



Production of Xanthan Gum from Nontraditional Substrates with Perspective of the Unique Properties and Wide Industrial Applications

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Abstract

Xanthan gum is a microbial product and important polysaccharide of high commercial value. It has many industrial applications, in food, chemical, pharmaceutical, textile and oil industries. This review summarizes different items of xanthan gum production, comprising the producing bacterium *Xanthomonas campestris*, substrates used, especially the recent approaches for using the cheaper and unconventional substrates like whey and whey permeate for reducing the cost of production which are important from the economic point of view. Xanthan gum serves as stabilizers and gelling agents emulsifiers, lubricants, binders, and thickening agents. The wide applications of Xanthan gum are attributed to its superior rheological properties including, high viscosity even at low concentrations, pseudoplasticity, solubility, stability over a wide range of temperatures and, pH values and compatibility with many salts. Xanthan gum has been considered an important source of polymeric material which gradually becoming economically competitive. Factors affecting xanthan production, growth conditions and the major applications of xanthan gum in various industries were also included.

Keywords: Xanthan gum; *Xanthomonas campestris*; Microbial polysaccharide; Low-cost xanthan production; Unconventional substrates; Xanthan wide applications

Introduction

Scientists in the 1950s, at U.S. Department of Agriculture (USDA) conducted an extensive examination of their culture collection for water-soluble gum producers of possible commercial importance. Polysaccharide B-1459 (xanthan) produced by *Xanthomonas campestris* NRRL B-1459 appeared to fulfill the most required properties. In 1960, the first industrial production of xanthan gum was carried out and became available commercially [1]. Xanthan was permitted as a food additive by the U.S. FDA, 1969, and by FAO/WHO in 1974 [2]. Xanthan has a high molecular weight and produced by *Xanthomonas campestris*. Many species of *Xanthomonas* can produce xanthan gum such as, *X. phaseoli*, *X. malvacearum*, and *X. carotae* [3]. In this review the focus will be on several items of xanthan production, comprised the producing organism *Xanthomonas campestris*, substrates used, with special emphasis on nontraditional substrates like whey and whey permeate since the high cost of Xanthan gum production from the conventional substrates like glucose and sucrose represents a limiting production factor. This led to searching for cheap substrates as whey, milk permeate and food

industry wastes [4]. In recent years some new techniques have been developed to use the novel cheaper substrates like milk or whey permeate for Xanthan production including preculturing with lactose fermenting organism as lactic acid bacteria or *Kluyveromyces lactis* for adapting the substrate for xanthan production [5]. The use of xanthan as prebiotic after hydrolysis is a novel approach for xanthan application [6]. Uses xanthan as a fat replacer for cheese manufacture was studied [7]. A novel approach for using xanthan gum in tissue engineering was developed [8]. This review was devoted to flow up and updates the information's concerning with xanthan gum production and its applications especially the substrates which recently involved in the process of xanthan production, factors affecting xanthan production and growth conditions. The major applications of xanthan gum in various industries will be also included.

Xanthan gum structure

The primary structure of xanthan was obtained by using an improved degradative technique and refined methylation analysis [9]. Xanthan gum is characterized by its high molecular weight ($15-50 \times 10^6$ Da). However, the molecular weight of the native molecule may be closer to $3-7.5 \times 10^6$ Da [10]. It was shown to consist of repeating pentasaccharide units consisting of two D-glucopyranosyluronic acid unit as shown in Figure 1.

