Production of xanthan gum using a rotating annular reactor : an exploratory study
by Dinesh Venkata

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering
Montana State University


Abstract:
Production of xanthan gum by fermentation of glucose by Xanthomonas campestris NRRL B-1459 is studied in this research. Xanthan gum was produced in a rotating annular reactor (RAR), also referred to as rototorque, and for the purpose of comparison the fermentation was also performed in a conventional stirred tank reactor (STR). Experiments were performed in both batch and continuous modes, in batch mode, an average maximum cell growth of 3.88 g/l was achieved in the RAR, 33% higher than in the STR, while xanthan gum production was 18.23 g/l, which was higher than in the STR by 19%. There was no significant change in glucose consumption in the RAR compared to the STR. However, product yield (0.82 g/g) was higher by 43% and cell yield (0.31 g/g) decreased by 29%. No significant difference in specific cell growth rate was observed, hr a continuous mode of operation, the RAR outperformed the STR with respect to xanthan gum production, product yield, and specific xanthan production rate. Experiments were performed at dilution rates of 0.05 h^-1, 0.10 h^-1, and 0.15 h^-1. Cell growth in the RAR was 15% higher than in the STR at D = 0.10 h^-1 and was less at other dilution rates, while xanthan gum formation and yield were higher in the RAR for all dilution rates. Cell yield in the RAR was higher at D = 0.10 h^-1 and 0.15 h^-1, however, it was less at D = 0.05 h^-1.

The higher xanthan production in the RAR, both in batch and continuous operation, is thought to be due to the formation of biofilm, even though the cell concentration and product concentration in the biofilm itself was a small fraction of that in the bulk phase. There is a speculation that the cells in biofilm undergo some kind of phenotype change that enable them to produce xanthan gum profusely. It is also believed that detached cells from biofilm have better xanthan growth characteristics in the bulk phase than those cells that were always in bulk phase, either in a RAR or in a STR.

# PRODUCTION OF XANTHAN GUM USING A ROTATING ANNULAR REACTOR – AN EXPLORATORY STUDY

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APPROVAL

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Dinesh Venkata

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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# ABSTRACT

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# CHAPTER 1

## INTRODUCTION

The empirical observation that sugar cane or beet syrup (first observed in 1813) may assume a quasi-solid texture was interpreted by Pasteur in 1861 to be the result of microbial fermentations. The chemical analysis of the fermentation product established that it was dextran. The second exopolysaccharide substance (EPS) to become an industrial reality was xanthan gum. Xanthan gum, an extracellular polysaccharide substance, is commercially produced by fermentation with *Xanthomonas campestris* NRRL B-1459, on a medium containing glucose. The genus Xanthomonas is defined as a gram negative, phytopathogenic bacterium. It has been suggested that many xanthomonas species could well be regarded as a single species comprising special forms of one species adapted to particular hosts. Most xanthomonads produce xanthan gum. Raw xanthan gum is light tan in color. Xanthan monomer has a chemical formula of $C_{37}H_{54}O_{51}$ and the gum has molecular weight in the range of 2 - 50 * $10^6$ dalton.

Allen Jeanes pioneered the study of the production of xanthan gum from strains of *X. campestris* in the 1950's at Northern Regional Research Laboratories of the US Department of Agriculture (USDA), where an extensive screening program was conducted over a wide collection of microorganism cultures that could biosynthesize water-soluble gums of likely commercial importance. Extensive research on xanthan was carried out in several industrial labs during the early 1960's, culminating in commercial production in 1964. The most important and financially lucrative use of xanthan initially was a rheological agent in tertiary oil recovery. A further significant event in the

development of xanthan was the acceptance of the polymer as a food additive by US Food and Drug (USFD) in 1969.

There has been extensive research going on in America, Europe and Japan to discover new EPS, which would have performance superior to the existing popular EPS. Some people question the relevance of this research considering the time and money spent on such researches. Industries on the other hand have screened quite a few EPS and have either rejected the use of those EPS or have them as standby. Thus it is a good idea to try and improve or optimize the production methods of existing EPS, which would be financially more rewarding. The present industrial process for xanthan gum production is energy-intensive and costly, mainly because the highly viscous xanthan broth causes agitation and aeration to be difficult in conventional stirred-tank fermentors. Consequently, conventional xanthan gum fermentation has low xanthan concentrations and low productivity. Due to high production costs, xanthan gum loses some market to plant-derived polysaccharides and synthetic polymers. In order to reduce production costs and improve competitive the position of xanthan in food industry etc., the conventional fermentation methods or bioreactors need to be improved. There have been many attempts to increase xanthan productivity and to lower energy costs by using new agitation designs (Funahashi et al., 1987) and new types of bioreactors (Herbst et al., 1992). Fermentations with water-in-oil emulsion (Ju and Zhao, 1993) and cell immobilization using porous Celite beads (Robinson and Wang, 1988), which reduce broth viscosity and improves aeration and oxygen transfer, have also been studied. Although a higher xanthan concentration was achieved in these processes, separation and recovery of xanthan gum from the oil emulsion or Celite particles was difficult.

