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Review

Production, recovery and applications of xanthan gum by *Xanthomonas campestris*

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ABSTRACT

Xanthan gum is a water-soluble exo-polysaccharide. It is produced industrially from carbon sources by fermentation using the gram-negative bacterium *Xanthomonas campestris*. There have been various attempts to produce xanthan gum by fermentation method using bacteria and yeast by using various cheap raw materials. This review explains the recent methods of production, recovery and applications of various industries such as food, agriculture, oil, paint and cosmetics.

Contents

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Nomenclature

C_N	nitrogen concentration (g L^{-1})
C_{O_2}	oxygen concentration
$C^*_{O_2}$	saturated oxygen concentration
C_P	product concentration (g L^{-1})
C_S	substrate concentration (g L^{-1})
C_{Sm}	maximum substrate above which growth is completely inhibited (g L^{-1})
C_X	biomass concentration (g L^{-1})
C_{Xm}	maximum concentration of biomass (g L^{-1})
C_ξ	other special adverse components or inhibitors concentrations, e.g., C_{CO_2}, C_{Poison} (chemical poison concentration) (g L^{-1})
f	function particular to the system used
k_La	oxygen mass transfer coefficient (s^{-1})
k_P	maximum specific production rate (l g S^{-1} gX^{-1} h^{-1})
k_X	maximum specific growth rate (l g N^{-1} gX^{-1} h^{-1})
K_S	saturation or Monod constant (g L^{-1})
m_{O_2}	dissolved oxygen consumption coefficient (mol O_2 gX^{-1} h^{-1})
m_S	biomass maintenance coefficient (g S^{-1} gX^{-1} h^{-1})
N	stirrer speed (RPM) (s^{-1})
r_X	rate of biomass production (g cells/h)
$Y_{P/S}$	product yield coefficient based on substrate (g product/g substrate)
$Y_{X/N}$	biomass yield coefficient based on nitrogen (g biomass/g nitrogen)
$Y_{O_2/X}$	oxygen used base on biomass
V_S	superficial air-flow rate (m s^{-1})

Greek letters

δ	exponent coefficient
μ	viscosity of fluid
μ_m	growth specific rate (h^{-1})

1. Introduction

Gum is the common term for hydro colloidal gels-polysaccharides that have an affinity for water and exhibit binding properties with water and other organic/inorganic materials. Traditionally, gums have been derived from a wide variety of plants. Chemically gums are carbohydrate polymers or polysaccharides (however, gelatin is a protein). Polysaccharides are present in all life forms. They have a number of unique chemical and physical properties. They serve as structural material to the plant kingdom, as energy reserves, adhesives and also information-transfer agents. Microbial polysaccharides are composed of regular repeating units of simple sugars like glucose, mannose, fructose, etc. These polysaccharides are sometimes termed as slime or exo-polysaccharides.

Dextran, discovered in early 1940s, was the first microbial polysaccharide to be commercialized. The second microbial polysaccharide commercialized was xanthan. Xanthan gum is a natural polysaccharide and an important industrial biopolymer. It was discovered in 1963 at Northern Regional Research Center (now called The National Center for Agricultural Utilization Research) of the United States Department of Agriculture (USDA) (Margaritis and Zajic, 1978). The polysaccharide B-1459, or xanthan gum, produced by the bacterium *Xanthomonas campestris* NRRL B-1459 was extensively studied because of its properties that would allow it to supplement other known natural and synthetic water-soluble gums. Substantial commercial production began in early 1964. The toxicological and safety properties of xanthan gum for food and pharmaceutical applications have been extensively researched. Xanthan is non-toxic and does not inhibit growth. It is non-sensitizing and does not cause skin or eye irritation. On this basis, xanthan has been approved by the United States Food and Drug Administration (FDA) for use in food additive without any specific quantity limitations (Kennedy and Bradshaw, 1984).

In the United States, the only available xanthan gum is food grade. It is relatively expensive due to glucose or sucrose being used as the sole carbon source and the very stringent purity standards of the Food and Drug Administration for foods. For food-grade xanthan gum, up to 50% of the production costs are related to downstream purification steps, many of which would not be necessary for non-food applications. Another cost reduction could be achieved by using less expensive substrates, such as waste agricultural products.

2. Structure and chemistry of xanthan gum

The primary structure of xanthan gum shown in Fig. 1 is a linear (1 → 4) linked β-D-glucose backbone (as in cellulose) 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 (Jansson et al., 1975; Melton et al., 1976). Approximately 50% of the terminal mannose residues are pyruvated and the non-terminal residue usually carries an acetyl group at C-6. X-ray diffraction studies on orientated xanthan gum fibers identified the molecular conformation as a right-handed, fivefold helix with a rise per backbone disaccharide residue of 0.94 nm, i.e., a fivefold helix with a pitch of 4.7 nm. In this conformation the trisaccharide side chain is aligned with the backbone and stabilizes the overall conformation by non-covalent interactions, principally hydrogen bonding. In solution the side chains wrap around the backbone thereby protecting the labile β-(1 → 4) linkages from attack. It is thought that this protection is responsible for the stability of the gum under adverse conditions.

The trisaccharide branches appear to be closely aligned with the polymer backbone. The resulting stiff chain may exist as a single, double, or triple helix (Milas and Rinaudo, 1979), which interacts

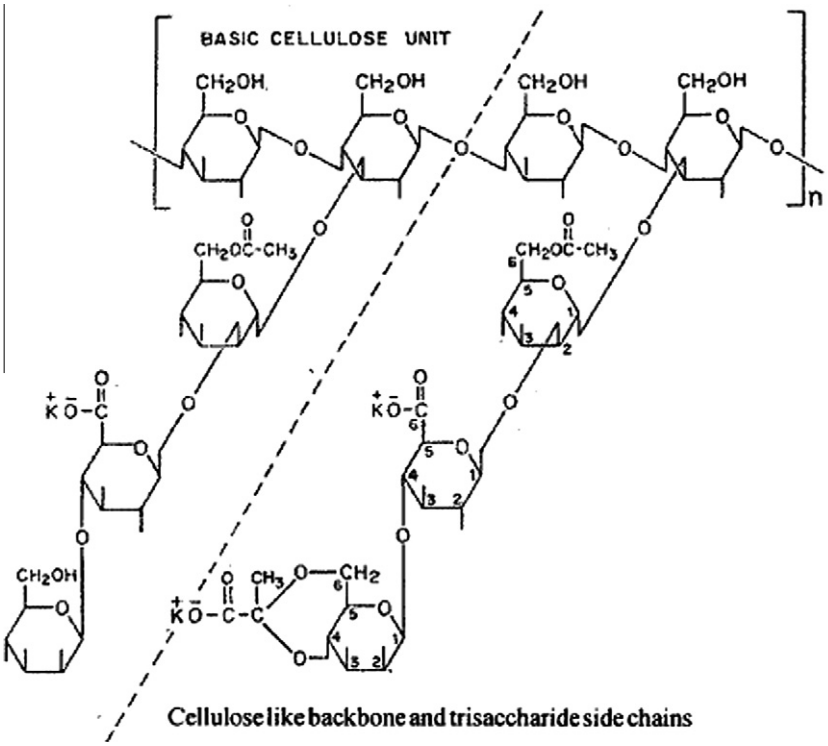

Fig. 1. Structure of xanthan gum.

with other polymer molecules to form a complex. The molecular weight distribution ranges from 2×10^6 to 20×10^6 Da. This molecular weight distribution depends on the association between chains, forming aggregates of several individual chains. The variations of the fermentation conditions used in production are factors that can influence the molecular weight of xanthan.

