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XANTHAN GUM: SECONDARY RAW MATERIALS FOR BIOSYNTHESIS, ISOLATION AND APPLICATION

Gabdulla F. Kurbanov, Artem O. Prichepa, Natalia Yu. Sharova
All-Russia Research Institute for Food Additives, Saint Petersburg, Russia

KEY WORDS:
Xanthomonas campestris, xanthan gum, biosynthesis, fermentation, secondary raw materials, waste products, biopolymer

ABSTRACT
The inevitable consequence of population growth is the development of agriculture and food production, which in turn has an impact on the volumes of secondary raw materials production. The processing of these materials can present significant challenges. One of the most effective solutions to this problem is the use of microbiological synthesis to create products with high added value. A notable example is xanthan gum, a biopolymer that has been utilized in a multitude of industries, including food, oil, pharmaceutical, and medicine. The value of xanthan gum is contingent upon its distinctive physicochemical properties, particularly its capacity to enhance the viscosity of solutions. The process of obtaining xanthan gum is conducted through the fermentation of liquid high-carbon media. The primary producer is the bacterium *Xanthomonas campestris*, a phytopathogen of cruciferous plants, which converts carbohydrates into a biopolymer of commercial value. This literature review examines several topics related to xanthan gum and its synthesis by *X. campestris*, with particular attention paid to the success of obtaining the target product using food production waste and secondary agricultural raw materials.

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КСАНТАНОВАЯ КАМЕДЬ: ВТОРИЧНОЕ СЫРЬЕ ДЛЯ БИОСИНТЕЗА, ВЫДЕЛЕНИЕ И ПРИМЕНЕНИЕ

Курбанов Г. Ф., Причепа А. О., Шарова Н. Ю.
Всероссийский научно-исследовательский институт пищевых добавок, Санкт-Петербург, Россия

КЛЮЧЕВЫЕ СЛОВА:
Xanthomonas campestris, ксантановая камедь, биосинтез, ферментация, вторичное сырье, отходы, биополимер

АННОТАЦИЯ
Неизбежным следствием роста населения является развитие сельского хозяйства и производства продуктов питания, что в свою очередь влияет на объемы производства вторичного сырья. Переработка этих материалов может представлять значительные трудности. Одним из наиболее эффективных решений этой проблемы является использование микробиологического синтеза для создания продуктов с высокой добавленной стоимостью. Ярким примером является ксантановая камедь — биополимер, который используется во многих отраслях промышленности, включая пищевую, нефтяную, фармацевтическую, и в медицине. Ценность ксантановой камеди обусловлена ее отличительными физико-химическими свойствами, в частности, ее способностью повышать вязкость растворов. Процесс получения ксантановой камеди осуществляется путем ферментации жидких сред с высоким содержанием углерода. Первичным продуцентом является бактерия *Xanthomonas campestris* — фитопатоген крестоцветных растений, который преобразует углеводы в биополимер, имеющий коммерческую ценность. В обзоре литературы рассматривается ряд тем, связанных с ксантановой камедью и ее синтезом *X. campestris*, при этом особое внимание уделяется успехам получения целевого продукта с использованием отходов пищевого производства и вторичного сельскохозяйственного сырья.

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1. Introduction

Modern agriculture is confronted with a number of significant challenges, one of which is the issue of secondary raw materials. These materials are generated as a consequence of the processing of crops and livestock. The food industry also produces materials with complex chemical compositions. The utilization of recycled materials in agriculture and the food industry is therefore not only an essential aspect of the "green circular economy", but also ensures competitiveness in the market [1]. The bioeconomy encompasses a number of interrelated aspects. One such aspect is the development of new technologies for the production of useful chemical compounds [2]. Biotechnology is the development of industrial processes based on the microbiological synthesis of molecules that can be adapted by humans to their needs. Through the vital activity of microorganisms, cheap raw materials rich in carbohydrates, proteins and lipids are transformed into new commercially important products [3]. As Manfred Kircher notes, microorganisms are an integral component of production systems that utilize renewable resources [4]. Indeed, the introduction of biotechnological processes into the production system is leading us towards the green economy of the 21st century. This practice has already been successfully applied to the recycling of animal and plant products [5,6]. Important biopolymers can be obtained from agro-industrial waste [7].

2. Objects and methods

A literature analysis was conducted using scientific literature search engines, namely PubMed and ScienceDirect. The literature search was carried out using the following keywords: xanthan gum, xanthan gum application, xanthan gum structure, *Xanthomonas campestris*, and xanthan gum biosynthesis.

The work analyzed 63 scientific sources in English.

Criteria for inclusion and exclusion were as follows.

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Inclusion criteria:
- ❑ Published scientific articles.
- ❑ Publication period 1980–2024.
- ❑ Publications from the Scopus\WOS databases.

Exclusion criteria:
- ❑ Publications before 1980.
- ❑ Publications not included in the Scopus\WOS databases.
- ❑ The topic of the article is related to methods for obtaining xanthan gum using secondary raw materials as the main source of carbon for further use in the food industry.

3. *Xanthomonas campestris*

The genus *Xanthomonas* was first described as a pathogen of tomatoes in South Africa in 1921 under the name *Bacterium vesicatorium*. Dawson grouped the bacteria described by various scientists under the name *Xanthomonas*, derived from the Greek words 'xanthos' meaning 'yellow' and 'monas' meaning 'essence', probably because of the yellowish color of the bacterial colonies as they grow. It is hypothesized that xanthan is produced as a response mechanism to stressful environmental conditions. The bacteria of the genus *Xanthomonas*, which synthesize xanthan, belong to the family *Pseudomonas*. They are gram-negative aerobic rods and include species such as *X. campestris*, *X. fragariae*, *X. albilineans*, *X. axonopodis*, *X. citri*, X. populi, *X. maltophilia*, *X. phaseoli*. The most commonly used plant for xanthan production is *X. campestris* NRRL B-1459 *Xanthomonas campestris* is an aerobic, gram-negative, mesophilic bacterium with an optimum growth temperature of 25–30 °C and is inactive at temperatures below 10 °C [8]. The bacterium has a filamentous structure called hypersensitive response and pathogenicity (HRP) pili attached to a type III protein secretion system, which provides the ability to transfer bacterial proteins into the plant, as well as motility in water [9].