The xanthan gum solution develops a relatively high solution viscosity of xanthan solution at low concentrations of xanthan, which presents a major challenge in the agitation of xanthan broth during fermentation. Xanthan solution, however, shows a high degree of pseudoplasticity, i.e. the viscosity decreases with an increase in shear rates. This allows efficient pumping of xanthan polymer solution at high pumping rates. This is highly desirable in downstream operations of xanthan gum production and in petroleum industry where xanthan is added to water-based drilling fluids. The resulting pseudoplastic solution has low viscosity near drill bits, where shear stress is very high, allowing faster penetration. The other main reason for the high cost production is the cost of down-stream operations, such as in recovery of the product. In fact, drying the gum itself can cost as much as twice the cost of fermentation process.[1] There has been some research done in reducing the cost of the recovery operations, but there is still a lot to be done. Up to 50% of the total xanthan production cost are linked to the downstream operations.[2]

In this work, use of a reactor which enhances biofilm to produce the xanthan gum was accomplished. Biofilms were first envisioned as solely a problem until researchers started looking at the beneficial aspects. For example, biofilm reactors remove dissolved and particulate contaminants from the effluent wastewater streams, determine water quality, etc. Biofilm reactors are used in some common fermentation processes, such as production of 'quick vinegar', where wooden vats are packed with wooden chips and alcohol is trickled down which leads to the formation of biofilm of *Acetobacter spp.*[3] Biofilms are also used for industrial and domestic wastewater treatment using rotating discs with microbial growth. The discs rotate in a vertical plane, with discs dipping in

trough of water. Microbial growth is alternately in contact with nutrients and air. The beneficial aspects of the biofilms have spurred this research to explore the feasibility of using biofilms for the commercial production of xanthan gum from *Xanthomonas campestris*.

The bacterium *Xanthomonas Campestris* has been shown to form a stable, well-established biofilm.[4] The biofilm was grown in a rotating annular reactor (RAR), also called a Rototorque. It is suggested enhancement of the biofilm may offer advantages over conventional planktonic growth procedures, in that an established biofilm may reduce the planktonic cells, reduce solution viscosity and substrate consumption rate may be increased.[5] Elimination of suspended cells in the xanthan broth might enable one to get a cell-free xanthan gum, allowing it to be used directly in oil recovery application and also to efficiently concentrate the xanthan fermentation broth by ultrafiltration without significant fouling of the membrane caused by cells and resulting debris.[2] On the negative side, the separation of xanthan gum from a thick and highly viscous biofilm might be more difficult.

Kelly in preliminary work[4] showed that *X. campestris* NRRL B-1459 forms a stable biofilm. Taking inspiration from this effort, the author has endeavored to explore the feasibility of an alternative reactor for the production of xanthan gum by studying the cell growth rate of *X. campestris* NRRL B-1459, product formation and substrate consumption. A reactor with high centrifugal force will help enhance mass transfer between gas and liquid (Log et al., 1988; Mohr and Khan, 1987).

The objective of this research is to explore the technical feasibility of enhancing the formation of biofilms to enhance the production of xanthan gum using *X. campestris*

NRRL B-1459. For this purpose a RAR has been used to study the cell growth, substrate consumption, xanthan production, cell yield, product yield and productivity. Comparison of the RAR performance to the conventional STR will also be reported here.

# CHAPTER 2

# LITERATURE REVIEW

## Properties and Applications of Xanthan Gum

Xanthan gum has some unique properties including the formation of high viscosity solution even at low concentrations. This enables the engineer to control the rheological properties of aqueous solutions. These properties make it a preferred food additive and provide for its extensive use in the oil industry.

### Effect of temperature

Solutions of most of the polysaccharides decrease in viscosity as temperature increases. Solutions of xanthan gum in a salt-free system, however, increase in viscosity. In the presence of small amounts of salt, 0.1% NaCl, the viscosity of xanthan gum is virtually unaffected by the temperature from 25°F to 200°F.