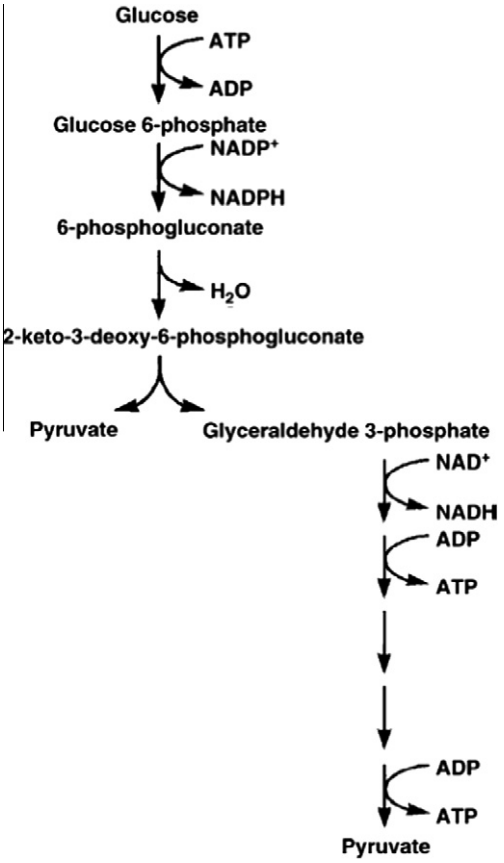

Fig. 2. Entner – Doudoroff pathway (Flickinger and Draw, 1999).

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 three parts: (i) Uptake of simple sugars and conversion to nucleotidal derivatives. (ii) Assembly of pentasaccharide subunits attached to an isopentyl pyrophosphate carrier. (iii) Polymerization of pentasaccharide repeats units and their secretion.

The xanthan backbone is formed by successive additions of D-glucose-1-phosphate and D-glucose from 2 mol of UDP D-glucose. Thereafter, D-mannose and D-glucoronic acid are added from GDP-mannose and UDP-glucoronic acid, respectively. *O*-Acetyl groups are transferred from acetyl-CoA to the internal mannose residue, and pyruvate from phosphoenol pyruvate 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, the step will be blocked.

In *X. campestris*, the Entner–Doudoroff pathway in conjunction with the tricarboxylic acid cycle pathway is the predominant mechanism for glucose catabolism (Fig. 2). 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 carbohydrate donors to form the polymer on an acceptor molecule.

3. Production of xanthan gum

First, the selected microbial strain is preserved for possible long-term storage by proven methods to maintain the desired properties. A small amount of the preserved culture is expanded by growth on solid surfaces or in liquid media to obtain the inoculum for large bioreactors. The growth of the microorganism and

xanthan production are 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 (Nasr et al., 2007; Rosalam et al., 2008; Borges et al., 2008; Papagianni et al., 2001). At the end of the fermentation, the broth contains xanthan, bacterial cells and many other chemicals. For recovering the xanthan, the cells are usually removed first, either by filtration or centrifugation (Flores-Candia and Deckwer, 1999). Further purification may include precipitation using water-miscible non-solvents (Isopropanol, ethanol, acetone), addition of certain salts and pH adjustments (Flores-Candia and Deckwer, 1999). After precipitation, the product is mechanically dewatered and dried. The dried product is milled and packed into containers with a low permeability to water.

The industrial scale production of xanthan is carried out using inexpensive substrates and nutrients. Carbohydrate sources such as sucrose, sugarcane molasses and whey (Silva et al., 2009) have been successfully used in the production medium. Whey also provides adequate nitrogen and some growth factors. Efficient conversion of carbon source to the desired polysaccharide production requires a high carbon to nitrogen ratio. Inorganic nitrogen sources like ammonium or nitrate salts are suitable, and a wide variety of complex nitrogen sources like yeast extract, soy-meal peptone and soybean whey are also useful for xanthan production. Beneficial production is achieved using cereal grains. Generally batch cultivation with complex media is favored. However, simple synthetic media can be used. The batch growth cultivation requires 2 days. Proper maintenance of *X. campestris* stock culture is important for consistency in the production of xanthan, as variation is a recognized characteristic in *Xanthomonas* species. During the initial growth phase polysaccharide accumulation starts and continues after growth. The pH decreases during the fermentation due to the formation of organic acids. If pH falls below 5.0, the formation of xanthan drastically reduces. Thus, it is necessary to control the fermentation medium at the optimum pH of 7.0 (Gumus et al., 2010; Kerdsup et al., 2009; Psomas et al., 2007; Silva et al., 2009) using a buffer or addition of base during process. Adequately designed agitation is necessary to disperse the introduced air evenly throughout the medium. Agitation of the medium is useful for enhancing the rate of transport of nutrients across the cell membrane, which in turn supports the growth rate of the microorganism.

The production of xanthan gum uses glucose or invert sugars, and most industries prefer batch instead of continuous (Letisse et al., 2001; Leela and Sharma, 2000). A typical xanthan production process starts with inoculums of *X. campetris* that are prepared in suitable fermentation medium in conventional batch processing

