Recent trends in functional characteristics and degradation methods of alginate

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Abstract. The total area of the Earth's oceans is 360 million square kilometers, accounting for approximately 71% of the Earth's surface area. It is a huge treasure trove of resources, containing abundant mineral resources, oil and gas resources, microbial resources, etc. The production of marine biomass is enormous, and as a third-generation renewable energy source, it has more sustainable development potential than terrestrial biomass. The main source of marine biomass is marine algae, so the development and excavation of marine algae resources is imperative. At present, alginate has become the second largest sustainable development resource in terms of production, second only to cellulose, and has enormous application value. The biological enzyme method for degrading alginate utilizes alginate lyase to β The elimination mechanism breaks the glycosidic bond, which has more degradation advantages than physical and chemical methods.

1. Source of alginate

Alginate, also known as alginic acid, has a molecular weight ranging from 20 to 250 kDa and is a linear polysaccharide composed of uronic acid monomers **Figure 1**. Alginate is abundant in brown algae plants and is generally distributed in the cell walls and intercellular spaces of kelp, sargassum, giant algae, and other brown algae accounting for approximately 10-45% of their dry weight[1]. In 1881, E. C. Stanford first discovered and successfully extracted alginate from the brown algae plant Laminaria[2]. In China, the production of alginate mainly relies on artificial breeding, and commercial alginate is mainly extracted from brown algae.

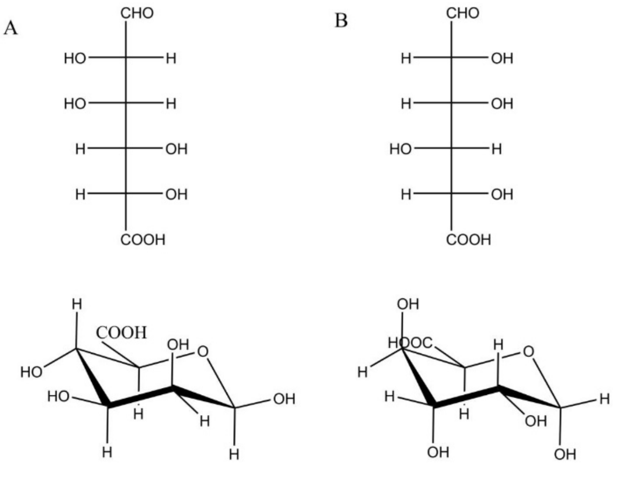

Figure 1. Structures of alginate monomer: (A) β-D-mannuronic acid, (B) α-L-guluronic acid.

2. Properties of alginate

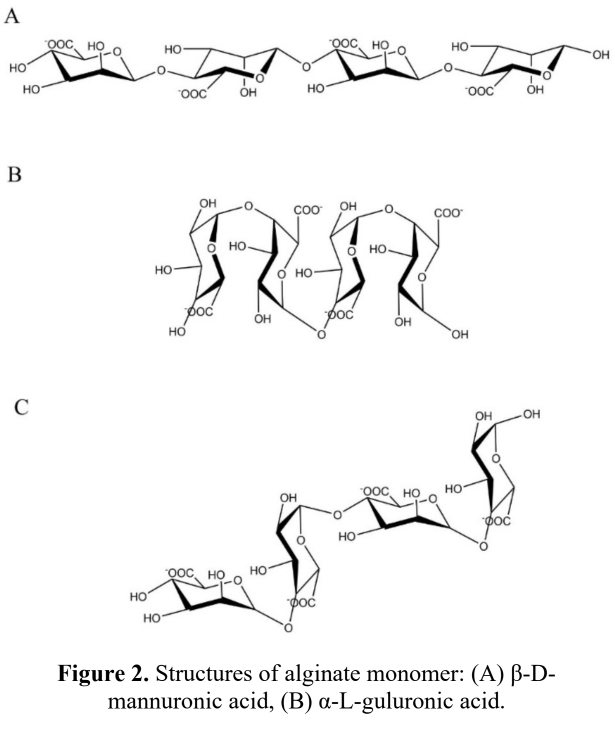

Figure 2. Structures of alginate monomer: (A) β-D-mannuronic acid, (B) α-L-guluronic acid.

Alginate is mainly composed of β- D-mannuronate acid, M and its epimer α- L-guluronic acid, G passed β- 1,4 glycosidic bond[3]. There are three main ways to polymerize alginate[4], : A Only a single M is polymerized to form a homopolymer of mannuronic acid (polyM) Figure 2A; B. Only a single G polymerization forms a polyG homopolymer Figure 2B; C. The heteropoly G/M fragment (polyM-G) is formed by alternating

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polymerization of M and G Figure 2C. Alginate, as a macromolecular polysaccharide, has special physicochemical properties. Alginate is colorless or light yellow in color, has no volatile odor, and is not easily soluble in organic solvents such as ethanol and chloroform. However, it is easy to form a co solution with macromolecular substances such as phosphates and proteins[5]. Alginate contains many free carboxyl groups and is prone to form alginate with metal ions such as sodium and calcium[6].

