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Mini Review

Marine polysaccharides and potential enzymes for its degradation

Bhupendra Baghel[1,#], Reeta Rani Singhania[1, 2,#,*], Anil Kumar Patel[1,2], Navneet Kurrey[3], Chiu-Wen Chen[1,2] & Cheng-Di Dong[1,2]*

[1]Institute of Aquatic Science and Technology, National Kaohsiung University of Science and Technology,
Kaohsiung City-81157, Taiwan

[2]Centre for Energy and Environmental Sustainability, Lucknow 226 029, Uttar Pradesh, India

[3]School of Engineering, Ajeenkya DY Patil University, DY Patil Knowledge City, Pune, Maharashtra, India

[4]Department of Marine Environmental Engineering, National Kaohsiung University of Science and Technology,
Kaohsiung City, 81157 Taiwan

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Marine polysaccharide degrading enzymes are pivotal for catalyzing glycosidic linkages' breakdown in polysaccharides, yielding low-degree oligosaccharides and monosaccharides. These enzymes show substantial potential in diverse biotechnological applications. Marine polysaccharides and their derivatives exhibit anti-inflammatory, antiviral, anticoagulant, and anticancer properties, and are used in food additives with potential health benefits such as prebiotic oligosaccharides. Research explores pharmaceutical applications of marine polysaccharide degrading enzymes, including novel drug delivery and therapeutic bioactive oligosaccharide production. These enzymes are crucial for depolymerizing marine polysaccharides, providing energy and nutrients to microorganisms, and contributing to nutrient cycling and ecological balance. The breakdown of marine polysaccharides is vital for the global carbon cycle and helps regulate atmospheric carbon dioxide levels, impacting environmental sustainability. This comprehensive review delves into marine polysaccharide degrading enzymes, emphasizing carrageenase, agarase, chitinase, chitosanase and alginate lyase. Challenges such as low yields, poor stability, and high costs prompt solutions through metabolic engineering, enzyme immobilization, and engineering, enhancing resistance and efficiency. These advancements amplify the value of marine polysaccharide-degrading enzymes in biotechnological and environmental contexts.

Keywords: Agarase, Alginate lyase**,** Carrageenase, Chitinase, Chitosanase, Fucoidan, Macroalga**e**, Ulvan

Introduction

The planet Earth's largest ecosystem is located in the oceans, which constitute 70% of the planet's surface. Because oceans contain the greatest carbon reservoir in the carbon cycle, marine systems have a major impact on atmospheric CO_2 concentration[1]. Ocean acidification, resulting from the absorption of excess carbon dioxide (CO_2) by seawater, leads to a decrease in pH levels and alterations in the oceans carbonate concentrations. This phenomenon presents massive risks to marine biodiversity, particularly to calcifying organisms such as corals, mollusks, and certain plankton species, which depend on calcium carbonate for shell or skeletal formation. An increase in ocean acidity disrupts the ability of these organisms to build and maintain their calcium carbonate structures, adversely affecting their growth, reproduction, and overall viability. Furthermore, non-calcifying organisms including fish, marine mammals, and certain algae are also impacted by ocean acidification indirectly[1].

Changes in seawater composition can disrupt marine food webs, modify species distributions, and impair physiological functions in these organisms, resulting in enduring ecological ramifications for marine ecosystems. Simultaneously, increased nitrogen levels in the ocean cause eutrophication, which in turn causes widespread algal growth that is known as "algae blooms," as the "Golden tides" (*Sargassum* genus) in the Atlantic Ocean or the "Green tides" (*Ulva* genus) in the world. Algal blooms and eutrophication have deep impacts on marine ecosystems. The rapid proliferation of algae due to nutrient enrichment disrupts the ecological balance, leading to hypoxic conditions and the death of marine life. Decomposing algae depletes oxygen levels, suffocating marine organisms and causing mass mortality events known as dead zones.

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*Correspondence:
E-Mail: cddong@nkust.edu.tw (CDD);
reetasinghania@nkust.edu.tw (RRS)

[#]Equal contribution

Additionally, harmful algal species produce harmful toxins to marine organisms and human health. Eutrophication accelerates biodiversity loss, damages fisheries, and undermines coastal economies[2]. These phenomena require comprehensive management strategies to reduce nutrient runoff and restore ecosystem health. Apart from the environmental problems and disposal expenses, the increasing amount of algal biomass resulting from these blooms presents significant biotechnological opportunities. Algal biomass holds significant potential across various sectors due to its diverse biochemical composition. In biofuel production, algae offer high lipid content suitable for biodiesel production, while their carbohydrates can be converted into ethanol or biogas. Pharmaceuticals benefit from algal compounds with therapeutic properties, including antioxidants and anti-inflammatory agents. Algal biomass also serves as a source of essential nutrients and bioactive compounds for food supplements.

Furthermore, algae possess intrinsic bioremediation capabilities, absorbing pollutants and heavy metals from aquatic environments. Harnessing algal biomass in these applications requires further research into cultivation techniques, processing methods, and sustainable utilization strategies. The usage of valuable chemical components inside algae is hindered by their access, a problem that has been extensively discussed in recent reviews, especially about lipids and protein fractions. The majority of plant and animal species on Earth may be found in this vast marine habitat[3]. There is a diverse range of marine resources available, even though they are mostly unexplored. Due to this, the marine environment is very promising for bioprospecting initiatives that aim for the discovery of novel characteristics and compounds. Over the past few decades, marine polysaccharides have been attracting research as a potentially useful class of biomaterials. Because marine organisms are so abundant in the ocean, it is comparatively inexpensive to extract these polysaccharides from them compared to those derived from plants[4] (Fig. 1). Marine macroalgae have been highlighted as a promising and sustainable biomass for biofuel and chemical compound production through microbial fermentation in recent investigations into potential feedstocks. This interest is especially strong in Far-East nations that have a long history of seaweed cultivation and are actively exploring large-scale seaweed farming as a means of producing bioethanol. Algal polysaccharides are already widely used as marine biomass resources since they make up a significant amount of the organic carbon found in marine ecosystems. Brown and red algal polysaccharide bioconversion methods show great promise for the generation of industrial chemicals and biofuel.

