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Production of Xanthan Gum from Agro -industrial Waste Substrate and its Wide Industrial Applications

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ABSTRACT

Xanthan gum is an extracellular water-soluble biopolymer produced by fermentation using Gram negative bacterium *Xanthomonas campestris*. Traditionally it plays an important role in various industrial applications. This review explores the production of xanthan gum using, food waste and disease infected plant materials, Agro- Industrial waste substrate, environmental waste and its Industrial applications.

Key words : *Xanthomonas campestris, Xanthan gum, Plant material, Industrial waste, Agricultural Waste, Food and Environmental waste, Fermentation.*

Introduction

Living organisms such as plants, animals, bacteria, and fungi synthesize biopolymers over their life cycle, termed as naturally derived polymers representing a vital plant biomass component. The biopolymers include proteins, nucleic acids, poly hydroxyalkanoates, polyphenols, and polysaccharides. Polysaccharides are carbohydrate biopolymers that contain monosaccharide ($(CH_2O)n$) units, which are further covalently attached by an O-glycosidic bond with branched or linear configuration (Torres *et al.,* 2019). They have naturally recycled carbon resources, eco-friendly because of their biodegradability: some of them are extensively used for packaging, food, pharmaceutical, biomedical industries, and other many industrial processes.

Plant biomass is the most abundant and applicative biomaterials in nature and is derived as polysaccharides (Gilani *et al.,* 2019). The microorganisms biosynthesized massive amounts of polysaccharides in the presence of carbon sources using plants, waste material as host sources, which excreted by the cells called exopolysaccharides (Dumitriu *et al.,* 2005; Dumitriu *et al.,* 2015; Ahmad *et al.,* 2015). A few of these polysaccharides such as starch, cellulose, alginate, xanthan gums are industrially synthesized and applied as stabilizers, binders, emulsifiers, thickeners, and gelling agents (Hussein *et al.,* 2018; Bass-Galia *et al.,* 2017). Among them, xanthan is the most economical and industrially useful microbial exopolysaccharide anionic biopolymer with repeated chains of cellulose monosaccharides and oligosaccharides *Xanthomonas* bacteria (a Gram-negative bacteria genus that exhibits several different species) (Kumar *et al.,* 2018; Becker *et al.,* 1998). Xanthan discovered in the 1950s

Abbreviations

XCC, Xanthomonas campestris pv. Campestris; NRRL, Northern Regional Research Laboratory; USFDA, United States Food and Drug Administration; GRAS, Generally Recognized As Safe by Allene Rosalind Jeanes at the United States Department of Agriculture, USA and approved by the Food and Drug administration (Fed. Reg. 345376) in 1969 as a nontoxic and riskless polymer permitted the use as thickener and stabilizer in many value-added food products (Lipnizki *et al.,* 2010; Tao *et al.,* 2012).

Xanthan has been approved by the United States Food and Drug Administration (FDA) (Murad *et al.,* 2019; Petri *et al.,* 2015). Further, xanthan gum is used as thickening and emulsifying agentin food preparations like in sauces, salad dressings, desserts, and fruit juicesand it is useful not only in the food and beverage industry but also in the pharmaceutical, agrochemicals, and cosmetic industries.

Gram-negative bacteria of the genus *Xanthomona*s produced xanthan gum, representing many different strains, as *X. arboricola*, *X. axonopodis*, *X. campestris*, *X. citri*, *X. fragaria*, *X. gummisudans*, *X. juglandis*, *X. phaseoli*, *X. vasculorium*. *X. campestris* (the strain *X. campestris* NRRL B-1459 being the most used) is the most common phytopathogen that is further employed for industrial production of biopolymer xanthan gum (Petri *et al.,* 2015; Janse *et al.,* 2005; Lopez *et al.,* 2004; Jeanes *et al.,* 1961). glucose, sucrose, starch, molasses (sugar cane, beet), and corn syrup are the most common, and cassava bagasse, green coconut shell, residue whey, olive mill wastewaters, citrus waste, glycerin, and vegetable leftovers are also used carbon sources in the fermentation process for xanthan production. (Palaniraj *et al*., 2011; Ronèevic *et al*., 2019).

Xanthomonas campestries, a plant-associated bacterium, further permitting its penetration and formation of local lesions, soft roots, scabs, cancers on different crop species and causes various diseases on the leaves, stems, and fruits. Black rots caused by *Xanthomonas campestries pvcampestries* (XCC) are the most severe damaging conditions of cauliflower and other crucifers crops, leading to economic losses.

Last few decades, disease-infected crop parts, food waste materials, shells, cereal husks, palm kernel shells, sugar cane bagasse, and other organic matter create significant issues and challenges towards maintaining a healthy pollution-free environment. Researchers are continuously working on using those infected materials to produce an adequate xanthan production with different bioactive applications. However, several issues need to be addressed to make xanthan gum biotechnologically cheap, use alternate raw materials, and further optimize fermentation processes.