### Effect of pH

In the presence of small amounts of salt, 0.1% NaCl, the viscosity of xanthan gum solution is independent of the pH over the range 1.5-13.

### Compatibility of xanthan gum solutions

Xanthan gum forms a stable solution with many acids and is compatible with most salts. The polyvalent metal ions make the xanthan solution unstable by causing gelation and precipitation of xanthan at high pH. Xanthan gum is resistant to attack from most enzymes. Xanthan solutions are very compatible with alcohols up to 60 vol% concentration, above this concentration the gum precipitates. Xanthan gum is insoluble in

most organic solvents, except formamide and ethylene glycol. Strong oxidizing agents degrade xanthan gum, as they do for any other gum.

Applications of Xanthan Gum

A combination of shear thinning property, thermal stability, salt stability, pH stability, thickening property, compatibility, pseudoplasticity, solubility in water, emulsifying properties, make xanthan gum very unique and is preferred in many applications. Some of them include petroleum,[26] textile, printing and dyeing, ceramic glazes, cleaners, slurry explosives, ink, paint, paper industries, pharmaceutical applications and widely used in food industry.

Although large amounts of xanthan are produced in the industrial scale, factors affecting its biosynthesis are not totally clear.[9] There have been empirical observations that indicate cell growth and xanthan gum production depends on substrate concentration, temperature, dissolved oxygen tension, agitation, pH, reactor type and strain of the bacterium. During fermentation of glucose by *Xanthomonas campestris*, 2 phases can be distinguished:

(i) Tropophase, in which there is fast cell growth but little xanthan formation.

(ii) Idiophase, in which no cell growth occurs but where large amounts of xanthan are produced.

Nutrients, also referred to as substrates, are substances used by organisms for metabolism. Nutrients can be broadly classified into (i) Essential nutrients (ii) Non-essential nutrients. Lack of essential nutrients hinders the growth, whereas lack of non-essential nutrients will not hinder the cell growth but the organisms would use them if available.

These essential and non-essential nutrients are available in the natural environment and are supplied in reactors. The nutrients can be supplied in various forms and the metabolic efficiency may vary with different forms. For example, the metabolic efficiency of glucose utilization is greater than $CO_2$[10] utilization. This is due to extra energy required to reduce the oxidation state of carbon in $CO_2$ to zero.

Care should be taken to supply the nutrients in right concentrations. Lack of sufficient nutrient would hinder the cell growth, however it does not mean the cell growth would be a maximum at very high nutrient concentrations. In fact, a very high nutrient concentration might poison the system.[11] Another important factor of nutrient that affects the cell growth is the way the nutrient medium is prepared. The nutrient broth has to be sterilized before it is available for the organism and it is a common practice to autoclave the nutrients. At those high temperatures in the presence of phosphate ion glucose forms substances that may be poisonous to some organisms.[11]

*X. campestris* cell growth depends on nitrogen concentration in the medium and xanthan production depends on the glucose concentration in the medium.[12] Rogovin et al.[12] and Flores et al.[13] have reported that the cell growth ceases as nitrogen concentration approaches zero, while xanthan production stops as the glucose concentration approaches zero. Prell et al.[14] reported a decrease in product concentration in cultivation at low nitrogen concentrations. There was a pronounced decrease in glucose concentration when the specific growth rate fell and the exponential phase passed into stationary phase. At the same time xanthan production was a maximum. They also observed that the utilization of the product occurred and the rate of glucose consumption increased simultaneously at low nitrogen concentrations after 70 hours. They explained this by proposing a possible

utilization of the polymer as a nitrogen source, because raw xanthan contains proteins. The inhibition of product occurred when concentration of nitrogen fell below 50mg/l.

Umashankar et al.[15] studied the influence of glucose concentration on the cell growth and concluded that cell density decreased for an initial glucose concentration less than 30g/l, and at higher glucose concentration substrate inhibition reduces cell growth. Other reports[12] have concluded that the optimum glucose concentration was between 25 and 30g/l. It was reported that approximately 25g of glucose per liter of medium was consumed regardless of the initial concentration. Umashankar et al.[15] in their studies have concluded that magnesium ions have an effect in activating sugar uptake, and the optimum concentration of magnesium for higher xanthan production and cell growth is 0.2g/l and 0.1g/l respectively. Lo et al.[16] have shown that it's not glucose concentration or nitrogen concentration alone that decides cell growth and xanthan production, but that ratio of glucose concentration to yeast extract (nitrogen source) concentration (G/YE ratio) is also critical. Higher G/YE ratios increased specific xanthan production rate but decreased cell growth. This was attributed to the lack of sufficient yeast extract concentration. They concluded that both yeast extract concentration and G/YE ratio are important in determining the fate of xanthan gum fermentation. To satisfy both factors they suggested a two-stage operation, with low G/YE ratio and moderate yeast extract in the first stage for higher cell growth and higher G/YE ratio in the second stage for higher xanthan production. Efforts to increase polymer concentration and glucose utilization by starting with 0.5 – 1.0% glucose concentration and then intermittently adding sterile glucose during fermentation resulted in the same yield and glucose utilization.[12] Some researchers,[17] on the other hand, have reported higher xanthan yield under nitrogen-