Properties of xanthan gum

Xanthan gum is characterized by high solubility in water, and this property is due to its molecule polyelectrolyte nature. Xanthan gave a high viscous solution even at low concentrations. These characteristics are beneficial in many industrial applications, particularly in the food industry where xanthan is used as a gelling agent, thickener, and to stabilize suspended preparations and emulsions. Xanthan produced solutions of pseudoplastic, or shear thinning, and with increasing shear rate

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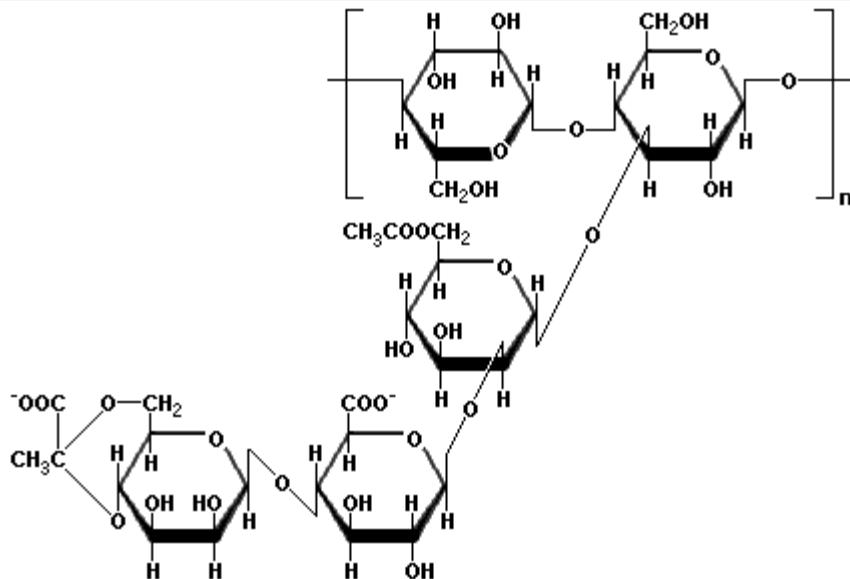


Figure 1 Xanthan gum structure.

the viscosity decreases. Xanthan viscosity is highly affected by temperature (both dissolution and measurement temperatures), the polymer concentration, pH [11] and concentration of salts, Rheological properties of Xanthan solution varies with polymer nature, as they depend on average molecular weight. The viscosity of Xanthan solutions increases with increasing the level of acetylation [12] and pyruvate content [10].

Xanthan gum Production

The growth of *Xanthomonas campestris* and xanthan gum production is affected by several factors including the type of fermentor used, the system employed (batch or continuous), the composition of the production medium and the culture conditions as temperature, pH, and dissolved oxygen concentration [13]. By the termination of the fermentation process, the production medium contains the produced xanthan, bacterial biomass, and many other chemicals. The cell biomass is usually removed first, either by centrifugation or filtration. For more purification precipitation may be involved using water-miscible solvents (ethanol isopropanol and acetone), with the addition of certain salts and pH adjustments [14]. After precipitation, the product is mechanically dewatered and dried.

Optimal conditions for large scale production of xanthan gum

For large scale production of xanthan, in a conventional batch system, inoculums of *X. campestris* are cultivated using the fermentation medium employing mechanically agitated vessels. The culture grown under aerobic conditions is held at a fixed temperature about 28–30°C, pH 7, the airflow level must be more than 0.3 (v/v) and agitation higher than 1 km⁻³. The fermentation run takes about 100 h and through which about 50% of the fermented glucose converts into xanthan gum. Inoculum preparation process involved several stages which require a set of

the fermenters ranging from 10 liters capacity for the initial seed up to 100 liters in production stage by which the inoculum size is increased to 10- fold [15]. Through the fermentation process, the microorganism would grow exponentially led to rapid consumption of the nitrogen source. Multi-steps downstream operations would follow after the fermentation stage.

Xanthan gum recovery

A large amount of alcohol usually uses in this process to precipitate the xanthan gum, which is then sprayed dry or maybe re-suspended on the water to be re-precipitated. For cells separated from the fermentation viscous medium, it is diluted to facilitate the separation process by centrifugation. This operation is a cost-intensive process [16]. It is favorable to add alcohol and salt which would improve precipitation by creating reverse effect charges. The produced xanthan gum in the wet solid state would treat for dewatering and washed to obtain the final required purity.