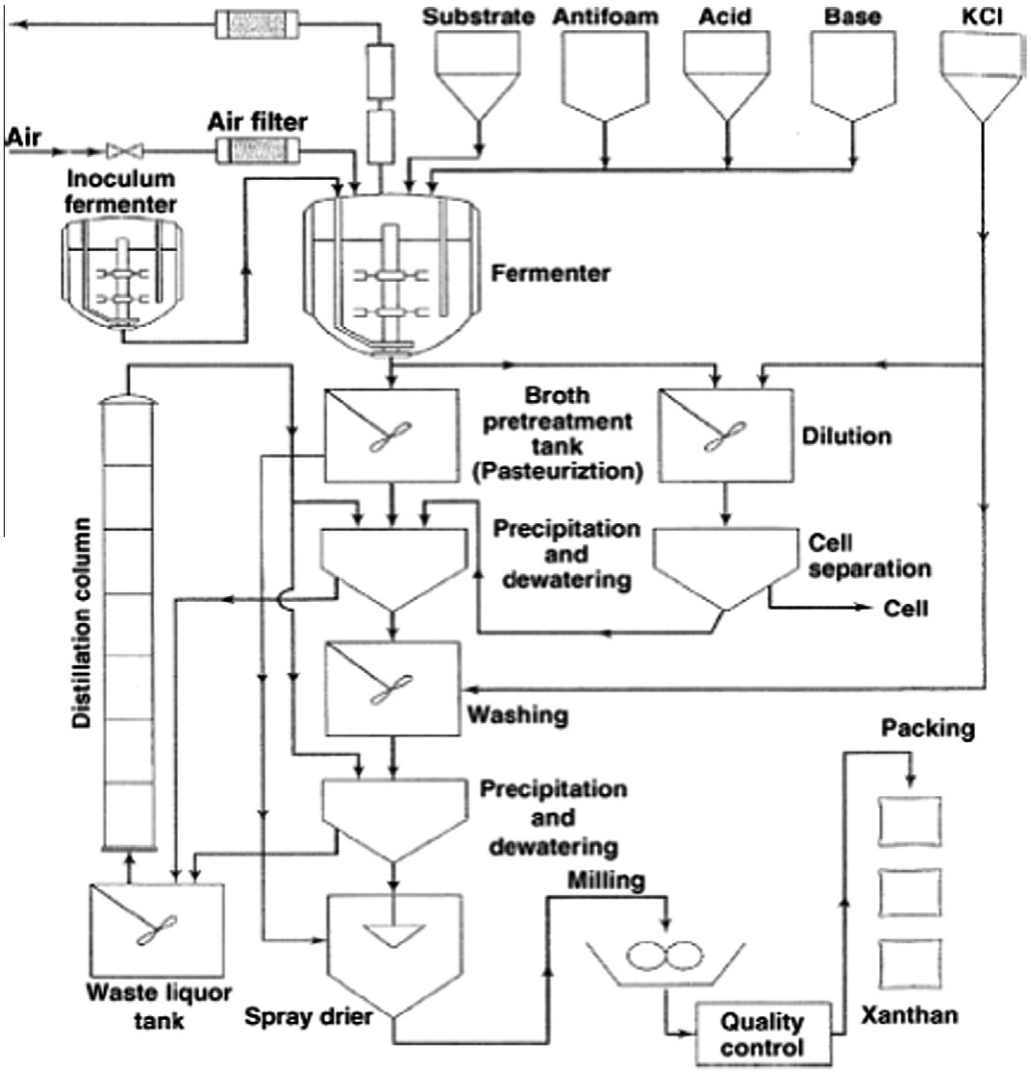

Fig. 3. Flow sheet of xanthan production in conventional stirred tank fermentor (Rosalam and Enland, 2006).

using mechanically agitated vessels. The aerated culture that undergoes aerobic process is held at the following operating conditions: temperature approximately 28–30 °C, pH ~ 7, the aeration rate must higher than 0.3 (v/v) and the specific power input for agitation higher than 1 kW m^{-3}. The fermentation process is carried out for about 100 h and converts an approximately 50% of the glucose into the product. Inoculum preparation includes several stages which require a set of the reactor ranging from 10 L for the initial seed up to 100 m^3 in production stage by which the volume is usually enlarged by 10- fold. As the fermentation evolves, cells would grow exponentially resulting in rapid consumptions of the nitrogen source. After fermentation stage, multi-steps downstream processes would follow. Fig. 3 shows an example of the xanthan gum process used by industry that includes multi-steps of downstream process (Rosalam and England, 2006). When industrial grade xanthan is required, the post-fermentation process treatment may be started with pasteurization on the fermented broth to sterile the bacterial and to deactivate the enzymes. This process usually uses a large amount of alcohol to precipitate the xanthan gum, and the precipitated xanthan gum is then sprayed dry or may be re-suspended on the water and then re-precipitated. When cell-free xanthan gum is required, cells centrifugation is facilitated by diluting the fermentation broth to improve the cell separation. The cell separation by dilution process from highly viscous xanthan solution is a cost-intensive process (Balows and Truper, 1991). A favored method is by adding alcohol and adding the salt would improve precipitation by creating reverse effect charges. The xanthan gum obtained in wet solid form would undergo the dewatering and washing to obtain the final purity required.

3.1. Factors affecting the xanthan production

3.1.1. Effect of carbon sources

In order to grow and be reproductive, cells must ingest nutrients necessary to manufacture membranes, proteins, cell walls, chromosomes and other components. The fact that different cells employ different carbon and energy sources shows clearly that all cells do not possess the same internal chemical machinery. Different growth phase and alteration of the growth medium, for example, by using different substrate and limiting nutrients, do not influence the primary backbone structure, but do affect the structure of side-chains, the molecular mass and the yield, thus xanthan gum produced from a batch culture process would represent a mixture produced at different growth phases and may vary with different culture conditions (Davidson, 1978). Glucose and sucrose are the most frequently used carbon sources. As different cultures would require different media and optimum conditions, many studies on nutrients required for the purpose of product side chain variation and optimization in xanthan gum biosynthesis have been reported (Davidson, 1978; Souw and Demain, 1979; Garcia-Ochoa et al., 1992; Letisse et al., 2001). The concentration of carbon source affects the xanthan yield; a concentration of 2–4% is preferred (Souw and Demain, 1980; De Vuyst et al., 1987a; Funahashi et al., 1987). Higher concentrations of these substrates inhibit growth.

Zhang and Chen (2010) derived xanthan gum from xylose/glucose mixture media and reported that the glucose was the preferred carbon source to produce xanthan while xylose was also utilized with a very low consumption rate. Partially hydrolyzed starches that have been utilized in xanthan gum production are from hydrolyzed rice, barley and corn flour (Glicksman, 1975). Acid whey (Charles and Radjai, 1977), cheese whey (Silva et al., 2009), sugarcane molasses (El-Salam et al., 1993; Kalogiannis et al., 2003), a mixture of mannose and glucose (Jean-Claude et al., 1997), waste sugar beet pulp (Yoo and Harcum, 1999), maize (Achayuthakan et al., 2006), cassava starch (Kerdsup et al., 2009) and peach pulp (Papi et al., 1999) also utilized in xanthan gum production. Yields and qualities of xanthan gum have been reported to be competitive, however, glucose still give the best in terms of product yield (Rosalam et al., 2008; Lo et al., 1997b), constancy of supply and product quality (Davidson, 1978). Leela and Sharma (2000) studied various types of sugars used as the carbon sources during fermentation of the wild-type of *Xanthomonas campestris* GK6. The obtained xanthan gum yield given as the declining order is glucose, sucrose, maltose and soluble starch. The result is shown in Table 1.

Table 1
Effect of carbon sources on xanthan gum production (Leela and Sharma, 2000).