limited conditions. Roseiro et al.[9] performed fermentation under nitrogen-limited conditions and have noticed higher broth viscosity than during carbon limited conditions. Also, they noticed a decrease in xanthan production under potassium limited, magnesium limited and sulfur limited fermentation, while phosphorus limited cultures increased xanthan yield. They reported that specific xanthan production rate in stationary phase depends on the initial nitrogen concentration in the medium. However, according to Lo et al.,[16] the rate and yield parameter of xanthan production seemed to be independent of the initial nitrogen concentration.

Agitation is another factor that has a profound effect on xanthan fermentation. The fermentation broth changes from a low viscosity in the initial stages of fermentation process to a highly viscous pseudoplastic solution. Since fermentation by *X. campestris* is an aerobic process, oxygen transfer rate plays an important role in determining cell growth. Agitation should be high enough to ensure high oxygen and nutrient transfer to the cells, good mixing of contents, and no temperature gradients. Li et al.[18] noted that the oxygen transfer increases due to the presence small amounts of xanthan, which acts as surface-active agent, increasing the gas-liquid surface area until stationary phase is reached. After the stationary phase is reached, the oxygen transfer decreases due to the formation of large amounts of xanthan, which increases the apparent viscosity, thereby causing the bubbles to coalesce and reduce gas-liquid surface area. Umashankar et al.[19] have studied the influence of agitation (between 300 rpm and 500 rpm) in the fermentation process and have concluded that the optimum agitation speed is 500 rpm. At this speed turbulence is high enough to make the environment congenial for oxygen and

nutrient diffusion. The high shear rate helped in higher product and cell yields, higher amounts of xanthan and cell formation due to increased glucose consumption.

Other research[13] explains the influence of agitation by observing fermentation in mycelial cultures and suggesting the involvement of micromixing. They proposed that there might be certain fluid elements containing more than one cell circulating inside the bioreactor. These elements may be several orders of magnitude larger than individual cells and therefore, oxygen transfer from the bulk liquid phase through the boundary of element to each cell could become rate-limiting. Higher agitation speeds reduce the size of the elements, thereby increasing specific oxygen uptake rate.

Other researchers[20] have slightly different explanations. Specific xanthan production rate decreases continuously after the exponential growth phase. The decrease is expected because it's partly growth associated. However, they noticed, in many instances, there has been a continual decrease throughout the production phase, where biomass concentration was approximately constant. This could be due to the resistance to oxygen and nutrient diffusion through the polysaccharide slime layer over the cells. Increasing agitation increases the specific xanthan production rate. Since xanthan broth is pseudoplastic, the region close to the impeller experiences shear thinning, thus offering less resistance to diffusion. However, the region away from the impeller could be almost stagnant or moving in laminar way, effectively reducing diffusion causing rapid consumption of oxygen and resulting in oxygen limitation. Higher agitation also increases the volume of zones of significant motion (caverns) around the impellers, and thus decreasing the size of stagnant regions elsewhere. It was found that specific xanthan production rate is proportional to $C_v/T_v$ ratio where $C_v$ refers to the cavern volume and $T_v$

refers to the total broth volume. Therefore it is suggested that it is actually cavern size and oxygen levels, rather than RPM, which determine xanthan production. Nakajima et al.[21] suggest the use of twin impellers to increase the high shear zone, thereby increasing the specific xanthan production rate.

Sufficient oxygen transfer and absence of stagnant zones are crucial demands for achieving high productivity Yang et al.[2], Amanullah et al.,[20] Shu et al.[22] have suggested a minimum dissolved oxygen tension (DOT) of 20% to prevent any adverse effect on xanthan production caused by oxygen limitation. Higher DOT has been found to increase the xanthan production,[13] because under these conditions xanthan production started earlier during the growth phase.