The complete genome sequencing of *Xanthomonas campestris* reveals the presence of various metabolic pathways, including glycolysis/gluconeogenesis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway, and others. The organism obtains its energy source through oxidative phosphorylation, carbon fixation, and the metabolism of methane, nitrogen, and sulfur.

X. campestris obtains carbon from the host and converts it to glucose through gluconeogenesis. Further studies have demonstrated that *Xanthomonas campestris* contains only the malic enzyme-PpsA pathway in gluconeogenesis, which is essential for virulence. Additionally, this plant pathogen contains a type III secretion system (T3SS), which is crucial for host infection. The T3SS is pivotal in pathogenesis as it facilitates the transport of effector proteins to suppress host defenses. The formation of a biofilm on plant surfaces by *X. campestris* exemplifies intercellular communication via the diffusible signal factor (DSF).

X. campestris can survive in soil for over a year and can be dispersed through any water movement, including rain, irrigation and surface water. The application of copper fungicides to healthy plants can reduce the spread of the bacteria in the field. However, once a plant is infected, *X. campestris* will eventually spread to the seed stem and suppress the growth of healthy progeny [10].

The identification of *X. campestris* strains based on host or disease symptoms is challenging due to the phenotypic variability observed in these bacteria. One of the characteristics of *Xanthomonas* bacteria is their ability to infect plants, with the capacity to infect more than 250 plant species, including major crops such as cabbage, tomatoes, beans, cotton and rice. Infection of cabbage with vascular bacteriosis can result in the death of up to 100% of the plants, while infection of wheat with bacteriosis can reduce yield by 44–90%. In cotton, yield losses range from 15% to 45%, which can also have a significant impact on the agricultural industry [11].

The genus *Xanthomonas* comprises bacteria that cause a variety of plant diseases, including spotting, tumors, rot and wilt. They also cause yellowing of bean leaves, spotting of cotton and leaf drop in carrots. Several studies have demonstrated that fermentation, acid treatment and heat therapy are effective in reducing bacterial contamination of seeds. The use of highly active producer strains obtained through applied genetics and breeding has played an important role in improving xanthan production.

4. Xanthan gum. Structure and properties

Xanthan is a biological polymer with a branched structure. The molecular weight ranges from $1 \cdot 10^6$ to $20 \cdot 10^6$ m/g [12]. The main chain consists of D-glucose residues linked by a β(1→4) bond. Such a disaccharide is also called cellobiose. The side chain consists of a trisaccharide linked by an α(1→3) bond. The trisaccharide is made up of mannose and glucuronic acid. Attached to the backbone is D-mannose, which may contain

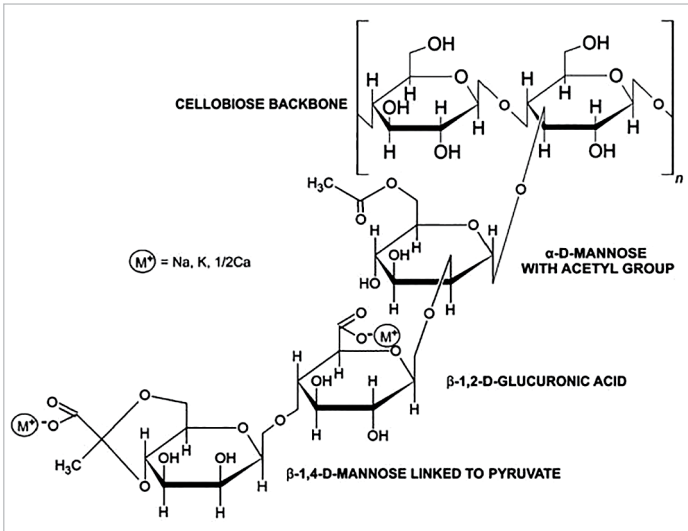

Figure 1. **Structure of xanthan gum [12]**
Рисунок 1. **Структура ксантановой камеди [12]**

an acetyl group at position 6. Mannose is followed by glucuronic acid, to which it is linked by a β(1→2) bond. The trisaccharide is completed by a mannose residue in a β(1→4) bond with glucuronic acid. Of particular importance for conformation is the pyruvate residue which terminates the side chain. The presence of acetyl residues promotes the association of side chain polymer molecules through hydrogen bonding. The presence of a pyruvate residue promotes the opposite effect through electrostatic repulsion [13]. Furthermore, the presence of acetyl and pyruvic acid residues is not required in every monomer. The content of pyruvic acid residues can be heterogeneous along the entire length of the polymer, with an increased content in local areas[14].

There are several conformations in which xanthan gum occurs: linear and helical. At room temperature the gum is a rigid and ordered chain. As the temperature increases, the chain begins to dissociate from the double helix into single molecules. When the temperature is raised to 60 °C, the single helix assumes the state of a single coil [15]. Xanthan can change its degree of ordering depending on the salt content of the solution [16].

The structure of xanthan gum is shown in Figure 1 [12].