Carbon sources	Xanthan yield (g L^{-1})
Glucose	14.744
Sucrose	13.234
Maltose	12.321
Fructose	5.232
Xylose	5.531
Arabinose	10.958
Galactose	7.129
Lactose	1.008
Inositol	1.502
Sarbitol	1.401
Soluble starch	12.10
Potato starch	9.754

Letisse et al. (2001) performed the fermentation using *X. camprestris* ATCC 13951 and sucrose as the carbon source. Amanullah et al. (1998) have extended the sequence feeding approach by introducing the glucose in a series of pulses after the supplied nitrogen had been exhausted in a conversional agitated fermenter. They have found that the yield improved significantly. Souw and Demain (1979) have reported that sucrose is better substrate for xanthan gum production. They have found that succinate and 2-oxoglutarate have stimulatory effects on xanthan gum production in sucrose-based medium. According to De Vuyst et al. (1987b), a relatively high value of the C/N ratio favors xanthan production. Due to the low level of β-galactosidase present in *X. campestris* the bacterium cannot use lactose as an efficient carbon source. Consequently bacterium grows poorly and produces little xanthan in a medium containing lactose as a sole carbon source (Frank and somkuti, 1979). Saied et al. (2002) reported that sucrose gave the highest yield (dry weight) 11.99 g L^{-1}, and glucose was the second 10.8 g L^{-1}.

3.1.2. Effect of nitrogen sources

Nitrogen, an essential nutrient, can be provided either as an organic compound (Moraine and Rogovin, 1973; Slodki and Cadmus, 1978; Pinches and Pallent, 1986) or as an inorganic molecule (Davidson, 1978; Souw and Demain, 1979; Tait et al., 1986; De Vuyst et al., 1987a,b). The C/N ratio usually used in production media is less than that used during growth (Moraine and Rogovin, 1971, 1973; Davidson, 1978; Souw and Demain, 1979; De Vuyst et al., 1987a,b).

Letisse et al. (2001) reported that two nitrogen sources containing either NH_4Cl or $NaNO_3$ (at 0.055% nitrogen equivalent) showed a slower cell growth rate at 0.07 h^{-1} than ammonium at 0.13 h^{-1}. Yet, the xanthan gum production rates have been increased by nitrate at 0.79 mmol/g cells/h cf. ammonium at 0.52 mmol/g cells/h, although the organic acid content (acetate and pyruvate) of the xanthan gum remained constant at 6.0% and 4.6%, respectively. Ammonium is therefore a better substrate for biomass accumulation, while xanthan gum yields are higher with nitrate used as the nitrogen source. Casas et al. (2000) have studied the effects

of temperature, initial nitrogen concentration and oxygen mass transfer rate. They have found that the degree of pyruvilation and acetylation and the average molecular weight of the xanthan gum increase with fermentation time for any operating conditions. Souw and Demain (1979) showed the best nitrogen source was glutamate at a concentration of 15 mM (higher concentrations inhibited growth). Small quantities of organic acids (e.g., succinic and citric) when added to the medium enhanced production. Lo et al. (1997b) used a moderately high yeast extract concentration in the medium in order to reach a high cell density before the culture enters the stationary phase. Saied et al. (2002) have recommended inorganic ammonium nitrate (11.19 g L^{-1}) as a economic source of nitrogen in the fermentation medium for xanthan production. The attainable amount of xanthan gum and its production rate in batch fermenter also seem to be affected by the amount of nitrogen source available at the beginning of the stationary phase (Pinches and Pallent, 1986).

3.1.3. Effect of temperature

The influence of temperature on xanthan gum production has been widely studied. Temperatures employed for xanthan gum production range from 25 to 34 °C, but culture at 28 and 30 °C is quite common (Table 2). Many authors (Borges et al., 2008; Gumus et al., 2010; Psomas et al., 2007; Silva et al., 2009; Kerdsup et al., 2009) showed that 28 °C was the optimal production temperature of xanthan gum. Psomas et al. (2007) observed the highest yield of xanthan gum production can be obtained with decreasing temperature from 30 to 25 °C or increasing from 30 to 35 °C. Cadmus et al. (1978) concluded that a higher culture temperature increases xanthan production but lowers its pyruvate content. Thonart et al. (1985) reported an optimum process temperature of 33 °C, proposing a temperature of 25 °C for growth and 30 °C for production. In addition, Shu and Yang (1990, 1991) concluded that the optimal temperature for xanthan production depended on the production medium used.

Esgalhado et al. (1995) reported that the optimal temperature for *X. campestris* growth was 25–27 °C and optimal temperature for xanthan gum production was 25–30 °C. Xanthan presents a confirmational transition depending upon a temperature (Milas and Rinaudo, 1979; Morris et al., 1977). Garcia-Ochoa et al. (1992) showed that the optimal temperature for the production medium was 28 °C.

3.1.4. Effect of pH

Most authors (Gumus et al., 2010; Kerdsup et al., 2009; Psomas et al., 2007; Silva et al., 2009) agreed that neutral pH is the optimum value for growth of *X. campestris*. During xanthan production, the pH decreases from neutral pH to values close to 5 because of acid groups present in xanthan (Borges et al., 2008). Psomas et al. (2007) observed that the pH values of the broth after 24 h fermentation were ranged from 7 to 8, after 48 h fermentation broth from 8 to 9.5 and after 72 h fermentation from 8 to 10 depending on the chosen combination of agitation and temperature. Esgalhado et al. (1995) showed that the optimum pH for culture growth was 6–7.5 and optimum pH for the xanthan production was 7–8. Garcia-Ochoa et al. (1996) suggested that the *Xanthomonas* can be cultured at a neutral pH. A study of the pH effects showed that pH control did enhance cell growth but had no effect on xanthan production.

3.1.5. Effect of mass transfer rate

Various types of bioreactors have been used to produce xanthan gum, but the sparged stirred tank is employed most frequently. In stirred reactors the rate of oxygen mass transfer is influenced by the air flow rate and the stirrer speed. When stirred tanks are used, airflow rate is generally maintained at a constant value, usually 1 L/L min. In contrast, the agitation speed used varies over a broad range. At low stirring speeds, oxygen limitation resulted in lower specific xanthan production rates. Specific xanthan production rate is directly related to the specific oxygen uptake rates (Suh et al., 1990, 1992; Amanullah et al., 1998). The agitation has more positive influence on xanthan production than the time. Higher production was also obtained at 1000 rpm at 50 h of fermentation (Amanullah et al., 1998). The agitation has positive effect on xanthan production which also increases upon time of cultivation (Peters et al., 1989). Cacik et al. (2001) observed the maximum xanthan production at 600 rpm, 35 °C and 72 h.

Borges et al. (2008) evaluated the production, viscosity and chemical composition of xanthan gum synthesized by bacterium *X. campestris pv pruni* strain 101 in bioreactor systems. Their results showed that xanthan gum production was dependent on k_La (21.4 h^{-1}), with a maximum yield of 8.15 g L^{-1} at 300 rpm, 3 vvm, 54 h of fermentation. Papagianni et al. (2001) studied the kinetics of growth and xanthan production by *X. campestris* ATCC 1395 in batch culture in a laboratory fermenter without pH control. Fermentations were carried out over a range of stirrer speeds (100–600 rpm) and the pyruvate content, as well as the molecular weight of the product was estimated. Increased agitation levels resulted in higher production rates and biomass levels, while product formation in this fermentation appeared to be partly growth associated. The chemical structure of xanthan was influenced by agitation, as the pyruvate content increased with increasing stirrer speeds. However, no significant effect was observed on xanthan molecular weight as the stirrer speed increased from 100 to 600 rpm.