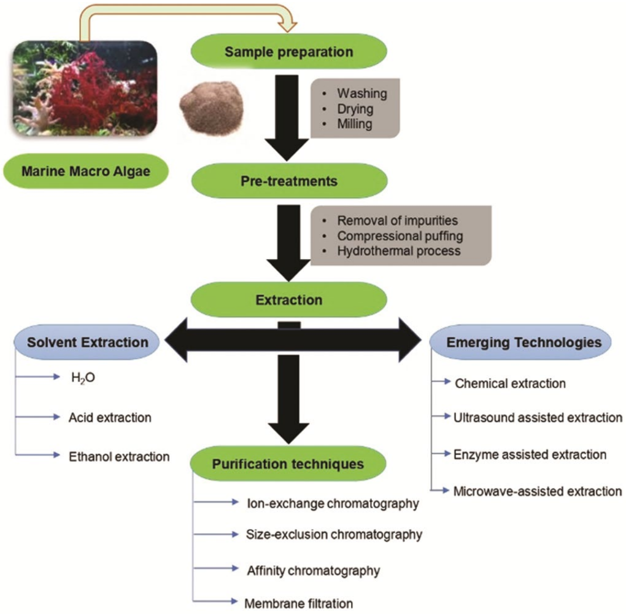

Fig. 1 — Process of extraction

Enzymatic machinery for such transformations is provided by marine microorganisms and bacteria associated with algae, which are thought of as integrated cell factories. The maritime environment poses diverse challenges ranging from nutrient-rich to nutritionally scarce places, altering the enzymes produced by marine microorganisms. The marine environment is characterized by its unique genetic architecture and life habitats. Enzymes involved in the assimilation of marine polysaccharides are not well characterized biochemically, despite the wide variety of macroalgal cell wall polysaccharides and their distinct enzymes. Notably, the metabolic pathways have not yet fully described the enzymes in charge of generating monosaccharides and converting unusual algal monosaccharides, such as 3,6-anhydro-L-galactose, for full assimilation.

Polysaccharides are the most abundant and complex organic substances in the ocean. Many marine polysaccharides have high levels of sulfation in contrast to terrestrial polysaccharides. Specifically, the cell walls of macroalgae comprise a broad variety of sulfated polysaccharides, including ulvans, fucoidans, agarans, and carrageenans. The sulfated polysaccharides which are produced from macroalgae, seaweeds, and various other primary producers are an important source of food for heterotrophic organisms and feature unique structural differences when compared to other natural polysaccharides[5]. A specific combination of enzymes is required to them for their breakdown. These enzymes can break the glycosidic linkages within the carbohydrate backbone known as glycoside hydrolases and the sulfate ester groups are referred to as polysaccharide sulfatases. The breakdown of marine polysaccharides yields a significant number of oligosaccharides that can be digested and may have therapeutic effects[6]. The potential of seaweed-derived sulfated polysaccharides as pharmacological agents against cancer is supported by several advantages they provide. Marine polysaccharides provide some biological activities such as anti-inflammatory, antitumor, anticancer, anti-immunomodulatory, antiviral, anticoagulant, and antioxidant. One of the marine polysaccharides, alginate is a biodegradable, non-toxic, bioactive and biocompatible polymer, useful for tissue engineering and drug delivery because of its stabilizing, thickening, and gel-forming properties. This comprehensive review explores and harnesses the available literature for the potential of marine organisms as sources for the isolation of enzymes specialized in degrading brown, red and green macro-algal polysaccharides. Metagenomic approaches for the discovery of novel enzymes have been elucidated. Here protein engineering and other advanced techniques have been discussed to enhance the catalytic activity, substrate specificity, and stability of marine polysaccharide degrading enzymes. Bioprocesses for their production have also been discussed which is not explored very well so far. (Fig. 2)

Marine polysaccharides

Marine polysaccharides serve as fundamental components in diverse organisms across the planet Earth. They come from a range of sources which includes shellfish, corals, sponges, seaweed, microalgae, and fungi. Various unexplored structures of marine polysaccharides have a variety of possible applications. Marine polysaccharides are discussed in the preceding section. (Suppl. Fig. S1)

Carrageenan

Sulfate polysaccharides called carrageenans are obtained from marine red algae (Rhodophyceae) and have a common D-galactose backbone that is alternatively connected by α-(1→3) and β-(1→4) glycosidic connections. The number and position of the sulfate ester groups along with the presence of 3,6 anhydro-ring determine its classification. The three majors commercially utilized carrageenans are κ, ι and λ carrageenans[7].

Fucoidan

Fucoidan, a sulfated polysaccharide, is present in several marine sources, such as brown algae (Phaeophyceae) or sea cucumber. Numerous

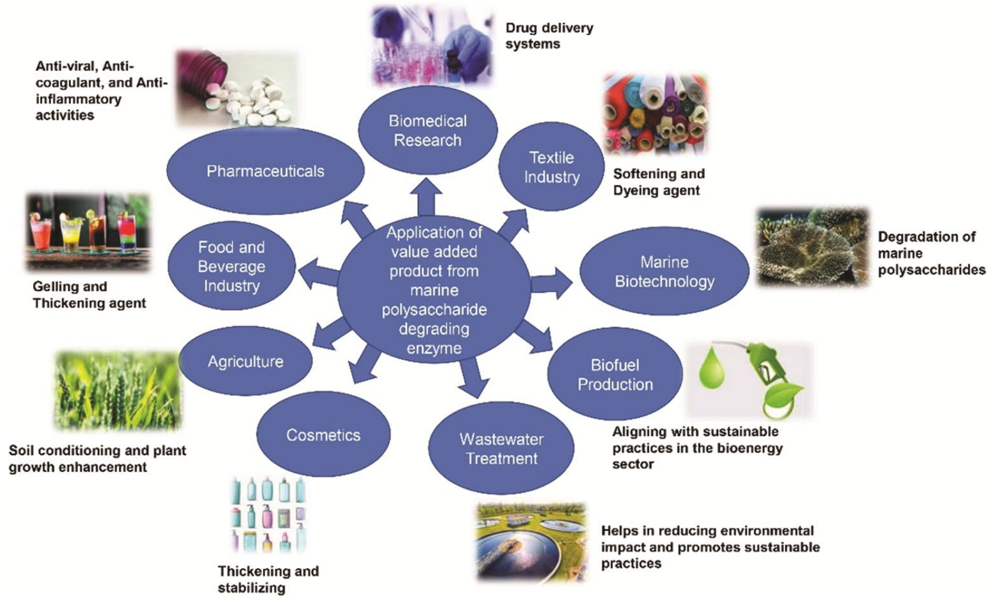

Fig. 2 — Applications of value-added products from enzymatic degradation of marine polysaccharides

monosaccharides, including mannose, galactose, glucose, xylose, and glucuronic acid, are frequently found in fucoidan[8]. Fucoidans have become the focus of extensive research because of their wide biological characteristics, which include strong antiviral, anticancer, and anticoagulant activities.

Ulvan

Ulvan, a cell wall polysaccharide found in Ulva species, constitutes approximately 9 to 36% of the dry weight of the biomass. Ulvan mainly consists of glucuronic acid, xylose, iduronic acid, arabinose, glucuronan, xyloglucan, cellulose, glucose, mannose, galactose, and sulfated rhamnose[9]. Major repeating disaccharide units describe its structure. Recent studies have revealed ulvan's unique biological properties, which make it an attractive polysaccharide that can be used in a variety of sectors, including food, and medicine.