5. Raw materials, components of the environment for xanthan biosynthesis and factors influencing the process

The biosynthesis of xanthan depends on many factors, including the strain of microorganism, as each strain consumes a different amount of substrate, affecting the structure of the gum, the bonds and the incorporation of polysaccharide functional groups. For instance, the studies [17,18] utilized date extracts exhibiting slight discrepancies in the fermentation process, in addition to differences in the preparation of the raw materials and the strains employed. The composition of the media assumes significance, yet it is also imperative to contemplate the degree of processing of the raw materials and the bioavailability. Media based on natural and recycled raw materials deplete their components at a more accelerated rate than their synthetic counterparts.

Furthermore, it is important to consider the method of isolating xanthan gum and the centrifugation speeds employed, as well as the duration of the centrifugation process. It is likely that the use of high speeds over extended periods may result in the precipitation of short polymer chains of xanthan gum, which could lead to higher product yields. However, this may also result in a potential reduction in the quality of the gum sludge.

Synthetic media containing purified components provide optimal conditions for the growth of *Xanthomonas campestris* biomass and the biosynthesis of gum. These media are frequently subject to rigorous standardization, which ensures uniformity and predictability of the properties of the gum. Due to the purity of the constituents of such media, it is possible to more easily control nutrients and cultivation conditions, which promotes the production of gum with the desired characteristics. The most essential nutrients for xanthan gum biosynthesis are an available carbon source, a nitrogen source, and minerals including phosphorus, calcium, magnesium, potassium, and iron, as well as some amino acids. However, synthetic media are quite expensive, so the use of media based on recycled materials is an interesting prospect. On such media, *Xanthomonas campestris* receives a variety of organic substances, mineral salts, and vitamins. These components can have an additional effect on the yield and impart properties to the gum that cannot be obtained on

a medium with a different composition. However, the use of such media requires optimization to claim stability of gum properties as well as yield. Table 1 presents examples of xanthan gum production using different substrates as the main carbon source.

6. Macro- and microelements, temperature and pH factors of the environment

6.1. Carbon sources

Carbon is a crucial macronutrient in the biosynthesis of gum, serving as the primary source of energy for microorganisms and facilitating their growth. The optimal carbon concentration in the medium during gum biosynthesis is 2–4%, with deviations from this range leading to growth inhibition. Currently, sucrose and glucose are the primary synthetic carbon sources employed for xanthan biosynthesis. It is also possible to use other pure substrates, such as xylose, galactose, maltose, arabinose, fructose, etc., but the yield will be significantly lower, including due to the inability to fully absorb some of the substrates. For example, *X. campestris* does not express β-galactosidase, which ferments lactose and therefore synthesizes low levels of xanthan in media containing lactose as a carbon source [19,28,32,36]. Concurrently, there is evidence that it is possible to utilize cheese whey, in which lactose is broken down into glucose and galactose. However, the lack of protein in this substrate renders it insufficiently rich or accessible [27,36].

Xanthomonas employs the Entner-Doudoroff pathway in conjunction with the tricarboxylic acid cycle pathway to metabolize glucose [8]. A wide variety of waste can act as a source of carbon. Among the most prominent examples are the use of winery wastewater and the hydrolysis of chicken feathers. Firstly, these approaches are noteworthy for their recycling potential, as they are waste generated in production. Secondly, it is important to highlight the concept of hydrolyzing kitchen waste and using it as a raw material. Despite the greater instability of the composition, the proposed option is worthy of consideration, as it can be optimized for the production of ready-made food, which will lead to better uniformity and repeatability of the composition. However, these approaches require special care during processing due to possible contamination by other microbial cultures. Nevertheless, they represent a potential component of completely zero-waste production. There are articles in which the yield obtained is two and even three times higher than with synthetic media. Firstly, this may be attributed to the producing strain itself. However, it is possible that the researchers employed disparate drying times and temperatures, which resulted in disparate outcomes due to incomplete dehydration. Alternatively, the researchers may have utilized disparate substrate concentrations. For instance, the article by Khosravi-Darani et al. [19] demonstrated a yield of 11.2 g/l, whereas the articles by Salah et al. [22] and Ben Salah et al. [18] exhibited a yield of 24.5 g/l and 43.35 g/l, respectively, when utilizing a date-based substrate. In this context, the comparison of certain data is problematic. However, the possibility of xanthan gum yield being comparable to or higher than that observed in synthetic, pure media is a highly promising avenue for further investigation and development.

6.2. Nitrogen sources

Nitrogen sources are also required by cells as a macronutrient for growth, as they are a building block for proteins in *Xanthamonas* cells. The most commonly used organic sources are peptone, yeast extract, corn extract, and soybean meal. Rapeseed cake, which is widely available in Russia, can also be considered an interesting alternative. The most commonly used nitrogen sources are yeast extract and peptone. Among inorganic nitrogen sources, ammonium salts and various nitrates are the most common, but ammonium salts are considered a more favorable op-

Table 1. **An overview of *X. campestris* strains for gum production using different substrates**

Таблица 1. **Обзор штаммов *X. campestris* для производства камеди, используя различные субстраты**