Factors affecting xanthan gum production

Effect of carbon sources: Traditionally sucrose and glucose are the most carbon sources used for xanthan production. Many studies on nutrients requirement of different cultures were achieved. Xanthan gum side chain is influenced by the conditions of production which should be optimized for xanthan gum synthesis [17]. The carbon source concentration has a profound effect on xanthan yield. The optimum concentration is about 2-4% [18]. The higher concentrations of the carbon source leads to inhibition of growth. Sucrose is a preferred substrate for xanthan gum production. Succinate and 2-oxoglutarate stimulate xanthan gum production in the sucrose-based medium [19]. A high level of the C/N ratio relatively favors the production of a good xanthan yield. *X. campestris* has a low level of β-galactosidase enzyme and subsequently, the microorganism cannot ferment lactose



efficiently as a carbon source. Therefore it grows scantily and produces low level of xanthan in media containing lactose as a sole carbon source. It has been mentioned that sucrose produces the highest yield (dry weight) 11.99 g l^{-1} , followed by glucose which yields 10.8 g l^{-1} [20,21].

Effect of nitrogen sources: Nitrogen, a vital nutrient, can be supplied either as an organic component [22] or as an inorganic compound [19-20]. The growth of the producing organism requires C/N ratio usually more than that used in production media [13, 19-20]. Ammonium salts are a proper nitrogen source for biomass yield, while nitrate is more suitable for the production of higher yields of xanthan gum [17]. Furthermore, the produced yield of xanthan gum and its production level in batch culture affected by the level of nitrogen source present at the onset of the stationary phase [23].

Effect of temperature: The effect of temperature on xanthan production has been widely investigated. Temperature degrees used for xanthan gum production fluctuated from 25 to 34°C ; however, culture at 28 to 30°C is familiar. Many investigators reported that 28°C was the optimal temperature for the production of xanthan gum [24,25]. A higher temperature may increases xanthan yield but reduces its pyruvate content [26]. In some reports an optimum production temperature was 33°C [27]; in other report temperature of 25°C was the optimum for growth and 30°C for production. The production medium plays an active role in the optimal temperature for xanthan production [28-29].

Effect of pH: The pH has a profound effect on xanthan production. The optimum pH for *X. campestris* growth range from (6 to 7.5) and the optimum pH for the xanthan production range from 7 to 8. [30] *Xanthomonas* can be cultivated at a neutral pH [31]. Most investigators recommended the neutral pH as the optimum value for the growth of *X. campestris* [29,32]. The pH decreases from pH 7 to values about 5 during xanthan production due to the acidic groups of xanthan molecules [33].

Effect of mass transfer rate: Different types of fermenters have been employed in xanthan gum production; however, the sparged stirred tank was often preferred. In stirred fermenters, the oxygen rate mass transfer is affected by the flow rate of the air and speed of stirring. The air flow rate is generally maintained at a constant value when stirred fermenters are used, usually v/v min. while the speed of agitation can be changed in a wide range. At reduced stirring speeds, the oxygen limitation leads to lower rates of specific xanthan production. The rate of specific xanthan production depends on the specific oxygen uptake rates [34]. The higher agitation rates have a more positive effect on xanthan production than the fermentation period. High xanthan yield was obtained at agitation rate reached 1000 rpm at 50 h of fermentation period [34]. Novel fermentation technique has been successfully adapted in the laboratory using hydrogen peroxide as an oxygen source to overcome gas-liquid mass transfer resistance in the xanthan gum fermentation medium [35].

