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REVIEW PAPER

The Enzymatic Process of Macroalgae for Conversion into High-tech Bioproducts

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Abstract Macroalgae are one of various groups of multicellular algae include some types of Rhodophyta (red), Phaeophyta (brown), and Chlorophyta (green) macroalgae. They are now a source for biorefineries, as they contribute to energy production as biomass. Algae are worth noting because of their high content in compounds with separate biological activities, including agar, agarose, and carrageenan in red algae; alginates, laminarin, and fucoidan in brown algae; and ulvan, sulfated galactans, and xylans in green algae. Skeletal polysaccharides of red algae are composed of cellulose 1,β-1,3-mannan and β-1,4-acrylic acid. More than half of the dry weight of brown algae is made up of the polysaccharides alginate, laminarin, and fucoidan, and this percentage can even exceed 70% in some species. They are converted to monosaccharides that can be easily used by using polysaccharide hydrolysis enzymes. This process has the potential to maximize biofuel yields. Compared with the enzymatic depolymerization of brown and red algae polysaccharides, the depolymerization of green algae polysaccharides has been less extensively investigated. However, the use of ulvan lyase is very promising because it can degrade ulvan with good specificity, high efficiency, and mild reaction conditions, and it can well maintain the rare sugar structure properties of ulvan. The depolymerization process of macroalgae by chemical hydrolysis requires high cost, causes environmental pollution, and has limited use due to problems such as low yield. Therefore, an environmentally friendly, energy efficient and economical enzymatic depolymerization process of macroalgae using degrading enzyme will be needed.

Keywords: macroalgae, enzymatic process, biorefinery, red algae, brown algae, green algae

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1. Introduction

1.1. Macroalgae are the current source for biorefinery

Algae are a heterogeneous group of photosynthetic organisms divided into two types: multicellular organisms and unicellular organisms. Macroalgae are one of various groups of multicellular algae, also called algae, that represent thousands of species of macroscopic, multicellular, marine algae and include some types of Rhodophyta (red), Phaeophyta (brown), and Chlorophyta (green) macroalgae [1]. Macroalgae provide essential habitat and shelter for many marine animals, and they also provide protection from predators and serve as a source of food [2]. Microalgae, such as planktonic algae, play an important role by absorbing and incorporating carbon from carbon dioxide, contributing as much as 50% of global carbon fixation, and another environmental function is the production of oxygen through photosynthesis [3].

Macroalgae also have potential as a renewable energy source. Biofuels produced from terrestrial crops such as sugarcane, corn and sugar beet impact large amounts of freshwater consumption, soil fertility and biodiversity and face environmental, social and ethical challenges due to land

reclamation and the release of harmful fertilizer compounds [4]. To overcome this, research has been conducted to convert algae into a fuel production feedstock. Algae can contribute to water quality improvement by removing nutrients from wastewater generated by aquaculture and can be used as a natural fertilizer in agriculture as a plant biostimulant to improve soil and crop quality and reduce the need for chemical fertilizers [5], which can produce more biomass per hectare than land crops [6]. Macroalgae's ability to take in CO_2, abundant carbohydrate content, and shortage of lignocelluloses increase its potential for use in biogas production [6]. In addition, there are many different kinds of algae that can produce energy-rich methane, so there is a wide range of sources of biomass, and it is suggested that development related to these production fields will play a central role in the future biomethane market [7]. In addition, the use of macroalgae on the beach, where eutrophication or overpopulation of marine ecosystems and marine algae can affect local tourism, as energy sources provides important environmental and economic benefits. Beach management is supported, and sea pollution can be reduced by large-scale algae growth [8]. Therefore, research is underway to use algae with this ability as a fuel production source, and the resulting biofuel is considered a "third-generation biofuel" [9].

Macroalgae are now a source for biorefineries, as they contribute to energy production as biomass. A biorefinery is a processing facility that converts biomass into energy and other beneficial coproducts (such as chemicals), defined by the U.S. Department of Energy as the overall concept of a processing plant that converts and/or extracts biomass raw materials into a variety of valuable products. The International Energy Agency (IEA) Bioenergy Task 42 defined a biorefinery as "the sustainable treatment of biomass into a spectrum of saleable products (groceries, fodder, substance, chemicals) and energy (fuel, electricity, heat)" [10]. A biorefinery can be a facility, process, factory or facility cluster for biomass conversion and can provide multiple chemicals by dividing biomass into multiple intermediates (carbohydrates, proteins, triglycerides) that can be further converted into added value products [11]. A biorefinery is further classified according to the type of feedstock used, the type of intermediate produced, the transition process, and the state of execution of the technology, so there are three different types of biorefinery [12]. Phase I Biorefinery, which uses only one feedstock, has stationary process ability and manufactures a single primary product; Phase II Biorefinery is similar to this class and can produce a variety of products; and Phase III Biorefinery utilizes different feedstock and processing technologies [13]. It can also be classified according to the chemical nature (composition) of the feedstock and is further divided into three categories [14]. This category includes Triglyceride Biorefinery, which uses vegetable oil, animal fat, algae-derived oil, and waste cooking oil as feedstock, and Sugar and Starchy Biorefinery, which uses sugar beet, sugar cane, wheat and corn, and Lignocellulosic Biorefinery that utilizes wood, straw, and grass, which is a source of wood [15]. The IEA Bioenergy Task 42 divided the classification system according to four main functions: feedstock, biorefinery platform, product and process [16]. Platform refers to the core intermediate between feedstock and end product, including biogas from anaerobic digestion, synthetic gases from gasification, C6 sugars obtained by hydrolysis of amylum, cellulose, and hemicellulose, C5 sugars obtained by hydrolysis of hemicellulose, food and feedstuffs, and lignin obtained from lignocellulose biomass. Products are grouped into energy-based systems that produce biofuels, power and heat or materials-based systems that produce food, feed, chemicals, and biomaterials, and processes are classified into four categories: mechanical/physical, biochemical, chemical, and thermochemical processes.

In these facilities, renewable biomass is used as an alternative to fossil fuels that have an adverse effect on the environment, and macroalgae are one of them, which has recently attracted attention as a feedstock for biorefineries [17]. It contains varying amounts of ash and carbohydrates, proteins, and lipids and can be converted into various fuels depending on the technology used and the type of algae [18]. In addition to the abovementioned characteristics, such as high CO_2 absorption capacity, environmental benefits, and rapid growth, macroalgae have great potential as an excellent source of biofuels. However, the biomass of this organism varies according to species, region, and season and is highly dependent on processing technology depending on production and product type, so certain limitations remain, such as lack of technology and unpredictability of the volume and quality of macroalgae. Therefore, to overcome this problem and use a promising biomass source in the future, many studies are in progress, such as the development of new microorganisms, the development of technologies for genetic modification and metabolic engineering, and the development and optimization of processes to improve yield.

1.2. Major algal polysaccharides

Algae are worth noting because of their high content in compounds with separate biological activities, including primary and secondary metabolites and complex organic compounds such as phytopigments (xanthophylls and carotenoids), polyunsaturated fatty acids, *etc*. Due to their biological activity and regenerative properties, they are compounds with promising potential applications in several industries, including food and pharmaceuticals, chemicals,

and cosmetics, and are considered viable and economical sources of biomass [19]. Along with this, macroalgae have also been highlighted in recent years as a promising source of new molecules and physiologically active compounds and have received increasing attention for use as medicines and functional foods due to a variety of health-promoting properties that can help reduce the risk and prolong the life of many chronic diseases [20].