Chitosan

Chitosan belongs to a group of linear polysaccharides consisting of β-(1→4) linked units of N-acetyl-2 amino-2-deoxy-Dglucose (glucosamine, GlcN) and 2-amino-2-deoxy-D-glucose (N-acetyl-glucosamine, GlcNAc). Certain fungal cell walls naturally contain chitosan, although this polymer is obtained commercially by deacetylating chitin. In processing facilities, chitin is extracted from the exoskeletons of crustaceans, including shrimps, lobsters, crabs, and krill. It often shares connections to lipids, minerals, proteins, and pigments[10].

Chitin

Chitin is the second most common polysaccharide after cellulose and is found in abundance in nature. It is made up of β-(1-4)-poly-N-acetyl-D-glucosamine. It is commonly found in the extracellular matrix of many different invertebrates, including fungi, nematodes, sponges, and mollusks. Chitin occurs in the crystalline organizations namely α-, β-, and γ- chitin[11].

Alginate

Alginate is a polysaccharide primarily found in brown seaweeds. It is commercially extracted from various species like Macrocystis, Eclonia, Sargassum, Durvillea, Laminaria, Lessonia, and Ascophyllum. It is made up of α-L-guluronate(G) and β-D-mannuronate(M) monomers that are linked together in a (1→4) configuration[12]. The excellent biocompatibility, biodegradability, and favorable physical and chemical properties, particularly its ability to form gel and maintain moisture have encouraged diverse biomedical uses of alginate such as wound dressing, drug delivery, and tissue engineering.

Agarose

Agarose is extracted from red algae (Rhodophyceae) and a linear polymer made up of β-D-galactose (d-Gal) and 3,6-anhydro-α-L-galactose (I-AHG) units connected through α-1-3 and β-1,4-glycosidic bonds. The main component in agar is agarose, which is synthesized by extracting agaropectin from agar[13]. Agarose is now used in several applications including therapeutic drug delivery, Cancer treatment, and tissue engineering.

Porphyran

Porphyran is a sulfated polysaccharide primarily present in red seaweeds that belongs to the genus *Porphyra*. It is water soluble which is present in the intercellular gaps and cell walls. It serves as a major component of porphyra[14]. It is made up of disaccharide units that alternate between 4-linked 3,6-anhydro-1-galactose and 3-linked d-galactosyl, which is followed by a 6-sulfate residue. It has antioxidant properties which is suitable for dry food packaging with high lipid content and inhibits the development of cancer which shows anticancer properties.

Marine polysaccharide degrading enzymes via marine microorganisms

Marine microorganisms, which include bacteria, fungi, and algae, are abundant sources of various enzymes that break down polysaccharides, providing a sustainable source of enzymes. Bioconversion and biorefinery techniques are suggested as sustainable means of valuing biomass derived from seafood processing waste, to address the environmental and economic issues associated with it. The investigation of enzyme reservoirs in marine microorganisms presents a viable approach toward the sustainable manufacture of enzymes that degrade polysaccharides in the marine environment. A proven and long-lasting method for acquiring enzymes is the large-scale fermentation of marine microorganisms to release enzyme resources. Additionally, focus is given to the development of economically feasible and energy-efficient enzymatic systems specifically designed for the processing of biomass from seafood waste. Enzyme-assisted bioconversions are emphasized as a potentially effective way to handle seafood processing wastes, with the ability to reduce environmental effects and promote the production of new compounds and the recovery of important resources.

Marine polysaccharide degrading enzymes encompass a diverse set of enzymes classified within glycoside hydrolases and polysaccharide lyases. These enzymes are capable of breaking α-(1→3) and β-(1→4) glycosidic bonds found in marine polysaccharides, resulting in the production of smaller oligosaccharides[15]. These enzymes are found extensively in a wide range of species, including bacteria, fungi, insects, algae, higher plants, and crustaceans. Marine polysaccharide-degrading enzymes hold immense potential across industrial, environmental, and biomedical domains. In industry, they catalyze the conversion of marine biomass into biofuels, pharmaceuticals, and food additives, offering sustainable alternatives to traditional production methods. Environmentally, these enzymes facilitate the bioremediation of marine ecosystems by breaking down pollutants and promoting nutrient cycling. Biomedically, they contribute to drug delivery systems, wound healing, and disease treatment through the synthesis of bioactive compounds. Harnessing the diverse capabilities of marine polysaccharide-degrading enzymes underscores their importance in advancing biotechnological innovations for a greener, healthier, and more sustainable future[16].

The utilization of waste biomass from the processing of seafood in the production of enzymes has attracted more attention. This fascination is a result of the seafood industry producing large amounts of by-products, such as fish heads, bones, fins, skins, and guts. Although these waste products present significant risks to human health and the environment, they also provide an important source for the production of microbial enzymes. Interestingly, these waste biomasses can serve as a nutrient-rich substrate that promotes microbial growth and enzyme synthesis. One area of focus has been investigating these waste products as cost-effective sources of carbon and nitrogen for the synthesis of enzymes, specifically lipases, chitinases, and proteases. Proposals that support the valorization of waste from seafood processing through bioconversion and bio-refinery techniques have come to light as viable solutions to the complex environmental and financial issues associated with these waste streams[17].

Production of marine polysaccharide degrading enzymes

A multi-step procedure is used in the traditional production of marine polysaccharide-degrading enzymes, which involves isolation and cultivating microorganisms having natural enzymatic properties. The first stage involves isolating microbial strains from various marine environments, including sediment samples or seawater. The objective of this step is usually to collect a wide range of microorganisms that are adapted to the marine environment, where polysaccharides are abundant sources of carbon[18]. One of the most important steps in finding viable candidates for biotechnological applications is screening microorganisms for the production of marine polysaccharide-degrading enzymes. To discover the microbial strains exhibiting the required enzymatic activities, a variety of strains *Pseudomonas carrageenovora*, *Bacillus* sp. Lc50-1, *Bacillus* sp. SYR4, *Catenovulum* sp. LP, *Cellulosimicrobium cellulans*, *Cellulophaga lytica* strain N5-2, *Vibrio* sp. NJ-2, *Agarivorans* sp. HZ105, *Agarivorans albus* YKW-34, *Bacillus* sp. TAG8, *Bacillus subtilis*, *Gongronella* sp. JG, *Bacillus cereus* acquired from marine environments are thoroughly analyzed. The selection of microbes capable of breaking down these complex polymers is made easier by the screening procedure, which frequently uses a particular culture medium enhanced with marine polysaccharides as substrates. Solid-state fermentation or submerged fermentation may be employed based on the microorganisms and the overall conditions[19]. Then, different biochemical and molecular assays are used to detect and quantify the enzymes that break down polysaccharides. Strains displaying promising enzymatic capabilities are further characterized for factors like enzyme stability, substrate specificity, and overall productivity. Identifying microorganisms with strong enzymatic activity against marine polysaccharides is the ultimate aim of this screening method, as it creates the way for further stages in the synthesis of enzymes and possible industrial uses. Promising microorganisms are identified and then cultivated in controlled environments, usually bioreactors, where ideal growth conditions are supplied. Suitable nutrient-rich media to promote microbial growth and enzyme synthesis are among these conditions. During the cultivation phase, the microorganisms might ferment and release enzymes that break down polysaccharides into the culture media. The culture medium is harvested after fermentation, and the broth that contains enzymes passes through several downstream processing steps. This includes the process of extracting crude enzyme extracts via centrifugation, filtration, or cell separation. To isolate the particular marine polysaccharide-degrading enzyme of interest, further purification procedures are subsequently carried out. For this, one may use chromatographic techniques, precipitation, or other separation procedures[20]. After the enzyme is purified, it undergoes a thorough characterization process that includes figuring out the ideal pH and temperature, as well as substrate specificity and stability profiles. These variables give information about the activity of the enzyme and direct future optimization techniques. Table 1 gives details of microorganisms and the bioprocess conditions for marine polysaccharide degrading enzymes.