Xanthan gum contains pyruvic acid as a part of a side chain in its molecule. The pyruvate is a highly oxidized component of xanthan. The quality of xanthan gum can be determined from the amount of pyruvate it contains. The polysaccharide is considered to have good quality (high pyruvate) if the pyruvic acid content is 4% or more, and the quality is considered poor if the gum has less than 3% pyruvic acid.[23] Since the pyruvate is a highly oxidized component of xanthan, deficiency of oxygen tension results in a lower concentration of pyruvate and thus forming lower quality xanthan gum. Peters et al.[24] reported a steep decrease in the degree of pyruvylation on xanthan side chains when the microbial oxygen demand was not met. There was no significant dependence on the growth rate. Flores et al.[13] have noted that higher dissolved oxygen concentration lead the bacterium to synthesize larger fractions of high molecular weight xanthan. Though the factors governing the degree of polymerization in xanthan gum biosynthesis is unknown, the observations indicate that dissolved oxygen concentration directly or indirectly has an

influence. In essence, higher dissolved oxygen concentration results in higher viscosity xanthan gum, which in turn enhances the shear-thinning behavior.

The other factor that affects the fermentation process is pH. Since protein configuration and activity are pH dependent, we expect cellular processes, reactions, and growth rates to depend on pH. There are different and opposing opinions about the role pH plays in xanthan fermentation. Organic acids are common contaminants in industrial broths, originating either from plant extracts or microbial activities in the broth. Roseiro et al.[25] have shown that presence of very small amounts of acid slightly decreased the cell growth compared to cultivations in the absence of the acid. Under those sublethal IBA concentrations, they noticed an increase in xanthan production rate, glucose consumption rate, specific xanthan production rate, and specific glucose consumption rate. On the other hand, Rogovin et al.[12] found an optimum pH centering around 7.2 and observed a decrease in product yield at slightly acidic conditions compared to slightly alkaline levels. Since xanthan fermentation is slightly acidogenic, the pH remains in the optimum range longer when initiated at somewhat alkaline pH. Conversely, when initiated at somewhat acidic pH, the acidity produced in the culture fluid drops the pH below the optimum value more rapidly. When the pH was not controlled, they noticed that the pH increased initially to about 7.5 followed by a decrease, due to the production of organic acids, to about 5.8.

There is unanimity about the effect of temperature on the xanthan fermentation. The literature[22] confirms the optimum temperature for cell growth as between 23°C and 25°C and for xanthan production as between 30°C and 33°C. Some researchers have done experiments on a 2-stage fermentation process, which helps achieving the optimal

temperatures for cell growth as well as xanthan production, while some researchers have performed experiments by a single stage process at 27°C that is a compromise between the two optimal temperatures. Shu et al.[22] have found that different temperatures yield gums varying in rheological properties (which depend upon the pyruvate content of xanthan polymer).

## Introduction to Biofilms

Microorganisms have evolved various adaptive mechanisms in response to changing environments. These adaptive responses from bacteria often result in the emergence of different phenotypes. Bacterial biofilms have been defined as adherent exopolysaccharide-embedded bacteria, or micro colonies, growing on surfaces.[6] Biofilms are biologically active matrices of cells and non-cellular material accumulated on solid surfaces[3]. Biofilm accumulation is the result of physical, chemical, and biological processes occurring in the system. Biofilms are one of the phenotypes bacteria are capable of expressing. Growth in biofilms has been shown to be the preferred mode of bacterial growth in nature as the sessile population exceeds that of planktonic biomass by 2-4 log units.[7] The mobile phase provides a mechanism for spread and colonization, while the adherent biofilms afford protection against protozoans, phages, and antibiotics in natural environments.[8] The two modes of growth complement each other.

The biofilm is formed by transport of microbes from the bulk liquid and subsequent attachment and growth. The transport could be by diffusion, sedimentation, convection, or cell motility. The conditions prevailing in the system decides which transport occurs. The conditions include temperature, pH, fluid dynamics, viscosity, etc. Conditions also

affect the longevity of the adsorption. The process of individual cells leaving the substratum is termed 'desorption'.

Surface chemistry affects the rate of non-specific adhesion of bacteria to inert surfaces. The substratum, growth condition, and physiology of the organism can affect the outcome of adhesion. The initial adhesion is usually described as a reversible phase involving the landing of the microorganism on the surface and the interaction between the specific receptor and bacterial adhesive. However, once the cells are secured on to the substratum, it becomes irreversible adhesion where Brownian motion ceases. Irreversible adhesion results in the formation of adherent bacteria on the surfaces. Growth of adherent bacteria on surfaces eventually leads to the formation of bacterial biofilms.