As a rich marine resource, macroalgae contain functional metabolites such as polysaccharides, proteins, peptides, lipids, amino acids, polyphenols and inorganic salts [21]. Among these components, the most abundant polysaccharides exist in macroalgae as cell wall structures, mycopolysaccharides, and storage polysaccharides [22]. Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units joined by glycosidic bonds and have a linear or highly branched structure [23]. Polysaccharides generally include storage polysaccharides such as starch and glycogen, which are used as energy sources, and structural polysaccharides such as cellulose and chitin, which are used in cell walls. Polysaccharides, also known as glycans, are the most plentiful form of carbohydrate substances in nature, and numerous studies have been conducted on various algal-derived polysaccharides due to their remarkable biological activity [24].

Macroalgae are sorted into three groups based on pigmentation and chemical composition: red algae, brown algae, and green algae [25]. Each of the red, brown, and green macroalgae has numerous species, including those listed in Table 1, some of which are used as industrial feedstocks. To date, no macroalgae species that are harmful to humans have been found yet. Cell wall and storage polysaccharides in algae vary by species, including agar, agarose, and carrageenan in red algae; alginates, laminarin, and fucoidan in brown algae; and ulvan, sulfated galactans, and xylans in green algae (Fig. 1). Algae polysaccharides have various physiological activities, so they are widely studied in the food, pharmaceutical, and cosmetic fields and are widely applied in commercial products such as stabilizers, thickeners, emulsifiers, beverages, and feed [26]. It is also of considerable interest due to its diverse therapeutic activities, such as anticoagulant, antiviral, antioxidant, anticancer, and immunomodulatory abilities [27]. In this review, we will discuss the enzymatic process for converting macroalgae into advanced bioproducts for use.

2. The Enzymatic Process of Red Algae for Biorefinery

2.1. Structural polysaccharides

A group of red algae as a phylum is viewed taxonomically.

Table 1. Species included in each of the red, brown, and green macroalgae

Type	Species
Red	*Cyanidioschyzon merolae*, *Atractophora hypnoides*, *Gelidiella calcicole*, *Lemanea*, *Palmaria palmata*, *Schmitzia hiscockiana*, *Chondrus crispus*, *Mastocarpus stellatus*, *Acrochaetium efflorescens*, *Audouinella*, *Polysiphonia ceramiaeformis*, *Vertebrata simulans*
Brown	*Eisenia bicyclis*, *Alaria esculenta*, *Durvillaea antarctica*, *Ecklonia cava*, *Saccharina japonica*, *Laminaria digitata*, *Postelsia palmaeformis*, *Nereocystis luetkeana*, *Saccharina latissima*, *Undaria pinnatifida*, *Undaria undarioides*, *Mastocarpus papillatus*, *Fucus vesiculosus*, *Sargassum echinocarpum*
Green	*Mesostigma viride*, *Spirotaenia condensate*, *Chlorokybus atmophyticus*, *Chlorokybus bremeri*, *Chlorokybus cerffii*, *Chlorokybus melkonianii*, *Chlorokybus riethii*, *Chloropicon*, *Chloroparvula*, *Trentepohlia*, *Monostroma kuroshiense*

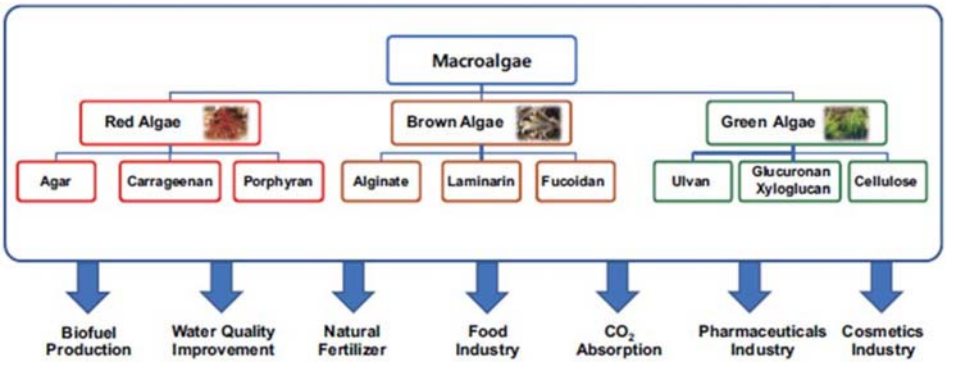

Fig. 1. Group classification and application of macroalgae. Macroalgae is classified into three groups, each containing representative polysaccharides. And these ingredients are used in many industries and applied environmentally and economically.

Most of them live in the sea, have red or reddish-purple color, and have phycocyanin and phycoerythrin as phycobilin pigments in addition to chlorophyll a and d. Most are multicellular bodies, and there are large individuals ranging from microscopic size to 1-2 m [28]. The photosynthetic product includes polysaccharides such as floridean starch, and the cell wall contains a large amount of floridean mucilage in which galactose forms calcium and sulfate esters [29]. Red algae are asexual and form tetraspores, monospores, and bisporangia, which are formed by meiosis in the biphasic sporophyte. In particular, tetraspores are annular, cruciform, or triangular, depending on the type. Sexual reproduction is a monophasic gametophyte that forms sperm and gonads and is formed by carpospores produced as a result of their fertilization. It is regarded as one of the main distinguishing characteristics of the taxon [30].

Therefore, the life history of red algae is based on the so-called polysiphonia-type generational alternation, in which three generations of carposporophytes created by the union of spores and gametes are alternately repeated [31]. It has also been found that this generational alternation has a slightly modified peculiar shape depending on the taxa and is used as another distinguishing trait for the taxon. Red algae are broadly divided into the subclass Bangiophycidae (Protoflorideophycidae), to which seaweed and purple hairs belong, and Florideophycidae, to which algae and serrata belong. The former includes Goniotrichales and Bangiales, and the latter includes Nemaliales, Gelidiales, Cryptonemiales, Gigartinales, Rhodymeniales, and Ceramiales [32]. Skeletal polysaccharides of red algae are composed of cellulose 1,β-1,3-mannan and β-1,4-acrylic acid. Storage polysaccharides consist of red algae starch, and viscous polysaccharides consist of agar, carrageenan, and porphyran [33].

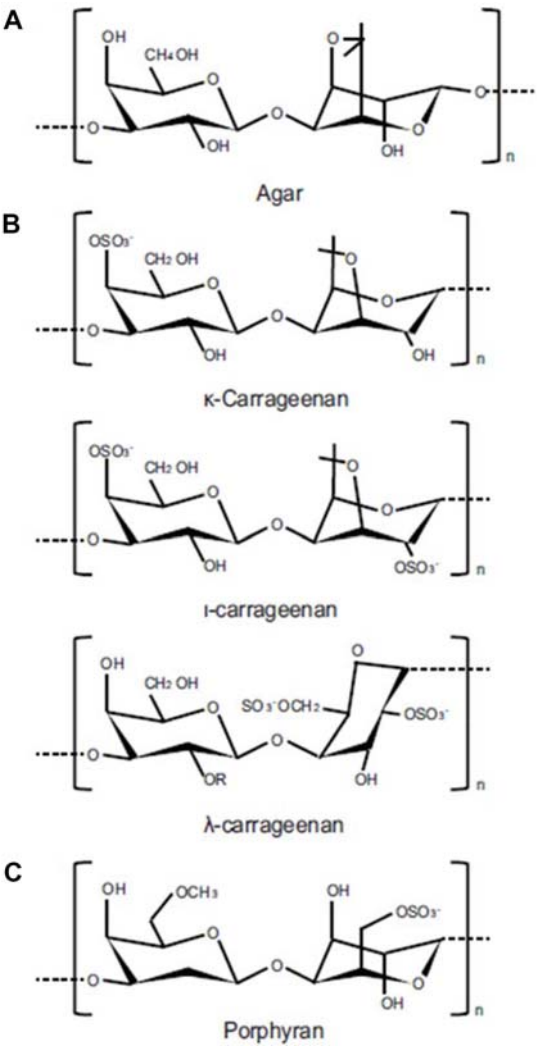

Fig. 2. Chemical structure of the (A) agar, (B) carrageenan, and (C) porphyran.

