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REVIEW

Xanthan gum: properties, production conditions, quality and economic perspective

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Summary
Xanthan gum is an important exopolysaccharide produced by *Xanthomonas campestris* in controlled conditions. These conditions must be carefully evaluated in order to obtain an optimal combination between yield and quality of the gum, and with production costs. The issue of this review is to provide a consolidated source of information on studies about xanthan gum production, such as new strains, supplementation of the fermentation medium, alternative carbon sources, effects of temperature, pH, stirrer speed and air flow. The quality of the final product is also discussed. Lastly, this review includes the overviews related to applications and commercial perspectives.

Keywords
xanthan gum; exopolysaccharide; *Xanthomonas campestris;* production; quality

Technically, gums are compounds with molecules of a high-molecular weight, high solubility in water and which can produce gels or highly viscous solutions at low concentrations. There is a wide variety of substances that present the "gummy" characteristics and can be referred to as gums, however, this term is employed in the industry to refer to plant and microbial polysaccharides and their derivatives [1]. The polysaccharides of microbial origin, namely exopolysaccharides, were discovered in the 1950s [2]. However, the last few decades have seen a rapid increase in interest in polysaccharides for food and non-food applications, mainly due to their great biotechnological usefulness [3]. They have a variety of structural and functional properties, which are determined by their chemical composition, various molecular bonds and groupings, molar mass and its distribution. Virtually all of them are non-toxic and obtained at low cost in large quantities, which determines their importance for industrial processes [4].

Xanthan gum is an exopolysaccharide mainly obtained from a plant pathogenic microorganism of the genus *Xanthomonas*, the strain *X. campestris* NRRL B-1459 being the mostly used [5–7]. *Xanthomonas* spp. occur as single straight rods. The cells are Gram-negative, motile, having a single polar flagellum [8]. The microorganism is strictly aerobic, thus oxygen being an essential nutrient both for microorganism growth and for xanthan production [6]. Its production is relatively expensive due to glucose and/or saccharose being used as the sole carbon source. However, there is a possibility of obtaining the carbon source from waste and residues from agriculture in order to reduce the production costs and to encourage the re-use of waste. Many studies were carried out on xanthan gum production and characterization facing the high industrial demand of xanthan gum, which is largely consumed by the following sectors worldwide: cosmetics, pharmaceutical, textile, petroleum and especially the food industry. Its safety use in foods was approved by the Food and Drug Administration (FDA) in 1969, and is based mainly on its unique rheological properties that allow

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the formation of viscous solutions at low concentrations and stability in a wide pH and temperature range [2].

Several factors influence the xanthan gum production, such as the carbon source, microorganism and operating conditions. In order to obtain the best configuration, it is necessary to carefully evaluate the settings of the bioreactor, considered as the best operating system (batch or continuous), the composition of the fermentation medium, and controlled production conditions of temperature, pH, agitation speed, aeration and fermentation time. All of these result in a good yield of a gum with high quality, in the sense of suitable rheological properties and structure.

Biochemistry of xanthan gum

Xanthan gum is composed of a backbone of repeating sub-units, branched or not, that consist of 3 to 8 monosaccharides [9]. However, the composition of xanthan gum depends on a number of factors during the production process. As a basic composition, xanthan gum is composed of D-glucose, D-mannose and D-glucuronic acid. Such composition is presented in the vast majority of works in the area, in varying proportions and with additional components. Quantitative variations are more common than qualitative variations among different polymers produced by microorganisms of the genus *Xanthomonas* [2]. The primary structure of xanthan gum is a linear (1→4) linked β-D-glucose backbone with a trisaccharide side chain on every other glucose at C–3, containing a glucuronic acid residue linked (1→4) to a terminal mannose unit and (1→2) to a second mannose that connects to the backbone