Agro-industrial wastes as a substrate for xanthan gum production: Waste is defined as any material, which has not yet been fully utilized, i.e. the left matter from production or consumption. However, waste is low-cost material and in many

cases cannot be avoided as a result of human activity. It includes materials from the plant; agricultural, industrial, and municipal sources. Waste may be solid or liquid disposed of residences, small scale industries, and business locations. In general, waste can be identified based on its bulk or physical properties organic components, and specific contaminants contents [36]. Agro-industrial wastes contain three main compounds, cellulose, hemicellulose and lignin, and other compounds (e.g. extractives) in fewer amounts. Cellulose and hemicellulose are complex carbohydrates of large molecules that can be hydrolyzed by enzymes, acids, or other chemicals to simple sugars, which can be fermented to produce several products as ethanol, fuels, enzymes, and biomass products [37-38]. The cost of xanthan production is one of the limiting factors in its large-scale fermentation processes, especially when compared with similar polysaccharides from plants and algae. To overcome this problem several approaches have been suggested to use cheaper substrates such as whey [24], citrus wastes [39], corn steep liquor [40], glycerol [41], chicken feathers [42], molasses and glucose syrup [43] and olive oil wastewaters [44].

Molasses: Molasses produced as a by-product of sugar industry. It can be produced from sugar cane and from sugar beet. It is defined as the runoff syrup produced from the terminal stage of the crystallization process when more crystallization of sugar is uneconomical [43]. Although beet and cane molasses are similar they exhibit marked variations in respect to fermentable sugars, nitrogenous compounds, ash and vitamin content [45]. Sugar beet molasses contains 74-77% (w/w) dry matter involving sugar, organic and inorganic compounds. Total sugars (mainly sucrose) represent about 47-48% (w/w) of molasses, ash 9-14% (w/w) and total nitrogen-containing compounds (mainly betaine and glutamic acid) 8-12% (w/w). Sugar beet molasses was used in a large scale as a substrate in fermentation process due to it contains a lot of nutrients required for growth such as pantothenic acid, inositol, and trace elements and some biotin [46].

Whey: Whey, produced from cheese processing can be conceded as a by-product of the dairy industry. It contains about 4-5% lactose, 0.8-1% proteins, small content of organic acids (lactic acid), mineral salts and vitamins. Whey represents a big waste disposal problem due to its high biological oxygen demand (BOD) and chemical oxygen demand (COD). Its disposal represents a costly and complex problem. When we take this point into account and the richness of whey with lactose as a carbon source in addition to its content of some other nutrients we can consider whey as a valuable substrate for production of several important value-added products such as xanthan gum [47]. Cheeses whey was employed for xanthan gum production by two strains of *Xanthomonas campestris*. The maximum xanthan yield (25 g l^{-1}) was obtained after three days fermentation period using the lactose-whey content as a sole carbon source [24], 0.1% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 2.0% (w/v) of K_2HPO_4 , produced about Genetically modified strains of *Xanthomonas campestris* by produced more quantities of xanthan gum and had the ability to grow on whey [48]. Also, the production of xanthan gum from milk permeates and uses it in the manufacture of yogurt and soy yogurt has been reported [4, 49].



Glucose syrup: Food syrup, made from the hydrolysis of starch. Maize is traditionally used as the source of the starch in the US, in which the syrup is called corn syrup. However, glucose syrup can be manufactured from other starchy crops, as potatoes, rice wheat, cassava and barley [50]. Glucose syrup containing over 90% glucose is used in industrial fermentation [51]. Xanthan gum was produced from glucose syrup as a sole carbon source using *Xanthomonas campestris*. This bacterium can ferment 42 DE syrup or high DE syrup. DE means dextrose equivalent. The selection of the appropriate substrate is a matter of balance between the raw material cost and the yield of the product [52].

Applications of xanthan gum

Industrial applications

Xanthan gum has many applications in several industries. This can be attributed to its superior characteristics, for instance, the long term suspension stability of emulsions in acid, alkaline, and salt solutions; temperature stability and pseudo-plasticity. In the chemical industry, Xanthan was extensively used. For instance, xanthan and locust bean gum can be used for the manufacture of deodorant gels. The capability of xanthan gum to form the required dispersion stability at rest and reduced viscosity upon application was used to attain a proper consistency to the toothpaste [15].