Recent advances in enzyme activity analysis

However, the biotechnological exploitation of marine microorganisms frequently necessitates their growth in pure cultures and the chemicals of interest. High-throughput sequencing provides a culture-independent assessment of microbial diversity[33]. High-throughput computational techniques that can conduct extensive screening have greatly advanced the catalyst search in recent years. The primary challenge in directed evolution is identifying uncommon functional variants in a mutant library. To prevent this, it is beneficial to employ a high-throughput screening or selection technique, which makes it possible to evaluate a larger number of library members and thereby increases the chances of success. Variants showing the desired traits are chosen in a screening assay, which measures a certain output from each library member. By connecting a variant's intended behavior to its survival, on the other hand, the selection makes sure that only the chosen mutations make it through the process. One

Table 1 — Comparative Account of Marine Polysaccharide Degrading Enzymes Production, Biochemical Characterization and Activity

Microorganism	Enzymes	Molecular mass (kDa)	Optimal pH and temp,	Activity (U/mg)	PL Family	GH family	Enzyme stability			Final Product
							Temp, (°C)	pH	Reagent	
Microbulbifer sp, SH-1[21]	Alginate lyase	66,4	9 and 40°C	12,908,26 U/mg	7		Below 30	5,0-9,0	-	Production of unsaturated oligosaccharides
Cobetia sp, NAP1[22]	Alginate lyase	35	8 and 45°C	-	7	-	30 to 70	-	2 M KCl, 70%	Production of Monosaccharides
Streptomyces anulatus CS242[23]	Chitinase	38	6 and 50°C	27,010	-	-	30 to 60	5 to 13	-	Conversion of colloidal chitin to GlcNAc and (GlcNAc)2
Streptomyces albolongus ATCC 27414[24]	Chitinase	47	5 and 55°C	66,2	-	-	35 to 65	3 to 9	1 mM EDTA, 97%; 1 Mm SDS,35%	Conversion of colloidal chitin to GlcNAc and (GlcNAc)2
Bacillus licheniformis B307[25]	Chitinase	36-42	6,0 and 60°	Intrecellular 2U/mg and extracellular 14,2 U/ml	-	-	30	6	-	-
Gynuella Sunshinyii[26]	Chitosanase	29,7	5,5 and 30°C	260,87	-	46	15 to 35	4 to 8,5	-	Controllable preparation of COSs with the specific DP
Kitasatospora setae KM-6054[27]	Chitosanase	29,19	6,0 and 30°C	1094,21 U/mg		46	15-55	3,0-10		Production of endo-type chitosanase with low degree of polymerization ranging from 2-4
Agarivorans gilvus WH0801 (AgWH50B) [28]	Agarase	105	7 and 40°C	1,523,2	-	50	10 to 40	6-8	10 mM EDTA, 0	Production of NAS4
Catenovulum maritimum STB14[29]	Agarase	156,09	8,0 and 35°C	206,1 U/mg		96	35-37	6		Cm-AGA is the first reported α-agarase with agarobiose (A2) and agarotetraose (A4) as the dominant products, suggesting the great potential of Cm-AGA in the efficient production of agaro-oligosaccharides with a low degree of polymerization
Pseudoalteromonas sp., *QY203*[30]	κ -Carrageenase	34	7,2 and 45°C	1,121,70	-	16	35 to 45	6 to 9	1 mM EDTA, 82%	Production of oligomers with DPs 2 and 4
Bacillus sp, *Lc50-1*[31]	λ-Carrageenase	37	8 and 75°C	105	-	-	55 to 75	6 to 9	2 mM EDTA, ~60%	Production of oligomers with DP2
Cellulophaga baltica[32]	ι–carrageenase	31	7,5-8 and 40°C	571	-	-	40	7,2	-	-

significant challenge with synthetic circuits is that their outputs frequently have various states, such as ON and OFF depending on the input conditions. As a result, to find functional variants, a selection or screening system must evaluate every conceivable condition. Though creative approaches to these kinds of screening/selection systems remain to be developed, they have great potential for advancing synthetic biology[34].

Droplet-based microfluidics is increasingly favored over traditional High-Throughput Screening (HTS) techniques due to its higher throughput and reduced assay volumes. Fluorogenic substrates that are specific to the droplet format are frequently used in these systems to monitor enzyme activity[35]. It is essential that the substrate is accessible in the produced enzymes and that the fluorescent probe product stays enclosed within the droplet. Therefore, selecting the right expression system is essential; ideally, this involves using enzymes that are expressed at the cell surface or extracellularly. While the majority of protein libraries examined thus far have employed periplasmic or cytoplasmic expression in Escherichia coli, requiring extra lysis steps or membrane transfer, there aren't many reported extracellular recombinant expression systems that work in tandem with droplet-based microfluidics.

Paper-based microfluidics, or microPADs, involves the manipulation of small fluid volumes on paper or other porous membranes using capillary action. Hydrophilic channels and zones are defined by patterning paper with hydrophobic barriers to build these devices. Paper-based microPADs are portable, process small fluid volumes, and perform multiplexed experiments, just like standard microfluidic devices. But what makes them unique is that they can be made without cleanroom facilities, with generally available, low-cost materials, and without the need for computer-controlled pumps. These qualities make them accessible and excellent for creating point-of-care (POC) diagnostic diagnostics[36]. Numerous assays, including colorimetric, electrochemical, chronometric, and distance assays, have been used to construct microPADs for enzyme analysis. Due to their activity, enzymes serve as essential analytes for pathogen detection, illness diagnosis, and general health monitoring. They also provide the benefit of signal amplification. As an illustration, the biomarker β-galactosidase has been used to identify E. coli in microPADs[37].

Enzymes catalyze or bind substrates through specialized interactions that result in the synthesis of new chemical entities through reversible or irreversible chemical processes. These interactions are known as biochemical transformations catalyzed by enzymes. The identification and binding of these molecular receptors to the chemically changed entities either via supramolecular interactions or a chemical reaction (chemo-dosimeter) is essential to the creation of small molecule fluorescence probes. A new luminescence band or a notable increase in luminescence quantum yield are the outcomes of this interaction's production of modifications in the luminescence spectrum[38].