The surface upon which the biofilm accumulates has to be conditioned first, but it usually does not take much time[3]. Concentration of nutrients on the substratum due to adsorption enhances the microbial growth. However, adsorbed molecules can inhibit microbial adsorption[3]. The role of the conditioning film in microbial adsorption to a surface is still a mystery. The significance of the nature of the substratum vanishes once the substratum is covered by a thin biofilm. The production of EPS becomes an important factor in determining the attachment of cells or biofilm growth. Upon adsorption, the cells in the biofilm may undergo phenotype changes.

Another process called 'detachment' is important to understand biofilm processes. Detachment is the loss of biomass from the biofilm due to erosion by fluid, abrasion due to collision between suspended particles and biofilm, and sloughing which is loss of a significant thickness of biofilm. Microbial cells in the dispersed state act indistinguishably from the bulk liquid phase and have low sedimentation velocities,

requiring centrifugation for cell separation. On the other hand, biofilms have a number of features which (a) Facilitates cell separation (b) Leads to high cell concentration within the reactor (c) Affects overall rates of growth etc. (d) Allows continuous fermentation to be operated at dilution rates beyond normal wash-out flow rate (e) Allows batch fermentors to be operated in a drain – and – fill basis.

# CHAPTER 3

# EXPERIMENTAL METHODS

## Materials and Methods

Experiments were conducted in two types of reactors, a stirred tank reactor (STR) or chemostat, and a rotating annular reactor (RAR).

### Stirred Tank Reactor (STR)

#### Construction

The present stirred tank reactor is a reactor with an agitator, in which the concentration is essentially constant throughout at any given point of time. The STR here has a capacity of 1.85 l. It is equipped with a 3-blade marine propeller fixed to the lid of the reactor. The lid is fastened to the tank by flange. The lid has ports to supply air/oxygen and nutrient medium, to inoculate and to sample, and to pump out the fermentation broth. The reactor has ports that facilitate monitoring temperature, pH, dissolved oxygen concentration. The agitator has a motor on the top of the reactor that drives the agitator and a regulator controls the speed. The agitation completely mixed the contents of the reactor, as visually observed, justifying the assumption for a chemostat that the effluent concentration is same as the broth concentration in the reactor. The oxygen is sparged just below the agitator to maintain dissolved oxygen concentration above the limiting concentration. Cell kinetics can thus be studied as a separate problem.

The reactor is maintained at a constant temperature by placing it in a heat exchanging water bath. The surface area per unit volume is considerably smaller than in a RAR, so the biofilm growth on the walls of the reactor may be neglected. The STR can be operated in batch mode as well as continuous mode.