Based on the above observations, the purpose of this review is to report on the production of xanthan gum using food, *Agro- Industrial* waste, disease infected plant materials, environmental waste materials along with their useful industrial application.

Structure of xanthan

Xanthan biopolymer molecules contain β-(1, 4)-linked glucopyranose backbone as in cellulose and in addition to trisaccharide side-chain on every other glucose residue linked through the C3 position, which is five sugar residues at side chain consist of two β-D-glucopyranosyl, two β-D-mannopyranosyl, and one β-glucopyranosyluronic acid residue (molar ratio of 2:2:1) **(**Melton *et al.,* 1976, Kang *et al.,* 1993) as shown in Fig. 1**.** Two mannopyranosyl residues are linked on either side to a glucuropyranosyluronic acid group.

The inner mannose residue connected to the backbone and acetylated, and the terminal mannose residue are pyruvated. The molecular weight of the xanthan gum molecules is very high (>3×106) which dissolves in water to form highly emulsifiable viscous solutions (Nurul *et al.,* 2015) and composed of Glucose 37%, mannose 43.4%, glucuronic acid 19.55%, acetate 4.5 %, and pyruvate 4.4% (Garcia-Ochoa *et al.,* 2000; Tako *et al.,* 1984). The glucuronic and pyruvic acid groups, xanthan gum with a highly negative charge, therefore the presence of anionic side chains on the xanthan gum molecules enhances hydration, which explores xanthan gum

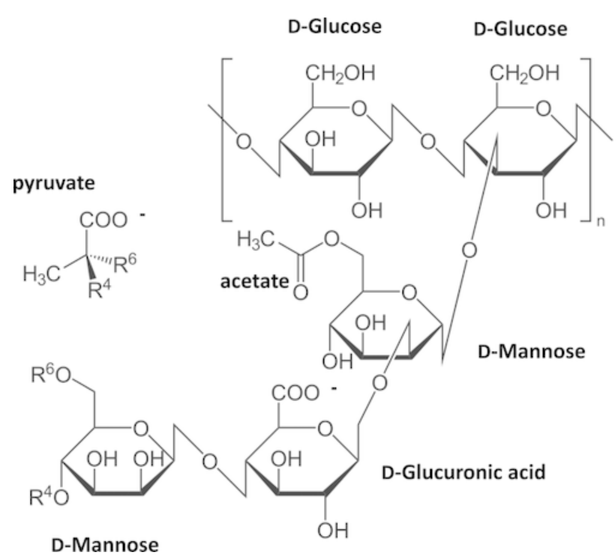

Fig. 1. Chemical Structure of Xanthan

soluble in cold as well as hot water without affecting PH value (Laneuville *et al*., 2015).

Production of xanthan gum

India has a wide range of climates from temperate to tropical, where fruits and vegetable crops are grown and are available round the year for fresh consumption. Over the period, the demand for vegetables increases day by day. Specific climatic conditions, biotic and abiotic factors affected crop growth and development. Several diseases and pests are also responsible for economic losses. Also, the crop and other substrate waste are low-cost. Substrates include materials from the plant, agricultural, industrial, and municipal sources: many researchers successfully developed some environmental and eco-friendly bioactive material using those materials. Several studies are available regarding xanthan gum production using microorganisms, carbon and nitrogen source, nutrient medium. Xanthan gum was prepared using bacterial strain *Xanthomonas campestris* and other *Xanthomonas sppby* fermentation process with useful carbon sources (Lopes *et al*., 2015), In order to obtain quality production of xanthan gum, it is necessary to carefully evaluate the settings of systems used for analysis, the composition of fermentation medium, and controlled production condition of temperature, pH, agitation speed, aeration, and fermentation time in the sense of suitable rheological properties and structure (Borges *et al*.,2008). For large scale production of high-quality xanthan gum, cost and time are the limiting factors during fermentation processes. To overcomes this problem there is to need to explore cheaper substrates. Cellulose, glucose, molasses, corn syrup, glycerol, starch, whey, and crop waste substrate are easily obtained and provide carbon source during fermentation. *Xanthomonas campestris isolated* from infected plant material is used for gum production process. *Xanthomonas campestris* causes infection to Brassicaceae (Cruciferae) family which includes cabbage, broccoli, cauliflower, kale, turnip, oilseed rape, mustard, radish, are causing black rot.

