat caused by global warming and 30% of CO₂ emissions.³¹ This increase in heat and CO₂ absorption can change sea temperature, cause deoxygenation and ocean acidification.^{32,33} As a result, these alterations in the marine environment can modify the chemistry and circulation of the oceans, raise sea levels, and reduce marine biodiversity.^{32,33} With these issues in mind, the term “blue economy” was introduced in 2012 during the Rio + 20 UN Conference on Sustainable Development to expand the blue aspect of the green economy, as a sub-field focused on marine activities and oceans environments.³⁴ In addition to a focus on ocean preservation, the blue economy consolidated the paradigm that sustainability also requires a balance between environmental protection, economic growth, and social justice and inclusion.³² There is still no unified definition of blue economy, but it revolves mainly around a sustainable ocean economy. Table 1 presents blue economy definitions established by leading representative organizations around the world.

The definitions shown in Table 1 overall agree that the blue economy is a concept related to the sustainable exploitation of the ocean and its resources. Nonetheless, while the preservation of marine environments is a priority, economic and

social variables are also considered for its design. Like the green economy, the blue economy can also be organized around (eight) main pillars, each with challenging principles and goals, as listed below.⁴³

1. Conserve oceans and marine life: The oceans absorb the excess heat and greenhouse gases and provide half the oxygen on earth.^{1,31} It also provides food, energy and allows the transport at a low (or at least fair) cost of a plethora of products and commodities. Therefore, the maintenance of a healthy ocean and the preservation of marine life is not only a matter of economy, but it is also vital for our survival.

2. Intelligent transport and shipping: Most of the shipping in the world is already by sea, but there is an increasing demand for more economic and green maritime shipping technology and effective sea routes.¹²

3. Improve aquaculture and fishing industry: With our current population growth and damage to the oceans due to climate change and pollution, it will be necessary to simultaneously maximize the efficiency and sustainability of fisheries and aquaculture's productive chains. These systems must be more efficient and generate less waste, also allowing marine species to regain sustainable biological levels.³⁴

4. Sustainable use of oceans resources: The industries discard more than half of the mass during processing. It is necessary to reduce seafood wastage to decrease the pressure on marine ecosystems and avoid pollution. The valorization of waste can, thus, be a source of revenue and job creation.⁴³

5. Renewable energy: The oceans can provide a reliable source of renewable energy in the form of waves and winds, and can help enable coastal communities to become energy self-sufficient.

6. Maritime and coastal tourism: This sector provides income for many disadvantaged communities, and is a critical

Table 1 Blue economy definitions, as established by leading representative organizations around the world

Organization	Definition	Ref.
Conservation International	“‘Blue economy’ refers to the range of economic uses of the ocean and coastal resources—such as energy, shipping, fisheries, aquaculture, mining, and tourism. It also includes economic benefits that may not be marketed, such as carbon storage, coastal protection, cultural values, and biodiversity”.	35
European Commission	“Blue economy encompasses all industries and sectors related to oceans, seas, and coasts, whether they are based directly in the marine environment (<i>e.g.</i> , shipping, seafood, energy generation) or on land (<i>e.g.</i> , ports, shipyards, coastal infrastructures)”.	36
The Center for the Blue Economy	“The ‘blue economy’ comprises the economic activities that create sustainable wealth from the world’s oceans and coasts”.	37
The Commonwealth of Nations	“The ‘blue economy’ is an emerging concept which encourages better stewardship of our ocean or ‘blue’ resources”.	38
The Ocean Foundation	“The (new) ‘blue economy’ refers to economic activities that are both based in, and which are actively good for the ocean, though definitions vary”.	39
United Nations	“A blue economy is a long-term strategy aimed at supporting sustainable economic growth through ocean-related sectors and activities while improving human well-being and social equity and preserving the environment”.	40
World Bank	“Blue economy is the sustainable use of ocean resources for economic growth, improved livelihoods and jobs, and ocean ecosystem health”.	41
World Wildlife Fund	“A sustainable blue economy is a marine-based economy that: (1) provides social and economic benefits for current and future generations, by contributing to food, security, poverty eradication, livelihoods, income, employment, health, safety, equity, and political stability; (2) restores, protects and maintains the diversity, productivity, resilience, core functions, and intrinsic value of marine ecosystems – the natural capital upon which its prosperity depends; (3) is based on clean technologies, renewable energy, and circular material flows to secure economic and social stability over time while keeping within the limits of one planet”.	42

source of income for several coastal countries. While irresponsible tourism can damage ecosystems, sustainable maritime and coastal tourism can help preserve and recover natural landmarks. This will be beneficial not only for the restoration of aquatic ecosystems but also for increasing touristic attraction and maximizing revenues.

7. Biotechnology: The biodiversity of sea life is a source for bioprospection and novel bioactive biomolecules, which can be produced biotechnologically through more sustainable systems (*e.g.*, recombinant technology, industrial enzymes, bioactive biomolecules for medical and industrial applications). There is a remarkable potential of application for these marine-based compounds for different medical and industrial applications.

8. Respect tradition: Although the preservation of marine life is a priority, it is also necessary to respect the cultures of coastal communities, discussing and elaborating strategies that allow for environmental protection while preserving traditions.

At this point, we must highlight that the blue economy is not a substitute for the green economy. Rather, it will be an additional and complementary guideline for action in a vulnerable and previously neglected ecosystem – the ocean. To elucidate the pillars of the green and blue economy and how they integrate, Fig. 3 shows a schematic summary of their main goals and principles.

In Fig. 3, the synergy between green and blue economies is evident, both in their principles and goals. The blue economy relies on perpetuating the actions of the green economy, while the green economy requires blue economy principles to advance towards more sustainable development goals. This synergy was very recently considered by the European

Commission (EC), when it proposed new guidelines for a sustainable blue economy, as part of the European Green Deal and the Recovery Plan for Europe. The commission emphasized the importance of the blue economy as crucial for achieving economic and environmental sustainability, arguing that “there can’t be green without blue”.⁴⁴ Therefore, it is necessary to keep these fundamentals in mind to address seafood wastage and protect marine ecosystems. They should be used as guidelines for establishing actions to save and manage resources and improve the efficiency and safety of the mining, drilling, shipping, fishing, and aquaculture industries.

While this perspective focuses on discussing circular economy models in the seafood industries, we must emphasize the need for policies to increase the safety and sustainability of maritime shipping, mining, and drilling, which are also crucial to fulfilling the precepts of the blue economy. For example, cargo ships can release toxic substances in the water and air, contribute to the introduction and migration of invasive species, and even physically damage coral reefs.^{45–47} Onshore and offshore drilling can generate hazardous or problematic discharges,^{48,49} in addition to the risk of total or partial destruction of natural ecosystems due to oil and gas spills.^{47,50–52}

Despite the relevance of the abovementioned activities, in our opinion, the complete synergy of green and blue economies will be achieved if implemented through a “biorefinery perspective”. Having this in mind, in the next section, we will discuss how biorefineries can integrate green chemistry into the seafood industry. Biorefineries are processing facilities that convert biomass (such as waste) into beneficial by-products.³ Considering the high volume of waste in fishery and aquacul-



Fig. 3 Principles and goals of the green and blue economy.

ture, a biorefinery model, *i.e.*, the creation of “marine (bio) refinery”, could benefit these industries while protecting the environment and maximizing revenues for society.

Biorefineries and sustainable marine industries

The integration of a biorefinery concept in traditional industries enables a transition to a bio-based economy model (bioeconomy), where renewable sources and the circular economy are the foundation.⁵³ The development of cost-effective, environmentally friendly, and circular technologies for processing biomass and waste, and converting it into value-added compounds are decisive factors to successfully implementing sustainable biorefineries, particularly those that contribute simultaneously to reducing industrial waste and energetic demand.⁷

Initially, the biorefinery model was conceived as a way to promote the switch from a petroleum-based to a bio-based economy, where renewable biological sources are central.^{7,53,54} Biorefineries are conversion facilities for biomass processing, adapted to their feedstocks, and generally decentralized and located close to its material sources.^{55,56} Today, this model is much broader, since biorefineries are designed to be highly efficient and accommodate even unrefined and hazardous raw materials (*e.g.*, industrial, agricultural and domestic waste) while generating innocuous and valuable by-products.^{54,57} Particularly for marine biorefineries, they aim to optimize the exploitation of ocean resources by recovering relevant substances from seafood residues, thus reducing waste creation and adding value to ocean-based processes.⁵⁸ Marine biorefineries, especially for algae, are already well-described and consolidated.^{58,59} For example, by applying a biorefinery approach, Baghel *et al.* had a rate of brown seaweed utilization of 93% in their processing.⁵⁸ They were able to develop an integrated strategy to recover 3.8 ± 0.2 g of protein concentrate, 10 ± 0.5 g of cellulose 32 ± 1.5 g of alginic acid, and 540 ± 5.5 mL of sap from 1 kg of wet brown seaweed biomass. This is a good example of an integrated process that allowed for recovering more by-products from the algae biomass, such as soluble algae products, protein concentrate, cellulose, and salts, in addition to having their effluents properly treated. Compared with conventional methods, the biorefinery model proposed by Baghel *et al.* allowed a reduction of the water footprint, and chemical and energy inputs, while providing extra revenue from the production of other commercially relevant substances.⁵⁸ Thus, considering the excessive amount of waste produced in fisheries and aquacultures, including biorefinery principles in these marine industries can turn a problem (seafood waste) into a source of energy and valuable (bio)products and, of course, an extra income.⁶⁰