2.1.1. Agar

Agar has been used for various purposes in the food, cosmetics, pharmaceutical, medical, and microbiological industries mainly because it forms a firm gel at low concentrations [27]. It has been consumed as a traditional healthy food, such as tokoroten (agar noodles), mitsumame jelly (cubic agar gels served with sweet red beans, dried apricot and other fruits, ice cream), yokan (sweet red bean paste with agar), and many other variants [34]. Nongelling agar has also found its application to make honey less spinnable as spread for toast. It has been used as a culture medium and matrix for electrophoresis [35]. Agar is a polysaccharide mainly consisting of agarose and agaropectin. The primary structure has been studied by Araki and his group. Agarose is a linear polysaccharide that can be extracted by treating agarophyte red algae with alkali. Araki published a series of papers on the chemical study of agar mainly using *Gelidium amansii* [36] and he proposed a chemical formula of agarobiose consisting of α-1,3-linked D-galactose and β-1,4-linked 3,6-anhydro-L-galactose (Fig. 2A). However, the structure of this repeating unit, agarobiose, is an idealised one, and an actual agarose contains a significant amount of the sulfated form, sometimes referred to as agarose sulfate, and sometimes agaropectin [37]. Actual polysaccharides extracted from red seaweed contain some sulfate, and the content of 3,6-anhydro-L-galactose is lower than 50%; therefore, alkali pretreatment has been known to increase the 3,6-anhydro-L-galactose content, which leads to a higher gelling ability of agarose [38]. The sulfate content and its location affect the conformation of agar polysaccharides in solution and the interaction with other biomolecules and thus influence bioactivities, such as antimicrobial, antithrombotic, anticoagulant, antioxidant, and antiviral activities [39].

2.1.2. Carrageenan

Carrageenan is a sulfated polysaccharide extracted from red seaweed (Rhodophyceae). Carrageenan is classified into three types, kappa (κ-), iota (ι-) or lambda (λ-) carrageenan, according to the number (one, two or three) of sulfate groups per repeat unit of disaccharide, respectively [40]. As shown in Fig. 2B, κ-carrageenan consists of a repeating unit composed of the disaccharide, β-(1-3)-D-galactose-4-sulfate and α-(1-4)-3,6-anhydro-D-galactose. ι-carrageenan possesses two sulfate groups in a disaccharide repeat unit: β-(1-3)-D-galactose-4-sulfate and α-(1-4)-3,6-anhydro-D-galactose-2-sulfate. λ-carrageenan consists of β-(1-3)-D-galactose-2-sulfate and α-(1-4)-D-galactose-2,6-disulfate, including three sulfate groups [41]. It should be noted that the represented structure is an ideal one, and the real samples contain some extent of different kinds of sequences. Under appropriate conditions, κ-carrageenan and ι-carrageenan in aqueous solutions undergo thermoreversible sol-gel transitions, while no gelation takes place in λ-carrageenan, which has more electrolyte groups [42]. Carrageenan is widely applied to the food industry as a gel or viscous agent and exhibits some physiological effects, such as antitumor activities. Such a wide application and an interest in the sol-gel transition phenomenon have promoted many fundamental studies on aqueous solutions of carrageenan [43].

2.1.3. Porphyran

The representative polysaccharide of red algae or porphyra is called porphyran. Porphyran is a sulfated carbohydrate produced from the Porphyra genus of red algae. It is a complex carbohydrate with a linear backbone made up of 3-linked beta-D-galactosyl units alternating with either 3,6-anhydro-alpha-L-galactosyl or 4-linked alpha-L-galactosyl 6-sulfate units. L-galactose, 3,6-anhydro-L-galactose, 6-O-methylated D-galactose, 6-O-sulfated L-galactose, and ester sulfate are among the ingredients. 1-4-linked L-galactose 6-sulfate makes up a portion of the ester (Fig. 2C). *Porphyra yezoensis* is a significant alga that is mostly grown in China, Japan, and Korea. *P. yezoensis* has a faster growth rate than other red algae and is less susceptible to disease. *P. yezoensis* is high in proteins (25-50%) and polysaccharides (20-40%) [44] and exhibits therapeutic properties such as tumor inhibition, antiviral activity, and antiulcer activity, which are linked to bioactive polysaccharides. *P. yezoensis* polysaccharide is a low-cost, high-sulfuric acid-group-rich porphyran with strong antioxidant potential [45].

2.2. Enzymes for utilizing red algae as biorefinery source

2.2.1. Enzymatic process of agar utilization

Agar consists of 70% agarose and 30% agaropectin. Enzymes that degrade agarose include β-agarase A and β-agarase B, which hydrolyze agarose into a neoagaro-oligosaccharide. A neoagaro-oligosaccharide is hydrolyzed to agarobiose by neoagaro-oligosaccharide 1,3-α-3,6-anhydro-L-galactosidase, and α-neoagaro-oligosaccharide hydrolase enzymes [46]. At this time, 3,6-anhydro-α-L-galactopyranose and D-galactopyranose are hydrolyzed from a neoagaro-oligosaccharide to be produced (Fig. 3A). There are two types of enzymes that degrade agarose: α-agarase and β-agarase. These two enzymes can degrade agarose into a neoagaro-oligosaccharide. α-Agarase has the gene name agaA and is an enzyme that can be obtained from the microorganisms *Alteromonas agarilytica* and *Thalassotalea agarivorans* [47]. β-agarase is divided into two types: β-agarase A and β-agarase B. A neoagaro-oligosaccharide is produced when agarose is hydrolyzed with α-agarase or β-agarase. A neoagaro-oligosaccharide is hydrolyzed with neoagaro-oligosaccharide 1,3-α-3,6-anhydro-L-galactosidase, or α-neoagaro-oligosaccharide hydrolase to produce agarobiose. At this time, 3,6-anhydro-α-L-galactopyranose and D-galactopyranose are released [48]. The neoagaro-oligosaccharide 1,3-α-3,6-anhydro-L-galactosidase enzyme has the gene name ahgA and can be obtained from *Zobellia galactanivorans*. The α-neoagaro-oligosaccharide hydrolase enzyme has the gene name *ahgA*. It can be obtained from a microorganism called *Vibrio* sp JT0107 [49].

