CHAPTER II

REVIEW OF LITERATURE

2.1. Introduction

Xanthan gum is a water-soluble microbial polysaccharide of great commercial significance. 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 focuses on various aspects of xanthan production, the downstream recovery of the polysaccharide, applications of various industries, the kinetics of growth, substrate and production, and the solution properties of xanthan.

2.2. Genus *Xanthomonas*

The genus *Xanthomonas* is classified under the family Pseudomonadaceae and the yellow-pigmented plant pathogen of this family have been unified in this genus (Schiegel, 1995). Cells are straight rods usually within the range 0.4 - 0.7 wide X 0.7 – 1.8 um long. They are gram negative, aerobic and motile by a single polar flagellum. The optimum temperature for growth is usually 25-30°C. Colonies are yellow, smooth and butyrous or viscid (Bradbury, 1984).

The oxidase test for genus *Xanthomonas* is negative or weak positive and catalase test is positive. They are chemoorganotrophic, able to use a variety of carbohydrates and salts of organic acids as sole carbon sources. Asparagine is not used as a sole source of carbon and nitrogen and this is used as a diagnostic test for *Xanthomonas* (Dye, 1962 cited in Bradbury, 1984). Certain species especially *Xanthomonas campestris* and *Xanthomonas fragia* are known to produce large amount of extracellular polysaccharides or xanthan gums when grown in media containing usable carbohydrates (Bradbury, 1984; Schiegel, 1995). Although not well
understood, the role of these extracellular polysaccharides is to maintain viability of bacterial cells in exudates and plant material under dry conditions and to protect them from enzymatic degradation (Schiegel, 1995).

Xanthans have a wide range of uses in food industry and in other industries because they are not toxic to man and animals. They are mainly used to increase the viscosity of aqueous solution such as diet soups, packed desserts, printing inks, paints etc. (Schiegel, 1995; Becker et al., 1998). *Xanthomonadins* (Yellow pigments) are brominated aryl polyenes found in all species of *Xanthomonas* and important characteristics for identification. However, there are non-pigmented strains, which sometimes occur. Therefore absence of *Xanthomonadins* does not exclude an organism from the genus (Bradbury, 1984). Phenotypic and genetic variations were observed in different *Xanthomonas campestris* pathovars. For example, biochemical and pathogenic variation was observed in strains of *Xanthomonas campestris* Pv. mangiferaeindicae collected from southern India. The isolates were different in their ability to liquefy gelatin, reduce nitrate and utilize carbon sources (Dayakar and Gnanamanickam, 1996).

Pathogenic variability was also observed in isolates of *Xanthomonas campestris* Pv. glycines collected from soybean cultivars from 6 states of USA and National Collection of Plant Pathogenic Bacteria in England. Based on the variability in pathogenicity, the isolates were classified into five races among which some avirulent strains were found (Hwang et al., 1998). Phenotypically distinct strains of *Xanthomonas campestris* Pv. mangiferaeindicae as yellow pigmented and a pigmented were isolated in Brazil (Pruvost et al., 1998). White pathovars of *Xanthomonas campestris* were also observed (Sugimori and Oliveira, 1994). Genetic variations in different isolates of several *Xanthomonas campestris* pathovars were also observed and measured using different molecular techniques such as RFLP and rep-PCR (Bragrad et al., 1995; Louws, et al., 1995; Norman et al., 1999; Restrepo et al., 2000).

### 2.3. *Xanthomonas Campestris* on Xanthan Gum Production

The strains for xanthan gum production are selected and improved by several conventional methods. The purpose of genetic modification could be to have improvements of the properties as required by the downstream application, or to suit with the medium supplied, or to improve the product yield, or to improve the
performance by reducing the fermentation time, or to simplify the recovering and purification in following processes. Attempts of mutation of specific genes involved in the xanthan gum synthesis have been made to simplify the repeating unit structure; however the xanthan gum yield was much lower than the one produced from wild-type strains. Betlach et al. have constructed a mutant lacking the glucuronic acid residues and the pyruvate. As a result, the xanthan gum solution produced by this strain is highly viscous. Another strategy to alter the structure of xanthan gum is by post synthetic enzymatic treatment, such as removal of the terminal β-D-glucuronosyl residue from xanthan gum eliminating the mannosyl side-chain terminus. This truncated xanthan gum by missing the terminal disaccharide is more viscous than the original xanthan gum (Betlach et al., 1987). The method of increasing xanthan gum production but varying of the quality of the product of xanthan gum to some extant has been successfully described by Pollock and Thorne (Pollock and Thorn, 1987).