In previous sections, we have highlighted that by applying the blue economy principles and a biorefinery approach, seafood waste can be reduced, and the use of natural resources optimized while increasing revenue and creating jobs. In tra-

ditional ocean-based industries, seafood (mainly algae, fish, and marine invertebrates) is obtained through capture by the fishery or farming with aquaculture. The products are usually transported fresh in localized distributions or undergo the first stage of preservation (*i.e.*, frozen, dried, or salted) and pre-preparation (*e.g.*, bleeding, eviscerating, beheading, filleting, and minced) for centralized distribution.⁶⁰ In conventional seafood industry models, after removing the primary products (seaweed for food applications and meat from fish and marine invertebrates), the residual biomass, wastewater, and lower-value animal parts (*e.g.*, skin, scales, fins, head, viscera, bones, shells) are discarded as seafood waste in the ocean or landfills.²

Seafood waste not only causes pollution and environmental risks but also generates costs for its disposal (if properly managed). However, seafood waste has a complex and highly nutritional composition, with elevated amounts of proteins, oils, pigments, natural polymers, and other bioactive biomolecules of high commercial value. Therefore, with proper and efficient secondary processing, it is possible to extract these valuable by-products from the rich marine residues and increase the economic and environmental sustainability seafood industry. Furthermore, after isolating all higher-value products, the remaining residues can also become feedstock for microbial conversion through the cultivation process to produce more biomolecules and chemicals (*e.g.*, alcohol, acids, biosurfactants, nutraceuticals, and biopharmaceuticals). The cultivation stage can also generate energy to supply the productive chain and ensure the system's energy self-sufficiency. Finally, depending on previous stages of processing, there may be solid residues that the industry can still use in the formulation of fertilizers and silage.

This thoughtful and intelligent process design, which applies green engineering principles, is critical for a successful waste management in a biorefinery approach. Having it in mind, in Fig. 4, we schematized a processing chain to eluci-

Valorization of seafood waste

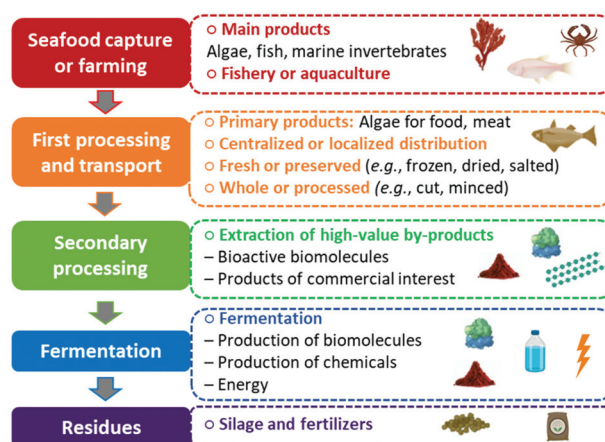


Fig. 4 Valorization of seafood waste with a biorefinery approach.

date what should be considered for the effective valorization of resources from seafood waste.

In Fig. 4, we schematized how a biorefinery approach could work for seafood waste valorization. Nonetheless, the feasibility of each stage of the biorefinery strongly relies on the composition and amount of the raw materials, as well as the type of industry they aim to supply. Hence, the first and crucial step in successfully designing a marine biorefinery is an in-depth knowledge of each resource, productive chain, operational limitations, and field of application. It is essential to know not only the composition of the feedstock (in this case, seafood waste from different sources), but especially the potential market value and application of the biomolecules, chemicals, and other by-products that can be isolated or converted from each type of waste. The following section will explore the different sources of seafood waste (*i.e.*, from fishing and aquaculture industries), the products they can generate, and their commercial applications.

Fishing and aquaculture industries waste and products

According to the United Nations Educational, Scientific, and Cultural Organization (UNESCO), researchers estimate that the ocean is home to 700 000 marine species.⁶¹ However, a few of them comprises the majority of the volume of commercial seafood, including as the most prevalent: marine invertebrates – shrimps, oysters, crabs, lobsters, scallops, and squids; and fish – different species of tuna, cod, herring, salmon, anchovy, flounder, and mullet.² Each type of aquatic species has a distinct composition and, therefore, different (bio)technological potential and applications.

The type of seafood, final product and processing parameters will directly impact the amount of waste that each fishery and aquaculture industry produces.⁶⁰ For example, only 45% of catfish mass is used for human consumption. For marine invertebrates, this number is even lower, *cf.*, prawns (40%), crustaceans (39%), shrimp (35%), crabs (32%), and mussel (14%).⁶² As for algae, certain species are entirely used as food (edible seaweed), generating almost no residues, while other species have no food applications, being cultivated or harvested only for the production of bio-compounds or fuel.^{63,64} However, it is important to highlight that the composition and concentration of bioproducts of interest in algae will be highly dependent of each algae species, as well as seasonal variations and types of biomasses (*i.e.*, parts of the algae). For example, the alginic acid content in brown seaweeds can vary from 17 to 47% of their dry weight depending on their species, seasonal variations, and parts of the algae.⁶⁵ *Ascophyllum nodosum* has 22 to 30% of alginic acid in their dry weight, while *Laminaria digitata* has 25 to 44% in their fronds and 35 to 47% in their stipes, and *Laminaria hyperborea* present 17 to 33% in their fronds and 25 to 30% in their stipes.⁶⁵

Overall, about 50% of the seafood mass is discarded.⁶² Still, these residues can be used by industries as a source of valu-

able by-products. If processed and preserved correctly, they can conform to the low-risk category 3 by-products of European Union (EU) regulation, *viz.*, “parts of animals that have been passed fit for human consumption in a slaughterhouse but which are not intended for consumption”.⁶²

Seafood waste is a remarkable source of by-products, mainly because it is a matrix rich in nutrients and bioactive molecules. Lipids comprise up to 60% of seafood residues (fish oil),⁶⁶ where the predominant oil is triacylglycerol, like most other natural oils.⁶⁶ Particularly, fish waste consists mainly of heads, frames, belly flaps, and parts of the viscera, such as liver and roe (containing valuable proteins and lipids).⁶⁰ A sizable drawback of fish waste is its fast deterioration, so industries must preserve these residues as soon as possible after capture or harvest.⁶⁰ Marine invertebrates waste also contains proteins (~40%), chitin (~25%), calcium carbonate matrix (~30%), lipids (~10%), and highly valuable carotenoids (*i.e.*, pigments) such as astaxanthin (which has an outstanding antioxidant potential).⁶⁰ As can be seen, there are many commercially relevant substances in seafood waste. In Table 2, we summarized the main classes of these by-products that can be obtained from fishery and aquaculture waste, listing their primary sources and industrial applications.

As detailed in Table 2, depending on the type of seafood waste, various by-products can be obtained and used in many industrial sectors, such as food, medicine, chemicals, materials, fertilizers, fuel, and animal feed. These can be extracted and isolated from algae biomass, marine invertebrate shells and viscera, the skin of octopus, fish residues (*e.g.*, bones, skin, scales and fins, viscera, head, and frame), or even from wastewater from the fishery and aquaculture industries. Moreover, the residues can be used as a carbon and nitrogen source to produce other high-value products through microbial bioconversion. Considering the great diversity of by-products that can be obtained from the different classes of seafood waste, in the following paragraphs we discuss the most representative examples according to their chemical nature.