Functional metagenomics is an emerging area of research that focuses on genes that encode specific proteins. This approach comprises fundamental techniques such as the screening of genes, their expressions, and eventually bioinformatic analyses like phylogenetic analysis, sequence testing, Pfam analysis, and structure prediction. Functional metagenomics provides multiple advantages such as target genes or active products can be achieved via screening without culture conditions. By accelerating the growth of novel drugs from marine and harsh environmental microbial resources. The recognition of enzymes that are only active under particular physiochemical conditions or the finding of novel members of already-existing families is made easier by functional metagenomics. Microorganisms in the environment are studied for their Carbon, Nitrogen, and Sulphur cycle metabolomics and functional metagenomics[39].

Metagenomics often uses function-based approaches, like enzyme activity-based screening conducted in culture plates. For example, cellulose screening assay for cellulase or starch-iodine test for amylase. Different function-driven screening approaches include detecting phenotypes, utilizing host strains for heterologous complementation, and employing induced gene expression techniques[40].

Advancement in sequencing technologies has increased greatly, favoring the reasonably priced and quick sequencing of the entire human genome in just one week. As such, the massive volumes of Next-Generation Sequencing (NGS) data require proficient computational and bioinformatics skills to handle, analyze, and interpret. Enzyme engineering becomes necessary due to the inherent trade-off associated with the high specificity of enzymes. For example, while a natural biocatalyst might possess the desired chemical reactivity, its active site might not effectively bind and transform an essential industrial substrate[41].

Robust properties of marine polysaccharide degrading enzyme

Polysaccharide enzymes from marine sources are expected to showcase excellent robust properties which enable them to be an excellent candidate for industrial application to withstand harsh conditions such as high salt concentration, low temperature or high-temperature resistance, and pH versatility. The properties of enzymes produced by marine microorganisms are elucidated in Table 2.

Salt tolerance

Enzymes that break down polysaccharides in the marine environment are ideally adapted to the salt

Table 2 — Novel Properties, kinetic parameters, hydrolysis efficiency, and applications

Enzyme	Source	Substrate	Optimal pH and temp (^{o}C)	Activity (U/mg)	K_m	V_{max}	K_{cat} (S^{-1})	$K_{cat/}$ K_m	Hydrolysis efficiency	Application
Alginate lyase[42]	-	Alginate	-	2416	0,49 mM	72 pmol,S^{-1}	59	120,41 mM^{-1},S^{-1}	-	Food, Nutraceuticals and Therapeutics
	-	polyM	-	2043	0,86 mM	95 pmol,S^{-1}	77	89,53 mM^{-1},S^{-1}	-	Food, Nutraceuticals and Therapeutics
	-	polyG	-	3559	0,24 mM	35 pmol,S^{-1}	29	120,83 mM^{-1},S^{-1}	-	Food, Nutraceuticals and Therapeutics
ι-carrageenase[43]	*Flavobacterium* sp, YS-80-122	-	7,6 and 30^{o}C	1,207,8	-	-	-	-	-	Production of oligosaccharides,
κ-carrageenase[44]	*Pseudoalteromonas* sp. *ZDY3*	-	8,0 and 45^{o}C	-	3,67 mg/mL	368 mmol,min^{-1}, mg^{-1}	-	53,0 ml,mg^{-1},s^{-1}	Polysaccharides get hydrolyzed to disaccharide and tetrasaccharide close to 100% in 180 min,	Saving reaction cost and energy, and using for continuous reaction,
κ-carrageenase[45]	*Escherichia coli* BL21 (DE3)	-	8,0 and 35^{o}C	1530	2 mg/mL	24 µmol,min^{-1}, mL^{-1}	-	-	-	Thickener, stabilizer, and emulsifier agent in the food industry, and pharmaceutical industries,
Chitinase[46]	*Exiguobacterium antarcticum* DW2	Colloidal Chitin	5,0 and 30^{o}C	7,3±0,3	0,68±0,07 mg/mL	15,26±0,59µmol,min^{-1}, mg^{-1}	11,70±0,45 s^{-1}	17,2 mL,mg^{-1},s^{-1}	Colloidal chitin was completely hydrolyzed after 30 min,	Molecular biology research, pharmaceutical industry, or food additives,
		Powdery Chitin		1,8±0,1	-	-	-	-		
		Regenerated Chitin		2,0±0,1	-	-	-	-		
		Powdery Shrimp Shell		1,3±0,2	-	-	-	-		
		Glycol Chitin		4,2±0,3	14,42±1,9 mg/mL	1,94±0,15µmol ,min^{-1}, mg^{-1}	1,49±0,12 (s^{-1})	0,1 mL,mg^{-1},s^{-1}		
		Chitosan		1,5±0,2	-	-	-	-		
		Glycol Chitosan		1,8±0,1	-	-	-	-		
		$GlcNAc_3$		3,1±0,1	-	-	-	-		
Chitosanase[26]	*Gynuella sunshinyii* (GsCsn46A)	-	5,5 and 30^{o}C	260,87	1,97 ± 0,09 mg/mL	385,65 ± 9,78 µmol,min^{-1}, mg^{-1}	-	-	The yields of three types of chitooligosaccharides products (degree of polymerization (DP): 2–7, 2–5, and 2–3) were 70,9%, 87,1% and 94,6%,	For functional food, agriculture, and biomedicine,
κ-Carrageenase[44]	*Pseudoalteromonas sp*, *ZDY3*	-	Wide pH and 35°C		3,67 mg/mL	368 µmol,min^{-1}, mg^{-1}	-	53 ml,mg^{-1},s^{-1}	The final products were κ-neocarrabiose and κ-neocarratetraose with different degrees of polymerization	Pharmaceuticals and diverse biological activities, including antiviral, antitumor, antioxidant
Chitinase[47]	*Paenibacillus sp*,	Colloidal chitin	4,5 and 50°C	0,85	4,28 mg/mL	14,29 µg,min^{-1}, mL^{-1}	-	-	14,33% monomer and 85,67% dimer of N-acetylglucosamine	Pharmaceuticals and induce broad-spectrum resistance in plants (*contd.*)

Table 2 — Novel Properties, kinetic parameters, hydrolysis efficiency, and applications

Enzyme	Source	Substrate	Optimal pH and temp (°C)	Activity (U/mg)	K_m	V_{max}	K_{cat} (S^{-1})	$K_{cat/}$ K_m	Hydrolysis efficiency	Application
Agarase[48]	*Cellvibrio sp*	Agaro-oligosaccharides	7,5 and 35°C	-	26,5 mg/mL	16,9 U/mg	25,2 S^{-1}	1,2×10^5 $S^{-1}M^{-1}$	Neoagarobiose, neoagarotetraose and neoagaro oligosaccharides with higher degree of polymerization in very less extent	-

environment because of their high salt tolerance. For instance, research on alginate lyase generated from a marine bacterium demonstrated that the enzyme had a high tolerance to salt, retaining more than 80% of its maximum activity throughout a wide pH range of 4.0 to 10[42]. Similarly, optimal temperature and pH were found to be altered by the presence of NaCl in β-1,3 xylanase, a salt-tolerant bacterium originating from marine environments that was recently discovered. Furthermore, a marine bacterium-derived maltopentaose-forming amylase showed salt tolerance, increasing its potential for a variety of commercial uses. These results highlight how marine polysaccharide-degrading enzymes may adapt to high salinity conditions, highlighting their potential for use in a range of biotechnological applications.