2.2.2. Enzymatic process of carrageenan utilization

κ-Carrageenan is hydrolyzed to neocarrabiose sulfate and neocarratetraose 4-O-disulfate by the enzyme κ-carrageenase [50]. The κ-carrageenase enzyme has the gene name *cgkA* and can be obtained from a microorganism called *Pseudoalteromonas carrageenovora* (Fig. 3B). Next, neocarrabiose sulfate is hydrolyzed to neocarrabiose by the enzyme neocarrabiose sulfate/neotetraose disulfate 4-sulfatase [51]. At this time, neocarrabiose sulfate releases sulfate and H^+ and is hydrolyzed to neocarrabiose. The enzyme neocarrabiose sulfate/neotetraose disulfate 4-sulfatase can be obtained from the microorganism *P. carrageenovora*. In addition, neocarratetraose 4-O-disulfate is hydrolyzed to neocarratetraose 4-O-sulfate by the enzyme neocarrabiose sulfate/neotetraose disulfate 4-sulfatase. At this time, neocarratetraose 4-O-disulfate, like neocarrabiose sulfate, releases sulfate and H^+ and is hydrolyzed to neocarratetraose 4-O-sulfate. The neocarrabiose sulfate/neotetraose disulfate 4-sulfatase enzyme can be obtained from the microorganism *P. carrageenovora*. In addition, neocarratetraose 4-O-sulfate is hydrolyzed to neocarrabiose sulfate and neocarrabiose by the enzyme neocarratetraose 4-O-monosulfate β-hydrolase. The enzyme neocarratetraose 4-O-monosulfate β-hydrolase is also obtained from the microorganism *P. carrageenovora* [52]. The decomposition process of ι-carrageenan can be divided into three main steps. The first is ι-carrageenan

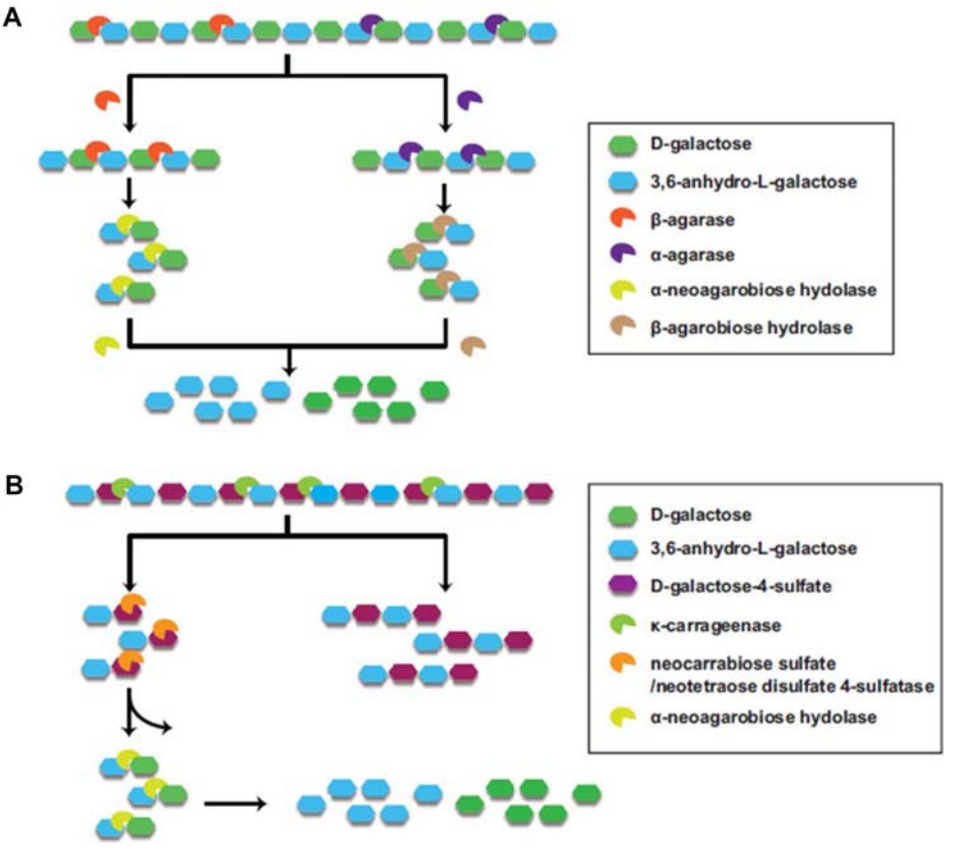

Fig. 3. Enzymatic hydrolysis model of red algae polysaccharides. (A) Enzymatic hydrolysis of agar polysaccharides into monosaccharides. (B) Enzymatic hydrolysis of porphyran polysaccharides into monosaccharides.

degradation. ι-carrageenan is hydrolyzed to ι-neocarratetraose sulfate and ι-neocarrahexaose sulfate by the enzyme ι-carrageenase. The ι-carrageenase enzyme has the gene name *cgiA* and can be obtained from the *Z. galactanivorans* microorganism [53]. λ-carrageenan is hydrolyzed by λ-carrageenase to produce neo-λ-carrahexaose. At this time, λ-carrageenan releases neo-λ-carratetraose. The λ-carrageenase enzyme has the gene name *cglA* and can be obtained from the microorganism *P. carrageenovora*. Next, neo-λ-carrahexaose is hydrolyzed to neo-λ-carratetraose and neo-λ-carrabiose by λ-carrageenase [54].

2.2.3. Enzymatic process of porphyran utilization

Porphyran is hydrolyzed to α-L-galactopyranose-6-sulfate-(1,3)-β-D-galactose and a neoagaro-oligosaccharide, by β-porphyranase A and β-porphyranase B enzymes. At this time, as it is hydrolyzed, H_2O, that is, water molecules, is released. Then, the sulfur portion of porphyran, α-L-galactopyranose-6-sulfate-(1,3)-β-D-galactose, is isolated, leaving a neoagaro-oligosaccharide oligosaccharide with no sulfur component [55]. The β-porphyranase A enzyme has the gene name porA, and the β-porphyranase B enzyme has the gene name porB. Both enzymes can be obtained from a microorganism called *Z. galactanivorans*. A neoagaro-oligosaccharide that is primarily decomposed from porphyran has a molecular structure very similar to that of an agarose. Therefore, a neoagaro-oligosaccharide is hydrolyzed to neoagarobiose and neoagarotetraose by the β-agarase B enzyme [56].

3. The Enzymatic Process of Brown Algae for Biorefinery Algae

3.1. Structural polysaccharides

Brown algae are a class of algae consisting mainly of complex, macroscopic seaweeds whose brown color comes from a carotenoid pigment, fucoxanthin, and in some species, various phaeophycean tannins. Less than 1% of the 265 genera and 2,000 possible species of brown algae are known from freshwater settings, while some marine species have been observed to inhabit brackish areas. This eukaryotic supergroup first arose around 1 billion years ago as a result of a secondary endosymbiotic interaction between an ancestral protist and a red algae [57]. In phylogenetic analysis, chromalveolates form a separate group from

mammals and fungi on the one hand, and red algae, green algae, and plants on the other [58]. So, independent of opisthokonts and archaeplastida, brown algae developed complex multicellularity [59]. The kelp species Laminaria (kombu), Undaria (wakame), and Marocystis are the most prevalent. More than half of the dry weight of brown algae is made up of the polysaccharides alginate, laminarin, and fucoidan, and this percentage can even exceed 70% in some species.