1. Carotenoids: The most prevalent liposoluble pigments in seafood waste are carotenoids.^{70–72,75,82,87–89,122} Astaxanthin alone comprises 74 to 98% of pigments mass in marine animal shells, with applications in the food and medical industries as a colorant, antioxidant, and vitamin A precursor.⁷⁵ This carotenoid also has promising properties against certain types of cancer, can enhance the immune systems, and protect cells against radiation and muscle degeneration caused by aging.⁷⁵ Other commercially relevant carotenoids with antioxidative properties present in seafood are β -carotene (vitamin A precursor),^{70,87} lutein,^{71,87} and zeaxanthin. β -Carotene is a natural antioxidant and an alternative source of vitamin A. This carotenoid is associated with aging prevention, benefits to night vision, improved immunity, skin, hair, and nails health, and fat metabolism. In addition to some food and nutraceutical applications, β -carotene has been used in cosmetics, such as skin tanning lotions and anti-aging creams.¹²³ The food industry applies carotenoids as natural colorants in substitution of their synthetic counterparts.^{124,125}

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However, there are still drawbacks to fish oil as a food additive and nutraceutical, mainly related to its intense flavors and the low stability of its components.^{75,93} Besides nutraceutical and food uses, fish oil has other relevant applications. They include feedstock for biodiesel production,¹²⁷ a consolidated use as drying oil in paints, coatings, and printing inks,¹²⁸ and for fat liquors production for application in the leather industry.¹²⁹ In addition to fish oil, shark liver oil is rich in squalene, a high-value marine product. Squalene is an organic molecule of the isoprenoids family (linear triterpene), with several nutraceuticals and therapeutical applications. They include antioxidant, detoxifier, hypocholesterolemia, cardioprotective, and anti-cancer properties.¹³⁰ Squalene is extensively employed as a principal component of parenteral emulsions for drug and vaccine delivery.¹³¹

3. Fermented products: The chemical or biological hydrolysis of seafood can generate seafood protein hydrolysates (SPH) that are formed by small fragments of peptides containing amino acid residues.⁷⁵ The SPH are used in food, pharmaceutical, and cosmetic formulations due to their bioactivities (e.g., antioxidant, antidiabetic, and immunomodulatory properties) and umami flavor. They also present chelating, hygroscopic, and surfactant properties. For example, the fermentation of seafood can generate different commercial products, such as the traditional fish sauce from Asian culinary made from SPH,^{97,106,126,132} and also transform seafood waste residues on silage for the production of animal feed and fertilizers.^{97,106–108}

4. Minerals: Marine invertebrates shells and fish bones, fins, and scales are a rich source of minerals and other micronutrients.^{75,93,94} For example, inorganic minerals such as hydroxyapatite, calcium, phosphate, zinc, selenium, and iron represent almost two-thirds of fish bones. The fish bone powder is a rich source of calcium (around 234 g calcium per kg of dry bone) and other minerals with potential health benefits in growth and metabolism.⁷⁵ Hydroxyapatite can be used for rapid bone repair after surgery or trauma and as a bone graft in dental applications.^{93,95,96,117,118} Calcium, phosphate, zinc, and iron are applied as food supplements, chemicals, or fertilizers.^{75,93,94}

5. Natural polymers: Chitin is the most prevalent natural polymer from marine invertebrate shells, while agar, alginate, and carrageenan are the most common polymers from algae biomass.^{63,75,90–92} It is also possible to process chitin into chitosan, a biodegradable and biocompatible polymer. Chitosan has antimicrobial, antioxidant, anticoagulant, anticancer, and film-forming properties.^{75,90–92} Because of these characteristics, the medical and dental fields use chitosan for material development. For example, the biomedical field uses chitosan for artificial tissue reconstitution (e.g., skin, bones, and cartilage). In the food industry, natural polymers are applied as biodegradable films and as a flavoring agent, while they are used in the development of drug delivery systems by the pharmaceutical industry.⁷⁵ The food, chemical, and pharmaceutical industries use agar and alginate as thickeners, plasticizers, and gelling agents.⁶³ Agars are also widely employed in microbiology for the cultivation of microorganisms on solid

medium.¹³³ Recently, agar-based films present an alternative to plastic as biodegradable and edible food packaging.¹³⁴ Analogous to agar, Funori is a polysaccharide mucilage made from the seaweed *Gloiopeltis* sp., common in Japan.¹³⁵ It is used as a weak water-soluble adhesive, and it can form a gel at concentrations as low as 1%. Funori has been also added to hygiene and cosmetic formulations and used as a conservation material.¹³⁶ Carrageenan, also called Irish Moss, is a natural polymer of the sulfated linear polysaccharide family obtained from red seaweed extracts. This biopolymer is currently used as a natural thickening agent in foods, beverages, gels, and lotions.¹³⁷

6. Organic solvents: Seafood waste can be used as feedstock to produce a series of organic compounds by fermentation, such as alcohols and acids. Most times, the microbial cultivation of seafood waste will aim to produce ethanol and energy,⁸⁵ but other organic (bio)solvents can be also obtained through an adequate choice of the microbial species.

7. Protein and peptides: This is the most diversified and highly valued group of seafood by-products, with applications in most industrial areas. It includes bioactive peptides (antioxidant and antihypertensive), collagen and gelatin, enzymes, peptones, and protein extracts. In addition to traditional uses in enzymatic reactions or as medicines, there are also novel and disruptive applications of these ocean biomolecules. For example, phycobiliproteins from red algae can be used to produce photovoltaic cells to capture low light underwater.¹³⁸ Regarding the most common protein and peptide-based products, they include:

(a) Bioactive peptides (antioxidant and antihypertensive) – Usually extracted from the liver and viscera of fish and are mainly applied as medicines.⁹⁷

(b) Collagen and gelatin – Obtained by thermal denaturation of collagen, they are proteins with applications in pharmaceutical, biomedical, leather, cosmetics, and tissue-engineering industries due to their unique structural and functional features. The fish skins, scales, and fins are rich in collagen, but it is also present in the meat and viscera of fish.^{75,112–116}

(c) Enzymes – Different classes of enzymes can be found on the viscera of fish and marine invertebrates, particularly digestive enzymes such as carbohydrases,⁹⁷ proteases (e.g., chymotrypsin, pepsin, and trypsin),^{99,100,119–121} lipases, and phospholipases.^{97,100} Other enzymes include lipoxygenase, myosin ATPases, polyphenol oxidases, transglutaminases.^{99,100,119–121} Most enzymes extracted from seafood are used for medical and industrial applications, but lipases and phospholipases are also employed by the food, chemical, and biofuel industries.^{97,100}

(d) Peptones – Peptones are semi-digested proteins that are used as a carbon and nitrogen source. They can be extracted from fish and marine invertebrates waste and used to produce animal feed, chemicals, and nutraceuticals.⁸⁴

(e) Protein extracts – Seafood is a rich source of animal protein. Protein extracts can be obtained from algae, fish, and invertebrate animal waste. They can be used as food additives, nutraceuticals, chemicals, pharmaceuticals, animal feed, and fertilizers.^{63,75}

To better elucidate the sources and applications of different seafood waste products, a schematic representation associating each product and its respective source(s) and application(s) is presented in Fig. 5.

As shown in Fig. 5, it is possible to use seafood waste to formulate many products, with direct or indirect application in most industrial areas. Consequently, there are many opportunities for implementing marine biorefineries as an ideal solution for seafood waste valorization. However, biorefineries implementation can be exceptionally complex and requires a study and validation of all processing stages. These stages usually include marine life capture, pre-processing of seafood, its conservation and transport, downstream processing of high-value by-products, fermentation, and formulation of products. In the next section, we present concepts and examples of marine biorefineries, elucidating how they work and their potential applications.

Marine biorefineries for seafood waste valorization

Traditionally, residues and side/by-products are considered low-value substances, which are regularly employed as feed, fertilizers, or simply discarded as waste.^{2,139} Nevertheless, if

properly extracted, isolated, and processed, they can become a valuable source of commercially relevant bioproducts, such as lipids, proteins, pigments, chitin, minerals, among others.⁶⁰ These substances have a wide range of applications, such as colorants, food supplements, nutraceuticals, pharmaceuticals, cosmetics, and others, as previously listed in Table 2.^{140,141} It is clear that the valorization of seafood waste (*e.g.*, fish and crustacean industries) is crucial for ecological reasons as well as a source of additional revenue for the productive sector. However, the conversion of residues into commercial goods relies on the successful implementation of large-scale biorefineries that create products with substantial market demand. Their manufacture should also be economically viable and environmentally friendly, ideally respecting the principles of a circular economy. To clarify these aspects, in this section, we propose a conceptual perspective panorama on a potential marine biorefinery (Fig. 6), including a careful analysis of the main steps required for seafood waste valorization, namely: extraction of primary high-value by-products; microbial cultivation for the production of other valuable biomolecules production; process design and integration.