A method of increasing xanthan gum production by some extent was successfully described. Despite positive strain developments, the overall increase of xanthan gum yield by new strains still seems to be unlikely (Becker et al., 1998). It may be concluded that modification of the microbial strains to increase the xanthan gum yields is not necessary since the synthetic production for xanthan gum is very efficient with high conversion of carbon sources into a product (50–85%) (Linton, 1990; Amanullah et al., 1998). This may suggest that improvement of xanthan yield and quality could be achieved by improving the fermentor and process design, changing the composition of the medium, and the media feeding strategy.

Due to differing supply of particular raw materials in various part of the world, other attempts have been made by researchers to extend the range of the substrates that can be efficiently used for xanthan gum production. Xanthomonas campestris generally does not use lactose efficiently because of the low level of β- galactosidase activity in the organism. Fu and Tseng (Fu and Tseng, 1987) had introduced a plasmid carrying β-galactosidase-encoding gene into Xanthomonas campestris. The resulting strain is able to produce xanthan gum in whey-containing medium, but the plasmid is not stable. It was concluded that the unmodified starch used as carbon source in producing xanthan gum may not be a correct method.
2.4. Synthetic Path Way of Xanthan

The synthesis of xanthan gum is believed to be similar to exopolysaccharide synthesis by other Gram-negative bacteria (Harding et al., 1995). The synthetic pathway can be divided into three parts:

- Uptake of simple sugars and conversion to nucleotidal derivatives
- Assembly of pentasaccharide subunits attached to an isopentyl pyrophosphate carrier
- Polymerisation of pentasaccharide repeats units and their secretion

![Figure 2.1 Entner–Doudoroff pathway](image)

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 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, the step will be blocked. In Xanthomonas campestris, the Entner–Doudoroff pathway in conjunction with the tricarboxylic acid
cycle pathway is the predominant mechanism for glucose catabolism (Fig. 2.1). 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, utilises various activated carbohydrate donors to form the polymer on an acceptor molecule. The oligosaccharide repeated units of xanthan are constructed by sequential additions of monosaccharides from sugar nucleotide diphosphates to isoprenoid lipid acceptor molecules. At the same time, acyl substituents are added from appropriate activated donors. It has been suggested that the construction of the exopolysaccharide follows a “tail-to-head” polymerisation (Flickinger and Draw, 1999). After the pentasaccharide repeated unit is formed, oligomers are formed by transfer to other lipid intermediates. Oligomer construction normally involves the addition of the longer oligosaccharide sequence to the isoprenoid lipid diphosphate. The inactive lipid carrier is dephosphorylated to yield isoprenyl phosphate, which can then re-enter the biosynthetic sequence.

Figure 2.2 The tricarboxylic acid cycle

The structure of repeating units is determined by the sequential transfer of different monosaccharides and acyl groups from their respective donors by highly
specific sugar transferases, the polymerase enzyme responsible for polymerisation of the pentasaccharides into a macromolecule. The final stages of exopolysaccharide secretion from the cytoplasmic membrane involve passing across the periplasm and the outer membrane and finally excreted into the extracellular environment. This mechanism must exist in all polysaccharide-producing bacteria for releasing polymer from the isoprenoid lipid prior to transport to its final destination (Fig. 2.2). The process requires an energy source and may be analogous to export lipopolysaccharide to outer membrane in which ATP is the energy supplier (Flickinger and Draw, 1999).

2.5. Modes of Fermentation

2.5.1. Batch Process

Although batch culture having fewer parameters to be controlled and well understood is commercially preferred. The problem of operation in batch culture is that the environment for cell growth keeps changing throughout the “growth cycle” and could give adverse conditions, such as toxic products or extreme pH and exhaustion of nutrients. While in continuous culture, the growth medium is continuously supplied to the culture vessel, extreme conditions will not occur as the medium is continuously diluted and removed from the vessel. Becker et al, have also pointed out that continuous process shows reasonably high conversion rates of substrate to polymer of 60–70%, but also mention problems of maintaining the sterility and the risks of emergence of fast-growing mutants that do not produce the desired product of xanthan gum (Becker et al., 1998).