3. The use of alginate

Alginate, due to its inherent physicochemical properties and biocompatibility, can be widely used in various fields such as food, chemical, biology, and medicine[7]. In food, alginate is generally considered a safe food additive, which can be added to certain foods to improve, modify, or stabilize the texture. For example, in ice cream or other dairy products, it serves as a stabilizer to stabilize the colloid and ensure the texture of cream[8]; In addition, it plays the role of thickening agent, adhesive or gel agent in dessert gel, pudding, sauce, etc. In addition, based on the characteristics of algin such as strong toughness, low hardness, high viscosity, etc., various components and functional factors between and in the cells of kelp and other marine algae are tightly wrapped by algin, which is difficult to extract and deep process, restricting the economic value of marine algae. Therefore, the degradation of alginate can release functional factors and components within marine algae, unleashing the high value of marine algae themselves. Alginate is also a healthy and edible green food, which can help treat diabetes[9] and prevent cardiovascular and cerebrovascular diseases[10]. It is called "longevity food". In the biological industry, the protective matrix formed by alginate is beneficial for cell culture, cell transplantation, and tissue regeneration[11]. It can also be used as a material for cell buffer to protect cells from physical pressure and reduce host immune system rejection reactions[12]. In the pharmaceutical industry, alginate has a long history and can be used as a hemostatic and wound repair material[13]. It can also be added as an auxiliary material to drugs, slowing down the absorption of drugs by the body, prolonging drug efficacy, and reducing drug side effects. In the chemical industry, it can be applied to papermaking, sewage treatment, and can also be used as various dyes in the textile industry[14]; In agriculture, it can be used as insecticides, growth promoting agents, water retaining agents, antiviral materials, etc; In the cosmetics industry, it can be used in the processing and production of daily necessities such as shampoo, toothpaste and facial mask. In addition, alginate, as a third-generation material for producing biofuels, can be used for the production and manufacturing of bioethanol[15], making it a widely studied hotspot both domestically and internationally due to its application value.

4. The degradation mode of alginate

Alginate oligosacc harides (AOS) are polysaccharides, oligosaccharides, and monosaccharide products with different degrees of polymerization obtained from alginate through different degradation methods. Brown algae oligosaccharides have many biological activities due to their small molecular weight, strong stability, and high water solubility.

At present, there are many methods for preparing brown algae oligosaccharides in industry, mainly using three methods Figure 3: (1) physical degradation method, mainly through high-temperature and high-pressure, ionizing radiation, ultrasonic fragmentation, etc.; (2) Chemical degradation methods, including acid hydrolysis, alkali hydrolysis, oxidation hydrolysis, etc; (3) Biological enzyme degradation method.

The physical degradation method mainly adopts methods such as high temperature and high pressure, ionizing radiation, etc. Usually, multiple physical methods are involved in the degradation together. The main principle is to achieve effective degradation of alginate by applying a certain external pressure. The physical degradation method has no environmental pollution and is simple to operate, but it has disadvantages such as high cost, easy to lose control of the reaction process, uneven product, and safety hazards caused by ionizing radiation[16].

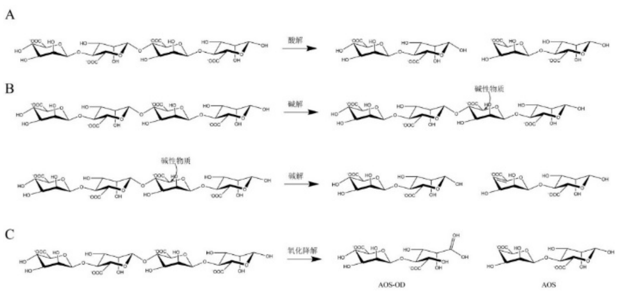

Figure 3. Composition of alginate oligosaccharide: (A) mannuronic acid oligosaccharide, (B) guluronic acid oligosaccharide, (C) heterozygous acid oligosaccharide.

The principle of chemical degradation method is to break the 1,4 glycosidic bond of algin through chemical reagent, so as to generate low molecular polymer. Acid hydrolysis usually uses dilute acids such as hydrochloric acid, sulfuric acid and formic acid to degrade algin into heteropolysaccharide fragments. Although AOS with different polymerization degrees can be obtained by acid hydrolysis at one time, the energy cost is high, and a large number of salt wastes that are difficult to recover are also generated. Alkali hydrolysis occurs under alkaline conditions β Elimination reaction: protons on algin C5 are induced by carboxyl groups, and electrons on C4 are transferred to C5, making β- The 1,4-neneneba glycosidic bond breaks, and algin is degraded into alginate oligosaccharides. The energy cost required for alkaline hydrolysis is low, but it has the disadvantages of intense reaction, large by-product pollution, and difficulty in controlling the biomass of the target product. Oxidative hydrolysis refers to the degradation of alginate into alginate oligosaccharides under high temperature or the addition of catalysts. The degradation process is simple and has low energy costs, but the yield of oligosaccharides is low and the reaction process is intense, making the reducing end of oligosaccharides easily damaged.