Garcia-Ochoa et al. (1997) used a constant airflow rate (1 L/L min) and examined the influence of stirrer speed on culture performance. When the stirrer speed was constant at <500 rpm, the production of xanthan was reduced because oxygen mass transfer became limiting with increasing viscosity of the broth. When stirrer speed was held constant at >500 rpm, the xanthan production was also poor because the cells were adversely affected by the intense mechanical agitation. To deal with this problem, the stirrer speed was varied during culture from lower values (200–

Table 2
Operational conditions used in making xanthan gum in different bioreactors.

Temperature (°C)	pH	Bioreactor	Speed *N* (rpm)	Aeration rate (vvm)	Volume (L)	References
28	5		300	3	3	Borges et al. (2008)
30	7		200	1	1.5	Gumus et al. (2010)
30	7	Batch	600	1	2	Psomas et al. (2007)
28	7.2		180			Silva et al. (2009)
28	–	Batch	200	1	2	Kurbanoglu and Kurbanoglu (2007)
30	7	Batch	200	0.5	5	Kerdsup et al. (2009)
29	6.9	Bubble column	500–900	0.3, 0.6	3.6	Pons et al. (1990)
28	7	Airlift	–	7.7–54	50	Suh et al. (1992)
28	7	Plugging jet Reactor		0.33	100	Zaidi et al. (1991)

300 rpm) at initiation of the fermentation to higher values later on. Similar effects of excessive agitation have been reported for numerous other fermentations (Moo-Young et al., 1993). Lo et al. (2001) found that, at 3.5% xanthan concentration the k_La in centrifugal packed-bed reactor (CPBR) (0.018 s^{-1}) was higher than that of stirred tank reactor (0.005 s^{-1}).

4. Recovery of xanthan gum

Xanthan gum is commercially produced by fermentation of glucose with *X. campestris*. The product is then recovered and purified using alcohol precipitation. The present process is energy-intensive and costly mainly because the highly viscous xanthan broth causes agitation and aeration to be difficult in conventional stirred-tank fermenters. The main steps of the recovery process are deactivation and removal (or lysis) of the microbial cells, precipitation of the biopolymer, dewatering, drying and milling. Processing must be done without degrading the biopolymer. Numerous methods have been developed to deactivate, lyse, or remove cells from the broth. Treatment with chemicals (e.g., alkali, hypochlorite, enzymes), by mechanical means, and thermal treatment are used. Chemical treatment at elevated pH can cause de-pyruvylation of the product. When enzymes are used, they must be removed from the medium and this adds to costs. Usually, the fermentation broth is pasteurized or sterilized to kill the cells (Smith and Pace, 1982; Garcia-Ochoa et al., 1993). These thermal treatments also enhance xanthan removal from the cells. When the broth is treated under proper conditions (80–130 °C, 10–20 min, pH 6.3–6.9) enhanced xanthan dissolution occurs without thermal degradation and disruption of cells is observed (Smith and Pace, 1982). The increased temperature also reduces the viscosity of the broth to ease removal of the insoluble by centrifugation or filtration. For highly viscous xanthan broths, viscosity reduction must precede filtration. Viscosity is reduced by dilution or heating. The fermentation broth is usually diluted in water, alcohol, or mixtures of alcohol and salts in quantities lower than those needed for xanthan precipitation (Smith and Pace, 1982; Garcia-Ochoa et al., 1993).

The high viscosity of xanthan broth is a major problem during removal of bacterial cell biomass from the fermented broth. Centrifugation and heat treatment are either ineffective at high viscosity or lead to degradation of xanthan during isolation of cell free gum. The enzymatic lysis of bacterial cells in microbial broth is preferred for removal of cells, as structure of the gum is preserved and effective at high viscosity (Suresh and Prasad, 2005).

Precipitation of polymer is achieved by decreasing the solubility of the dissolved colloid using methods such as addition of salts, water-miscible non-solvents and concentration by evaporation. The alcohols (methanol, ethanol, isopropanol) and acetone, which are non-solvents for the polysaccharide, can be added to the fermentation broth not only to decrease the solubility until phase separation occurs, but also to wash out impurities such as colored components, salts and cells. Recovery options that have been studied include precipitation with organic solvent such as ethanol (Zhang and Chen, 2010; Nasr et al., 2007; Gonanzalez et al., 1989), isopropyl alcohol (IPA) (Galindo and Albiter, 1996), mixtures of salts and alcohol (Torrestiana-Sanchez et al., 2007; Psomas et al., 2007; Garcia-Ochoa et al., 1993) and precipitation with trivalent or tetravalent salts (Kennedy and Bradshaw, 1984). Also, the use of ultra filtration has been reported (Torrestiana-Sanchez et al., 2007; Lo et al., 1997a).

The quantity needed depends on the nature of the reagent. Total precipitation of the gum is possible only when 3 volume of IPA or acetone or ethanol (Zhang and Chen, 2010; Salah et al., 2010; Rottava et al., 2009; Silva et al., 2009) are added per volume of the broth. If lower alcohols such as ethanol are use, $\geqslant$4 volume of alcohol is needed per broth volume (Borges et al., 2008). Gumus et al. (2010) precipitated xanthan gum by using 2 volume of isopropanol per volume of the broth. Addition of salts in sufficient concentration also causes precipitation or complex coacervation due to ion binding of the cations of the added salt to the ionized groups on the polyanion. This leads to charge reversal at the instance when all the available anionic groups are bound to a cation. Polyvalent cations such as calcium, aluminum and quaternary ammonium salts are especially effective in precipitation. Precipitation does not occur with monovalent salts such as sodium chloride (Pace and Righelato, 1981). The addition of a non-solvent reagent promotes precipitation not only by decreasing the water affinity of the polymer, but also by enhancing the binding of the cations, which are present. Thus, xanthan precipitates with lesser amounts of reagents when alcohol and salt are used in combination (Garcia-Ochoa et al., 1993).

Lo et al. (1996) developed a new recovery process that is energy efficient, environmentally benign and cost effective using ultra filtration (UF), an alternative method to alcohol precipitation that recovers xanthan gum from dilute fermentation broth. Even under high-shear-rate conditions, UF did not give any observed adverse effects on the rheological properties and molecular weight of the xanthan polymer. Thus, UF can be used to concentrate xanthan broth from fermentation by a factor of five or higher, thereby reducing the amount of alcohol needed for xanthan recovery by at least 80%.

A xanthan gum-containing fermented solution is subjected to an enzyme treatment for solubilizing the microbial cells present in the fermented solution. While the fermented solution has undergone the enzyme treatment is maintained at a temperature of 50–80 °C and xanthan gum is precipitated by adding an hydrophilic organic solvent incapable of dissolving xanthan gum to the fermented solution. When a rotary turbine is used, the precipitate can be cut with a shearing cutter to recover a finely divided fibrous product.