2.5.2. Continuous Process

The continuous process gives a cost competitive system, and with suitable growth conditions, considerable yields of polysaccharides can be maintained for more than 2000 h (Evans et al., 1967), thus the continuous process could be the choice rather than the batch mode. Although conventional methods can be improved by continuous fermentation, there is still a classic problem of the product containing cells and cell debris that lowers the filterability of the xanthan solution thereby limiting its application. The production of cell-free xanthan gum is therefore desirable. In 1966, Esso Production Research Company found that the continuous film fermentation reactions can be readily carried out by continuously depositing a suitable substrate on the surface of a rotating drum or moving belt or similar device, and applying a culture
containing selected microorganisms to the film, and then continuously removing the fermentation product after sufficient elapsing of residence time. Tests have shown that such a process makes it possible to use the substrate in higher concentrations, permits surprisingly effective utilisation of the substrate, reduces the time required for carrying out the fermentation reaction, minimises the variation in product quality, and simplifies the recovery of the fermentation products (Glicksman, 1967).

2.5.3. Solid State Fermentation

The history and evolution of Solid-State Fermentation (SSF) in Brazil during the past 15 years have been discussed. SSF processes and applications are presented hereby pointing out the advantages and the perspectives for the use of this technique. Brazilian economy is strongly dependent on the various kinds of agro-industrial production such as coffee, sugar cane, soybean, etc., which also generates huge quantities of agro-industrial residues such as sugarcane bagasse, apple pomace, coffee husk and pulp, soybean defatted cake and declassified potatoes. Following the global trends on SSF research for the past 15 years, the Laboratory of Biotechnological Processes (LBP) of Federal University of Paraná (UFPR) started a very promising journey through the development of SSF processes using agro-industrial residues for protein enrichment, biological detoxification, production of biomolecules such as enzyme, organic acids, food aroma compounds, biopesticides, mushrooms, pigments, xanthan gum, hormones (gibberellic acid (GA3)), etc. The basic aim has been to develop a laboratory scale bioprocess and optimize the production applying biochemical engineering principles (Carlos and Luciana, 2003).

2.6. Commercial Production of Xanthan Gum

Most commercial production of xanthan gum uses glucose or invert sugars, and most industries prefer batch instead of continuous (Harding et al., 1995; Letisse et al., 2001; Leela and Sharma, 2000). Quality assurance and easy to control are the reasons why the xanthan gum production uses invert sugars, instead of polysaccharides, and batch process instead of continuous operation.
A typical commercial production process starts with inoculums of *Xanthomonas campestris* that are prepared in suitable fermentation medium in conventional batch processing 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 be higher than 1 kW/m³ (Glicksman, 1975). The fermentation process is carried out for about 100 h and converts an approximately 50%
of the glucose into the product. Inoculums preparation includes several stages which require a set of the reactor ranging from 10l for the initial seed up to 100 m$^3$ in production stage by which the volume is usually enlarged by 10 folds. 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. Figure 2.3 shows an example of the xanthan gum process used by industry that includes multi steps of downstream process.

When industrial grade xanthan is required, the post fermentation process treatment may be started with pasteurisation on the fermented broth to sterile the bacterial and to deactivate the enzymes. This process usually uses a large quantity 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 favoured method is by adding alcohol and adding the salt that 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. Alcohol used for xanthan precipitation is recovered by distillation column. Washing may be carried out to improve the quality of product hence it would entirely remove particulate matters, such as cell debris, microgels, organic residues and pigments. Concentrated xanthan gum is then re-dissolved and washed with water/KCl to reduce viscosity, precipitated and dewatered again until satisfied with the produced quality. Finally, the precipitated xanthan gum is spray-dried in batch or continuous driers. The dry xanthan gum is milled to the desired mesh sizes for control of disperse ability and dissolution rate as well as to get the handling much easier.

Papagianni et al., (2001) studied the production of xanthan from Xanthomonas campestris ATCC 1395 in a laboratory fermentor 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. They reported that the 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.

The production of Xanthan gum by *Xanthomonas campestris* ATCC 1395 using sugar beet molasses as carbon source by Stavros Kalogiannis *et al.*, (2003) gave the following results. Maximum xanthan gum production (53 g/l) was observed after 24 h at 175 g/l molasses, 4 g/l K$_2$HPO$_4$ and at neutral initial pH. Results indicated that K$_2$HPO$_4$ serves as a buffering agent as well as a nutrient for the growth of *Xanthomonas campestris*. Sugar beet molasses appears to be a suitable industrial substrate for xanthan gum fermentations.