Strain	Main substrate	Inoculum, %	Temperature, °C	pH	Mixing speed, rpm	Incubation time, h	Yield g/l	Source
Xanthomonas campestris GK6	Glucose	5–10	28–30	7–7,5	200	96	14,744	[19]
	Sucrose	5–10	28–30	7–7,5	200	96	13,234	[19]
	Maltose	5–10	28–30	7–7,5	200	96	12,321	[19]
	Soluble starch	5–10	28–30	7–7,5	200	96	12,1	[19]
	Arabinose	5–10	28–30	7–7,5	200	96	10,958	[19]
	Galactose	5–10	28–30	7–7,5	200	96	7,129	[19]
	Fructose	5–10	28–30	7–7,5	200	96	5,232	[19]
Xanthomonas campestris PD656	Dry apple pomace	5	30	6,6–7,6	200	120	52,1	[20]
Xanthomonas campestris NRRL B-1459	Date extract	5	30	7	180	72	43,35	[17]
X. campestris NRRL B-1459	Date palm sap	5	28	7	180	48	24,5	[21]
Xanthomonas campestris PD656	Dry citrus juices	5	30	6,6–7,6	200	120	32.9	[20]
Xanthomonas campestris EBK-4	Medium with processed ram horns (3%)	10	28	7	200	60	25,6	[22]
Xanthomonas campestris ATCC13951	Winery wastewater	10	30	7	475	96	24,18	[23]
	Peach pulp	10	28	6,0	150	72	22,5	[24]
Xci/NIGEB-386	Pegah Dairy Whey Powder	10	28	7,2	200	120	22,7	[25]
Xanthomonas campestris pv. *Campestris strain*, ATCC33913	Moist olive pomace	10	28	7	250	76	21,64	[26]
X. campestris PTCC1473 и X. pelargonii PTCC1474	Cheese whey	5	28	7	250	48	16,4	[27]
Xanthomonas campestris PTCC1473	Cheese whey	10	32	7	550	72	16,3	[28]
	Date extract	5	28	6	200	72	11,2	[18]
Isolate *Xanthomonas* sp. MO-03	Chicken feather hydrolyzate	5	30	7	200	54	14,56	[29]
X. campestris LRELP-1	Kitchen waste hydrolyzate	10	30	7	300	120	11,73	[30]
Isolate *Xanthomonas campestris*	Pineapple waste	5	30	7	120	72	10,34	[31]
X. campestris MTCC2286	Sugar cane molasses	5	30	7	180	48	10,3	[32]
X. campestris CCTCC M2015714	Glycerin, crude glycerin	10	30	7	300	90	7,9	[33]
Xanthamonas campestris (NCIM 2954)	0.5% Tapioca pulp treated with sulfuric acid	5	28	6,8	200	72	7,1	[34]
X. campestris NCIM 2961	Beer grains (brewery waste)	5	32	6	200	72	5,71	[35]
X. campestris mangiferaeindicae 2103	Crude glycerin biodiesel	20	28	7	700	120	5,59	[32]
X. campestris pv. *manihotis* IBSBF1182	Milk permeates or deproteinized cheese whey	10	28	7	180	96	1	[36]

tion for gum biosynthesis. At the same time, there is evidence that the use of ammonium phosphate as a nitrogen source can increase the pyruvate content in the gum structure [37].

It is essential to regulate the nitrogen level at the initial stages of xanthan gum biosynthesis, as this promotes rapid cell growth. Conversely, in later stages, carbon levels are of greater importance. Other promising organic nitrogen sources include meat peptones, casein peptones, and soy peptones. However, they are inferior to monosodium glutamate as the main source of nitrogen, which gives a very high biomass yield. Ammonium citrate is also a promising option, although its use can be a highly contentious one, as it has the potential to increase the number of citrate ions. The use of ammonium acetate has been reported to result in very low biomass yields [20].

6.3. Effect of precursors (enhancers)

Potassium salts, calcium salts and phosphates, zinc and magnesium sulfates are frequently added during the synthesis of gum. The presence of phosphorus and magnesium directly influences the growth of *Xanthamonas campestris*, while the presence of sulfur and phosphorus directly influences gum production. Furthermore, phosphates are required to reduce pH fluctuations in the culture [38]. In addition to the aforementioned sources of microelements, boric acid, zinc chloride and iron (III) chloride are also employed in the composition of the medium for gum biosynthesis. It has been reported that the use of organic acids during the biosynthesis process can stimulate the production of xanthan gum. Citrate is known to stimulate the production of xanthan gum by *Xanthamonas campestris*, however this leads to a change in the pH of the environment. It has been demonstrated that a citrate concentration in the culture fluid of between 0.09% and 0.18% is optimal. Furthermore, citrate can be employed as a chelating agent to prevent the precipitation of salts during the sterilization of the medium. Pyruvate is also of significance, as it affects the structure of xanthan gum.

There is evidence that a high nitrogen content can increase the pyruvate content in the gum structure [37].

Acetic acid can enhance the dissolution of xanthan because it is a weak carboxylic acid, in which xanthan gum can dissolve.

6.4. Effect of temperature and pH

It can be observed that temperature plays an important role in the biosynthesis of xanthan gum. The influence of temperature is associated not only with the creation of optimal conditions for the production of biomass, but also with the very structural state of xanthan gum. The optimal temperature for the growth of *Xanthamonas campestris* is considered to range from 25 °C to 30 °C. Concurrently, the biosynthesis of xanthan gum can occur between 25 °C and 35 °C. However, increasing the temperature to the upper limits of the range causes acetates and pyruvates to be incorporated into the xanthan gum chain less effectively, resulting in a decrease in the average molecular weight and viscosity. It is postulated that the optimal temperature is approximately 28 °C [38]. However, there are instances where higher temperatures have been employed during the fermentation process[17,20,23,30,32,33,35]. This is attributable to the distinctive features of the strain or technological process, as fermentation in certain media necessitates disparate temperature parameters.

The acidity of the environment is also of significant importance for both the growth of the microorganism and the production of gum. However, the optimal values for these processes do not align perfectly. The optimal pH for the growth of *X. campestris* bacteria is between 6 and 7.5. Conversely, the pH for gum production can range from 6.0 to 8.0, with a neutral value of 7.0 being the most commonly preferred [39]. It is also crucial to regulate the pH during the fermentation process. Any deviations from the optimal pH are to be avoided, as a decrease in pH towards the acidic side leads to the loss of pyruvic acid groups [40], and an increase towards an alkaline environment leads to the loss of acetyl groups [41].