3.1.1. Alginate

Brown macroalgae contain alginate, a naturally occurring polymer, in both their intercellular matrix and cell walls. This term generally refers to alginic acid and its salt, but it can also be used in all derivatives of alginic acid. Alginate is hydrophilic and forms a viscous gum when hydrated. There are two structural isomer residues in this linear polysaccharide: β-D-mannuronic acid (M) and α-L-guluronic acid (G) connected through 1,4-glycosidic linkages [60]. Consequently, three different types of blocks might make up the polymer: homopolymeric sections of successive Ms, Gs, or heteropolymeric sections of units of M and G placed at random (Fig. 4A). The proportion of GG, MM, and MG defines a physical characteristic of alginate. Alginate with a high ratio of M blocks has a higher viscosity, and alginate with a high ratio of G blocks has a higher gelling property. Typically, the M to G ratio is 1:1. However, the order in the polymer chain as well as the relative amounts of M and G rely on a variety of variables, including the type of algae, growth circumstances, season, and part of the algae [61]. That is, each species that produces alginate may have a different alginate composition, and the ratio of mannuronic to guluronic acid may be different [62].

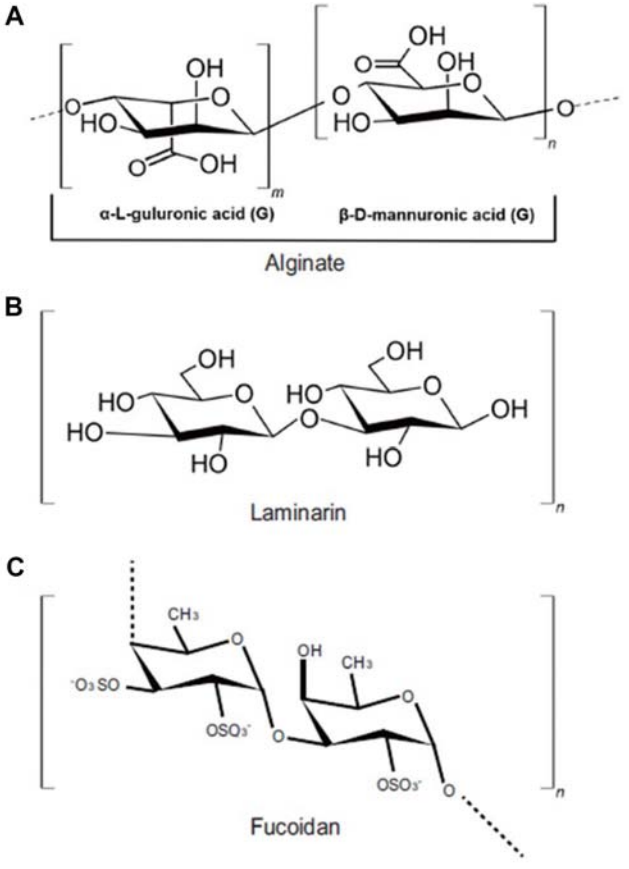

Fig. 4. Chemical structure of the (A) alginate, (B) laminarin, and (C) fucoidan.

3.1.2. Laminarin

Laminarin, also called laminaran, is a major storage carbohydrate found in brown algae. It is an active substance that is taken out of the dry thallus of brown seaweeds such as *Eisenia bicyclis*, *Ecklonia kurome*, and *Laminaria japonica*. Its molecular composition consists of (1-3)-β-D-glucan with β-(1-6) branching and various reducing ends that may contain mannitol or glucose residues (Fig. 4B). Different laminarin solubilities are predetermined by the degree of branching. In contrast to highly branched laminarin, which is soluble in both warm and cold water, low degrees of branching only allow for solubility in water [63]. Thus far, two types of laminarin have been recognized. An M chain is one that is connected to D-mannitol at the chain's reducing end. When mannitol is missing at the reducing end, laminarin is a G chain. Depending on the isolated species and the environmental conditions that are anticipated to have a direct impact on the biological properties, the ratio of the two forms of laminarin may change [64].

3.1.3. Fucoidan

Fucoidan is a biologically active substance that has recently become popular as a natural immune enhancer. In addition to echinoderms and a few lower plants, it is frequently found in brown seaweed. *Undaria pinnatifida*, *L. japonica*, *Cladosiphon okamuranus*, and *Fucus vesiculosus* are the most common seaweed species from which fucoidan is commercially accessible [65]. It has also been found in various forms in animal species such as sea cucumber [66]. Fucose repeating units make up the majority of this long-chain sulfated polysaccharide's composition, along with a few additional monosaccharide residues (Fig. 4C). Since fucoidan was first separated in 1913, researchers have examined the structure of fucoidans from various brown seaweeds. The chemical structure is a complex sulfuric acid polysaccharide containing esterified sulfuric acid as a main component in L-fucose and is also bonded to small amounts of glucose, galactose, mannose, uronic acids, *etc*. [67]. That is, the name fucoidan does not mean substances having the same structure but is a generic term for polymer

polysaccharides whose main component is fucose. In addition, an important feature of fucoidan is that its molecular weight varies. Various structures are shown depending on the type of algae or the stage of development of algae. For example, similar climatic conditions and habitats are known to have similar structures. Fucoidan, extracted from *Saccharina latissimi*, *Analipus japonicus*, *Laminaria digitata*, *Chorda filum*, and *C. okamuranus*, has a 1→3 binding structure of a pyranose residue, and a polysaccharide derived from *Ascophyllum nodosum* has a structure in which the α-L-fucopyranose residue is modified 1→3 to 1→4 [68]. Therefore, fucoidans constitute a highly variable and versatile subgroup of fucans.

3.2. Enzymes for utilizing brown algae as biorefinery source

3.2.1. Enzymatic process of alginate utilization

Alginate lyases are present in algae, fungi, viruses, and various marine and terrestrial bacteria, especially in bacteria [69]. Alginate is degraded into alginate oligosaccharides by endo-alginate lyases (Fig. 5A). In accordance with the M-block (poly-D-mannuronate) and B-block (poly-L-guluronate) that comprise alginate, these enzymes are categorized as poly-D-mannuronate lyase and poly-L-guluronate lyase, respectively. After that, alginate oligomers are decomposed into 4-deoxy-α-l-erythro-hex-4-enopyranuronate (DEHEP),