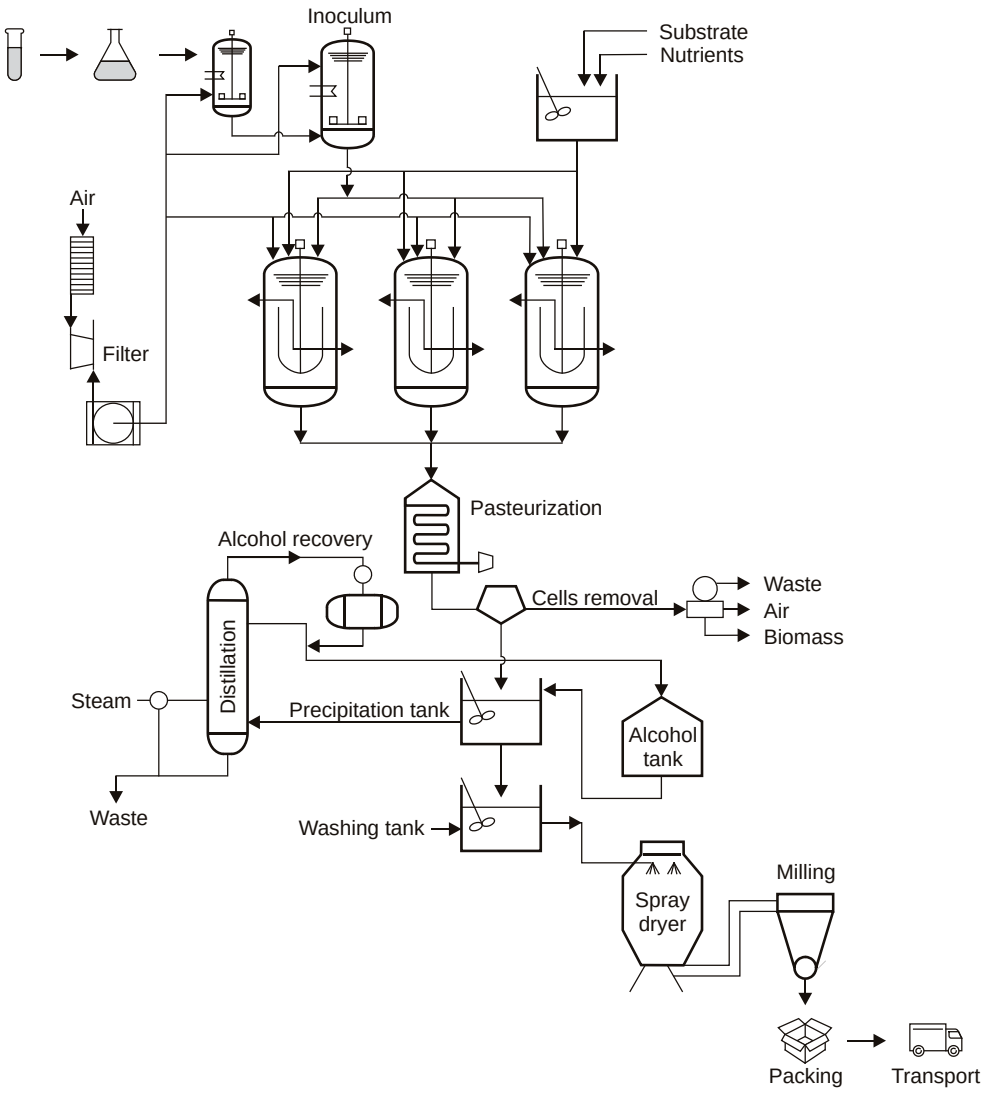

Fig 1. Flowchart of xanthan gum production in a series of stirred tank fermentors.