Esabi Basaran Kurbanoglu and Namudar Izzet Kurbanoglu, (2003) in their investigation on xanthan production using Ram horn hydrolysate from a local isolate of *Xanthomonas campestris* EBK-4 in batch culture found that xanthan production increased by RHH. A concentration of 3% v/v RHH resulted in the highest xanthan concentration of 25.6 g/L in 48 h. This value was 49% higher than that of control medium (17.1 g/L) in the absence of RHH in 60 h. The pyruvate content increased with increasing RHH concentrations. The application of RHH resulted in enhancement of xanthan production.

Moosavi-Nasab *et al.*, (2008) carried out experiments on production of xanthan gum using date syrup by *Xanthomonas campestris*. Fermentation was carried out with date syrup and sucrose syrup at 28°C and pH 6.8 in a rotary benmarin shaker (240 rpm). The results showed that EPS concentration increased with an increase in fermentation time with a maximum yield of 0.89 g/100 mL after 96 h which was much higher than that of the sucrose-containing medium (0.18 g/100 mL). The effect of pH on the yield of the purified xanthan was also examined. The optimum pH for xanthan production was determined to be 5.5. The xanthan obtained through this study was compared with commercial xanthan, which showed a very similar chemical composition that was confirmed by Thin Layer Chromatography (TLC) and Fourier Transform Infrared Spectroscopy (FTIR). The results revealed that date syrup has a good potential as a substrate for xanthan production.
Marceli Fernandes Silva et al., (2009) studied xanthan gum production using cheese whey as carbon source by two strains of *Xanthomonas campestris*. The maximum production of xanthan gum were observed after 72 h using cheese whey as sole carbon source, 0.1% (w/v) MgSO$_4$$\cdot$7H$_2$O and 2.0% (w/v) of K$_2$HPO$_4$, yielding approximately 25 g/L. This value is quite higher than some results presented in the literature using glucose as substrate. Although the production was similar for the two strains, chemical composition and ionic strength presented several differences in chemical characteristics between the polysaccharides produced.

Sandra Faria et al., (2010) investigated on xanthan gum Production using *Xanthomonas campestris pv. NRRL B-1459*, under controlled conditions with diluted sugar cane broth at different initial sucrose concentrations (15.0, 25.0, and 35.0 g L$^{-1}$). The kinetic models used in this study provided estimations of microbial growth, substrate consumption, and product formation, and therefore these parameters were quantified in the fermentation experiments. Higher yield of xanthan per amount of sucrose (0.58 g/L) and productivity (0.63 g/L h) were obtained using initial sucrose concentrations of 25.0 and 35.0 g/L, respectively. The models were used to predict the kinetic parameters for a medium containing an intermediate and a larger initial sucrose concentration (27.0 and 40.0 g/L). When tested experimentally, the measured fermentation parameters were in close agreement with the values predicted by the model that presented the best adjustment, demonstrating its validity.

Gilani et al., (2011), produced Xanthan gum from *Xanthomonas campestris* PTCC 1473 using cheese whey as carbon source. Maximum xanthan gum productions of 16.5 g/L were observed after 72 h using cheese whey as sole carbon source at agitation rate 550 rpm. This value is higher than some results presented in the literature using waste material as substrate. FTIR studies also carried out for further conformations of compatibility.

The production of xanthan gum from *Xanthomonas campasteris* using industrial semi defined medium by Enshasy et al., (2011) gave the following results. The maximum xanthan gum production of 19.9 g/L was achieved by excluding ammonium nitrate from the industrial medium and making new medium formulation composed of (sucrose, soybean meal, ammonium phosphate and magnesium sulphate). Further
optimization in the production process was achieved by transferring the process to 16-L bioreactor and cultivation under controlled pH condition. The maximal volumetric and specific xanthan production \( [Y_{PX}] \) obtained were 28 g/L and 11.06 g/g, respectively. Thus, the semi-defined medium formulation developed in this work could be better and alternatively used for large scale production process for xanthan production when compared to other published media in respect to yield and cost.