Infected cabbage and radish leaves used for isolation of *Xanthomonas campestriesstrain NRRL B-1459 andfermented* using carbon sources. Further precipitated with hexadecyltrimethylammonium bromide and purified by repeated precipitation with ethanol (Sutton *et al.,* 1970), which may influence the production cost compared with other nitrogen sources such as yeast extract and ammonium salts. It showed that the highest broth viscosity was achieved at the optimum concentration of cabbage extract of 100 g/l (Purwadi *et al.,* 2009; Nitschke *et al.,* 1995; Kassim *et al.,* 2011). Several other plants are also used for isolation along with cabbage, such as cauliflower, mango (El Hadi *et al.,* 2018). Also, Sesame, papaya, radish, orange peels, sugar cane leaves, Melon, rice, citrus, pepper leaves were used for isolation of the microbe and xanthan gum was produced by fermentation with optimum temperature 30 °C, incubation period 72 hours and at pH 6.0. Further, response surface methodology with kinetic modeling was adapted for the process optimization and its influence on scale-up production (Makut *et al.,* 2018). Various natural carbon sources were used in a fermentation media for the biosynthesis of xanthan gum by a different strain of *Xanthomonas campestries* viz: hydrolyzed starch, corn syrup, hydrolyzed rice, barley and cornflour, acid whey, molasses, coconut juice, sugar cane, beet molasses, date juice palm, corn steep liquor, and cheese whey, etc**.** (De Vuyst *et al.,* 1987a; Kalogiannis *et al*., 2003). Using diluted sugar cane broth with 27.0 g/l sucrose: 2.0 g/l Brewer'syeast: and 0.8 g/l NH_4 NO_3, fermented at 750 rpm in the batch process, xanthan gum was produced (Faria, 2011). In industries, 2-4 % (glucose or sucrose) is used as a carbon source, whereas 0.05-0.1 % (ammonium nitrate, peptone, yeast extract, urea) is used as a nitrogen source. Molasses is runoff syrup produced as a by-product of the sugar industry. Using pre-treated molasses in 1% acidified molasses and acidified aerated molasses with 1% yeast extract as a nitrogen source for xanthan precipitation (Murugesan, 2012). Sugar beet molasses contains 74-77% (w/w) dry matter involving sugar, organic and inorganic compounds, which produced 20g/l xanthan gum during fermentation. Other non-traditional agro-waste substrate gives better xanthan gum production like cassava starch 1.86g/l, (Kerdsup *et al.,* 2009; Gunasekar *et al.,* 2014) sugar beet molasses 20 g/l, (Afendra *et al.,* 2002) ram horn hydrolysate 6.3 g/l, (Basaran *et al.,* 2007) date palm juice 43.35g/l, (Ben *et al.,* 2010) cheese whey 36g/l, (Zabot *et al.,* 2011) apple pomace 52.1g/l; (Stredansky *et al.,* 1999). Whey 17.3 g/l, (Gilani *et al.,* 2011) date extract 11.2g/l (Fatemeh *et al.,* 2011). Sugar sources used as the carbon sources during fermentation of the wild-type of *Xanthomonas campestris* GK6 and the obtained yield of xanthan produced using glucose 14.744g/l, sucrose 13.234g/l, maltose 12.321g/l, fructose 5.232g/l, xylose

5.531g/l, arabinose 10.958g/l, galactose 7.129g/l, lactose 1.008g/l, inositol 1.502 g/l, sorbitol 1.401g/l, soluble starch 12.10g/l, potato starch 9.754 g/l (Leela and Sharma *et al.,* 2000)

A small amount of the preserved culture is expanded by growth on concrete surfaces or in liquid media to obtain the inoculum for large bioreactors. The growth of the microorganism and xanthan production is influenced by factors such as the type of bioreactor used, the mode of operation (batch or continuous), the medium composition, and the culture conditions like temperature, pH, dissolved oxygen concentration, and mass transfer rate. The production medium contains gum, bacterial biomass, and other inert chemicals during the fermentation process. Further the biomass is removed with centrifugation or filtration using water-soluble solvents like ethanol, isopropanol, and acetone, precipitation occurred along with salts and pH. Adding alcohol and the salt would improve precipitation. Obtained wet solid gum and further underwent dewatering and washing to obtain the xanthan (Flickinger *et al*., 1999; Nasr *et al*., 2007; Rosalam *et al.,* 2006; Borges *et al.,* 2008; Papagianni *et al.,* 2001).

Commercially or industrially, xanthan gum is produced with glucose or sucrose. The growth of bacterial strain (*Xanthomonas campestries*) and xanthan gum yield is impacted by various factors, including fermenter with the system employed (batch or continuous), the nutrient medium's composition, and the culture conditions as temperature, pH, dissolved oxygen concentration, and mass transfer rate. The production medium contains gum, bacterial biomass, and other inert chemicals during the fermentation process, wherein further the biomass is removed by centrifugation or filtration (Flickinger *et al*., 1999).