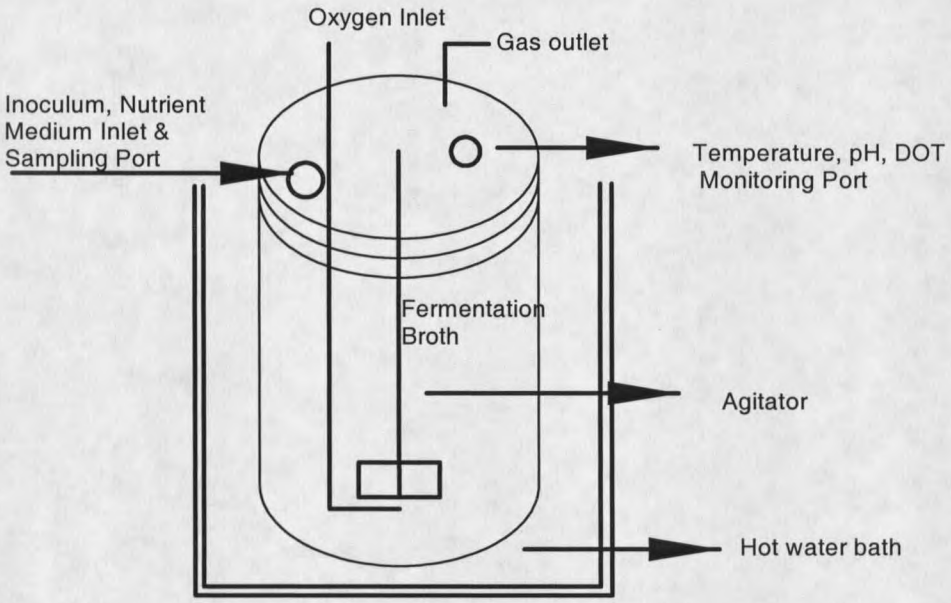



Figure 1 – Schematic Diagram of Stirred Tank Reactor

## Rotating Annular Reactor

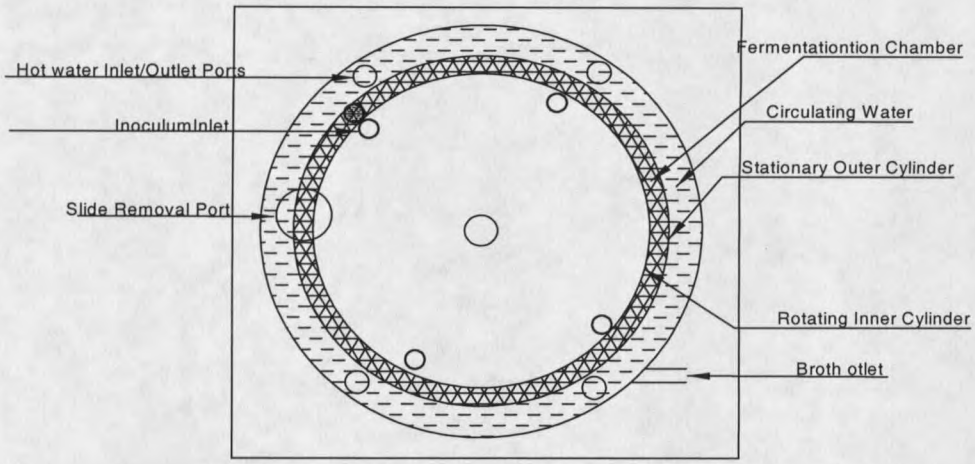
### Construction

A rotating annular reactor (RAR) has two concentric cylinders, one a rotating inner polycarbonate cylinder and the second a stationary outer glass cylinder. The inner, rotating cylinder has four draft tubes bore through it at an angle. These tubes cause mixing of the broth by virtue of centrifugal force. The drafts provide the necessary vertical mixing thus ensuring a uniform mixing without concentration gradients. It is assumed that the concentration of effluent is same as that inside the reactor. The peripheral surface of the inner cylinder has 20 polycarbonate slides, which can be removed for sampling. A direct coupling to motor drives the inner cylinder, while a regulator fixes RPM at a desired constant value manually. At constant RPM, the shear stress induced on the surface of the cylinder is constant. Centrifugal force has been used to enhance mass transfer between gas and liquid, to separate dextran from sucrose during enzymatic reaction and to achieve high-density cultivation of cells[2].

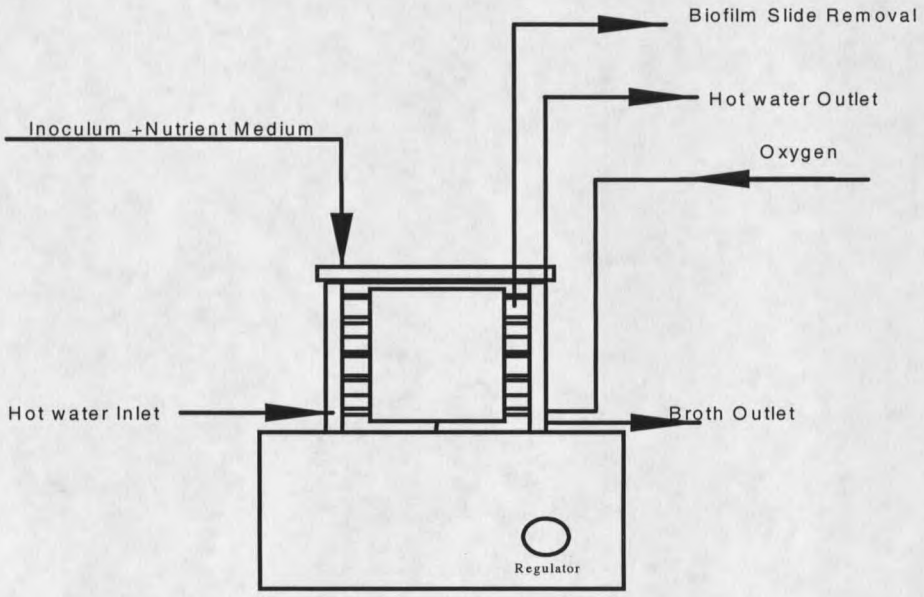
The inner wall of the stationary cylinder and the surface of the rotating cylinder are the areas considered for biofilm formation. Biofilm also grows on the top and bottom of the inner cylinder and the bottom (or floor) of the reactor, but have been neglected in the experimental analysis due to lack of proper sampling techniques and inconsistency of the biofilm. The total surface area considered is 1511 sq.cm. (707 sq.cm. on inner cylinder and 804 sq.cm. on outer cylinder).