Savvides et al., (2012) reported that *Xanthomonas campestris* ATCC 13951 can produce xanthan gum by using whey as a growth medium, a by-product of dairy industry. Production of 28 g/L was obtained when partially hydrolysed b-lactamase was used, which proved to be one of the highest xanthan gum production reported so far. Preservation methods such as lyophilization, cryopreservation at various glycerol solution and temperatures have been examined. The results indicated that the best preservation method for the producing strain *Xanthomonas campestris* ATCC 13951 was the lyophilization. Taking into account that whey permeate is a low cost by-product of the dairy industry, the production of xanthan achieved under the studied conditions was considered very promising for industrial application.

Shang-Tian Yang et al., (1996) produced Xanthan Gum from *Xanthomonas campestris* immobilized in a novel, Centrifugal Packed-Bed Reactor (CPBR). The bioreactor was operated in repeated batch mode to study the feasibility and performance of long-term xanthan gum production using the immobilized cells in the fibrous bed. Consistent xanthan production rate and gum quality were obtained for eight consecutive batches studied during a total operation period of over 3 weeks. The volumetric xanthan productivity achieved in the reactor was ~ 1 g/L h based on the total liquid volume and ~ 3 g/L h based on the fibrous-bed volume. The high productivity in CPBR was attributed to the relatively high cell density, ~ 7 g/L, in the reactor. This was because of the relatively low cell viability (~ 60%) and limitation in oxygen transfer in CPBR, which can be improved by increasing the medium recirculation rate and the rotational speed of the fibrous matrix.

Rosalam Yang et al., (2008) made experimentations on xanthan gum production from *Xanthomonas campestris* DSMZ using glucose as a carbon source in an immobilised batch and a Continuous Recycled Packed Fibrous-bed Bioreactor-
Membrane (CRPBFBM). The immobilisation technique gave higher xanthan gum concentration of 20 g/L than the free moving cell without immobilisation of 18 g/L at the dilution rate of 1.44 d\(^{-1}\). The highest xanthan gum production rate of 0.475 g/L-h was achieved in a continuous recycled packed fibrous-bed bioreactor.

Seong D Yoo and Sarah W Harcum (1999) investigated xanthan gum production from *Xanthomonas campestris* using Waste Sugar Beet Pulp (WSBP) as a supplemental substrate. These results indicate that optimal cell growth might optimize WSBP degradation. Xanthan gum production from the WSBP supplemented cultures was significantly greater than the unsupplemented production medium. Based on a preliminary analysis, the use of WSBP for xanthan gum production has the potential to be a cost-effective supplemental substrate to produce non-food grade xanthan gum.

Kianoush Khosravi-Darani *et al.*, (2011), in their investigation on production of xanthan from date extract using bacterium *Xanthomonas campestris* PTCC1473 in submerged fermentation (SmF) found that the cell growth decreased with carbon source concentration up to 50 g/L. The highest cell growth and xanthan production were achieved at 40 g/L concentration of carbon source. The nitrogen source concentration did not cause a significant effect on cell growth but the highest concentration of xanthan was produced in 0.2 g/L of nitrogen source. The ratio of carbon to nitrogen content had a significant impact on xanthan production. In the optimum condition, maximum concentration of produced xanthan yield and productivity was seen at 11.2 g/L and 8.19 g/kg day, respectively.

Xanthan gum production from waste date using *Xanthomonas campestris* PTCC1473 in submerged fermentation was investigated by Moshaf *et al.*, (2011). They concluded that xanthan production increased with increasing level of phosphor. Low level of nitrogen led to higher xanthan production. Xanthan amount, increasing agitation had positive influence. The statistical model identified the optimum conditions nitrogen amount = 3.15 g/L, phosphor amount=5.03 g/L and agitation=394.8 rpm for xanthan. To model validation, experiments in optimum conditions for xanthan gum were carried out. The mean of result for xanthan was 6.72±0.26. The result was closed to the predicted value by using RSM.
Miroslav Stredansky and Elena Conti (1999) achieved xanthan polymer yields ranging from 32.9 to 57.1 g/L, as referred to the liquid volume impregnating the various substrates by solid state fermentation under optimized conditions from *Xanthomonas campestris*. The solid substrates used were agro-industry wastes or by-products, including spent malt grains, apple pomace, grape pomace, and citrus peels, which can be obtained at a very low cost. The products were analysed by NMR spectroscopy, revealing a composition consistent with that of commercial xanthan.