7. Biosynthesis of gum: a detailed account of the main stages and modes of the biotechnological process

7.1. Preparation of raw materials

The biosynthesis of xanthan gum involves a number of steps. The first of these is the preparation of the nutrient medium. Synthetic media are often used in this process, as they are easier to prepare than secondary agricultural products. However, they are significantly more expensive. In order to use secondary raw materials as a nutrient medium for biosynthesis, it is necessary to carry out mechanical processing and hydrolysis. Hydrolysis can be chemical, using 1% sulfuric acid or enzymes. For enzymatic hydrolysis, a variety of enzymes may be employed, including cellulase, amylase, glucoamylase, and pectinase [42].

7.2. Inoculum value

Firstly, an inoculum is prepared that involves a high concentration of cells and a low concentration of xanthan gum. This is done in order to reduce the lag phase during the main fermentation. At the time of transfer of fresh bacteria into the biosynthesis medium, they have not yet formed a xanthan shell, and as a result, the transport of substances inside is facilitated [43]. An inoculum that is transferred too early may not contain sufficient cells. Conversely, the inoculum culture introduced during the dying phase may lack the requisite number of cells for active division. The most common inoculum age is 36–48 hours. After approximately 48 hours, the culture begins to enter the stationary phase, during which active division and biomass growth will not be observed.

In this context, the mass fraction of the inoculum also plays a role in the biosynthesis of xanthan gum. In most cases, the applied inoculum comprises 5–10% of the total inoculum mass. This parameter also depends on the duration of inoculum cultivation. In addition, there are complex environments for gum biosynthesis that require specific conditions. For instance, during the biosynthesis of gum on a medium where the primary source of energy is glycerol, the optimal mass fraction of inoculum is 20% [32].

7.3. Fermentation process

The primary fermentation process is conducted within bioreactors, which are equipped with stirring and bubbling mechanisms. Nevertheless, the utilization of bioreactors with airlift mixing has been announced. The yield of xanthan gum is contingent upon a multitude of factors, including pH, oxygen content, temperature, medium composition, osmotic shock, aeration system, mixing system, and bioreactor design [44].

During the cultivation process, the concentration of pyruvate and acetyl increases within the xanthan gum structure, as does the molecular weight. Once the substrate has been completely utilized and the cells begin to die, fermentation ceases, thus indicating that continuous fermentation is preferable in order to achieve the maximum fermentation time and production.

Both batch and continuous cultivation can be employed to produce xanthan. Batch cultivation is associated with a high percentage of substrate conversion to gum, although it requires more than two days. Batch cultivation is effective in terms of substrate conversion, with an estimated range of 75–80%, while continuous cultivation is estimated to be 65–70% [45]. In laboratory settings, batch cultivation is the preferred method, whereas on an industrial scale, continuous cultivation is more commonly employed. The utilization of secondary raw materials within this mode is also feasible, although in most cases, pre-processing of the raw materials is required.

The typical duration of the process is approximately 72–96 hours, although laboratory conditions may result in a shorter timeframe. However, there are also cultivation processes that can extend up to 120 hours. The aforementioned factors influence the outcome of the cultivation process, which is dependent on the chosen mode of cultivation and the composition of the medium, as well as the availability of the main substrate. It is therefore not possible to predict optimal conditions, and experiments should be conducted to identify the most suitable conditions.

7.4. Mass transfer during the fermentation process

Xanthomonas campestris is an aerobic microorganisms, therefore another crucial factor is the availability of oxygen to the environment. The speed of the mixer ensures access to oxygen, which is of great importance in the process, as the growth of *X. campestris* and the increase in gum content in the medium result in a significant increase in viscosity. This affects mass transfer, including the dissolution of oxygen and the uniform distribution of nutrient concentrations [46].

Inoculum growth and laboratory experiments require the use of shaker incubators to ensure the mixing of the liquid and the supply of air to the volume. The optimal stirring speed for flasks is 180 to 200 rpm, while the most common stirring speeds for fermenters are 500 to 700 rpm. Additionally, the aeration rate must be considered when working with fermenters. This parameter is most often found in values ranging from 0.5 to 1.4 l/l × min [10]. In this instance, the biosynthesis of gum is directly proportional to the presence of oxygen in the medium. However, an excessive aeration rate can result in hydrodynamic stress, which in turn leads to a reduction in the maximum mass of the resulting product [47]. One potential solution to the problem is to consider modifying the aeration mode and mixing speed during the fermentation process. This could help to circumvent the challenges of hydrodynamic stress at the outset of the cultivation process and oxygen deficiency during biomass accumulation. This, in turn, necessitates the need to optimize conditions and to implement a process of constant regulation of the air supply and mixing speed [32,48].

7.5. Isolation of xanthan from culture liquor

The culture liquid derived from *Xanthomonas* fermentation may contain up to 3% (w/w) xanthan gum and up to 0.1–0.3% (w/w) dried cells. Additionally, approximately 0.1–1.0% (by weight) of unused media components may be present. These figures necessitate the removal of approximately 95% of the culture fluid mass. The following steps are required to obtain a pure polymer:

1) Deactivation and removal of cells or their lysis.
2) Precipitation of the polymer.
3) Dehydration.
4) Drying.
5) Grinding.

The culture liquid obtained after fermentation can be pre-treated by heating until intense evaporation in a water bath or by heating to approximately 80 °C, or with chemical agents (hypochlorite, alkalis, enzymes, ketones, alcohols). The use of chemical agents can cause pH changes that reduce the pyruvate in xanthan gum, and enzymes can be quite expensive. The most common method of heating is temperature-based, as at elevated temperatures, xanthan gum dissolves, reducing the viscosity of the culture fluid. In such instances, it is recommended to add potassium or sodium chloride to reduce the viscosity of the solution, facilitating effective precipitation or filtration in the future. However, excessive temperatures can lead to degradation of the gum. Nevertheless, the following conditions are met: the optimal temperature range for this process is 80–130 °C, with a duration of 10–20 minutes; the pH should be maintained within the range of 6.3–6.9. While ultrasonic methods can be employed, they have the potential to alter the structure of the gum, resulting in the fragmentation of its chains. Consequently, these methods are not universally applicable.