Substrate specificity

Marine enzymes responsible for the degradation of polysaccharides exhibit substrate specificity, implying their distinct affinity for particular polysaccharide types. Given the structural diversity of polysaccharides, their effective breakdown necessitates a diverse array of enzymes with specificities tailored to their respective substrates. Noteworthy examples include alginate lyases for the degradation of alginates, carrageenases for carrageenans, and fucoidanases for fucoidans. While detailed studies have been conducted on enzymes degrading cellulose, chitin, and xylan, the enzymes engaged in the degradation of marine-derived polysaccharides are still undergoing exploration. The CAZy (Carbohydrate-Active enZymes) database categorizes carbohydrate-active enzymes based on enzyme class and genetic relationships. This classification includes glycoside hydrolases (GHs), glycosyl transferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), and enzymes exhibiting auxiliary activity (AAs). The metabolic pathways involved in the degradation of marine polysaccharides remain insufficiently understood, with ongoing exploration into the enzymatic repertoire provided by marine microorganisms. These microorganisms offer specialized enzymatic tools for the sequential degradation of diverse marine polysaccharides[3]. The substrate specificity of AlgNJ-04 was evaluated with three different substrates: polyM, polyG, and sodium alginate. The enzyme demonstrated increased activity levels about polyG (3359 U/mg) and sodium alginate (2416 U/mg); however, it showed decreased activity about polyM (2043 U/mg). These results demonstrate that AlgNJ-04 can function on a variety of substrates and imply that it has a wider substrate specificity[42].

Cold-adaptation

The cold-adaptive nature of marine polysaccharide-degrading enzymes allows them to function effectively in cold conditions. The capacity to adjust to cold stimulation, high-temperature sensitivity, and catalytic activity at low temperatures are the hallmarks of cold-adapted enzymes. These enzymes are mostly found in plants, animals, and microbes that live in the deep sea, mountains, and polar regions[49]. A study performed in the Arctic Ocean's Kongsfjorden on complex polysaccharide-degrading bacteria that were cold-adapted revealed that these bacteria have extracellular enzymes that could hydrolyze large molecular weight polysaccharides. The study aimed to investigate the functional/phenotypic and phylogenetic variety of these bacteria, particularly enzyme activity. Another study on β-glucosidase derived from a marine bacterium indicated the enzyme's maximum activity at 25°C, which remained unaffected by organic solvents or NaCl. The property of being cold-adapted offers great promise for the large-scale synthesis of cold-adapted enzymes, such as xylanases and laccases, which have uses in a variety of biotechnological processes[49].

Ecological role

Enzymes that break down marine polysaccharides play a crucial role in sustaining the biological balance within marine environments. These enzymes are essential for breaking down marine polysaccharides, thereby providing marine life a major source of energy as well as nutrition. The complex process of polysaccharide synthesis and breakdown is essential to the global carbon cycle and helps control atmospheric carbon dioxide concentrations. Moreover, the valuable utilization of marine biomass depends on the exploitation of enzymes that can break down marine polysaccharides. However, a comprehensive understanding of the phylogenetic diversity of enzymes targeting marine polysaccharides is currently lacking, impeding our knowledge of the ecological roles of diverse marine bacteria and the ocean ecosystem. Precise knowledge of enzyme functions is essential for the targeted degradation of marine polysaccharides in vitro. In summary, marine polysaccharide-degrading enzymes contribute significantly to carbon and nutrient cycling in marine environments and play a crucial role in unlocking the high-value potential of marine biomass[3].

The breakdown and cycling of marine polysaccharides are largely dependent on the interactions between marine bacteria and organic materials and algae, which are known to be abundant producers of enzymes that break down polysaccharides. The microbial community's makeup, substrate availability, and enzyme specificity are some of the variables that affect these interactions. In particular, the complex dynamics of microbial interactions—which are aided by enzymes that break down polysaccharides—become much more significant during algal blooms. The complexity of these interactions is shown by the diverse ways that different bacterial groups respond to the fluctuating availability of algal polysaccharides at different stages of the bloom.

Protein engineering for enhanced activity

Protein engineering has become a potent tool for improving product spectrum, increasing enzyme stability, and boosting enzymatic activity in a variety of applications, including the biosynthesis of natural products. Recent studies have demonstrated how protein engineering can be used to increase enzymatic activity and enzyme colocalization to improve the production of natural products. This strategy has proven especially helpful in the development of biocatalysts for the biosynthesis of natural products. Furthermore, protein engineering has been used to improve enzyme activity assays, making it possible to assess an enzyme's activity using advanced, automated assays. One of the most successful methods for creating and evaluating proteins that have been modified to have increased activity is the application of directed evolution with machine learning. Significant promise exists for the development of more effective and adaptable biocatalysts in a variety of industrial and scientific fields because of these advancements in protein engineering.[50].

Rational design

For a rational design of a protein, knowing the target protein's structure, function, and mechanism is crucial. Mutations designed to improve or alter the protein's activity are based on earlier evaluations of its structure and activity. However, directed evolution does not need detailed knowledge of the protein's mechanism for the engineering process. Several instances illustrate the utilization of rational design in enhancing the thermostability of proteins. For example, thermostability of α-amylase was raised by substituting asparagine sidechains with a specific group[51].

Site-directed mutagenesis

Rational design by site-directed mutagenesis (SDM) is an advanced technique in genetic engineering that involves actively inducing point mutations. In this method, one of the standard amino acids is substituted for a particular amino acid at a precise place. However, a significant challenge associated with SDM is the requirement for detailed and comprehensive knowledge about the three-dimensional structure and operational mechanism of the target enzyme[52].

Directed evolution

Directed evolution is a powerful technique used to modify the properties of enzymes introducing mutations into their DNA sequence. This technique can be applied to marine polysaccharide degrading enzymes to increase their catalytic activity, substrate specificity, or thermostability, which could lead to opportunities for their commercial applications. Directed evolution resembles the process of Darwinian evolution inside a controlled environment like a test tube. In contrast to natural evolution, which aims to reproduce and survive, directed evolution selects for the desired biological function by implementing mutation and recombination rates significantly more than natural evolution[53]. For example, the mutation of chitosanase from

Streptomyces sp. N174 involved the deletion of residues A198, A199, H200, and D201. This modification resulted in a shift of its hydrolysis product from chitooligosaccharide with a degree of polymerization of 3 to 5, to specifically producing chitopentasaccharide.