[10]. On approximately half of the terminal mannose residues, a ketal linkage is joined by a pyruvic acid moiety and acetyl groups are often present as 6-O substituents on the internal mannose residues [11]. The synthesis of xanthan gum is believed to be similar to exopolysaccharide synthesis by other Gram-negative bacteria. The synthetic pathway can be divided into the following parts: uptake of simple saccharides, their conversion to nucleotidal derivatives, assembly of pentasaccharide sub-units attached to an isopentyl pyrophosphate carrier, polymerization of pentasaccharide repeat units and their secretion. The xanthan backbone is formed by successive additions of D-glucose-1-phosphate and D-glucose from 2 mol of uridine diphosphate glucose (UDP-D-glucose). Thereafter, D-mannose and D-glucuronic acid are added from guanosine diphosphate mannose (GDP-mannose) and uridine phosphate glucuronic acid (UDP-glucuronic acid), respectively. O-Acetyl groups are transferred from acetyl-CoA to the internal mannose residue, and pyruvate from phosphoenolpyruvate is added to the terminal mannose. Each of these steps requires specific substrates and specific enzymes for completion. If either the substrate or the enzyme is absent, then the step will be blocked [12]. In *X. campestris*, the Entner-Doudoroff pathway in conjunction with the tricarboxylic acid cycle pathway is the predominant mechanism for glucose catabolism. A small portion of glucose is routed via the pentose phosphate pathway. For glucose uptake, two discrete systems exist. The biosynthesis of xanthan, as in most polysaccharide-producing bacteria, utilizes various activated saccharide donors to form the polymer on an acceptor molecule [13, 14].

COMMERCIAL PRODUCTION PROCESS

Several studies from different authors are available regarding the conditions of xanthan gum production [11, 15–17] and the microorganism used to produce the gum [2, 18–20], stating that variations affect significantly the results in terms of yield and quality. The xanthan gum is produced through aerobic fermentation at a temperature between 27 °C and 30 °C, and the production is stimulated by the presence of organic acids. In the commercial process, the microorganism can convert approx. 70% of the substrate to gum. In order to achieve maximum efficiency at a high quality of the product, the process conditions must be carefully evaluated. The starting point of the optimization of the production process is usually a set of bench-scale experiments, as these are easily controllable and flexible, generating significant results for upscaling. These results can be useful to select the most suitable strain, the nutrients required for fermentation, pH, temperature, stirrer speed and air flow rate. In a commercial scale, the xanthan gum is normally produced by a batch fermentation process [21] followed by fermentation, thermal treatment, cells removal, recovery with alcohol (normally ethanol), drying and milling of the gum. This process is illustrated in Fig. 1 for a batch reactor in a series. Kinetics models were described for batch processes in several studies [22–26]. Simulation of a continuous fermentation system demonstrated also applicability of this approach to xanthan gum production [17]. Another study showed that it was possible to achieve maximum productivity of xanthan gum utilizing a single-stage continuous system [27].

Microorganism

Xanthomonas is a genus of Gram-negative, aerobic, short rod-shaped bacteria belonging to the family *Pseudomonadaceae*. This genus includes several producers of xanthan gum, which are mainly plant pathogens [28, 29]. Xanthan gum production is highly influenced by the microorganism used, individual pathovars determining the composition of the polysaccharide [18]. Several studies related to xanthan gum production with different *Xanthomonas* spp. were published [20]. The result showed that 9.67 $g \cdot l^{-1}$ of xanthan gum were produced by *X. campestris* pv. *campestris* 1078. In another study, *X. arboricola* pv. *pruni* strain 106 was used and, as the best result achieved, 19.5 $g \cdot l^{-1}$ xanthan gum was produced, which was heavily influenced by pH and air flow during the process [19]. An excellent result in terms of yield, 26.4 $g \cdot l^{-1}$, was obtained by performing the production process by *X. arboricola* pv. *pruni* strain 31, but the gum produced showed the lowest viscosity [16]. Different pathovars of *X. campestris* were evaluated regarding the production process of xanthan gum [30, 31]. For *X. campestris* pv. *campestris*, the output varied between 0.0 $g \cdot l^{-1}$ and 15.3 $g \cdot l^{-1}$; for *X. campestris* pv. *juglandis*, the output varied between 0.0 $g \cdot l^{-1}$ to 7.3 $g \cdot l^{-1}$; and for *X. arboricolas* pv. *pruni* and *X. axonopodis* pv. *manihotis* pathovars the output reached 7.4 $g \cdot l^{-1}$ to 9.0 $g \cdot l^{-1}$. All these studies demonstrated that by varying the strain, as well the process conditions, different yield and gum characteristics can be obtained.