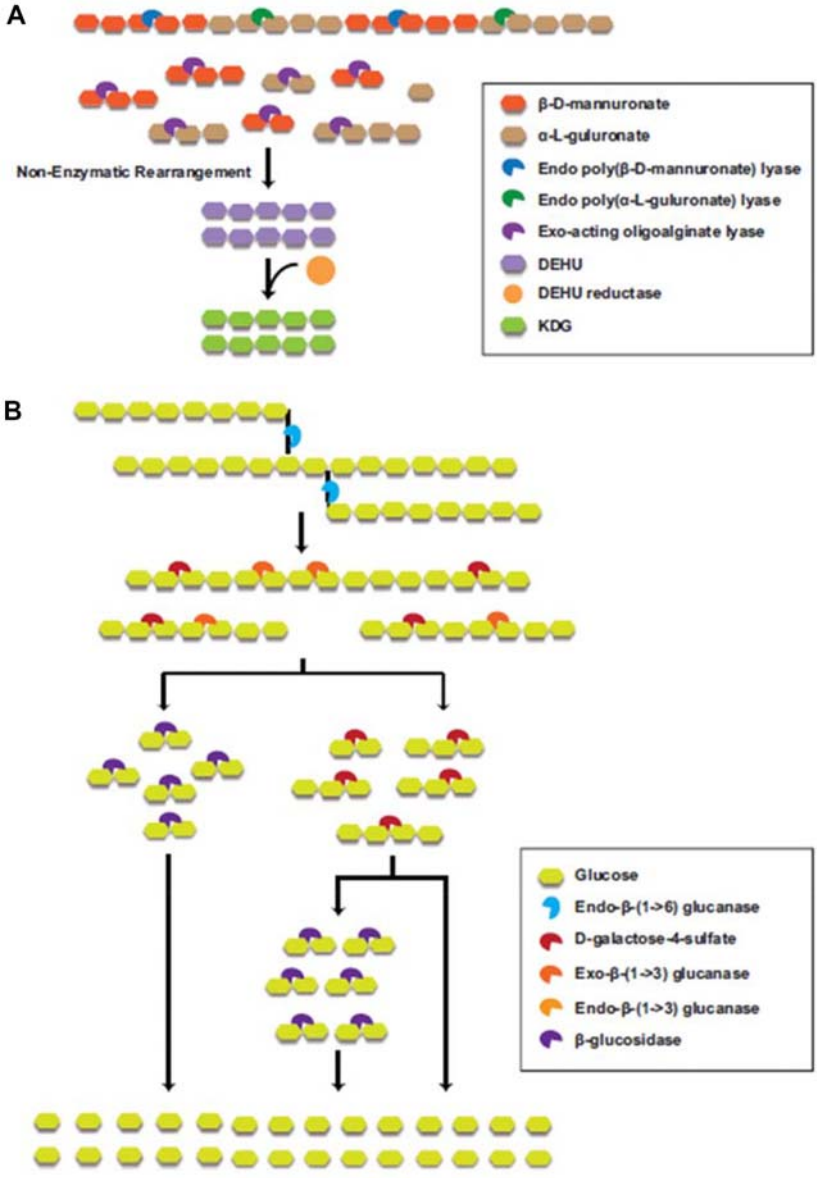

Fig. 5. Enzymatic hydrolysis model of brown algae polysaccharides. (A) Enzymatic hydrolysis of alginate polysaccharides into monosaccharides. (B) Enzymatic hydrolysis of laminarine polysaccharides into glucose.

an unsaturated monosaccharide, by exo-acting oligoalginate lyase. The following step involves nonenzymatically converting DEHEP into 4-deoxy-l-erythro-5-hexoseulose uronic acid (DEHU), which is then reduced by DEHU reductase to 2-keto-3-deoxy-glucose (KDG) [69]. KDG kinase phosphorylates KDG to 2-keto-3-deoxy-6-phosphogluconate (KDPG), and then KDPG enters the ED (Entner-Doudorof) pathway [70].

3.2.2. Enzymatic process of laminarin utilization

Laminarin is hydrolyzed by four different enzymes: endo-β-(1→6) glucanase, endo-β-(1→3) glucanase, exo-β-(1→3) glucanase, and β-glucosidase. Endo-β-(1→6) glucanase breaks the β(1→6) bond of the branched laminarin and converts it into a debranched form (Fig. 5B). Linear laminarin is broken down into laminaritriose and laminaribios by two enzymes: endo-β-(1→3) glucanase and exo-β-(1→3) glucanase. At this time, endo-β-(1→3) glucanase cleaves in the middle of the laminarin skeleton, while exo-β-(1→3) glucanase separates glucose from the nonreducing end of laminarin oligosaccharide. Decomposed laminarin oligosaccharide is broken down into glucose, a monosaccharide, by β-glucosidase [71]. In particular, β-glucosidase has been found in many natural organisms, so it is a relatively easy compound to be used during enzymatic hydrolysis from brown algae [72].

3.2.3. Enzymatic process of fucoidan utilization

Fucoidanases are hydrolytic enzymes that cleave down fucoidan connections from nonreducing ends of fucoidans such as 1,3-α-L-fucan. They are found in bacteria such as *Pseudoalteromonas* spp., fungi, sea urchin, sea cucumber, and brown algae. Fucoidanases are divided into fugosidase and endohydrolase based on the decomposition method [73]. Fucoidans make it difficult to produce only fucose, a monomeric form of fucoidans, because fucoidans consist of several types of monosaccharides. As fucoidanases have lower activity levels than other enzymes and their structures are unclear and complex, in-depth analysis studies are still insufficient. However, Fucoidanase's catalytic mechanism is drawing attention along with its various medical and nutritional effects [74].

4. The Enzymatic Process of Green Algae for Biorefinery

4.1. Structural polysaccharides

The main component of green algae biomass is cell wall polysaccharides, accounting for 54% of the total dry weight. Of these, ulvan is the most abundant, accounting for 9-36% of the dry weight of algae. Ulvan is biosynthesized by the marine algae ulva spp. and Enteromorpha spp [75,76]. In addition to ulvan, three other cell wall polysaccharides are found in ulva species: glucuronan, xyloglucan, and cellulose. Together with ulvan, glucuronan, xyloglucan, and cellulose account for up to 45% of the total dry weight. Xyloglucan and glucuronan are soluble polysaccharides similar to ulvan. However, compared to ulvan, it is a relatively insignificant cell wall polysaccharide component. In addition, ulvan is the only one of the four cell wall polysaccharides (ulvan, glucuronan, xyloglucan, cellulose) present in Ulva that contains both rhamnose and iduronic acid [77].

4.1.1. Ulvan

Ulvan polysaccharides consist primarily of L-rhamnose-3-sulfate (Rha3S), D-xylose (Xyl), L-iduronic acid (IdoA), and D-glucuronic acid (GlcA), with the sulfate group linked to rhamnose [78]. Ulvan is composed primarily of two major repeating disaccharide units linked by β-1,4 linkages. α-L-iduronic acid (1→4)-α-L-rhamnose and β-D-glucuronic acid (1→4)-α-L-rhamnose-3-sulfate are the main repeating units. Instead of the L-iduronic acid and D-glucuronic acid components, xylose (sulfated or nonsulfated) units can form β-D-xylose (1→4)-α-L-rhamnose-3-sulfate and β-D-xylose-2-sulfate (1→4)-α-L-rhamnose-3-sulfate monomers [79] (Fig. 6A). Nonetheless, the general structure of ulvan is made up of repeating disaccharide units connected by an α-(1→) glycosidic bond. Ulvan disaccharides are made up of monomers of [glucuronic acid - sulfated rhamnose], [iduronic acid - sulfated rhamnose], [xylose - sulfated rhamnose], and [sulfated xylose - sulfated rhamnose] that are linked together by α- or β-(1→4) glycosidic bonds.

4.1.2. Glucoranan and xyloglucan

Several types of green algae have β-(1,4)-d-polyglucuronic acid (algae glucuronan) on their cell walls (Fig. 6B). Algae glucuronan is coextracted with ulvan, a major water-soluble polysaccharide that is normally extracted from the cell wall of ulva sp [80]. Xyloglucan is a component of most plant primary cell walls. Xyloglucan is a water-soluble polysaccharide composed of a 1,4-β-glucan main chain and xylose and galactose side chains. Xyloglucans are distinguished by a major β-D-(1→4)-glucan backbone that is branched by α(1→6)-linked D-xylopyranosyl or β-D-galactopyanosyl (1→2)-D-xylopyranosyl residues. Xyloglucan is closely related to cellulose microfibrils by hydrogen bonding, providing a load-bearing network of the cell wall and protecting the cell wall from collapse due to osmotic stress [81,82].