Following pretreatment, centrifugation is typically employed to sediment the cells and some of their metabolites, insoluble solid compounds. An alternative approach is filtration through a filter with pore sizes of 0.45 μm, given that the *X. campestris* cell is 0.4–0.7 μm wide and 0.7–1.8 μm long. Centrifugation at 4 °C is the preferred method, with speeds ranging from 10,000g to 20,000 g, depending on the desired purity. Some researchers employ ultracentrifugation at speeds above 25,000 g, which allows for higher yields due to the precipitation of small gum chains. However, in production, this method is not able to provide a sufficiently high yield relative to the costs. The use of filtration is also acceptable, although it is employed to a lesser extent due to the high viscosity of the solution. Following centrifugation, the supernatant is collected, from which extraction is carried out by the addition of alcohols (methanol, ethanol, isopropanol) or acetone. Some studies employ the use of chilled alcohols or acetone. However, alcohols and acetone are not solvents for xanthan gum, which leads to phase separation and leaching of impurities. The most commonly used ratios are 1:3 of supernatant to alcohol or acetone, but a 1:4 ratio is recommended for complete precipitation. Following this, repeated centrifugation is employed at speeds ranging from 7000 to 14000 g for 5–20 minutes at room temperature. It is also permissible to use salts in the formation of complexes. These include polyvalent cations such as calcium, aluminum and ammonium salts. However, subsequent purification from salts is required if the product is to be used in the food industry. If filtration was used, it is necessary to refiltrate through a filter with pore sizes of 100 microns. Ultrafiltration is also available, but in production conditions it is cheaper to use other methods [8,49].

Subsequently, the xanthan gum must be dried. The conditions employed for this process vary considerably, with the most common being drying at 50–70 °C for 24–48 hours. Once dried, the gum can be ground using a stainless-steel sieve, ultrasonic treatment or other suitable methods.

8. Applications of xanthan gum

Xanthan gum has a multitude of industrial applications and is poised for significant growth in numerous fields. This is largely due to its unique physicochemical properties, including high viscosity even at low concentrations in solution, solubility in both cold and hot water, and stability over a wide pH range. Xanthan gum is utilized as a stabilizer, thickener, and emulsifier for emulsions and suspensions. Among the areas of application, the food industry, hygiene and cosmetology, the agricultural industry, and pharmacology are especially distinguished. However, as previously mentioned, xanthan gum has found other areas of application: the paper and textile industries, and the oil industry. Moreover, the structure of xanthan gum can be subject to numerous modifications, which expands the range of potential applications of this biopolymer [50].

The applications of xanthan gum are shown in Figure 2 and described in more detail in Table 2.

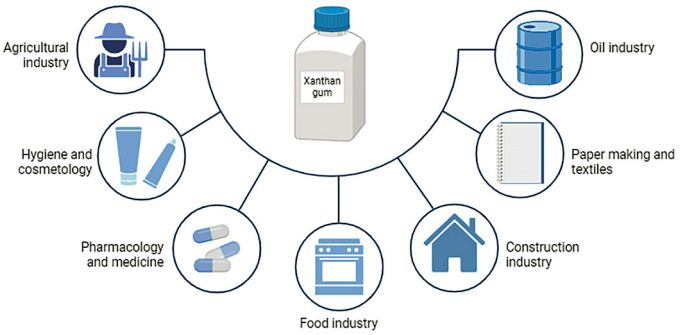

Figure 2. **Areas of application of xanthan gum**
Рисунок 2. **Области применения ксантановой камеди**

8.1. Food industry

Xanthan gum plays a pivotal role in baked goods, acting as a gelling agent that enhances the structural integrity of the dough, imparting elasticity and optimal texture and shape to the final product. Furthermore, it facilitates the increase in the viscosity of the dough, which in turn facilitates the unfolding of the dough during the baking process and also enhances its resistance to breaking [54].

In beverages, gum is employed primarily as a stabilizer, preventing the separation of fruit purée and other solids from the liquid portion of the drink. This maintains a uniform appearance of the drink and prevents separation over an extended period. Additionally, xanthan gum contributes to the creation of a thicker, creamier texture for the drink, enhancing mouthfeel and increasing satisfaction [8].

In sauces, salad dressings and soups, xanthan gum acts as a stabilizer and thickener. It helps to maintain the smoothness and structure of the sauce, preventing it from separating and coloring, and giving it a creamier texture. Thanks to xanthan gum, sauces and dressings are easier to mix and distribute on the surface of dishes, and they also better retain their shape and consistency during storage and serving [32].

In dairy products such as yoghurt, ice cream and milkshakes, xanthan gum is employed as a stabilizer and thickener. It assists in maintaining a uniform and stable product texture, prevents separation of whey and other liquid components, and improves viscosity and creamy texture [12].

In desserts such as puddings, mousses and jellies, gum acts as a stabilizer and thickener. It helps to maintain the shape and texture of the dessert, preventing separation and lightening of its components, and also gives it a creamier and smoother consistency [8].

In prepared foods such as canned foods, soups and semi-finished products, xanthan is employed to enhance the texture, structure and stability of the product. It facilitates the maintenance of its shape and consistency throughout the production, storage and heating process, and improves its appearance and mouthfeel [8,55]. Xanthan gum is also employed in the modification of traditional recipes with the objective of stabilizing them, improving their quality and appearance [56].

In the field of food packaging, xanthan gum is regarded as an ingredient that facilitates the formation of a robust and resilient film, thereby preventing the penetration of air, moisture, and other external factors that could potentially compromise the quality and freshness of the product [57].