Vidhyalakshmi *et al.*, (2012) studied the production of xanthan by *Xanthomonas* species from agro-industrial waste in solid state fermentation. A dry weight of 2.9 g / 50 g of substrate when fermented by *Xanthomonas citri*, 2.87 g by standard strain *Xanthomonas campestris* (MTCC) 2286 and 1.5 g by *Xanthomonas oryzae* of xanthan were yielded from agro-industrial wastes by SSF. The recovered xanthan was checked for its purity and composition by chemical analysis and structural analysis by FTIR. TLC and HPLC confirmed the sugars in isolated xanthan while FTIR ensured the presence of uronic acids. This work emphasizes the possibility of using agricultural wastes as lower cost alternative substrates for xanthan production which is a widely used food additive.

2.7. Factors Affecting the Xanthan Production

2.7.1. 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 in the declining order is glucose, sucrose, maltose and soluble starch. The result is shown in Table 2.1.

Letisse et al. (2001) performed the fermentation using Xanthomonas campestris 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 fermentor. 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 Xanthomonas 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, and glucose was the second 10.8 g/L.

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2.7.2. 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 is 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 NH₄Cl or NaNO₃ (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, 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 being 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 that 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) as an economic source of nitrogen in the fermentation medium for xanthan production. The attainable amount of xanthan gum and its production rate in batch fermentor also seem to be affected by the amount of nitrogen source available at the beginning of the stationary phase (Pinches and Pallent, 1986).

2.7.3. 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 2.2). Many authors (Borges et al., 2008; Gumus et al., 2010; Psomas et al., 2007; Silva et al., 2009; Kersdorp 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 Xanthomonas 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.
### Table 2.2 Operational conditions used in making xanthan gum in different bioreactors

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Bioreactor</th>
<th>Speed N(rpm)</th>
<th>Aeration rate (vvm)</th>
<th>Volume (L)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>6.9</td>
<td>Bubble Column</td>
<td>500-900</td>
<td>0.3-0.6</td>
<td>3.60</td>
<td>Pons et al. (1990)</td>
</tr>
<tr>
<td>28</td>
<td>7.0</td>
<td>Plugging jet Reactor</td>
<td>-</td>
<td>0.33</td>
<td>100</td>
<td>Zaidi et al. (1991)</td>
</tr>
<tr>
<td>28</td>
<td>7.0</td>
<td>Airlift</td>
<td>-</td>
<td>7.4-54</td>
<td>50.0</td>
<td>Suh et al. (1992)</td>
</tr>
<tr>
<td>30</td>
<td>7.0</td>
<td>Batch</td>
<td>600</td>
<td>1</td>
<td>2.00</td>
<td>Psomas et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>Batch</td>
<td>200</td>
<td>1</td>
<td>2.00</td>
<td>Kurbanoglu and Kurbanoglu (2007)</td>
</tr>
<tr>
<td>28</td>
<td>5.0</td>
<td>-</td>
<td>300</td>
<td>3</td>
<td>3.00</td>
<td>Borges et al. (2008)</td>
</tr>
<tr>
<td>28</td>
<td>7.2</td>
<td>-</td>
<td>180</td>
<td>-</td>
<td>-</td>
<td>Silva et al. (2009)</td>
</tr>
<tr>
<td>30</td>
<td>7.0</td>
<td>Batch</td>
<td>200</td>
<td>0.5</td>
<td>5.00</td>
<td>Kersdup et al. (2009)</td>
</tr>
<tr>
<td>30</td>
<td>7.0</td>
<td>-</td>
<td>200</td>
<td>1</td>
<td>1.50</td>
<td>Gumus et al. (2010)</td>
</tr>
</tbody>
</table>

#### 2.7.4. Effect of pH

Most authors (Gumus et al., 2010; Kersdup et al., 2009; Psomas et al., 2007; Silva et al., 2009) agreed that neutral pH is the optimum value for growth of *Xanthomonas 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 ranging 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.

2.7.5. 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 the 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 Xanthomonas campestris pv pruni strain 101 in bioreactor systems. Their results showed that xanthan gum production was dependent on kLa (21.4 h^{-1}), with a maximum yield of 8.15 g/L at 300 rpm, 3 vvm and 54 h of fermentation.

Papagianni et al. (2001) studied the kinetics of growth and xanthan production by Xanthomonas campestris ATCC 1395 in batch culture in a laboratory fermentor 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 (200–300 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 kLa in Centrifugal Packed-Bed Reactor (CPBR) (0.018 s⁻¹) was higher than that of stirred tank reactor (0.005 s⁻¹).