Nutrients

Fermentation may involve solid substrates or a liquid medium. The submerged fermentation has the following advantages: easy control, steri-

Tab. 1. Medium composition of the inoculum for xanthan gum production by different authors.

Nutrient	References					
	[32]	[33]	[34]	[35–37]	[21]	[37]
Glucose [g·l^{-1}]		42	10	10	20	
Saccharose [g·l^{-1}]	20					20
CH_4N_2O [g·l^{-1}]						0.10
Citric acid [g·l^{-1}]		2				
NH_4NO_3 [g·l^{-1}]	0.86					
KH_2PO_4 [g·l^{-1}]	2.5					
K_2HPO_4 [g·l^{-1}]		5				1.0
$MgSO_4 \cdot 7H_2O$ [g·l^{-1}]		0.25				
NH_4Cl [g·l^{-1}]		1.94				
H_3BO_3 [g·l^{-1}]		0.006				
Na_2HPO_4 [g·l^{-1}]	2.5					
$ZnSO_4$ [g·l^{-1}]		0.012				
$FeCl_3 \cdot 6H_2O$ [g·l^{-1}]		0.0024				
CaCO3 [g·l^{-1}]					20	
$CaCl_2 \cdot 2H_20$ [g·l^{-1}]		0.018				
Malt extract [g·l^{-1}]				3		
Yeast extract [g·l^{-1}]	3		5	3	10	
Peptone [g·l^{-1}]				5		
Tryptone [g·l^{-1}]			10			

Tab. 2. Fermentation medium composition for xanthan gum production by different authors.

Nutrient	References					
	[30]	[38]	[21]	[25]	[39]	[33]
Saccharose [g·l^{-1}]	40			50	1.125	
Glucose [g·l^{-1}]		30	40		42	42
Citric acid [g·l^{-1}]	2.1	2.0	2.1			
NH_4NO_3 [g·l^{-1}]	1.144				0.217	1.125
$(NH_4)_2SO_4$ [g·l^{-1}]		3.33		0.2		
$(NH_4)_2HPO_4$ [g·l^{-1}]				1.5	0.25	0.217
KH_2PO_4 [g·l^{-1}]	2.866	7.2	2.866	5.0		
K_2HPO_4 [g·l^{-1}]				2.5		
$MgCl_2$ [g·l^{-1}]	0.507		0.507			
$MgSO_4 \cdot 7H_2O$ [g·l^{-1}]		0.24		0.3	20	0.25
Na_2SO_4 [g·l^{-1}]	0.089		0.089			
H_3BO_3 [g·l^{-1}]	0.006	0.0072	0.006	0.006		
ZnO [g·l^{-1}]	0.006	0.006	0.006			
$ZnSO_4$ [g·l^{-1}]				0.002		
$FeCl_3 \cdot 6H_2O$ [g·l^{-1}]	0.020	0.0042	0.020	0.0024		
$CaCO_3$ [g·l^{-1}]	0.020	0.029	0.020			
$CaCl_2 \cdot 2H_2O$ [g·l^{-1}]				0.002		
HCl [ml·l^{-1}]	0.13	0.16	0.13			
Yeast extract [g·l^{-1}]		75				
Peptone [g·l^{-1}]		0.34				
Soybean flour [ml·l^{-1}]					15	15