8.2. Hygiene and cosmetology

Xanthan is employed in toothpastes to confer the requisite viscosity and stability, enhance fluidity, and facilitate the formation of a stable, creamy lather. It is an optimal binder for all types of toothpastes, including gel and pump varieties. The shear-reducing property of xanthan gum enhances the flow of these products, rendering them simple to remove from tubes or pumps. Furthermore, xanthan gum gives toothpastes a shiny, glassy appearance and ensures their stability on the brush. In creams and lotions, xanthan gum is used to give them the correct viscosity and stability, improve their flow and provide suspension to insoluble solids. It also promotes the formation of a stable, creamy foam, making them more comfortable to use and feel pleasant on the skin. In shampoos, xanthan gum is employed to impart the requisite viscosity and stability, as well as to enhance the flow. It facilitates the suspension of insoluble solids and the formation of a stable foam, which renders shampoos more convenient to use and ensures an even distribution of the product throughout the hair [32].

8.3. Pharmacology and medicine

Xanthan gum is employed in the manufacture of medicinal tablets as a filler, providing the tablets with the requisite structure and volume. It is also utilized in the form of supportive hydrogels, which regulate the release of drugs within the body.

Table 2. **Use of xanthan gum in different areas and its functions**

Таблица 2. **Использование ксантановой камеди в различных областях и её функции**

Application area	Application	Function	Source
Food industry	Bakery products	As a gelling agent, it can be used to improve the elasticity of a gluten-free product and to bind water.	[32]
	Beverages	To impart uniformity and suspension of pulp in drinks; as a thickener for specialty drinks	
	Sauces, salad dressings, soups	Improved stability; ensuring the viscosity of the fluid flow; giving consistency	
	Dairy	To give stability to yoghurts; moisture retention, temperature stability	
	Dessert	To increase elasticity and viscosity in fillings and creams; giving consistency	
	Ready meals	To improve stability; moisture retention; stabilization of emulsions	
	Meat products	To improve stability; moisture retention	
	Cling film	Application possible due to pseudoplastic properties, as well as temperature and pH stability	
Hygiene and cosmetology	Toothpastes	Provides serving consistency as well as stability	[8]
	Creams and lotions	Stabilization of emulsions and giving creams their consistency	
	Shampoos	Suspending insoluble substances, imparting consistency	
	Cleaners	Improved stability, increased contact time on uneven surfaces	
Pharmacology	Drugs	For tablets it delays the release of the drug, for suspensions and emulsions it improves their stability; prevention of phase separation	[51,52]
	Drug delivery	A promising agent for drug delivery in case of binding with nanoparticles or with specific modification of structure	
Agricultural industry	Liquid animal feed	As a stabilizer	[32,53]
	Pesticides and other treatment solutions	Suspension of active components; control of splashing and adhesion to the surface	
Other areas	Polishing pastes	Suspension of abrasive components	[8]
	Textile	Control color migration, improve processing	
	Paper	For suspension and rheological properties	
	Ceramics	For suspending solid particles	
	Oil production	For salt and pH resistance	
	Water based paints	To improve rheological properties	

Xanthan gum is employed to stabilize suspensions of insoluble materials, such as barium sulfate for X-rays and complexed dextromethorphan in cough preparations. It is also utilized in colonic drug delivery systems, where it provides stable and efficient delivery of active ingredients [52,58].

Additionally, there is a growing tendency towards the utilization of modified forms of xanthan gum. The majority of modified forms of xanthan gum are employed in the form of hydrogels as a carrier of medicinal molecules [58,59].

Xanthan gum can be employed as a scaffold for a variety of nanoparticles. This design can be utilized for diverse purposes, contingent on the nanoparticles attached [60]. Chromium nanoparticles with a xanthan gum framework can be utilized to detect heavy metal ions [61].

8.4. Agricultural industry

In liquid animal feeds, such as milk replacers for calves and piglets, xanthan gum is employed to stabilize the suspension of insoluble substances, thereby ensuring uniform distribution of nutritional components and improving feed digestibility. In sauce-based pet foods, xanthan gum is frequently utilized in conjunction with other stabilizers and binders, including LBG and guar gum, in order to guarantee a uniform texture and product stability [8].

In the field of agriculture, xanthan gum is employed in pesticide, herbicide, and insecticide formulas to enhance the fluidity and stability of solutions. It facilitates the maintenance of uniformly suspended particles of active ingredients in solution, which results in more effective and uniform coverage of the treated surface and an increase in the contact time between the pesticide and the target plants or pests [32,53,57].

8.5. Other applications

Xanthan gum is the preferred thickener in polishing compounds used on metal and other surfaces due to its pH stability and flow properties. It provides uniform coverage and ease of removal, which are essential characteristics for such compounds [8].

In the textile industry, xanthan gum can be employed as a stabilizer or additive to ensure uniformity and texture stability in textile fibers or yarns, and to enhance the properties of dyes or impregnations [8].

In the paper and board industry, xanthan gum can be employed as a stabilizer to maintain the suspension of additives or coatings while ensuring uniform distribution and print quality. In the ceramics industry, xanthan gum can be utilized as a stabilizer or thickener in ceramic glazes or paints, providing ease of application and uniform coverage, as well as improved texture and shade [8].

In the dyeing industry, xanthan gum can be employed in water-based paints as a thickener or stabilizer, thereby ensuring uniform distribution of pigments or fillers, maintaining the quality of the paint and facilitating its application [8].

In the field of construction, xanthan gum can be employed as an additive in cement mixtures with the objective of enhancing resistance to high temperatures [62].

Emulsions based on xanthan gum in combination with other gums can be employed in the 3D printing of emulsions, which in turn can be utilized in a variety of industrial applications [63].