The optimum fermentation time for xanthan gum production by Xanthomonas campestris (NRRL-B-1459) using 10% sugar beet molasses as a carbon source was studied by Marzieh Moosavi- Nasab et al., (2010). Maximum xanthan gum production in fermentation media (9.02 g/L) was observed after 4 days incubation at 25°C and 240 rpm agitation speed. A solution of 10% sucrose was used as a control medium. Results indicated that the optimum period for xanthan gum production in this condition was 4 days.

Selva Mohan and Babitha (2010), in their study on the effect of the nutrient factors for the production of xanthan polymer by Xanthomonas malvacearum isolated from infected cotton leaves showed the highest yield of polymer 0.621 kg of sucrose at 48 hours. Xanthan production and biomass with varying carbon source, nitrogen source, different pH and different temperature were investigated. Physical characterization of Xanthan was made using solubility study. Chemical characterization of Xanthan is Osazone test revealed crystal shaped structure on viewing under microscope.

Gomashe et al., (2013) optimized the xanthan gum production from Xanthomonas campestris. Their studies revealed that the production of Xanthan gum is influenced by different nitrogen sources. Xanthan production increased with the increased yeast extract concentration, probably due to facilitated nitrogen uptake. The
optimum pH was found to be 7.0 and sufficient supply of oxygen was needed for the Xanthan gum production.

Moshaf et al., (2014) studied the production of xanthan gum by Xanthomonas campestris PTCC1473 using the second grade date palm from an experimental Response Surface Methodology (RSM) coupled with a Central Composite Design (CCD). The optimum conditions for xanthan gum production were 3.15 g/L for nitrogen source, 5.03 g/L for phosphorous source, and 394.8 rpm for agitation rate. A higher yield of biomass production was obtained at 13.74 g/L for nitrogen source, 4.66 g/L for phosphorous source, and 387.42 rpm for agitation rate. In the next stage, scale-up from the shake flasks to the 1-L batch fermentors was carried. By using the optimum conditions for xanthan gum, the biomass and xanthan gum concentrations after 72h in three levels of air flow rate (0.5, 1 and 1.5 vvm) were obtained as 3.98, 5.31 and 6.04 g/L, and 11.32, 15.16 and 16.84 g/L, respectively. Overall, the second grade date palm seemed to exhibit promising properties that can open new pathways for the production of efficient and cost-effective xanthan gum.

Seyyed Vahid Niknezhad et al., (2015) optimized the production of xanthan gum with Xanthomonas campestris and Xanthomonas pelargonii using the carbon source cheese whey lactose. The highest xanthan concentration of 16.4 g/L was achieved at 65.2 g/L of cheese whey (39.1 g/L of lactose), 14.8 g/L of phosphate (KH₂PO₄), and 1.1 g/L of magnesium (MgSO₄·7H₂O) at 48 h of fermentation using Xanthomonas campestris. The corresponding optimum cheese whey, phosphate, and magnesium concentrations in cultures of Xanthomonas pelargonii were 80.0, 6.7, and 0.8 g/L, respectively, which resulted in a xanthan production of 12.8 g/L. The xanthan gum yield (g of xanthan / g of lactose) was 0.42 for X. campestris and 0.27 for X. pelargonii.

2.8. Recovery of Xanthan Gum

Xanthan gum is commercially produced by fermentation of glucose with Xanthomonas 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 fermentors. 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,
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 used, ≤ 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 caring 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 a 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 finely divided fibrous product.
2.9. 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 Xanthomonas 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 behaviour 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 Xanthomonas 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 cell mass (C_X), the product concentration (C_P) and the substrate concentration (C_S).

Gilani et al., (2011) investigated the effects of media temperature, agitation rate and molasses concentration on the yield of fermentation in xanthan gum production. The maximum production of xanthan gum of 17.1 g/L was achieved at 32°C, 500 rpm and media with 30 g/L of total sugar. In batch culture, several kinetic models for the biochemical reactions were extensively studied. The growth kinetic parameters were evaluated by unstructured model and derived from the related equations. Based on Malthus and Logistic rate equations, the maximum specific growth rate, μ_max, and initial cell dry weight, X_0, were defined. Luedeking-Piret and Modified Luedeking-Piret models were applied for the product formation and substrate consumption rates.