Personal care applications

Xanthan gum improves the rheological properties of shampoos and liquid soaps preparations and enhances stability, richness and creamy lather [13]. It is the binder of choice for all toothpastes, including gel and toothpaste, of pumping capability. Ribbon quality and ease of extrusion are also improved [15].

Pharmaceutical applications

Xanthan gum stabilizes suspensions of a variety of insoluble materials such as barium sulfate (X-ray diagnoses), complexed dextromethorphan (for cough preparations) and thiabendazole. It can keep liquids homogeneous and non-layered [15].

Food applications

The majority of xanthan applications directed to food industries to suspend and thicken fruit juices and chocolate preparations. United States food and drug administration (FDA) have permitted xanthan gum according to toxicological studies for use in food. Many of modern foods require the advantage of improving texture, appearance, flavor enhancing, viscosity and water-control characteristics [53]. Xanthan gum improves all these characteristics and furthermore controls the rheological behavior of the end product. It provides solutions with pseudoplastic traits and modulates the 'gummy' mouthfeel than other gums [15].

Dairy

Preparations of xanthan, with other polysaccharides like galactomannans carrageenan, and guar gum are superior stabilizers for many industrial dairy products including milkshakes, ice milk, ice cream, sherbet, and water ices.

Xanthan with methyl-carboxy methyl cellulose employed for the manufacture of frozen dairy products and with CMC for direct preparation of acidified yogurts. Similar products are used for dessert puddings, acidified milk gels, and others. These dairy products characterized by its high stability, delicate and fresh taste, preferable viscosity, proper heat transfer during preparation, good flavor release, heat-shock resistance and ice-crystal prevention [1,13]. Xanthan, guar and LBG mixture is very important to sliceability body firmness and flavor enhancing of creamy cheese. In Addison, xanthan improves the body texture of cottage cheese dressings by controlling syneresis [21]. EPS-producing culture to improve Karish cheese texture, as it received higher body and texture scores after aging for 7 and 15 days [54].

Bakery products

Xanthan gum was employed in bakery products manufacture to elevate the water holding capacity during backing process and storage. This led to an increase in the shelf life of backed products. Xanthan gum was used as an egg replacer in some bakery products especially the egg-white without affecting the organoleptic properties of the resultant products. It contributes for improving smoothness, air incorporation, and retention in bread mixes, biscuits, and cakes. The supplement with xanthan gum can reduce calorie and gluten content in bread and extend shelf stability, freezing and thawing stability in cream and fruit fillings and control syneresis process.

Beverages

During beverages processing, xanthan gum was added as a bodying agent and suspending material in drinks contains particles. This contributes to obtaining a good product appearance and texture in addition to improving mouth-feel to attain pleasing taste, rapid solubility, and compatibility with most components. Xanthan gum in reconstituted beverage plays an active part in enhancing body and quality with rapid viscosity development.

Conclusion

This review focuses on different aspects of xanthan gum production. It is a water-soluble unique polysaccharide produced industrially from carbon sources including sucrose, glucose and agro-industrial wastes by fermentation using the bacterium *Xanthomonas campestris*. It has wide applications in food, chemical, cosmetics, pharmaceutical, textile, and oil industries. This due to its various physicochemical properties, including stable viscosity at varying pH levels and temperatures, and its associated pseudoplastic behavior at low concentrations. Xanthan has been approved by the United States Food and Drug Administration (FDA). The polysaccharide is used as suspending, stabilizing, thickening and emulsifying agent, for food and non-food industrial applications for use as a food additive without any specific quantity limitations.

Significant Statement

This review discovered the recent novel approaches for Xanthan gum production from unconventional substrates that can be beneficial for economical production of this important

product of wide applications in several industries. This study will help the researchers to uncover the critical areas of xanthan gum production which led to reduce the cost of production.

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