The degradation principle of the biological enzyme degradation method is to use alginate lyase to β The elimination mechanism breaks the glycosidic bond and forms unsaturated uronic acid between C4 and C5 of the sugar ring. Compared with physical and chemical degradation methods, biological degradation method has more degradation advantages, such as mild reaction conditions, high degradation efficiency, strong controllability, fewer by-products, and high product singularity. It is currently the main method for directed preparation of brown algae oligosaccharides, and has a broad prospect[17].

5. Alginate lyasei

Alginate lyase is a kind of enzyme belonging to the polysaccharide lyase (PL) family, which degrades large molecules of fucoidan to small molecules of oligosaccharide. It is a key tool enzyme for enzymatic hydrolysis of fucoidan to prepare functional oligosaccharides. Alginate lyases have a wide range of sources and have been discovered in animals, fungi, bacteria, viruses, etc. Most of them come from bacteria. Different alginate lyases exhibit substrate specificity for different compositions of alginate[18], which can be divided into three categories based on their substrate specificity: polymannuronate lyases (EC 4.2.2.3), polygururonate lyases (EC 4.2.2.11), and bifunctional lyases that can cleave two substrates. According to the different ways in which alginate lyases degrade alginate, they are divided into endonucleases and exonucleases. The endonuclease method degrades alginate from within the alginate polysaccharide to produce low molecular weight oligosaccharides, while the exonuclease is also known as oligoalginate lyase (EC 4.2.2.26), which begins to cleave from one end of the substrate molecule and produces monosaccharides[19]. There are significant differences in the structure of alginate lyases from different families. According to its crystal structure, it mainly includes β Jelly roll (β jelly roll), (α/α) N Barrel shaped structure (α/α Barrel) and β Spiral structure (β Helix) Class 3.

Alginate lyase passes through β-Eliminating mechanisms acting on β-The 1,4 glycosidic bond breaks two adjacent uronic acid monomers in the fucoidan molecule [20-22], resulting in the formation of double bonds between C4 and C5 of the sugar ring where the hydrolyzed 4-O glycosidic bond is located, forming a non reducing terminal containing 4-deoxy-L-erytirohex-4-dilute alcohol pyranose uronic acid (DEH).

The overall mechanism is the assumption put forward by Gacesa [23] et al. that algin lyase catalyzes the degradation of algin in three steps: first, the negative charge on the carboxyl group is transferred through the neutralization of the salt bridge; Secondly, the proton at C5 is attracted and transferred; Finally, the electron transfer on the carboxyl group results in the formation of double bonds between C4 and 5, leading to β Elimination reaction occurs. When the proton on C5 is captured, it can remain on the same or opposite side of C-4-O, resulting in the formation of two different configurations at C-4 and C-5, cis and trans Figure 4.

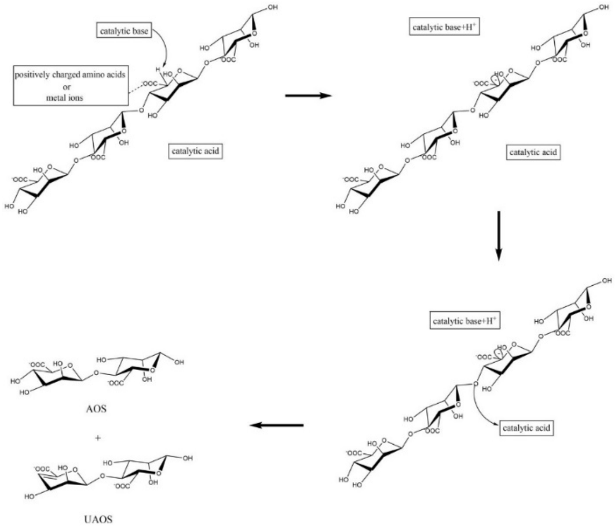

Figure 4. Mechanism of alginate lyase.

Alginate lyase has very important biological functions [24]. The most direct application of alginate lyase is the enzymatic degradation of alginate to prepare functional oligosaccharides. It can be used in various fields to prepare oligosaccharides with different degrees of polymerization based on the alginate lyase. Its production process is simple, environmentally friendly, and the reaction process is easy to control. The alginate lyases derived from microorganisms have a wide range of sources and rich enzyme systems. However, in the early stage, there was little research on alginate lyases in the field of molecular biology at home and abroad, mainly focusing on the exploration of the bacterial species themselves, such as bacterial screening, gene mining, and analysis of degradation substrates and product structures. With the continuous progress of technology, research on the heterologous expression of alginate lyase genes, protein structure analysis, catalytic mechanism of active centers, and modification strategies has gradually become a mainstream trend.

6. Summary and Outlook

The production of marine biomass is enormous, and as a third-generation renewable energy source, it has more sustainable development potential than terrestrial biomass. Alginate, as the second largest sustainable development resource, has significant application value. Studying the functional characteristics and biological activities of alginate can better apply it in various fields and enhance its economic value. In addition, by summarizing the degradation method of alginate - biological enzyme method, the source, degradation mode, and mechanism of alginate lyase were introduced, providing an important basis for the development and application of alginate oligosaccharides with more abundant biological activity functions.

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