The perspective panorama in Fig. 6 represents potential marine biorefineries within a zero-waste model, exemplifying how a biorefinery can work towards an efficient valorization of seafood waste. The processing of seaweed will vary depending

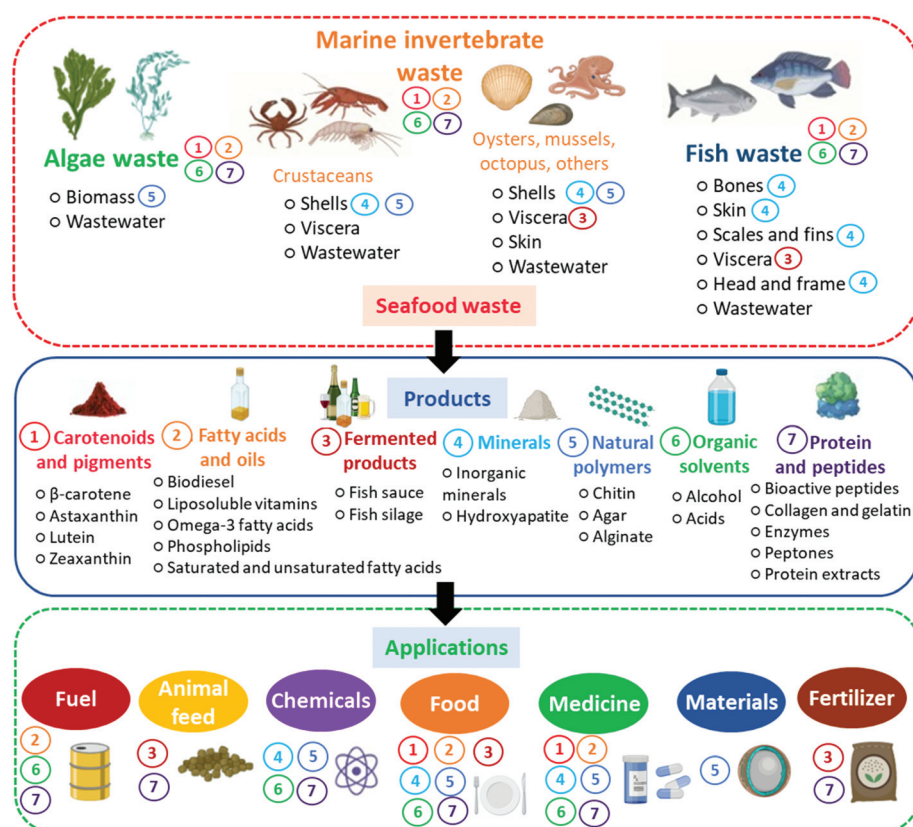


Fig. 5 Products from seafood waste and their sources and applications. Products are correlated with their sources and applications by the numbers from 1 to 7 next to them.

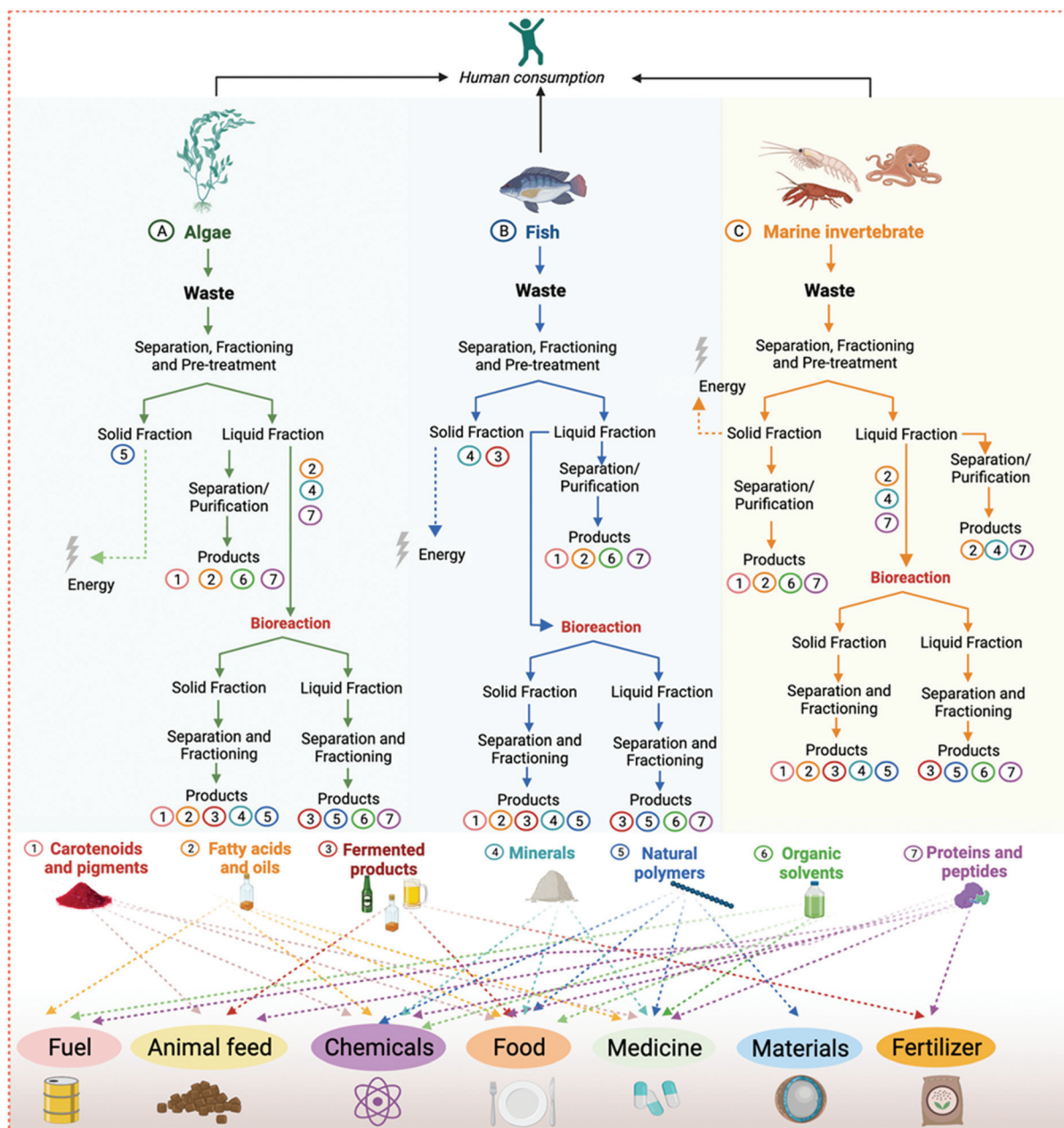


Fig. 6 Perspective panorama of potential marine biorefineries.

on its type, considering that some are edible and can go entirely/partially for food applications, while others are cultivated for the extraction of bioproducts such as alginate, agar, and carrageenan.⁶³ As for fish and marine invertebrates, after removing their “meat” (*i.e.*, the flesh of an animal used for food purposes) for human consumption, the residues can go through the separation of liquid and solid fractions.⁶⁰ Usually, the seafood solid residues are employed directly in the production of fertilizers or silage for animal feed. Nonetheless, this is not ideal at the beginning of the process, considering they are commercialized as low-value products. In a biorefinery approach, solid residues must undergo separation, fraction,

and pre-treatment stages, where promising substances are recovered and the solid fractions pre-processed for further extraction of these high-value biomolecules.⁶⁰ Particularly for shell-waste, it is necessary to design a pre-treatment for their deproteinization and demineralization.^{142,143}

The liquid fraction of marine invertebrates and fish waste, particularly from their viscera, is a rich carbon and nitrogen source (*e.g.*, proteins, carbohydrates, lipids, and minerals).^{144–146} Therefore, it is possible to directly extract some valuable products (*e.g.*, bioactive molecules, proteins, colorants, lipids), while the rest can become feedstock to produce biomolecules through fermentation (*i.e.*, microbial

cultivation).⁶⁰ Like the solid seafood fractions, the microbial biomass from fermentation can be processed for the recovery of commercially relevant substances. In a similar way, the liquid fraction of the fermented broth can also be processed to obtaining high-value biomolecules (e.g., proteins, pigments, polymers) or organic compounds (e.g., fuel and chemicals). In addition to generating high-value substances, fermentation processes can be included to produce energy for the industrial complex. The solid fractions (biomass) of different stages can be also processed for energy production.

After the pre-treatment of the solid fractions, they will go to the extraction stages. The recovery steps will vary according to the nature of target biomolecules and their final applications, as well as the source and composition of the crude fractions. The separation methods include liquid–liquid extraction, ultra-filtration, evaporation, precipitation, and potentially chromatography according to the degree of purity required. To maintain the sustainability of the biorefinery, it is also essential to include adequate solvent recycling procedures, ideally using solvents produced in the fermentation stage, or biosolvents obtained from renewable sources with minimal ecological impact. After extracting all high-value products, the remaining solid residues can finally be used to produce silage and fertilizers. It is important to emphasize that it is also possible to recover chitin and inorganic minerals after deproteinization and demineralization of shells (especially for crustacean-waste).