The reactor has a port to pump out the fermentation broth, four process ports, and sampling port. There is no provision to monitor pH, temperature, and dissolved oxygen concentration. Those parameters are monitored by taking samples that can also be used for other analyses.

The RAR is provided with an outer annulus to control the temperature of the fermentation. The reaction compartment is isolated from the heating compartment by fastening the bolts on the lid tightly. The hot water is pumped from the bottom of the annulus and the overflow is recycled into the temperature bath reservoir. The RAR can be operated in both batch mode and continuous mode.




(a) RAR top view




(b) RAR front view

Figure 2. – Schematic Diagram of Rotating Annular Reactor (RAR)

Microorganism

The *Xanthomonas campestris* NRRL B-1459 strain used in this research project was obtained from the USDA, Permit 33098 (valid from 2/25/1997 to 12/21/1999). It was maintained in cryofreeze medium (2%Peptone + 20%Glycerol) at –70 °C. Storing *X. campestris* over a long period of time (about an year) does not deteriorate the ability to produce xanthan gum, nor does it affect product yield and viscosity change[32].

Nutrient Medium

Taking into consideration the facts furnished in the literature, the following nutrient medium was prepared and used. 17.5g/l glucose, 3g/l yeast extract, 2g/l $K_2HPO_4$, 0.1g/l $MgSO_4.7H_2O$ and tap water for micronutrients (Shu et al.[22] (Batch Process) except for glucose concentration and Silman et al.[33], (Continuous Process)).

To prepare the media, first a solution of yeast extract, $K_2HPO_4$, $MgSO_4.7H_2O$ was prepared. To avoid the Browning effect with glucose, a separate concentrated glucose solution was prepared. The pH of the extract solution was adjusted to 7 by adding HCl, and both solutions were then autoclaved at 120°C and 15 psig for 45 min. After autoclaving, both solutions were allowed to cool to room temperature and were mixed aseptically in a biohood.

Inoculum

Cells from a fresh culture of *Xanthomonas Campestris* NRRL B-1459 were put into 100 ml liquid medium. This solution was incubated for 24 h at 28 °C in an incubator-shaker. This solution was used to inoculate the fermentor.

Cleaning Procedures

Reactor

Stirred Tank Reactor: At the end of an experiment, Clorox® was added to the reactor with the broth and left for 2 h Then the reactor was disassembled and was rinsed with cold water. This was followed by soaking in Microcleaner® for 1 h and thorough scrubbing. The reactor was finally rinsed and air-dried. Also, before autoclaving, the reactor was thoroughly rinsed to clean it from dust.

Rotating annular reactor

After completion of an experiment the contents of the reactor were transferred to a flask through effluent tubing and the reactor was visually inspected for any irregularities. Clorox® was added to the reactor and the flask with culture and left for 2 h Then the flask and disassembled reactor were rinsed with cold water and soaked in Microcleaner® for 1 h Finally the parts were rinsed with water and air-dried. As with the STR, the RAR was rinsed thoroughly before autoclaving for next experiment.

Ancillary Fittings

All the tubing and other ancillary items were soaked in Microcleaner® for 1 h and scrubbed wherever necessary and possible, then rinsed thoroughly with water and air-dried.

Batch Operation - STR

To the autoclaved reactor, sterile nutrient medium is added and freshly prepared culture is used as inoculum. The fermentation broth is agitated at a constant speed and the temperature of the water bath is maintained constant. The oxygen is supplied at desired rate by passing it through a flow meter. The supply rate had to be increased manually (to keep the dissolved oxygen concentration greater than 40 vol.%) especially during the latter part of the fermentation when the viscosity of the broth is high thus decreasing the dissolution of oxygen. To avoid contamination of the culture, oxygen passes through a microfilter just before it enters the reactor. At regular intervals of around 6 h, samples are taken for analysis (as described later in Analytical Techniques section).

Continuous Operation - STR

Prior to continuous operation, the fermentation is conducted in a batch mode until the stationary growth phase is attained. Then the sterile nutrient medium is pumped from a carboy into the reactor and the fermentation broth is pumped out at the same rate. At regular intervals the broth that is being pumped out is used for analysis.