lization of the medium, sterile aeration, possible variations of the medium and the surface of the microorganism fully exposed to the medium facilitating metabolic exchange. Knowledge about the nutritional needs of the microorganism is very important. The main objective of the nutrients is to provide only the necessary organic and chemicals compounds for standardization of the process, gum quality and cost reduction. Tab. 1 shows the components used by different authors to prepare the inoculum. In order to synthesize the gum, *X. campestris* requires macronutrients – carbon and nitrogen, and micronutrients such as potassium, phosphate and calcium salts. The most common carbon sources are glucose and saccharose. However, in the last decades, the use of agro-industrial wastes have been studied as a carbon source. These studies are described in the next chapter. The effect of glucose concentration on the production of xanthan gum by *X. campestris* was studied, too [32]. According to the study, glucose contents between 30 g·kg^{-1} and 40 g·kg^{-1} were the best range for producing the gum. Also, controlling of glucose to keep its content between 30 g·kg^{-1} and 40 g·kg^{-1} prevented the inhibition of cell growth and the cessation of xanthan gum production. Through this strategic supply of glucose, the concentration of xanthan gum reached 43 g·l^{-1} after 96 h of fermentation. Nitrogen, phosphorus and magnesium directly influenced the bacterial growth, while nitrogen, phosphorus and sulfur directly influenced the xanthan gum production [33]. The addition of phosphates in a minimum concentration of 4 g·l^{-1} increased xanthan gum production, because phosphates served as a buffering agent, reducing the pH fluctuations of the culture [34]. Tab. 2 shows the components used by different authors to prepare the fermentation medium.

Alternative carbon sources

Industrial processes generate waste agriculture products with the potential to cause damage to soil and water if they are not treated and/or disposed

properly. These by-products may generate new processed goods of commercial interest such as alcohols, enzymes, organic acids, amino acids, and may also serve as a source of carbon and/or nitrogen to biotechnological processes [40]. Several processes have been developed to utilize residues such as cassava bagasse [11, 41], green coconut shells [42], residue of apple juice [38], bark cocoa or whey [43], whey [16, 44, 45], sugar cane [34, 46, 47], olive mill wastewaters [48], sugar beet pulp residue [49], citrus waste [50, 51], glycerin and vegetable leftovers [52]. In the aforementioned studies, utilize the cassava bagasse as substrate, acid hydrolysis was used. In a specific research [39] two fermentation broths were used, containing glucose and hydrolysed bagasse as carbon source. Four different strains of *Xanthomonas* spp. were studied. The results showed very good yields for three of the strains tested with the hydrolysed bagasse as a substrate. In another work [41], the cassava bagasse was used as a carbon source for all samples. The samples were supplemented with different nitrogen sources. As a result, an average yield of 14 $g \cdot l^{-1}$ was obtianed. That study showed that cassava bagasse hydrolysed by acidic way and supplemented with nitrogen source could be a suitable substrate for the production of xanthan gum using *X. campestris*.

Effect of pH on production conditions

It is generally assumed that neutral pH is optimal for polysaccharide synthesis and the growth of the microorganism during fermentation process. For xanthan gum, neutral pH is ideal for the growth of *X. campestris*, however, pH decreases during fermentation to around 5.0 due to acid groups present in the biopolymer. The effect of pH and temperature for growth and gum production by *X. campestris* has already been evaluated [53, 54]. Better results were achieved with pH between 6.0 and 7.0, and at a temperature of 25–27 °C related to growth, and pH around 8.0 and temperature 30 °C related to xanthan gum production and its viscosity. A study showed that the pH control improved the growth of the microorganism but had no effect on the production of xanthan gum [6]. In summary, most authors agreed that pH control in the range from 6.0 to 8.0, with the use of alkali such as KOH, NaOH or $(NH)_4OH$, was advantageous for the xanthan gum production [6, 53, 55].