Using the rotary turbine, precipitate would be cut with a cutter and recover the final product. During the recovery process, deactivation and removal (or lysis) of the microbial cells, precipitation of the biopolymer, dewatering, drying, and milling are required without degrading the biopolymer (Balows *et al.,* 1991).

Commercial applications of Xanthan Gum

Xanthan is a water-soluble microbial polymer with specific rheological properties: gum has diverse industrial applications. The significant applications of xanthan gum are in the food industry, personnel care products, biomedical, agrochemicals manufacturing, pharmaceuticals, textile, and in many other fields and attributed to its superior characteristics as an instance with long term suspension stability of emulations in acid, alkaline, and salt solutions.

Agrochemicals Applications

Xanthan gum is used as biodegradable green adjuvant to improve spray applications in agriculture, which constitute a promising alternative to synthetic adjuvants, which improved performance on the leaf (Lewis *et al*., 2016. Xanthan gum has been studied as an elicitor together with fungicides on barley against *Bipolarissorokiniana*. Xanthan gum is also used in cleaners, coatings, polishes, and agricultural flowables (Antoniazzi *et al.*, 2006; Katzbauer *et al.*, 1998).

Food industry

Xanthan is approved *as safe* (GRAS) by the United States Drug and food administration based on toxicology tests in human foods. Xanthan gum improves the viscosity, water-holding, flavor releasing properties that exhibit an excellent pseudoplastic behavior. It is mainly in salad dressings 0.1–0.5%, provides easy pourability with good cling and suspends. In bakery products, xanthan gum (about 0.05–0.3%) is used to increase the water holding capacity and binding during baking, storage and it also extends the shelf life of baked goods and refrigerated doughs. Xanthan gum is used as an egg replacer in soft baked products (Murad *et al.,* 2019), especially the egg-white, without affecting taste. It also contributes to improving smoothness, air incorporation, and retention in bread mixes, biscuits, cakes, muffins. Xanthan gum reduces the calorie and gluten content in bread and other baked items and increases shelf stability, freezing, and thawing stability in cream and fruit fillings (Murad *et al*., 2019; Salehi *et al.,* 2019). In beverages, the gum used is around 0.05–0.2%, acts as a binding agent, and suspends material in drinks containing particles. It contributes to pleasing mouth-feel with the excellent suspension of insoluble and compatibility components. In prepared foods, 0.1–0.3% of xanthan gum used and it acts as a stabilizer to avoids syneresis. Also, in soups, sauces, and gravies, the gum is 0.05–0.5% provides good temperature stability (Murad *et al.,* 2019).

Along with food, xanthan gum majorly contributed to dairy products 0.05–0.2% and acts as an excellent stabilizer for ice cream, ice milk, sherbet, milkshakes, and water ice. It also improved the body texture of cottage cheese dressings by controlling syneresis (Murad *et al.,* 2019; Frank *et al.,* 1979; Sharma *et al.,* 2006)

Pharmaceuticals and biomedical 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 (Murad *et al.,* 2019; Palaniraj *et al.,* 2011; Sharma *et al.,* 2006). In drug release applications, xanthan is used as an excipient as supporting hydrogels and tablets combined with other biopolymers: xanthan gum helps to tune the desired properties to release drugs, proteins, or biosolutes. The potential of xanthan for biomedical applications in the form of such luminescent composites is enormous (Gilbert *et al.,*2013; Vianna-Filho *et al.,* 2013).

Personal care applications

Xanthan gum improves the flow properties of shampoos and liquid soaps to enhances the stability, richness, and creamy lather (Rosalam *et al.,* 2008). Also, xanthan is an excellent binder for all kinds of toothpaste, including gel and pumpable types (Rosalam *et al.,* 2008; Palaniraj 2011; Murad *et al.,* 2019).

Other industrial applications

In the chemical industry, Xanthan is extensively used to manufacture deodorant gels and beneficial viscus supporting roles. There are many diversified applications in every sector: liquid cleaners, paints, pigments, construction chemicals, oil well drilling, ceramics, mineral suspensions, latex emulsions, fire foams, fertilizers/pesticides, liquid polishes (car care), inks, foundry core washes.

Conclusion

This review focuses on different aspects of xanthan gum production using waste materials with various carbon sources and from agro-industrial wastes by fermentation using the bacterium *Xanthomonas campestris* and about its wide industrial applications in several industries. It can be concluded that there is yet a scope of improvement, research gaps need to be addressed to produce xanthan gum in cost effective manner viz: isolating high yield xanthan gum-producing microorganisms, use of alternate and low-cost raw materials and further optimization of fermentation processes to make it cost effective, high yielding and better-quality xanthan production.

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