5. Production kinetics and models

Design and scale up of production bioreactors require an understanding of the process kinetics. A number of kinetic models of varying complexity have been developed for the xanthan gum fermentation (Faria et al., 2010; Pinches and Pallent, 1986; Quinlan, 1986; Schweickart and Quinlan, 1989; Garcia-Ochoa et al., 1998). These models generally attempt to predict the growth and production profiles. Because *X. campestris* is an aerobic bacterium and the fermentation is accompanied by a substantial increase in viscosity, oxygen mass transfer rate varies a lot and this has a major impact on the process. Selection and development of the appropriate kinetic models particularly for certain microorganisms should begin with understanding of the behavior and habitat of the microorganisms. Two main options are available for kinetic developments, batch or continuous operation, as the time course of the microbial growth would differ in each operation.

Studies of the unstructured kinetic and the structured kinetic models have been described in a batch process (Faria et al., 2010; Luong et al., 1998; Garcia-Ochoa et al., 1995, 2004). Many authors used the unstructured kinetic model to describe the synthesis of xanthan gum by *X. campestris* sp. (Faria et al., 2010; Luong et al., 1998; Garcia-Ochoa et al., 1995; Moraine and Rogovin, 1971). These unstructured kinetic would include a balance on the cells mass (C_X), the product concentration (C_P) and the substrate concentration (C_S).

A more comprehensive kinetic model could also include the mass transfer limitation caused by increased viscosity, mechanical design of equipment and variation of the adverse conditions with

time, such as cell population density, by which would contribute to increase cells stress and endogenous rate. Garcia-Ochoa et al. (1998) have proposed the metabolic structured kinetic model for xanthan gum production by *X. campestris* which is based on the assumptions studied by Pons et al. (1989). Garcia-Ochoa et al. (2004) proposed a chemical structured kinetic model by involving both carbon source metabolism and nitrogen metabolism into cells.

Many authors (Faria et al., 2010; Luong et al., 1998; Garcia-Ochoa et al., 1995, 2004) used unstructured kinetic model and batch processing. The kinetic model and system discussed here are commonly used. In this case, interest is centered on the population growth rather than the substrate utilization and the xanthan gum production as both relate to the microbial growth. Other than substrates and nutrients would limit the microbial reproductive is the reactor design, and agitation rates, and changes of viscosity (Casas et al., 2000). The general form of microbial growth kinetics may be expressed by the following equation.

$$r_X = dC_X/dt \propto f\ (C_X, C_S, C_P, C_N, C_{O_2}, C_\xi) \tag{1}$$

where r_X is the biomass rate, f the function particular to the system used, C_X the biomass concentration, C_S the substrate concentration, C_P the product concentration, C_N the nitrogen concentration, C_{O_2} the oxygen concentration and C_ξ is other special adverse components or inhibitors concentrations, e.g., C_{CO_2}, C_{Poison}.

Weiss and Ollis (1980) have expressed growth rate as a function of biomass using the logistic equation which also known as the Verlhurst–Pearl or so called the autonomous equation. The equation can be written as:

$$dC_X/dt = \mu_m C_X(1 - C_X/C_{Xm}) \tag{2}$$

The modified logistic growth kinetics for describing the batch kinetics of microbial growth during the biosynthesis of extra and intra-cellular polymers proposed by Mulchandani et al. in a paper published by Luong et al. (1998) is given as:

$$dC_X/dt = \mu_m C_X(1 - C_X/C_{Xm})^\theta \tag{3}$$

The relationship is valid as long as $[1 - C_X/C_m)^\theta]$ in nonnegative, i.e., $\theta > 0$. The constant θ could be defined as an index of inhibitory effects that accounts for the deviation of growth from the exponential growth. For a very large θ, the generalized logistic equation kinetic approaches the exponential growth equation:

$$dC_X/dt = \mu_m C_X \tag{4}$$

Luong et al. (1998) proposed a combination of Monod and logistic or modified logistic as follows:

$$dC_X/dt = \mu_m(C_S/K_S + C_S)[(1 - C_X/C_{Xm})C_X] \tag{5}$$

The two substrate evolutions with time are expressed in term of stoichiometric coefficients (Garcia-Ochoa et al., 1995).

$$dC_S/dt = -1/Y_{P/S}(dC_P/dt) \tag{6}$$

$$dC_N/dt = -1/Y_{X/N}(dC_X/dt) \tag{7}$$

where $Y_{P/S}$ is the product yield coefficient based on substrate, $Y_{X/N}$ is the biomass yield coefficient based on nitrogen. Unfortunately for the biomass in xanthan production, the rate is not of the Monod type, therefore the equation proposed by Luong et al. (1998) could not suitable to represent the synthesis of xanthan gum. Garcia-Ochoa et al. (1995) have proposed the rates as follows:

$$dC_X/dt = k_N C_N C_X \tag{8}$$

$$dC_P/dt = k_P C_S C_X \tag{9}$$

The logistic equation is given from the combination of Eqs. (7) and (8).

$$dC_X/dt = k_X C_{Xm}/Y_{X/N}[C_X(1 - C_X/C_{Xm})] \tag{10}$$

When nitrogen is the limiting factor, microbial growth has ceased after the nitrogen source had exhausted, therefore the parameter C_{Xm} is replaced by:

$$C_{Xm} = C_{X0} + Y_{X/N}C_{N0} \tag{11}$$

$$dC_X/dt = k_X(C_{X0}/Y_{X/N} + C_{N0})C_X(1 - C_X/C_{X0} + Y_{X/N} + C_{N0}) \tag{12}$$

Carbon source is used for maintenance and for growth, thus:

$$dC_S/dt = -m_S C_X - [(1/Y_{X/S})(dC_X/dt)] \tag{13}$$

Dissolve oxygen is described by the following equation:

$$dC_{O_2}/dt = k_L a\left(C^*_{O_2} - C_{O_2}\right) - (m_{O_2}C_X + (1/Y_{O_2}/X)(dC_X/dt)) \tag{14}$$

where the oxygen mass transfer coefficient was given as:

$$k_L a = 3:08 \times 10^{-4}\ V_S^{0.43}\ N^{1.75}\ \mu^{-0.39} \tag{15}$$

where N is the rotational speed of the stirrer, V_S is the air flow rate and μ is the apparent viscosity.

So far from the unstructured kinetics models have been reviewed, no authors have evaluated the microbial growth causes by the adverse condition, e.g., carbon dioxide contents and other inhibits chemical produced by the microbial itself. However, a modified of the combination logistic and Monod kinetic given by Luong et al. (1998) (shown in Eq. (16)) which represents the cell growth kinetic of *Alcaligenes eutrophus* sp., has described the effect of substrate concentration to the growth rate. The inhibition effects consideration proposed by Mulchandani et al. might partially describe adverse conditions, and if so, a combination of the substrate concentration effect (Eq. (16)) and inhibition effects (Eq. (17)) into the microbial kinetic rate suggested by Garcia-Ochoa et al. (1995) could completely describe the adverse conditions effect to the microbial growth rate

$$r_X = \mu_m C_S/K_S + C_S(1 - C_S/C_{Sm})^\delta C_X \tag{16}$$

$$r_X = \mu_m C_S/K_S + C_S(1 - C_X/C_{Xm})^\theta \tag{17}$$

$$dC_X/dt = k_X(C_{X0}/Y_{X/N} + C_{N0})C_X(1 - (C_X/C_{X0} + Y_{X/N} + C_{N0})\theta) \times (1 - C_S/C_{Sm})^\delta C_S \tag{18}$$

All of the available kinetic models describe the time course of growth, the consumption of the carbon source and production of xanthan. Some of the models also describe the variation in the nitrogen source concentration (Quinlan, 1986; Schweickart and Quinlan, 1989; Garcia-Ochoa et al., 1995) and oxygen consumption (Pinches and Pallent, 1986). In some cases, the models assume the nitrogen source to be the growth-limiting factor and the carbon source to be the xanthan production-limiting nutrient.