Effect of temperature during fermentation and thermal treatment

In several studies, temperature ranges were evaluated in order to find the best temperature in relation to the yield and rheological characteristics of the xanthan gum. Some studies reached the best gum yield at the fermentation temperature of 28 °C [24, 26, 56]. In addition, at high temperature (close to 34 °C), xanthan gum produced had low acetate and pyruvate contents and low average molecular weight, which caused that its aqueous solutions had low viscosity [24]. At a low temperature, 25 °C, xanthan gum with a high acetate content and a high average molecular weight was synthesized, producing solutions with high viscosity. Other studies were done to evaluate the fermentation temperature, with a consensus that the optimum temperature was close to 28 °C. Similar to the fermentation temperature, the thermal treatment to devitalize the microorganism, broth sterilization and removal of cells from xanthan gum, can generate distinct rheological properties [57]. However, no sufficient studies are available that address practical differences that can be found by varying the temperature of thermal treatments in combination with other operating conditions. Pasteurization of the fermented broth at a high temperature often caused thermal degradation of the microbial exopolysaccharide. When the broth was treated under appropriate conditions (80–130 °C, 10–20 min, pH 6.3–6.9), dissolution of the xanthan gum occured without thermal degradation, and cell lysis was observed [58]. Increasing the temperature also caused a decreases in viscosity of the medium, facilitating the removal of insolubles by centrifugation or filtration [59, 60]. In an instructive experiment, composition of the medium was determined, then it was divided into several tubes in order to submit them to different sterilization temperatures. From the twelve tubes subjected to the heat treatment, six of them had their pH adjusted and the amount of salt determined. The study showed that pH had a large effect on the degree of degradation and pH 7.0 seemed to ensure the best thermal stability. The degree of degradation increased with the temperature and time of treatment of the broth as soon as the temperature was higher than 60 °C. The presence of external salt had no important role at qualitative level. The last conclusion of the work was that thermal treatment always had a degradative effect on the polymer (causing a decrease of the molecular weight) even if, in some conditions, increase of the broth viscosity was observed [57].

Stirrer speed and air flow

The conditions of stirrer and air flow are very important in the production of xanthan gum, once the microorganism involved is strictly aerobic. Another factor that reinforces the importance of

these conditions is the fact that, during xanthan gum production, the viscosity of the broth increases substantially, which is the result of the extracellular accumulation of the biopolymer, which produces the significant decrease of oxygen mass transfer rate [6]. The dissolved oxygen becomes the limiting nutrient, and the oxygen mass transfer rate can become the rate-controlling step for the overall process. Several studies presented the relationship between stirring speed and air flow rate with the yield and rheological characteristics of the gum produced. Another study showed that the more dissolved oxygen, the more xanthan gum is produced [6]. Nevertheless, high levels of dissolved oxygen require high aeration speeds, which results in hydrodynamic stress and can cause the cell damage and affect negatively the yield of gum. In another study, constant airflow rate (1 $l \cdot min^{-1}$) was used and the influence of stirrer speed on culture performance was examined. When the stirrer speed was constant at < 8.3 Hz, the production of xanthan gum was reduced because oxygen mass transfer became limiting with the increasing viscosity of the broth. When stirrer speed was held constant at > 8.3 Hz, the xanthan gum production was also poor because the cells were adversely affected by the intense mechanical agitation [25]. To deal with this problem, the stirrer speed was varied during culture from lower values (3.3–5 Hz) at initiation of the fermentation to higher values later on. There was a dependence between agitation and the pyruvate content of xanthan, when stirring at 1.7 Hz was compared with 10 Hz (1.5% at 1.7 Hz and 3.5% at 10 Hz) [22]. On the other hand, the molecular weight appeared to be practically non-influenced by the increase in the stirrer speed between 1.7 Hz and 10 Hz. In both cases, the molecular weight of the polysaccharide was around 5×10^5 $g \cdot mol^{-1}$. Finally, the stirrer speed strongly influenced xanthan gum production. As a result of these studies, we could conclude that stirring and air flow are key factors to obtain good yields and rheological characteristics of xanthan gum. However, other aspects should be also considered when optimizing such operation conditions, for example, the maintenance of high levels of dissolved oxygen that requires higher power consumption, due to a higher stirring speed and air flow, raising the cost of the process