Chitin can be converted into chitosan by its partial deacetylation (above 50%).¹⁴⁷ During deacetylation, the *N*-acetyl-D-glucosamine monomers of chitin are converted into randomly chained *N*-acetyl-D-glucosamine and D-glucosamine monomers. This process can occur in highly alkaline environments (e.g., 40 to 50% NaOH) or in the presence of chitin deacetylase enzymes (the biocatalytic reaction converts chitin + H₂O into chitosan + acetate). The quality and size of chitosan will be strictly dependent on the purity and molecular weight of the starting chitin, as the methods and conditions employed for deacetylation. These marine-based polymers (particularly chitosan) can be used for the manufacture of (bio)materials or the production of glucosamine. These applications have made chitosan a highly valued compound, with an annual market of USD 2.49 billion in 2020, with an expected Compound Annual Growth Rate (CAGR) of around 12% during 2021–2026.¹⁴⁸

Although Fig. 6 represents the potential “ideal scenario” for a marine biorefinery (i.e., with circular processing and zero waste), a closed and waste-free system is not always economically or even environmentally viable. For example, by optimizing the processing routes for a marine biorefinery (using fish, crustaceans, and cartilaginous species), Antelo *et al.* found that it was better to use specific parts of the fish than the whole animal, from both environmental and economic perspectives.¹⁴⁹ These researchers also demonstrated that some products (e.g., biopeptides, chondroitin sulfate, and fish enzymes) have a higher potential than others due to their elevated commercial prices and low environmental impacts.¹⁴⁹ Nevertheless, they concluded that there is still a need to improve the recovery of chitin, gelatin, and fishmeal from

seafood waste as, while profitable, it had a severe environmental cost due to its high energy and large water consumption.¹⁴⁹ In addition, Antelo *et al.* observed that many of the optimal valorization pathways still generated waste, even if in small quantities.¹⁴⁹

In our opinion, although a circular process and zero waste are preferred, the pursuit of a “perfect” marine biorefinery should not prevent industries from implementing systems capable of reducing waste or aggregating value to their products. With this information in mind, in the following subsections, we will give examples of seafood waste valorization strategies for marine biorefineries designs, namely, for the extraction, microbial cultivation, and process integration stages.

Extraction of by-products from seafood waste

As previously demonstrated, seafood waste is a complex matrix with a mix of natural polymers, macromolecules, and minerals. Therefore, the extraction of by-products from these residues is usually a multi-step process that involves different separation techniques. The design of the extraction platform will vary depending on the characteristics and properties of the raw substrate and target compounds, as well as desired purity degree of commercial goods. For example, it is possible to use simple physical methods to recover fish oil from seafood waste. Nonetheless, the recovery of chitin and proteins from seashells is more complex, requiring additional demineralization and deproteinization steps to break the strong chemical bonds between the components of the biological matrix.^{142,143} Furthermore, it is possible to add a cultivation step to improve the recovery of chitin, proteins, lipids, and pigments from seafood.¹⁴⁵ Besides helping in the extraction of by-products, microbial cultivation can also allow the production of other relevant biomolecules (e.g., adding carotenogenic microorganisms to produce carotenoids). With so many possibilities and variables, it is necessary to carefully evaluate each processing stage before implementing a new biorefinery. In this subsection, we will explore different strategies for the recovery of by-products from seafood waste. Moreover, the differences between working with algae, fish, or seashell residues will also be discussed.

Saturated and unsaturated lipids are the most prevalent by-products of fish waste.⁶⁶ For their recovery, it is possible to apply even physical separation methods (e.g., heat, filtration, and centrifugation).¹⁵⁰ However, the high water content of fish residues can create emulsions, reducing the value of the oil recovered, while complicating subsequent downstream processing.¹⁵⁰ For this reason, chemical methods are also often employed, such as liquid–liquid extraction. For example, Khoddami *et al.* extracted oils and fatty acids from sardine waste (mainly head, liver, and intestine) using liquid–liquid extraction with chloroform and methanol.¹⁰⁵ Furthermore, the degree of purity will also influence the extent and complexity of the separation of lipids from fish waste. Although we can separate aqueous and hydrophobic phases employing simple physical methods or liquid–liquid extractions, further processing is needed to isolate lipids with similar physical–chemical

properties. For example, saturated and longer alkyl chain lipids generally have a higher fusion point than shorter and unsaturated lipids. Therefore, these distinct physicochemical properties can be exploited for their separation and isolation. Although this processing approach would be more than enough to obtain fish oil for the food and cosmetic industry, it may not be enough for a pharmaceutical formulation, which requires specific lipids with a high purity degree. Lipid refining processes include active filtration using adsorbents, distillation, chemical purification, and membrane separation.¹⁵⁰ Each of these purification methods has its advantages and drawbacks, and your selection will depend on the substrate and intended application of the target lipids.

From seashell waste, it is possible to extract carotenoids,¹⁵¹ proteins,¹⁵² chitin,¹⁵³ and inorganic minerals.⁷⁵ However, it is usually necessary to carry out initial demineralization and deproteinization procedures to extract them, considering the strong linkage between chitin and proteins, calcium carbonate, lipids, and pigments in the shell.^{142,143} It is possible to recover these compounds by a conventional chemical route using acids for demineralization and alkali treatment for deproteinization. For example, Charoenvuttitham *et al.* extracted chitin from black tiger shrimp shells using NaOH and different mixtures of acids. With the conventional method using 0.25 M hydrogen chloride (HCl), these researchers obtained 86.5% of chitin from protein and minerals, while achieving 88.1% of purity with a new approach using a solution of formic acid and citric acid at 1 : 2 (v/v).¹⁵⁴ Alternatively, some authors have been associating chemical and physical methods to improve chitin deproteinization. For example, Kjartansson and Zivanovic used sonication after adding the acid (HCl) and base (sodium hydroxide, NaOH) to increase the deproteinization of chitin from shrimp (*Pandalus borealis*) shells.¹⁵⁵ However, this approach also resulted in lower yields, which the authors attributed to higher concentrations of depolymerized materials in the wash water.

As can be seen with the previous examples, acidic and alkaline chemical treatments are the routine protocols for the recovery of products from shells, mainly due to their low cost, simplicity, high yields, and the possibility to reuse or recycle its components.¹⁵⁶ However, the problem with using these traditional chemical extraction methods is that they still rely primarily on large amounts of toxic solvents that pollute and persist in the environment,^{157,158} as well as with concerns about handling them. Of course, these traditional methods are not sustainable solutions for a transition to a blue economy. Therefore, academics and industries seek substitute practices with lower risks and environmental impacts.

Non-conventional biotechnological protocols (*e.g.*, microbial-based or biocatalytic) could be alternatively applied.^{142,159} Researchers have been proposing the use of biological methods to recover marine products as an alternative to conventional chemical extraction,¹⁶⁰ for example, using enzymes (*e.g.*, proteases) for the deproteinization step during the chitin extraction. Ghorbel-Bellaaj *et al.* used a crude metal-

loprotease from *Pseudomonas aeruginosa* A2 for the deproteinization of shrimp waste. After two hours of hydrolysis at 40 °C, there was up to 85% of chitin deproteinization. The purity of the sample was also high and comparable to a commercial α -chitin.¹⁶¹ In a similar strategy, Manni *et al.* used proteases produced by *Bacillus cereus* SV1 to remove 88% of the proteins from chitin from shrimp waste.¹⁶² These are good examples of how enzymatic reactions can be employed to extract chitin from seashell waste.

Instead of using crude or isolated proteases, it is also possible to cultivate the seashell residues with microorganisms capable of producing proteases. Following this approach, Ghorbel-Bellaaj *et al.* evaluated the aptitude of six protease-producing *Bacillus* species (*i.e.*, *B. pumilus* A1, *B. mojavensis* A21, *B. licheniformis* RP1, *B. cereus* SV1, *B. amyloliquefaciens* An6, and *B. subtilis* A26) to extract chitin from shrimp shell waste during microbial cultivation.¹⁶⁰ The researchers used shrimp waste as an alternative carbon and nitrogen source in the liquid culture medium, inducing the bacteria to produce proteases to break the strong bonds between the components of the shells and access its nutrients.¹⁶⁰ The proteases of all strains were able to generate at least 80% of deproteinization of chitin, but only a maximum of 67% of demineralization. However, this study found that supplementing the medium with 5% (w/v) glucose improved demineralization rates without decreasing deproteinization.¹⁶⁰ With a similar method, Doan *et al.* analyzed the deproteinization of chitin from shrimp waste with submerged cultivation using the alkaline protease-producing strain *Brevibacillus parabrevis*, achieving 95% of protein removal after four days of cultivation.¹⁶³ Ghorbel-Bellaaj *et al.* also employed a protease-producing microorganism (specifically, *Pseudomonas aeruginosa* A2) to enhance chitin extraction from shrimp. Using this method, they reached 96% of demineralization and 89% of deproteinization.¹⁶⁴ These successful examples make us believe that biological processes (using isolated microbial-produced proteases or protease-producing microorganisms) can be successfully applied to extract chitin from shell waste.