Enzyme immobilization strategies

Enzyme immobilization has emerged as a key strategy for large-scale applications, offering a way to improve the stability and efficiency of marine enzymes that break down polysaccharides. It is necessary to have a thorough understanding of the fundamental concepts regulating enzyme immobilization and how they apply in industrial settings to explore possible immobilization techniques. Enzymes are attached to a solid support in this technique, which is usually a carrier material made of various materials, such as polymers, inorganic compounds, or biological matrices. Enzyme immobilization methods come mainly under two categories: physical and chemical methods. The physical method consists of adsorption, entrapment, and encapsulation, while the chemical method consists of cross-linking and covalent bonding processes[54].

Adsorption

The adsorption mechanism depends upon weak bonds, including Van der Walls forces, Electrostatic, and hydrophobic interactions. Enzymes are dissolved within a solution, and the solid support comes into contact with the enzyme solution for a specified period under conditions conducive to preserving enzyme activity. Following this, any unattached enzyme molecules are removed from the surface by rinsing or washing with a buffer solution. Adsorption-based immobilization is valued for its simplicity, cost-effectiveness, and no reagent requirement. Furthermore, because it does not alter the support material, it usually maintains enzyme activity. Still, there are limitations to this approach. Since enzymes have weak physical interactions that bind them to support, they are easily detached or leached in response to changes in temperature, pH, or ionic strength. Additionally, due to the possibility of enzyme leaching, biosensors that use adsorbed enzymes frequently experience decreased stability during use and storage. Performance may additionally be further affected by the non-specific adsorption of other proteins or materials onto the transducer surface, which can lead to contamination and signal interference[55].

Entrapment

Entrapment is a process in which the presence of the enzyme causes a solid or gel to form or change, usually as a result of interactions between molecules that eventually integrate into the support structure. The presence of the enzyme is expected to direct or affect the process, even though it is not directly involved in the production of this solid or gel. By altering the polymer network, this method facilitates the optimization of parameters like the desired pH and polarity, which affect the microenvironment around the enzyme. Entrapment immobilization provides the enzyme with increased stability. Compared to adsorption techniques, it considerably minimizes enzyme leakage, while it does not remove it. Although, entrapment immobilization has several drawbacks. These include difficulties with mass transfer, limited enzyme loading capacity, and the possibility of carrier material degradation. Furthermore, if the polymer network has big pores, there could be a considerable enzyme leakage. Techniques for entrapment immobilization include the sol-gel process, microencapsulation, photopolymerization, and electropolymerization[56].

Encapsulation

Encapsulation is a process that offers exceptional mechanical strength and storage stability, all while maintaining the enzyme in its original conformation as well as it offers covering various biomolecules in different polymeric matrices. It resembles entrapment as both techniques contain cells and enzymes in solutions inside predetermined areas. The specific goal of encapsulation is to trap specific enzymes and cell solutions in tiny, permeable vesicles. Semipermeable membranes allow small substrates and products to pass through while these membranes keep big enzymes from entering or leaving the capsules. By enclosing biocatalysts in a protected habitat, the encapsulation approach protects them from potentially damaging external factors. Biocatalyst's longer periods of activity are prolonged by encapsulation, which prevents direct contact with the environment[57].

Cross-linking

Enzyme immobilization via cross-linking is a straightforward method that involves covalently connecting the molecules of the enzymes without requiring an additional carrier. Using linker molecules as intermediates between two enzyme entities, this technique creates covalent bonds between the molecules of the enzymes which results in reducing the possibility of enzyme leakage by creating a strong chemical bond between the molecules of the enzyme[56]. The objective of this process is to minimize the amount of enzyme leakage and enhance the operational stability of enzymes. Two approaches that are widely used in this method are Cross-Linked Enzyme Aggregates (CLEA) and Cross-Linked Enzyme Crystals (CLEC). Using one of two commonly used cross-linking agents (glutaraldehyde) to create covalent bonds between neighboring enzyme molecules, the free amino groups of lysine residues at the reactive sites of the molecules are mostly involved in the reactions. Glutaraldehyde is added to cross-link enzyme crystals in the CLEC-based method after they have crystallized. When enzymes are immobilized using Cross-Linked Enzyme Crystals (CLEC), their mechanical characteristics are often improved, making them more effective and stable than untreated enzymes. Significantly, enzymes immobilized in CLECs exhibit enhanced stability and efficiency; the process of immobilization involves the production of crystals. Cross-Linked Enzyme Aggregates (CLEA) is an improved method that has been developed to overcome the drawbacks of CLEC, mainly due to its dependence on crystal formation in non-aqueous environments. Enzyme aggregates are formed in the CLEA-based technique with the addition of salts, organic solvents, or non-ionic polymers. These aggregates provide a more adaptable and superior immobilization technique by successfully maintaining the catalytic characteristics of the enzyme while functioning in aqueous solutions[55].

Covalent binding

Covalent binding is the most common method for enzyme immobilization. In this method, the enzyme is affixed to a carrier through the establishment of covalent bonds between the carrier and specific functional groups on the enzyme. Functional groups that play a role in bond formation include amino groups, carboxylic groups, phenolic groups, sulfhydryl groups, thiol groups, imidazole groups, indole groups, and hydroxyl groups. Covalent immobilization can take place through the formation of covalent bonds using either long spacer arms or multipoint attachment. The former imparts a moderate constraint on the enzyme configuration, while the latter is presumed to offer higher stability. The process of immobilizing enzymes via covalent bonding involves a two-step procedure. Before enzyme covalent attachment, the surface of the carrier is activated using linker molecules, such as glutaraldehyde or carbodiimide. These linker molecules serve as bridges between the carrier surface and the enzyme. The selection of the linker is contingent upon the type of carrier, be it inorganic materials, biopolymers, or synthetic polymers, as well as the specifics of the immobilization protocols[56].

Biofuel production pathways

To minimize conflict with food resources, marine macroalgae, which are rich in algal polysaccharides, have been suggested as a viable feedstock for biofuel production. Furthermore, it has been discovered that marine polysaccharide-degrading bacteria, like *Flammeovirga pacifica* WPAGA1 and *Penicillium oxalicum* overexpressed BGL under constitutive and inducible promoter, resulted in 65 folds higher yield compared to wild type[58], or can use crude agarose, a marine polysaccharide, as their only carbon source. This emphasizes the possibility of using marine polysaccharides as an abundant source of biofuel. Moreover, the study shows that low-polymerization oligosaccharides can be produced by marine polysaccharide-degrading enzymes, which may be used in the manufacturing of biofuel.