Quality control

In light of the above considerations, the microorganism used and the operating conditions applied in xanthan gum production influence the yield, chemical composition and quality of the polymer. For commercialization of xanthan gum as a food additive, the Food and Drug Administration [61] establishes, on the basis of Federal Register for 21 CFR 172.172.695 – Xanthan gum, that gum needs to be derived from *Xanthomonas campestris* by a pure-culture fermentation process and purified by recovery with isopropyl alcohol. Xanthan gum was approved for use in foods after extensive animal testing for toxicity in 1968. It is accepted as a safe food additive in the USA, Canada, Europe, and many other countries, with E number E415 [62]. It needs to contain D-glucose, D-mannose and D-glucuronic acid as the dominant hexose units, and should be produced as sodium, potassium or calcium salt. Furthermore, it needs to be produced by a process that renders it free of viable cells of *X. campestris* and it should meet the following specifications: residual isopropyl alcohol not to exceed 750 $mg \cdot l^{-1}$ and an aqueous solution containing 0.1 $g \cdot l^{-1}$ of the additive and 0.1 $g \cdot l^{-1}$ of potassium chloride stirred for 2 h to have a minimum viscosity of 0.6 Pa·s at 24 °C, as determined by Brookfield viscometer, model LVF (Brookfield Engineering Laboratories, Middleboro, Massachusetts, USA) at 1 Hz , and the ratio of viscosities at 24 °C and 66 °C to be 1.02–1.45. Usually, the measurement of viscosities is made with solutions containing gum at 2.5–20 $g \cdot l^{-1}$ and shear rate from 25 s^{-1} to 450 s^{-1} [20, 39, 47, 63].

APPLICATIONS OF XANTHAN GUM

Since xanthan is a water-soluble microbial polymer with specific rheological properties, this gum has diverse industrial applications. Even in agriculture, xanthan gum has been studied as an elicitor together with fungicides in prevention of *Bipolaris sorokiniana*, which attacks barley cultivars [64]. However, the major applications of xanthan gum are in food industry as emulsifier and thickening agent in a variety of products such as juice, fruit pulp and powder beverages, chocolates, desserts, jellies, dairy products, margarine, yoghurt, bakery products, frozen foods, sauces and gravies. Xanthan gum is used in combination with other gums like locust bean gum or guar, to reduce the production costs [65]. Xanthan gum provides texture, viscosity, flavour release, appearance and water-control properties as so required by the food products nowadays. Besides these properties, xanthan gum also improves rheology of the final products by its pseudoplastic behaviour in solutions and as a result of more Newtonian characteristics [11]. A study was performed in order to evaluate viscosity of bacterial exopolyssacharides improvement by repeatedly exposing strains, in-

cluding *X. campestris*, to the antibiotic ampicillin. After the process, no differences were observed in the monosaccharide composition produced by the mutant and parent strains, but high-viscosity mutant strains exhibited higher molecular weights of the product [66]. Another important application of xanthan is the enhanced oil recovery. Even in low concentration, the gum forms high viscosity solution that shows pseudoplasticity. As the oil is held in the tiny pores of the small sand stone, the pumping of xanthan gum solution in the rocks is necessary, ensuring the efficient extraction of oil [67].

Economic perspective

The market capitalization of xanthan gum is approximately 270 million US dollars, and projections for 2015 exceed 400 million US dollars [68]. In order to supply the various sectors of consumption, 86 000 t of xanthan gum are produced every year at a cost of approx. 400 million US dollars per year [69]. From the total volume of xanthan produced in the world, 65% is used by the food industry, 15% by the oil industry and around 20% by other applications. Such demand is increasing and it is estimated an annual growth of 5–10% [11]. The major manufacturers of this raw material can be found in China (FufengGroup, Shandong Province and Deosen Biochemical, Zibo City) and in Austria (Jungbunzlauer, Pernhofen). According to the Brazilian Department of Trade and Industry, the local market consumes about 30 000 t of xanthan gum per year. Such demand is supplied in whole by international industries as no production factories for xanthan gum are in Brazil. As can be observed, local production of this product would contribute significantly to the development and empowerment of some countries [70].

CONCLUSION

Due to the increasing use of xanthan gum in the global market, many studies were carried out aiming at improvement of production microorganisms, medium composition and production conditions. *Xanthomonas campestris* and its production yield can be further increased at suitable physical conditions i.e. temperature, pH, agitation, carbon and nitrogen sources. The development of these processes with the use of alternative media are the subject of patent protection. For studies of operating conditions, the literature refers to commercial carbon sources such as glucose and saccharose. Several studies described alternative sources of carbon, aiming at the use of by-products and/or industrial waste, and the reduction in costs of production of this biopolymer.

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