Microbial cultivation processes that generate acids can also be used to replace or enhance chemical deproteinization and demineralization of chitin from seashells. For example, Castro *et al.* improved chitin extraction from crab (*Allopetrolisthes punctatus*) by applying preliminary a lactic ensilation using *Lactobacillus plantarum* sp. 47 followed by chemical deproteinization and demineralization.¹⁶⁵ For that, bacteria were inoculated into the biomass and cultivated in a semi-solid medium until reaching the lactic acid peak production (*i.e.*, 17 mg lactic acid per gram of silage with 10% inoculum, 15% sucrose, 85% biomass). After 60 h of processing, the chitin was recovered from the biomass using 0.4 M NaOH and 0.5 M HCl, allowing removal of 99.6% of minerals and 95.3% of proteins from the biopolymer.¹⁶⁵ The additional cultivation step for chitin extraction increased both quality and yield when compared to conventional chemical methods.¹⁶⁵ In another study, Bhaskar *et al.* evaluated the effect of *Pediococcus acidolactici* cultivation for the recovery of chitin and carotenoids

from shrimp waste.¹⁶⁶ Using this bioprocess, chitin demineralization and deproteinization yields reached 72% and 98%, respectively, while 4.3 g of carotenoids were also recovered, after 72 h of submerged cultivation.¹⁶⁶ Lactic fermentation appears as another suitable bio-strategy for recovering biomolecules from seashells.

Despite the benefits of microbial cultivation procedures in the recovery of seashells bioproducts, namely, lower risks and environmental impacts, and high efficiency and specificity, these bioprocesses have not been applied on an industrial scale yet.¹⁵⁶ As with other industrial residues, one of the main difficulties in using shellfish waste as a substrate for microbial cultivation is the great variability of properties and quality of residues, which will have a strong impact on the outcome of the process.¹⁵⁶ For example, Kjartansson and Zivanovic observed that chitin obtained from North Atlantic shrimp (*Pandalus borealis*) had more impurities than the same compound from freshwater prawns (*Macrobrachium rosenbergii*), even using the same extraction method.¹⁵⁵ These researchers demonstrated that the composition and structural arrangements of chitin in shells have a major impact on extraction process efficiency. The *Pandalus borealis* have low lipid content (0.3 to 0.5%), high protein (33 to 40%), and high ash (32 to 38%) dry weight,¹⁶⁰ while other species, such as the *Metapenaeus monoceros* shrimp, have more lipids (6%), lower protein (25%), and higher ash (46%) content. These are considerable differences between species that will directly impact chitin recovery. In addition, there are also seasonal variations that will also change the composition of seafood. These changes will influence purification outcomes and difficult the scaling of microbiological extractions of chitin.¹⁶⁰

To summarize seashell waste deproteinization and demineralization strategies, in Table 3 we compiled the different methods, detailing the major reactants or systems, as well as deproteinization and demineralization yields.

As clearly shown in Table 3, it is possible to achieve high deproteinization and demineralization yields using the most distinct methods. This picture is transversal to the other biomolecules, especially if the extraction is applied to the valorization of different seafood residues. For this reason, there is no

standard and universal procedure for the recovery of marine substances, and the efficiency of each process is always dependent on the type of seafood waste (e.g., type of organism, concentration, and nature of target biomolecule) and the operational extractions procedures (e.g., extraction time, temperature, biomass, and solvent ratio). Hence, we believe that using biorefinery and circular economy approaches, viz., introducing efficient bio-based pre-treatments, integrating downstream operation units, and including adequate solvent recycling protocols, can overcome some of the current ecological and economical concerns.

Chemical extraction will appear to be the simplest and most convenient compared to biological alternatives. Unfortunately, if the chemical operations are still carried out following traditional methods (e.g., using acids or alkalis) the sustainable environmental appeal of the marine biorefinery will not be achieved. Therefore, we suggest applying strategies that allow the efficient recovery of by-products and simultaneous recycling of the solvents, as well as the use of “green” solvents [e.g., biosolvents, ionic liquids (ILs), and deep eutectic solvents (DES), supercritical fluids], which can be fully, or at least partially, obtained (using sustainable technologies) from renewable raw materials.

In the last two decades, some research groups have proposed the use of ILs and DES as an alternative for the extraction of biomolecules from seafood waste.^{156,167} ILs are a diverse group of salts with low melting points that are easily tailorable to obtain distinct structures with useful properties for industrial applications (e.g., low vapor pressure, high thermal stability, low toxicity, and solvation capability).¹⁶⁸ As ILs, DES are also solvents with outstanding properties suitable for industrial use. However, instead of chemical reactions, they are produced with eutectic mixtures of Brønsted or Lewis acids and bases.¹⁶⁹ Additionally, ILs and DES have previously demonstrated great aptitude to act as dissolution agents for biopolymers such as cellulose, lignin, and starch.^{170,171}

Besides the environmental advantages of using ILs and DES, it is possible to exploit their unique properties to integrate different downstream stages, such as demineralization, deproteinization, and extraction. For example, Qin *et al.* used

Table 3 Different seashell waste sources, types of methods for deproteinization and demineralization, reactants and systems, and deproteinization and demineralization percentages for chitin extraction from seashell waste

Source	Type of method	Reactants or systems	Deproteinization (%)	Demineralization (%)	Ref.
Shrimp	Chemical	NaOH, formic acid, citric acid	88 (total purity)		154
		NaOH, HCl	87 (total purity)		
	Chemical and physical	HCl, NaOH, sonication	98	97	155
	Enzymatic	Metalloprotease from <i>P. aeruginosa</i> A2	85	Not significant	161
		Protease from <i>B. cereus</i> SV1	88	N/A ^a	162
	Microbiological	Protease-producing <i>B. spp.</i>	80	67	160
		Protease-producing <i>B. parabrevis</i>	95	N/A ^a	163
		Protease-producing <i>P. aeruginosa</i> A2	89	96	164
		Lactic acid bacteria <i>P. acidolactici</i>	98	72	166
Crab		Lactic acid bacteria <i>L. plantarum</i> sp. 47	95	97	165

^a N/A – not available.

imidazolium-based ILs to completely dissolve crustacean shells and recover chitin powder of high molecular weight and purity.¹⁷² This is an interesting approach since it allows not only to obtain pure chitin powder but also other chitin-based products, namely, chitin fibers and films directly from the IL solutions of dissolved shrimp shells. Tolesa *et al.* also employed ammonium-based ILs for the extraction of chitin and conversion to chitosan from shrimp shells.¹⁷³ After treating shrimp shells with ILs, 14% of chitin was extracted from the original biomass and successfully converted to chitosan under ILs alkaline conditions. As for DES, Zhongji *et al.* proposed the direct extraction of chitin from shrimp shells using DES, namely, mixtures of choline chloride as hydrogen bond acceptor and thiourea, urea, glycerol, and malonic acid as hydrogen bond donors. Their results demonstrated that a mixture of choline chloride–malonic acid improves the chitin isolation by almost 20%.¹⁶⁷ In a similar study, Saravana *et al.*¹⁷⁴ used different DES to extract chitin from shrimp shells (*Marsupenaeus japonicas*). Curiously, in this work, the high purity of chitin was also obtained using a mixture of choline chloride–malonic acid with a yield of 19.41%.

Although most studies focus on the recovery of natural polymers, it is possible to recover other classes of biomolecules from seafood waste. For example, some researchers have applied alternative IL-based extraction methods for the recovery of marine pigments from seashells waste, such as astaxanthin.⁸⁹ Using a combination of ultrasound and a solution of imidazolium-based ILs with biosolvent (ethanol) as extractant agents, Bi *et al.* reported that astaxanthin extraction yields from shrimp wastes increased 98%.¹⁷⁵

Nunes *et al.* evaluated another non-conventional extraction strategy to recover astaxanthin-rich extract from crab shell wastes using microwaves for pretreatment and supercritical fluid for extraction (ethanol as co-biosolvent).⁸⁹ This new methodology improved 12-fold the extraction efficiency when compared to the traditional soxhlet protocol. This result demonstrates that the strategy of integrating non-conventional technologies was effectively capable of intensifying astaxanthin extraction from crab wastes.