6. Applications of xanthan gum

6.1. Food applications

The major applications of xanthan gum are in food industry (Table 3) as a suspending and thickening agent for fruit pulp and chocolates. United States Food and Drug Administration have approved xanthan on the basis of toxicology tests for use in human food. Many of today's foods require the unique texturization, viscosity, flavor release, appearance and water-control properties. Xanthan gum improves all these properties and additionally controls the rheology of the final food product. It exhibits pseudoplastic properties in solutions, and has less 'gummy' mouth feel than gums with more Newtonian characteristics.

Table 3
Applications of xanthan gum and its function.

	Usage in %	Function	References
Food application			
Salad dressings	0.1–0.5	Provides easy pourability and good cling; suspends	Sharma et al. (2006)
Bakery products	0.05–0.3	Binds water; improves texture	Sharma et al. (2006)
Beverages	0.05–0.2	Enhances mouth feel: suspends fruit pulp	
Prepared foods	0.1–0.3	Stabilizes; avoids syneresis	
Soups, sauces and gravies	0.05–0.5	Gives good temperature stability; prevents separator	
Dairy products	0.05–0.2	Inhibits syneresis; stabilizes emulsions	Sharma et al. (2006) Rosalam and England (2006)
Meat products	0.2–0.5	Binds water; inhibits syneresis	
Personal care application			
Toothpaste	0.7–1.0	Provides easy pumpability and gives good stand on	Rosalam and England (2006)
Creams and lotions	0.2–0.5	Stabilizes emulsions; gives creamy consistency	
Shampoos	0.2–0.5	Controls rheology; suspends insolubles	
Industrial applications			
Agricultural chemicals	0.1–0.3	Suspends active ingredients; controls drift and cling	Flickinger and draw (1999)
Cleaners	0.2–0.7	Provides good pH-stability; extends contact time	
Polishes	0.2–0.7	Suspends abrasive components	
Water based paints	0.1–0.3	Controls rheology and penetration	
Textile and carpet printing	0.2–0.5	Improves processing; controls color migration	
Adhesives	0.1–0.3	Controls rheology and penetration	
Paper industry	0.1–0.2	Acts as suspension aid and rheology control	
Ceramic glazes	0.3–0.5	Suspends solids effectively	
Oil drilling	0.1–0.4	Provides good stability against salt, temperature and	Katzbauer (1998)
Enhanced oil recovery	0.05–0.2	Functions as mobility control agent	Byong (1996)
Pharmaceuticals			
Suspensions and emulsions	0.1–0.5	Provides excellent stability and good flow	
Tablets	1.0–3.0	Retards drug release	

6.1.1. Bakery

In the bakery products production, xanthan gum is used to increase water binding during baking, storage and extends the shelf life of baked goods and refrigerated doughs. In soft baked goods xanthan gum can also be used as an egg replacer, in particular the egg-white content can be reduced without affecting appearance and taste. Xanthan gum also contributes smoothness, air incorporation and retention and recipe tolerance to batters for cakes, muffins, biscuits and bread mixes. Xanthan gum improves volume, texture, reduced-calorie baked goods and gluten-free breads. Adding xanthan gum to either cold or hot processed bakery and fruit pie fillings improves texture and flavor release. The added benefits in cream and fruit fillings are extended shelf stability, freeze–thaw stability and syneresis control (Sharma et al., 2006).

6.1.2. Beverages

Xanthan is used as bodying agent in beverages and squashes. When these drinks contain particles of fruit pulp, inclusion of xanthan helps in maintaining the suspension, resulting in good product appearance and texture. Xanthan contributes to pleasing mouth-feel, rapid and complete solubility at low pH with excellent suspension of insolubles and compatibility with most components. Xanthan gum in dry-mix beverage bases provides enhanced body and quality to the reconstituted drink, along with rapid viscosity development.

6.1.3. Dairy

Blends of xanthan gum, carrageenan, guar, LBG and galactomannans are excellent stabilizer for ice cream, ice milk, sherbet, milk shakes and water ices. Xanthan with methyl-carboxy methyl cellulose works for frozen dairy and with carboxy methyl cellulose for directly acidified yogurts. Similar blends are used for dessert puddings, acidified milk gels and others. These economical blends provide optimal viscosity, long-term stability, improved heat transfer during processing, enhanced flavor release, heat-shock protection and ice-crystal control (Sharma et al., 2006; Rosalam and England, 2006). The xanthan, guar and LBG blend is vital to sliceability, firm body and flavor release of cream cheese. Also, xanthan thickens cottage cheese dressings by providing good drainage control.

6.1.4. Dressings

Xanthan gum's stability to acid and salt, effectiveness at low concentrations and highly pseudoplastic rheology make it the ideal stabilizer for pourable no-oil, low-oil and regular salad dressings. Dressings with xanthan gum have excellent long-term emulsion stability and a relatively constant viscosity over a wide temperature range; they pour easily but cling well to the salad. As a partial replacement for starch in regular and reduced calorie spoonable dressings, xanthan gum imparts desirable body, texture and freeze–thaw stability, as well as improved flavor release and eating sensation (Sharma et al., 2006).

6.1.5. Pet food

Xanthan, along with LBG or guar produces a homogeneous gelled product (for blood chunks or semi-moist pet foods. In liquid milk replacers for calves and piglets xanthan gum is used to stabilize the suspension of insoluble substances. Xanthan is often used in combination with LBG and guar gum as stabilizer and binder in the formulation of canned gravy based pet food.

6.1.6. Syrups and toppings

The outstanding solution properties of xanthan are utilized in syrups, toppings. Buttered syrups and chocolate toppings containing xanthan have excellent consistency and flow properties and because of their high atrest viscosities, appear thick and appetizing on products such as pancakes, ice cream and cooked meats (Sharma et al., 2006; Rosalam and England, 2006). Frozen nondairy whipped topping concentrates have firm texture, high overrun and excellent freeze–thaw stability.

6.1.7. Relish

The addition of xanthan gum to relish improves the drained weight and virtually eliminates the loss of liquor during handling.

In portion pack relish, xanthan gum keeps the relish and liquor uniformly distributed during filling and prevents spattering.

6.1.8. Sauces and gravies

Low levels of xanthan gum provide high viscosity in sauce and gravies at both acid and neutral pH. Viscosity is also extremely stable to temperature change and is maintained under a variety of long-term storage conditions. These sauces and gravies cling to hot foods and have excellent flavor release and appearance.