4.1.3. Cellulose

Cellulose from green algae exhibits a pattern similar to that of native land plants. Thousands of D-glucose subunits

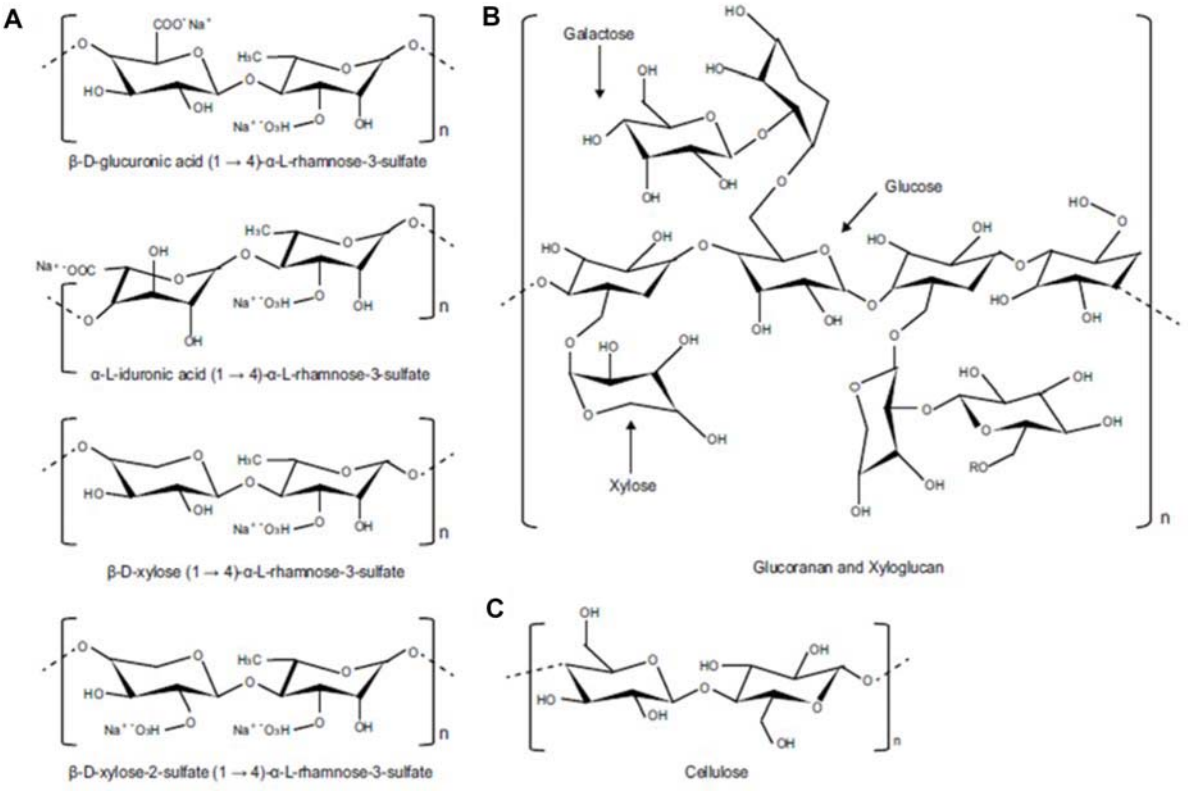

Fig. 6. Chemical structure of the (A) ulvan repeat disaccharide, (B) xyloglucan, and (C) cellulose.

make up cellulose (Fig. 6C). Beta 1-4 glycosidic bonds connect the glucose subunits in cellulose. Cellulosic microfibers are formed by the formation of intramolecular and intermolecular hydrogen bonds between cellulose chains. Microfibrils combine to form fibrils, which then aggregate to form cell wall fibers [83]. In cellulose, the orientation of glucose molecules is reversed. They have beta orientation in which the hydroxyl group of the anomeric carbon or carbon number one is directed above the plane of the glucose ring. The hydroxyl groups of the remaining carbon atoms are directed down the plane of the ring. All alternative glucose molecules in cellulose are inverted to make beta 1-4 glycosidic bonds. The hydroxyl group of carbon 1 is pointed upward, whereas that of carbon 4 is directed downward. One of these molecules is flipped so that the two hydroxyl groups are in the same plane, creating a beta 1-4 glycosidic bond. That is, all alternate glucose molecules in cellulose are reversed [84].

4.2. Enzymes for utilizing green algae as biorefinery source

4.2.1. Enzymatic process of ulvan utilization

The use of ulvan lyase can degrade ulvan with good specificity, high efficiency, and mild reaction conditions, and it is very promising because it can well maintain the rare sugar structural properties of ulvan. This is the first step in ulvan's complete disintegration. Ulvan lyases are ulvan-degrading enzymes that are classified into five families according to their amino acid sequence in the CAZy database (http://www.cazy.org//): PL24, PL25, PL28, PL37 and PL40. These enzymes are endolytic enzymes capable of cleaving ulvan into oligosaccharides with unsaturated reducing ends by acting on the ulvan through the β-elimination mechanism [85]. Ulvan β-elimination is achieved through Ulvan lyase. When the initial cleavage step of ulvan is catalyzed, the α-1,4-bond between rhamnose-3-sulfate and glucuronic acid or iduronic acid is cleaved to form an unsaturated uronic acid residue at the nonreducing end. This residue is hydrolytically removed by glucuronyl hydrolases of the GH88 or GH105 family (EC 3.2.1.-) to form 5-dehydro-4-deoxy-D-glucuronate [86].

4.2.2. Enzymatic process of glucuronan and xyloglucan utilization

Glucuronan lyase randomly cleaves glucuronan polysaccharides, polymers of linked β-(1-4)-D-glucuronic acids, by a β-elimination mechanism to generate unsaturated oligoglucuronan. There are no detailed reports of xyloglucan hydrolase [87].

4.2.3. Enzymatic process of glucuronan and cellulose utilization

The enzymatic hydrolysis of cellulose is efficiently carried out by the enzyme cellulase, which consists of exo-glucanase, endoglucanase and β-D-glucosidase (cellobiase) enzymes. Exoglucanase (EC 3.2.1.91) hydrolyzes cellulose β-1,4-glycosidic bonds at the ends of cellulose chains. Endoglucanase (EC 3.2.1.4) randomly cleaves cellulose's β-1,4-glycosidic bonds, forming short-chain oligosaccharides with free reducing ends. β-D-Glucosidase hydrolyzes soluble cellobiose and other cellodextrins to produce glucose molecules. Endoglucanase and exoglucanase hydrolyze cellulose to form a D-glucose dimer, β-cellobiose. When the β-cellobiose molecule is released, β-glucosidase (EC 3.2.1.21) attacks and cleaves the β-cellobiose disaccharide into two glucose molecules [88].

5. Conversion into Biofuels and Health Functional Ingredients from Macroalgae

5.1. Application of polysaccharides in macroalgae for biofuels

As the demand for bioproducts gradually increases, a stable supply of raw materials is required to satisfy this growing demand. Due to their ability to grow starch, which is a widely used raw material thus far, has been limited, macroalgae have been newly proposed as alternative raw materials and are in the spotlight as sustainable raw materials [89]. Sea tangles, sea mustard, and brown algae account for the largest amount of seaweed produced worldwide [30]. As such, macroalgae are very abundant and contribute 35 to 60% to the dry weight of carbohydrates, making them an ideal biomass source [90]. Currently, algal biomass is treated as waste due to the high growth rate of algae and accumulates in very large quantities, making it an inexpensive and easily accessible raw material source for producing biofuels and high value-added fine chemicals. The use of green macroalgal biomass in a process known as 'ABE' fermentation to produce ethanol, n-butanol, acetone, and ethanol was recently reported. In addition, this kind of biomass can be used to produce other useful byproducts. As one of the major cell wall polysaccharides, ulvan is a source of rhamnose, which can be used as a precursor for the synthesis of 1,2-propanediol.