As presented above, alternative extraction methods and novel solvents, namely, ILs, DES, biosolvents, and supercritical fluids, have great potential to improve the recovery of seafood waste by-products while also reducing hazards and environmental pollution, integrating different processing steps, and enhancing yields. However, our opinion is that the most efficient route to enhance the valorization of seafood waste is to associate the chemical processing of biomass and recovery of by-products with additional bioprocessing stages, in which the microbial cultivation is applied both to digest the raw materials and to produce other high-value biomolecules. With this in mind, in the next subsection, the use of seafood residues as feedstock for the production of bioproducts is discussed.

Microbial production of biomolecules in the seafood industry

Different industries have exploited the application of marine waste to produce value-added bioproducts.¹⁷⁶ Seafood waste,

viz., heads, tails, fins, chitinous materials, or viscera, represents a rich source of pigments, proteins, lipids, and chitin, which can be recovered or used as low-cost substrates to produce several bioproducts. In most cases, these substrates are subjected to an initial pre-treatment to transform them into suitable feedstocks for cultivation.¹⁷⁶ For example, viscera or heads from fish waste are cooked, pressed to remove water, and dried. This pre-treated solid fraction (rich in high contents nitrogen, phosphorus, and calcium)¹⁷⁶ have been successfully used as a fertilizer for the production of ice lettuce (*Lactuca sativa* L.),¹⁷⁷ tomato crop,¹⁷⁶ and biopharming¹⁷⁸ (Fig. 6). On the other hand, the nutrient-rich aqueous supernatant is often used as a basal medium in cultivation procedures to produce several biomolecules.^{144,145}

Microbial cultivation using fish waste as a source of carbon and nitrogen is considered a low-cost, safe, and sustainable technique to obtain a wide range of valuable compounds.¹⁴⁵ For example, fish waste (from *Sardinella anchovia*, *Lepophidium profundorum*, *Trachurus lathami*) was used as a substrate for the extracellular production of L-lysine by *Corynebacterium glutamicum* ATCC 21543, achieving 30 g L⁻¹ of this amino acid after 72 h of submerged cultivation.¹⁴⁴ Horn *et al.* studied the bacterial growth of *Lactobacillus plantarum* in a medium containing cod viscera hydrolysates, observing a 10% increase in biomass yield.¹⁷⁹ Fish waste hydrolysate (rich in protein and amino acids) can also be used as a low-cost nitrogen source for the culture medium. Martone *et al.* observed that soluble fish protein hydrolysates obtained from hake (*Merluccius hubbsi*) filleting waste allowed the growth of different bacteria similarly to the conventional Luria–Bertani (LB) medium. The microorganisms included *Halobacterium salinarum*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus epidermidis*.

Seafood waste can also be used for the production of ethanol *via* yeast fermentation.¹⁸⁰ Alfonsín *et al.* used *Saccharomyces cerevisiae* to metabolize the seaweed *Euchema spinosum* industrial waste, converting its carbohydrates into ethanol with a 75% efficiency.⁷⁷ Using the same microorganism, Korzen *et al.* produced bioethanol from *Ulva rigida* biomass,⁷⁸ obtaining 196 mg of glucose per gram of dry weight of algae biomass, while every gram of glucose produced 333 mg of bioethanol. It is important to note that, to facilitate the conversion of carbohydrates from seaweed, these researchers assisted the cultivation process with a sonication unit. In our opinion, this is not only a good example of how algae biomass can be used as a suitable feedstock for the production of alcohol *via* fermentation but also to demonstrate the importance of process integration (*viz.* ultrasonic-assisted fermentation).

From the above examples, we can conclude that the complex but nutritional-rich composition of seafood waste makes them promising nutrient additives (particularly as a nitrogen and carbon source) for microbial culture and cultivation.

Beyond the use of seafood waste for microbial cultivation, the enormous potential of exploiting marine ecosystems as a “biotechnology source” for a wide range of medically and industrially relevant biomolecules and microorganisms should

be remarked. For example, fluorescent proteins (widely applied as biosensors and biomarkers in research and medicine) were originally isolated from jellyfish and coral reefs.^{181,182} As presented in Table 2, there are also many medically and industrially relevant enzymes isolated from sea life, such as carbohydrases, lipases, phospholipases, and proteases. Furthermore, microalgae isolated from the sea have numerous applications for the biological production of added-value chemicals and products. For example, Fagundes *et al.* developed a biotechnological approach for squalene production using agro-industrial wastewater and the microalgae *Phormidium autumnale*.¹⁸³ The resulting biomass presented 0.18 g kg⁻¹ of squalene and a high content of unsaturated fatty acids (52%). Interestingly, the research group estimated that at an industrial level, and depending on processing capacities, production rates from 727 to 72 750 kg per year of squalene can be obtained using this method.¹⁸³ In fact, we must highlight the importance of these marine-based biotechnological processes in restoring fish habitat and population. For example, to illustrate the impact of replacing shark hunting with the biotechnological production of squalene, between 635 to 4446 sharks would be needed to supply the same amount of squalene that a small industry could produce annually (727 kg).¹⁸³ Aiming at the implementation of sustainable marine biorefineries within the principles of blue economy, this is a perfect representation of the path we must follow. The use of microalgae or other biotechnological approaches to produce biomolecules is probably one of the most promising alternatives to improve productivity and reduce the environmental and ecological impacts of obtaining substances of marine origin.

In addition to advances in R&D at the academic level, there are already industries investing in the production of valuable biomolecules using marine microorganisms such as microalgae. Veramaris® (a joint venture of DSM and Evonik) implemented in 2019 an industrial plant for the production of omega-3 fatty acids EPA and DHA from natural marine algae (strain *Schizochytrium* sp.) for sustainable animal nutrition in salmon farming.^{184,185} The high nutritional composition of this oil allows a better and healthier fish growth in aquaculture without depleting fish oil and burdening marine resources. This joint venture was established as the largest fermentative manufacturing using algae, following a responsibility purpose to help marine life, as claimed by Veramaris®: “using a pioneering omega-3 from natural algae”, Veramaris® “enables partners along the value chain to become independent of marine resources, making them leading stewards of the ocean”.

The use of biotechnology to obtain bioproducts more efficiently and sustainably has been called “white biotechnology”.^{59,185} Although this is not a recent phenomenon (the food and detergent industries have been using enzymes to improve their processing and products for years), recent advances in molecular biology, genetic and metabolic engineering have allowed the development of disruptive industrial and medical applications using microorganisms, biomolecules, and other bio-based products, including those

obtained from marine ecosystems.¹⁸⁶ White biotechnology is closely associated with modern and greener biorefineries, appearing as the key to consolidating more sustainable and profitable practices in the seafood industry.¹⁸⁷

The sustainable extraction of by-products directly from seafood waste and the production of valuable biomolecules using seafood residues or microalgae are significant advances towards a blue ocean-based economy. However, it is only possible to create a complete marine biorefinery when integrating these different stages one to another into a cohesive and sustainable production system.

Integration of processes in a marine biorefinery

Establishing integrative platforms for the efficient sequential or simultaneous recovery of by-products from complex seafood waste is fundamental in making these processes more economical and sustainable. The choice of target substances and techniques employed should be rational and based on measurable economic and environmental parameters. To elucidate how biorefineries can be delineated and managed, we will discuss representative examples of laboratory and industrial-scale marine biorefineries for the valorization of seafood waste from various sources (*e.g.*, algae, marine invertebrates, and fish).

There are already several studies on the integration of different stages for seafood processing. For example, Deng *et al.*¹⁵² proposed an integrative and low-cost biorefinery platform to separate crustacean shells into several fractions of high-added value bioproducts. This integrative platform includes two biocatalytic steps using recombinant aspartic proteases (for the hydrolysis of proteins) and recombinant chitinase (for chitin hydrolysis), and a liquid extraction using bio-solvents (ethyl acetate) for the recovery of astaxanthin. The integrated process resulted in 0.45 g protein hydrolysate, 0.17 g chitin oligomers, 101.3 µg astaxanthin, and 0.33 g mineral residues for 1 g shrimp shell waste biomass, demonstrating its efficiency for chitinous waste processing.¹⁵² In another approach, Prabhu *et al.* developed a biorefinery to extract different products from the green algae *Ulva ohno*, aiming to reduce waste creation and to maximize the recovery of the product from their biomass.¹⁸⁸ These researchers were able to convert 90% of the algae biomass in the form of six by-products, namely, salts, starch, lipids, ulvan (cell wall polysaccharide), proteins, and cellulose. As for fish, Abdollahi and Undeland used a biorefinery approach to extract fish oil and recover gel-forming proteins from salmon and herring residues. To this end, the pH-shift process, heat-induced isolation, and emulsion breaking techniques were properly integrated to extract, in parallel, high-quality gel-forming proteins, and fish oil.¹⁸⁹ These are remarkable results at laboratory scale, but it is necessary to evaluate the viability of scaling up these “marine biorefineries” to realize their potential as practicable industrial operations.