Lignocellulosic biofuel production

Lignocellulosic biomass, consisting of cellulose, hemicellulose, and lignin, presents itself as a promising feedstock for the production of biofuels like ethanol and butanol due to their abundant availability and ubiquitous nature[59,60]. Fermentable sugars are released during the enzymatic hydrolysis of lignocellulosic biomass's polysaccharide component, which may produce chemicals and renewable energy. However, the complete hydrolysis of lignocellulosic polysaccharides into their monomeric components needs the utilization of carbohydrate-active enzymes (CAZymes) with all the components of cellulase required to convert cellulose into glucose[61]. Industrial processes for lignocellulosic biomass degradation have to operate at high temperatures to achieve high yields, but enzymatic hydrolysis offers an eco-friendly and efficient method for the hydrolysis of lignocellulosic biomass. The three enzyme combinations that are most effective for producing biofuel via LC biomass are ligninases, hemicellulases, and cellulases. However, the cost of producing ethanol from lignocellulose biomass is currently high, and further study is required to optimize the procedure and reduce the cost of production.

Seaweed biofuel production

Currently, large-scale production of seaweed-derived biofuels lacks economic viability but holds promise for the future. Biofuels that could be obtained from seaweeds contain ethanol, butanol, methane, and biodiesel, along with bio-oil, hydrogen, and various hydrocarbon derivatives. Growing interest in utilizing microalgae and macroalgae for biofuel stems from their abundant biomass potential and cultivation methods that do not compete with farmable land or freshwater resources. The generation of biodiesel is more important than that of biogas and bioethanol among seaweed-based biofuels. Third-generation biofuel sources, separate for their independence on farming land, contain advanced gaseous biofuels like biomethane and biohydrogen which are derived from microalgae and macroalgae (seaweeds) showing great promise for achieving advanced biofuel goals. Seaweeds, on the other hand, are mostly used for bioethanol rather than biogas or biodiesel because of the differences in their chemical structures. Seaweeds, with their high carbohydrate content, are better suitable for producing bioethanol, whereas microalgae, with their abundance of lipids, are better suited for biodiesel[62].

Advances in biofuel production using marine enzyme

Biodiesel is a suitable energy source that helps in lowering greenhouse gas emissions like CO_2, CO, SO_2, and HC, benefiting the environment over fossil fuels. Four primary biodiesel production methods include pyrolysis, micro-emulsion, transesterification, and direct use/blending of oils[63]. Base-catalyzed alcoholysis (transesterification) is considered the most cost-effective due to lower temperature and pressure requirements, leading to higher product yields. Presently, biodiesel is predominantly produced via the esterification of fatty acids or transesterification (alcoholysis) of oils using alcohols, with or without a catalyst.

Urban biorefineries have been recognized as essential given the immediate demand for substitutes for fossil fuels and petroleum products in our society's economy. By concentrating on two sustainable designs that are suitable for the Wallonian context, these refineries provide several options. First of all, they involve the processing of final products in combination with biological waste to create urban biorefineries that are energy-independent environmentally friendly and, Second, integrated urban biorefineries supply energy in sync with adjacent industrial operations[64].

Challenges and future directions

There are various challenges in the way of producing marine polysaccharide-degrading enzymes. Identification and isolation of microorganisms that can effectively produce these enzymes is a major problem, particularly in marine environments where a variety of frequently uncultivable microbes are present. Since different microbial strains can produce different amounts of enzymes under different conditions, optimizing growth conditions to increase enzyme yields poses another major challenge. The sustainable exploitation of marine resources poses significant challenges in the face of growing concerns about the environment. Overexploitation, habitat destruction, and pollution threaten marine ecosystems, compromising their resilience and biodiversity. Balancing resource extraction with conservation efforts necessitates integrated management approaches, incorporating ecosystem-based strategies. Addressing these challenges requires interdisciplinary research, policy interventions, and international collaboration to mitigate environmental degradation. Sustainable resource management practices, technological innovations, and ecosystem monitoring are pivotal for safeguarding marine ecosystems and securing their long-term viability amidst increasing anthropogenic pressures. Furthermore, the issue of scaling up the production process for industrial applications remains a challenge, necessitating economical and efficient methods to produce enzymes on a large scale. To achieve the potential of marine polysaccharide-degrading enzyme production, a multidisciplinary strategy involving molecular biology, bioprocess engineering, and microbiology is needed to overcome these challenges. There are several issues with using marine polysaccharide-degrading enzymes that need to be solved. Optimizing the enzymatic processes for large-scale industrial applications is a major challenge. Crucial challenges include achieving cost-effective production and maintaining the stability of these enzymes in a wide range of environmental conditions. Other issues include the creation of robust enzyme formulations that can resist industrial processes and the development of effective immobilization procedures.

A more thorough understanding of these enzymes' substrate selectivity and catalytic processes is necessary to tailor them for particular industries and applications. Overcoming these obstacles will enable the utilization of marine polysaccharide-degrading enzymes to their maximum potential across a range of industries, including bioenergy and pharmaceuticals.

The field of marine polysaccharide-degrading enzymes is expected to solve several important issues through future studies and innovations. Exploring metagenomic techniques is one way to find new enzymes with special qualities and uses. Molecular knowledge of the structural and functional variety of these enzymes will direct work toward improving catalytic efficiency and substrate selectivity through enzyme engineering. Furthermore, the stability of marine polysaccharide-degrading enzymes and the optimization of industrial processes will depend on developments in enzyme immobilization techniques. Future studies could also focus on creating sustainable and environmentally friendly production techniques that use marine macroalgae as a plentiful source of renewable resources for the synthesis of enzymes.

The benefits and challenges associated with marine polysaccharide-degrading enzymes' sustainability and environmental implications are closely linked. Positively, these enzymes help break down complex polysaccharides in marine ecosystems effectively and provide a sustainable way to utilize marine biomass. This mechanism is essential to the carbon cycle because it promotes the cycling of nutrients and preserves ecological equilibrium. Sustainable practices may benefit from the potential uses of marine polysaccharide-degrading enzymes in a variety of industries, including bioenergy and bioremediation.

Conclusion

Marine enzymes from bacteria, fungi, and other organisms exhibit significant potential for diverse industrial applications, particularly in green technologies. They enzymatically degrade various marine polysaccharides, with optimal conditions varying based on source and targeted polysaccharide. The CAZy database categorizes these enzymes, offering insights into glycoside hydrolases, glycosyl transferases, polysaccharide lyases, carbohydrate esterases, and enzymes with auxiliary activity. Recent research focuses on discovering, characterizing, and modifying marine polysaccharide-degrading enzymes, yielding bioactive oligosaccharides and polysaccharides with health-related functionalities. A critical aspect is the immobilization of these enzymes, enhancing their stability and reusability in industrial processes. This immobilization element extends their application potential. Marine polysaccharide-degrading enzymes hold promise for green technologies and various industrial sectors. Future research is anticipated to concentrate on enzyme engineering, biofuel production, and exploring novel properties, thereby expanding their applications in scientific and industrial contexts.

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