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 that 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. Xanthomonas 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 that 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.

\[ r_x \text{ } k = \frac{dC_x}{dt} \propto f \left( C_x, C_s, C_p, C_N, C_{O_2}, C_\xi \right) \]

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 \( C_\xi \) 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 is also known as the Verhulst–Pearl or so called the autonomous equation. The equation can be written as:

\[ \frac{dC_x}{dt} = \mu_mC_x \left( 1 - \frac{C_x}{C_{Xm}} \right) \]

(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:

\[
\frac{dC_x}{dt} = \mu_m C_x \left(1 - \frac{C_x}{C_{xm}}\right)^\theta
\]  

(3)

The relationship is valid as long as \((1 - \frac{C_x}{C_{xm}})^\theta\) in nonnegative, i.e., \(\theta > 0\). The constant
\(\theta\) could be defined as an index of inhibitory effects that accounts for the deviation of
growth from the exponential growth. For a very large \(\theta\), the generalized logistic
equation kinetic approaches the exponential growth equation:

\[
\frac{dC_x}{dt} = \mu_m C_x
\]  

(4)

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

\[
\frac{dC_x}{dt} = \mu_m \left(\frac{C_s}{K_s} + C_s\right) \left[\left(1 - \frac{C_x}{C_{xm}}\right) C_x\right]
\]  

(5)

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

\[
\frac{dC_s}{dt} = \frac{1}{Y_{ps}} \left(\frac{dC_P}{dt}\right)
\]  

(6)

\[
\frac{dC_N}{dt} = \frac{1}{Y_{Nx}} \left(\frac{dC_x}{dt}\right)
\]  

(7)
where $Y_{ps}$ is the product yield coefficient based on substrate, $Y_{xs}$ 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) is not suitable to represent the synthesis of xanthan gum. Garcia-Ochoa et al. (1995) have proposed the rates as follows:

$$\frac{dC_x}{dt} = K_NC_N C_x$$

$$\frac{dC_p}{dt} = K_p C_x C_x$$

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

$$\frac{dC_x}{dt} = \frac{K_x C_{xm}}{Y_x N} \left[ C_x \left(1 - \frac{C_x}{C_{xm}}\right)\right]$$

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

$$C_{xm} = C_{x0} + Y_{x/N} C_{N0}$$

$$\frac{dC_x}{dt} = K_x \left( \frac{C_{x0}}{Y_x N} + C_{N0}\right) C_x \left(1 - \frac{C_x}{C_{x0} + Y_x + C_{N0}}\right)$$

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

$$(dC_s)/dt = -m_s C_x \left[(1/Y_{s/x}) \right]((dC_x)/dt)$$
Dissolve oxygen is described by the following equation:

\[
\frac{dC_{O_2}}{dt} = k_L a (C_{O_2}^* - C_{O_2}) - \left( m_{O_2} C_x + \left( \frac{1}{Y_{O_2}} \right) \frac{dC_x}{dt} \right) (14)
\]

where the oxygen mass transfer coefficient was given as:

\[
k_L a = 3.08 \times 10^{-4} V_S^{0.43} N^{1.25} \mu^{-0.39} \quad (15)
\]

where \( N \) is the rotational speed of the stirrer, \( V_S \) is the air flow rate and \( \mu \) is the apparent viscosity.

So far from the unstructured kinetics models that 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 modification of the combination logistic and Monod kinetic given by Luong et al. (1998) (shown in Eq. (16)) which represents the cell growth kinetic of \textit{Alcaligenes eutrophus} sp., has described the effect of substrate concentration to the growth rate. The consideration of inhibition effects 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

\[
r_x = \frac{\mu_m C_s}{K_s} + C_s \left( 1 - \left( \frac{C_s}{C_{sm}} \right) \delta \right) C_x \quad (16)
\]

\[
r_x = \frac{\mu_m C_s}{K_s} + C_s \left( 1 - \left( \frac{C_s}{C_{sm}} \right) \theta \right) \quad (17)
\]
\[
\frac{dC_x}{dt} = k_x \left( \frac{C_{x0}}{Y_x \frac{N}{N}} + C_{N0} \right) C_x \left( 1 - \left( \frac{C_x}{C_{x0}} + Y_x + C_{N0} \right)^\theta \left( 1 - \frac{C_s}{C_{sm}} \right)^\delta \right) \]

(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.