Xanthan is a widely used additive in oil recovery processes due to its ability to control water and stabilize slurries under extreme conditions of temperature and high salt concentration. Even in small quantities, xanthan provides high viscosity to solutions, which gives them pseudoplasticity and improves the process of extracting oil from small pores in pipes. In the petroleum industry, xanthan is used in drilling fluids, pipe cleaning and hydraulic fracturing due to its stability in high salt concentrations and protection from high temperatures. In the tertiary oil recovery process, xanthan is employed to create a thickened solution that effectively removes residual oil from porous rock. However, it is important to note that when using xanthan to enhance oil recovery, it is necessary to remove particles such as cells that can clog porous oil-bearing formation [32].

9. Conclusion

A conclusion was reached based on the results of information research, analysis of scientific publications and comparison of experimental data obtained by scientists from different scientific groups. They show that the use of secondary raw materials is a promising solution to the problem of processing agricultural waste, with the production of biopolymers, such as xanthan, being one such solution. The distinctive properties of xanthan gum have been known for a considerable period of time. However, the developed methods for its production are based on the fermentation of individual simple carbohydrates, such as glucose, sucrose, maltose, arabinose, galactose, and fructose. Alternatively, it can be produced as part of soluble starch. The above substrates are expensive sources of carbon, while agricultural processing products provide a relatively cheap and effective alternative. Biotechnological processes, including the fermentation stage of various secondary raw materials, exhibit relatively high rates. The range of secondary raw materials is diverse and encompasses almost all sectors of the food industry. Thus, extracts, juices, se-

rums, hydrolysates, molasses, fruit pulp, brewer's grains, and raw grains represent a non-exhaustive list of promising secondary raw materials for the production of xanthan by biotechnological means. The composition of the culture medium for the productive biosynthesis of xanthan, as well as the parameters (temperature, pH of the medium, air exchange to prevent hydrodynamic stress) that affect the biosynthesis and subsequent purification of the cultural liquid, are controlled in order to optimize the release of xanthan gum. The secondary raw materials mentioned in the review include important macroelements, such as carbon and nitrogen, which are essential for the balanced nutrition of the microbial producer.

It is important to highlight the impact of specific microelements on xanthan biosynthesis, particularly precursors. The presence of these in trace quantities can lead to the formation of the target metabolite.

The purification and isolation of xanthan is a multi-stage process that employs solvents. The quantitative yield is contingent upon a number of factors, including the rheological properties of the culture liquid.

The physicochemical properties of xanthan gum, including its high viscosity even at low concentrations in solution, solubility in water at different temperatures, and stability over a wide pH range, determine the scope of its application. Xanthan gum is widely used in the food industry, in hygiene and cosmetology products, pharmacology and medicine, the agricultural industry, and other areas.

Consequently, there are prerequisites for the development of xanthan gum technologies that utilize not only expensive individual carbohydrates as a carbon source, but also secondary raw materials. Furthermore, the quantitative content of the culture liquid indicates that this is a priority.

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AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	**Принадлежность к организации**
Gabdulla F. Kurbanov, Research Assistant, Laboratory of Biotechnology and Bioengineering, All-Russia Research Institute for Food Additives 55, Liteiny pr., 190000, St. Petersburg, Russia Tel.: +7–937–164–45–66 E-mail: tatanka.sn@gmail.com ORCID: https://orcid.org/0000-0002-3958-7326	**Курбанов Габдулла Фаритович** — лаборант-исследователь, лаборатория биотехнологий и биоинженерии, Всероссийский научно-исследовательский институт пищевых добавок 190000, Санкт-Петербург, Литейный пр., 55 Тел.: +7–937–164–45–66 Email: tatanka.sn@gmail.com ORCID: https://orcid.org/0000-0002-3958-7326
Artem O. Prichepa, Research Assistant, Laboratory of Biotechnology and Bioengineering, All-Russia Research Institute for Food Additives 55, Liteiny pr., 190000, St. Petersburg, Russia Тел.: +7–931–588–40–14 E-mail: prichepa.a@yandex.ru ORCID: https://orcid.org/0000-0002-1037-5629	**Причепа Артем Олегович** — лаборант-исследователь, лаборатория биотехнологий и биоинженерии, Всероссийский научно-исследовательский институт пищевых добавок 190000, Санкт-Петербург, Литейный пр., 55 Тел.: +7–931–588–40–14 E-mail: prichepa.a@yandex.ru ORCID: https://orcid.org/0000-0002-1037-5629
Natalya Yu. Sharova, Doctor of Technical Sciences, Professor of the Russian Academy of Sciences, Deputy Director for Research, All-Russia Research Institute for Food Additives 55, Liteiny pr., 190000, St. Petersburg, Russia Тел.: +7–900–624–11–09 E-mail: n.sharova@fncps.ru ORCID: http://orcid.org/ 0000-0002-4208-9299 * corresponding author	**Шарова Наталья Юрьевна** — доктор технических наук, профессор РАН, заместитель директора по научной работе, Всероссийский научно-исследовательский институт пищевых добавок 190000, Санкт-Петербург, Литейный пр., 55 Тел.: +7–900–624–11–09 E-mail: n.sharova@fncps.ru ORCID: http://orcid.org/ 0000-0002-4208-9299 * автор для контактов
Contribution	**Критерии авторства**
Gabdulla F. Kurbanov: conducting information research, creating drawings and tables. **Artyom O. Prichepa:** conducting information research. **Natalya Yu. Sharova:** systematization of information data results, editing the article.	**Курбанов Г. Ф.:** проведение информационных исследований, создание рисунков и таблиц. **Причепа А. О.:** проведение информационных исследований. **Шарова Н. Ю.:** систематизация результатов информационных данных, редактирование статьи.
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