In addition to several academic works, some industries have already applied biorefinery concepts for seafood waste valorization. For example, 525 Solutions, a research & develop-

ment company, proposed a technology that employs ILs in a biorefinery approach to recover chitin from seashell waste.¹⁹⁰ Because it can extract natural polymers under mild conditions, this technology produces high molecular weight and pure chitin that cannot be obtained with traditional methods. This company demonstrates that it is possible to develop and implement, at an industrial scale, disruptive technologies following the marine biorefineries precepts and accomplishing green and sustainable practices while maintaining economic viability. Still, even at already established biorefineries, there is space for improvement.

Although the biorefinery precept envisages improving economic value and environmental preservation, if their design and implementation are not careful, it can generate a system that leans towards one or the other. For example, García-Santiago *et al.* assessed the environmental impacts and gross benefit of a cartilaginous fish biorefinery operating in Spain.¹⁹¹ They found that the extraction of high-value by-products generated a more eco-efficient process (*i.e.*, produced more with fewer resources), but it had a worse environmental performance. García-Santiago *et al.* concluded that the poor ecological results were because the proposed biorefining processes were still not optimized, mainly in terms of equipment and energy consumption.¹⁹¹ Therefore, this study shows the importance of evaluating different economic and environmental parameters to design the best strategies for waste valorization. Even though we normally infer that more eco-efficient systems are automatically more environmentally friendly, this is not always the case. This controversial outcome may occur due to the complexity and energetic demand for the recovery of certain products outweigh the environmental cost of just treating the waste. For some substances (*e.g.*, bioactive proteins and peptides), their high commercial value and unique potential for medical and industrial applications can justify their recovery.¹⁴⁹ However, for low-value compounds that can also be obtained from other sources (*e.g.*, gelatin), the cost of processing, energy, and materials can surpass the selling point of the bioproducts.¹⁴⁹ Therefore, a sustainable biorefinery requires much more than just thoughtless processing of waste. It requires an intelligent strategy oriented towards continuous evaluation and optimization. Furthermore, the examination must include adjustment parameters that effectively represent its economic and environmental performance. Thus, our opinion is that the development and assessment of a marine biorefinery must take into account the multiple processing steps, raw materials, products, and distinct evaluation parameters.

Fortunately, there are several well-established methods and tools for assessing the environmental impacts of an industrial process. Some ecological parameters are straightforward and estimate the effect of the systems on single environment issues, such as energy, carbon, and water footprints.¹⁹² These can provide an overall idea of the burden of the production chain on energy consumption, climate change, and freshwater scarcity, respectively. However, to determine all the environmental impacts on micro and macro scales, an association of different footprints and more in-depth assessment methods

are needed. For example, the ecological footprint combines several parameters to determine the productive space and resources required for a given process to produce its commodities and absorb its generated waste.^{192,193} There is also the life cycle assessment (LCA), a comprehensive method to determine all direct and indirect environmental impacts of the complete cycle of a production system.^{194,195} This approach is also referred to as “cradle-to-grave” because it covers all manufacturing phases (*i.e.*, from raw material obtention to manufacturing, distribution, use, and disposal).¹⁹⁴ Both the footprints and LCA can be applied depending on the process complexity or implementation phase. For example, a carbon and water footprint can be useful tools for quick feedback during the outline of a new process. However, an LCA is ideal at the latest stages of system development for a reliable assessment of its environmental impacts.

As for the techno-economic analysis, there is also a range of models to estimate the commercial viability of a new industrial complex. Some of the most popular parameters include capital expenditure (*i.e.*, required initial investment), net present value (*i.e.*, how much monetary value the project will add to the company), internal rate of return (*i.e.*, the liquid profit per year), and the payback time (*i.e.*, the time required to recover the investment).^{53,196} Although it can be overlooked, the market viability analysis must also take into account the public's response to healthy and ecological products. For example, Altintzoglou *et al.* showed that consumers had an increased interest in products with information regarding their health and environmental benefits.¹⁹⁷

Many models and parameters can determine the economic viability and environmental impact of an industrial process. However, it is essential to choose an appropriate combination of methods to accurately predict the outcomes of a production chain. With these examples and issues in mind, it is clear to us that ocean-based industries must integrate and optimize all pre-processing, extraction, cultivation, and formulation stages to obtain an integrated, efficient, sustainable, and profitable marine biorefinery. It is also mandatory to assess different economic and environmental parameters when outlining and implementing each phase of their marine (bio)processing. These steps are crucial for the transition of seafood industries from a linear extractivist model to a circular and sustainable blue economy model, whose fundamental principles are to improve environmental protection, the economy, and public welfare. Nonetheless, although changing seafood production systems is fundamental, it should not be an isolated measure to preserve our marine resources. We should also enforce green and blue economy principles in different industrial, commercial, and social sectors to maintain a healthy and bountiful ocean.

Beyond biorefineries towards a blue economy

Establishing sustainable marine biorefineries is just a fraction of what is necessary to protect our aquatic ecosystems.

Although biorefineries can help reduce seafood wastage and marine pollution, they cannot contain overconsumption and global warming damages to the ocean. As presented in the latest report from The Intergovernmental Panel on Climate Change (IPCC) from the UN on August 9th, 2021, global warming has reached unprecedented levels.¹⁹⁸ This report warns us that “this is code red for humanity”.¹⁹⁹ For the oceans, the IPCC informs us that we can expect more frequent marine heatwaves, ocean acidification, and reduced oxygen levels in the water.¹⁹⁸ Furthermore, sea-level rise is likely to be inevitable for at least a hundred years, even if we take drastic measures to halt CO₂ emissions in this decade.¹⁹⁸ However, the report is also hopeful that it is not too late for action. UN Secretary-General António Guterres was direct in his suggestion to solve the issue: “*The solutions are clear. Inclusive and green economies, prosperity, cleaner air, and better health are possible for all if we respond to this crisis with solidarity and courage*”.²⁰⁰ Thus, our future relies on the effective implementation of green and blue economy models not only as an alternative but as the only path to preserve our ecosystems and the quality of life.

It should also be considered that an estimated 90% of marine species remain undiscovered,²⁰¹ and UNESCO indicates that half of ocean life will be at the brink of extinction by 2100.²⁰² By degrading the oceans and decreasing marine biodiversity, we are not only impacting current ocean-based production systems. Altogether, we are also losing uncountable future and disruptive biotechnological applications of bioprospecting marine life.

In 2022, it will be ten years since the concept of “blue economy” was first introduced during Rio + 20.³⁴ We suggest policymakers, academics, and activists continue to push forward green and blue economies practices and discuss the issues presented in this perspective during the Rio + 30 in 2022. We also believe that marine biorefineries can play a decisive role in improving the sustainability of ocean-based industries towards a blue economy. However, an integrated and joint effort from all economic and social sectors is necessary to maintain a healthy ocean fit for commercial activities. After all, an ocean economy cannot exist without an ocean.

Conclusions

In this perspective, we assessed the importance of the ocean economy and the urgency of preserving our aquatic environments and resources. We intended to propose how marine biorefineries can benefit the seafood industry and help the transition towards a sustainable and circular production system. This shift could launch the ocean economy into a new cycle, where sustainability, social justice, and revenue are not antagonistic but essential segments of the same structure. This perspective also evaluated the most promising by-products that can be obtained from distinct sources of seafood waste and how and where they can be applied. The design and integration of processing stages of a marine biorefinery were

carefully examined in the final sections, where we demonstrated how these aspects are crucial to ensuring the implementation of efficient, profitable, and greener marine biorefineries.

As a final message, we believe that despite their complexity, marine biorefineries will be effective systems for seafood waste valorization. They can be additional sources of revenue to ocean-based industries while helping to prevent seafood wastage and sea pollution. Biorefineries embody the most meaningful precepts of the green and blue economies and already have solid industrial applications. Their dissemination, as in the case of the marine sector, should be encouraged and supported by governmental and economic sectors, in particular, considering the current concerns with the shortage of strategic natural resources and the negative consequences of climate change.

Author contributions

JFBP, NVV, and CUM: conceptualization, investigation & visualization; NVV, CUM, and AAO: data curation and writing – original draft; JFBP, RPSO, CMNM, APJ, and VCSE: writing – review & editing; JFBP: supervision and project administration.

Conflicts of interest

There are no conflicts to declare.

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