6.2. Industrial applications

Industrial xanthan gum products are manufactured to meet formulation criteria such as long term suspension and emulsion stability in alkaline, acid and salt solution; temperature resistance, and pseudo plasticity. Xanthan has wide applications in the chemical industry (Table 3). A mixture of xanthan and locust bean gum can be used in deodorant gels. A special gel of xanthan can form in presence of borate. This combination has been used for preparation of explosives. The ability of xanthan to impart the desired dispersion stability at rest, and low viscosity under application is used to obtain a right consistency to the toothpaste.

6.2.1. Oil industry

The special rheological properties of xanthan are technologically suitable for the 'enhanced oil recovery' (EOR) application. In the petroleum industry, xanthan gum is used in oil drilling (Katzbauer, 1998), fracturing, pipeline cleaning and work-over and completion. Due to xanthan gum is excellent compatibility with salt, and resistance to thermal degradation, it also useful as an additive in drilling fluids. Xanthan gum is used in micellar–polymer flooding as a tertiary oil recovery operation. In this application, polymer-thickened brine is used to drive the slug of the surfactant through porous reservoir rock to mobilize residual oil; the polymer prevents bypassing of the drive water through the surfactant band and ensures good area sweeping (Byong, 1996).

6.2.2. Agricultural products

In the agriculture industry, xanthan has been used to improve the flow-ability in fungicides, herbicides and insecticides formulations by uniformly suspending the solid component (Flickinger and draw, 1999). It helps control spray drift and cling, which increase the contact time between the pesticide and the crop.

6.2.3. Cleaners

Xanthan gum's flow properties and broad pH stability make it the thickener of choice in products such as highly alkaline drain, tile and grout cleaners; acidic solutions for removing rust and metal oxide; graffiti removers; aerosol oven cleaners; toilet bowl cleaners; and metal cleaning compounds. Xanthan gum provides cling to vertical surface, as well as easy removal.

6.2.4. Coatings

The pseudo plastic properties of xanthan gum provide excellent texturing in ceiling-tile coatings and paints with high-solids content, ensuring in-can stability, ease of application to the wall and retention of the texture finish. Xanthan gum thickens latex paints and coatings, and uniformly suspends zinc, copper and other metal additives in corrosion coatings.

6.2.5. Paper

Xanthan gum is used as a suspension aid or stabilizer in the manufacture of paper and paperboard, particularly when intended for contact with food.

6.2.6. Personal care applications

Xanthan gum improves the flow properties of shampoos and liquid soaps and promotes a stable, rich and creamy lather (Rosalam and England, 2006). It is an excellent binder for all toothpastes, including gel and pumpable types. Ribbon quality and ease of extrusion are improved as well.

6.2.7. Pharmaceutical applications

Xanthan gum stabilizes suspensions of a variety of insoluble materials such as barium sulfate (X-ray diagnoses), complexed dextromethorphan (for cough preparations) and thiabendazole.

7. Recent developments and future studies

Development and improvement have been studied for the xanthan gum production. In the last few years, the membrane processes have been increasingly used for concentrating of high viscous broth (Rosalam et al., 2008; Torrestiana-Sanchez et al., 2007; Lo et al., 1996, 1997a). Ultrafiltration was also reported that would save up to 80% the energy required for recovering of xanthan gum from the fermentation (Lo et al., 1997a). Torrestiana-Sanchez et al. (2007) also reported that Membrane-assisted precipitation reduced the amount of precipitating solutes used while greatly increasing membrane flux, resulting in a large improvement in separation productivity.

As the cell free xanthan gum has eliminated the membrane fouling, Yang et al. (1996) have developed a novel of centrifugal packed-bed reactor (CPBR) used for viscous xanthan gum production. Rosalam et al. (2008) also produced cell free xanthan gum by using continuous recycled packed fibrous-bed bioreactor membrane (CRPBFBM). *X. campestris* cells were immobilized in a rotating fibrous matrix by natural attachment to the fiber surfaces. Continuously pumping and circulating the medium broth through the rotating fibrous matrix would ensure a good transfer of the gas and liquid with the cells. Fibrous matrix support would give a good separation of the xanthan from the cells has said immobilized most cells onto the fiber surfaces. Rosalam et al. (2008) and Yang et al. (1998) have also studied a good device for cells adsorption used as a matrix support. Attempting with different woven materials; cotton towel, cotton fabric, and 50% cotton and 50% polyester, the result showed that cotton with rough surfaces is the preferred material. Rosalam et al. (2008) also observed the xanthan gum purity from the CRPFBMB was found to be greater than 98%. Lo et al. (1996) also reported the same finding that the cell free xanthan gum broth by immobilizing in fibrous matrix supports did not significantly foul the membrane during the entire period.

Sereno et al. (2007) processed xanthan gum by extrusion and subsequent drying produces a biopolymer showing particulate, rather than molecular behavior in aqueous solution. This form of xanthan disperses very readily to give a viscosity that is strongly dependent on salt concentration. It is suggested that the extrusion process melts and aligns xanthan macromolecules. A xanthan solution can be prepared from this particulate material by dispersing and subsequent heating far more readily than can be achieved with non-processed xanthan. Nasr et al. (2007) used 2D micromodel for microbial oil recovery investigation and showed that the most efficient concentration of xanthan for enhanced oil recovery (EOR) is 1000 mg L^{-1}. Some authors (Kerdsup et al., 2009; Salah et al., 2010; Rottava et al., 2009; Gumus et al., 2010; Kurbanoglu and Kurbanoglu, 2007) developed mutant strain of *Xanthomonas* sp. for higher yield and productivity of xanthan gum. Recently cassava starch (Kerdsup et al., 2009), date palm-juice by products (Salah et al., 2010) and ram horn hydrolysate (RHH) (Kurbanoglu and Kurbanoglu, 2007) also utilized for higher productivity of xanthan gum.

The CPBR, CRPFBMB and cells adsorption method have potential to be developed further for continuous operation. Other developments are the axial-flow hollow fiber cell culture bioreactors, the fibrous-bed bioreactor for continuous production of developmental endothelial locus-1 by osteosarcoma cells (Chen et al., 2002), and the ceramic membrane reactor, which possibly help in development of the continuous xanthan gum production.

8. Conclusions

Xanthan is the most commercially produced industrial gum, obtained by fermentation, with an annual worldwide production of 30,000 tons, which corresponds to a market of $408 million (Kalogiannis et al., 2003). The current major markets for xanthan gum are food and related industries. Many investigations explained the various factors such as recovery methods, micro organisms and raw material cost involved in the production of xanthan gum, and capital investment. However, there are still several issues that need to be addressed in order to produce xanthan gum biotechnologically within the targeted cost, such as the development of high-performance xanthan gum-producing microorganisms and the lowering of the costs of raw materials and fermentation processes. The biotechnological processes for the production of xanthan gum from cheap raw materials should be improved further to make them effective one.

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