Brown algae are in the spotlight as a sustainable raw material in bioindustry. Laminarin, one of the sugars found in brown algae, has the ability to be used to produce bioethanol. A marine bacterium called *Saccharophagus degradans* has the capacity to decompose marine material and generate bioproducts. A new yeast strain was created using transformation techniques by introducing Gly5M, a laminase from *S. degradans*, and the β-glucosidase encryption gene. 5.2 g/L of ethanol was produced from 20 g/L of laminarin solution through the developed strain. Unlike the previous ethanol production through laminarin, this study allowed laminarin to be produced directly into bioethanol using yeast cell surface display methods [91]. Alginate bioproducts, one of the primary carbohydrates found in brown algae, are difficult to produce using industrial microorganisms along with a general metabolic process. However, the marine bacterium *Vibrio* sp. dhg not only has an enzyme that can efficiently utilize alginate but can also produce products by simultaneously using mannitol as a material along with alginate. Alginate and mannitol exist in different oxidation states, which is advantageous for ethanol produced in an oxygen-limited environment. It is significantly more productive and efficient to produce bioproducts in combination with brown algae alginate and mannitol than to limit production to a single raw material [92]. *Vibrio* sp. dhg originally has both aldehyde dehydrogenase and alcohol dehydrogenase, allowing acetyl-CoA reduction to create ethanol. As a result of ethanol fermentation using a medium in which alginate and mannitol are mixed at a ratio of 1:2, only 1.6 g/L ethanol is produced from 30 g/L mixed solution, and the production rate is gradually reduced. In addition, a considerable amount of byproducts, such as lactic acid and succinate, which interfere with ethanol production, were produced [89]. To solve this problem, the *Zymomonas mobilis*' pyruvate decarboxylase (pdc) and aldehyde dehydrogenase (aldB) genes were introduced into *Vibrio* sp. dhg to be recombined to produce more ethanol [70]. Additionally, ethanol production was carried out through recombinant *Vibrio* sp. dhg with VP15, one of the powerful synthetic promoters. As a result, ethanol production increased by nearly 4.8 times to 7.7 g/L, and the amount of byproducts also decreased. Endogenous frdABCD (fumaric acid reductase), ldhA (lactose dehydrogenase), and pflB (pyruvate-formatease) genes formerly harbored by *Vibrio* sp. dhg were eliminated to enhance ethanol synthesis and reduce carbon loss [89]. As such, it is anticipated that a variety of bioproducts can be produced from brown algae using cutting-edge microbial platform technology that researches the distinctive metabolic routes of microorganisms. By 2025, bioproducts are predicted to account for 22% of the chemical industry's market share, hence the advancement of these technologies is predicted to have a large economic influence.

The green algae *Ulva lactuca* has been studied as a potential feedstock for acetone, ethanol, and ethanol fermentation. More than 90% of sugar is solubilized through hydrolysis using cellulase and hot water treatment. Attempts were made to synthesize acetone, butanol, and ethanol (ABE) from hydrolysates and *Clostridium acetobutylicum* and *Clostridium beijerinckii.* Without supplementation, the

U. lactuca hydrolysate-based medium was fermentable. *C. beijerinckii* used all of the sugars in the hydrolysate and produced a high yield of ABE (0.35 g ABE/g consumption). *C. acetobutylicum,* on the other hand, primarily produces organic acids (acetic acid and butyric acid). These findings highlight the enormous potential of *U. lactuca* as a fermentation feedstock. Intriguingly, the main fermentation product (9.7 g/L) in the control culture of *C. beijerinckii* on rhamnose and glucose was 1,2 propanediol [93]. Butanol production in ABE fermentation with *U. lactuca* has been reported. The green algae *U. lactuca* was harvested manually and mechanically, dried, and ground first. The pulverized *U. lactuca* was then acid hydrolyzed to extract carbohydrates for the preparation of an algal sugar solution. Finally, butanol was synthesized by fermenting an algal sugar solution containing *C. beijerinckii* and *Clostridium saccharoperbutylacetonicum*. The fermentation broth contained 4 g/L butanol and removing excess solids from the hydrolysate prior to fermentation resulted in a 75% increase in productivity. Both examples demonstrate the potential of green algae as feedstocks for ABE fermentation-based biobutanol production [94].

5.2. Application of polysaccharides in macroalgae for health functional ingredients

There are increasing publications reporting the potential health benefits of macroalgae on neurodegenerative disorders, metabolic disorders, and cancer. In macroalgae, there exist various kinds of potential health functional ingredients including polysaccharides, proteins and peptides, lipids, vitamins, minerals, and polyphenols [95]. The investigation of health beneficial functions of these ingredients is thoroughly ongoing these days, and those of polysaccharides in macroalgae are mostly focused on gut microbiota related health benefits such as prevention of chronic diseases and immunomodulating effects. It is known that many algae-derived polysaccharides are not digested by humans but utilized by gut microbiota to provide a wide range of physiological benefits to the host, which is called the 'prebiotic effects' [96]. The most well-known prebiotics are the polysaccharides from brown algae, alginates, laminarin, and fucoidan. Since there are several well-studied oligosaccharides as prebiotics, it might be possible to discover and produce new types of health beneficial oligosaccharides from macroalgae by using enzymatic processes [97]. However, it will be very challenging because the number of cases of possible oligosaccharides regarding of their structure, size, and sugar unit is infinite. An application method that is free from this problem would be to utilize sugar units from macroalgae. β-D-mannuronic acid (M2000) which is produced by enzymatic and chemical procedure from alginate is known as a promising immunosuppressive drug with non-steroidal anti-inflammatory drug properties together with cardioprotective, anti-diabetic, and anti-tumoral efficacy [98]. There is also a research group investigating the efficacy of 3,6-anhydro-L-galactose on skin and apply it as a novel cosmetic compound [99].

6. Conclusion

Macroalgae is currently a compound that is potentially applicable in many industries and is considered a renewable source of biomass. Macroalgae can be classified into three groups: red algae, brown algae, and green algae, and the enzymes that act to use the polysaccharides contained in each macroalgae as feedstock for biorefinery are also divided. Among the various polysaccharides of red algae, viscous polysaccharides consist of agar, carrageenan, and porphyranes, and the enzymes that break them down include α-agarase and β-agarase, carrageenase, β-poranase A and β-poranase B. In the case of brown algae, alginate, laminarin, and fucoidan account for a large portion of polysaccharides and are decomposed by alginate lyases, endo-β-(1→6) glucanase and endo-β-(1→3) glucanase, exo-β-(1→3) glucanase and β-glucosidase and fucoidase. Polysaccharides of algae are rich in ulvan, glucuronan, xyloglucan, and cellulose, and enzymes that make them available as biomass include ulvan lyase, glucuronan lyase, exoglucanase, endo-glucanase and β-D-glucosidase (cellobiase). Macroalgae, which contains these ingredients, is gaining attention in many industries and can be seen as a valuable source for biofuel production, and research using it is ongoing.

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Ethical Statements

The authors declare no conflict of interest.
Neither ethical approval nor informed consent was required for this study.

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