



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
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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 rotatorque, 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, in 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.

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MONTANA STATE UNIVERSITY
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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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TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES.....	ix
ABSTRACT	xii
1 INTRODUCTION.....	1
2 LITERATURE REVIEW.....	6
Properties And Applications of Xanthan Gum	6
Introduction to Biofilms	14
3 EXPERIMENTAL METHODS	17
Materials and Methods	17
Stirred Tank Reactor (STR)	17
Rotating Annular Reactor (RAR).....	19
Microorganism	22
Nutrient Medium	22
Inoculum.....	23
Cleaning Procedures.....	23
Batch Operation - STR.....	24
Continuous Operation - STR.....	24
Batch Operation – Rotating Annular Reactor	26
Continuous Operation – Rotating Annular Reactor	26
Analytical Techniques	28
Cell Dry Weight	28
Glucose Assay by Glucose Oxidase Method	28
Xanthan Concentration.....	29
Frozen Bacterial Stock	30
Scraping of Biofilm.....	30
4 MATHEMATICAL ANALYSIS.....	32

Assumptions in the mathematical models	32
Cells.....	34
Cell Growth.....	35
Batch Operation.....	36
Mathematical model for batch cultivation	36
Continuous Operation	41
Mathematical model for continuous cultivation.....	42
 5 RESULTS.....	 46
Batch Operation.....	46
Continuous Operation	54
 6 DISCUSSION AND CONCLUSIONS.....	 56
 7 RECOMMENDATIONS FOR FUTURE RESEARCH.....	 62
 NOMENCLATURE.....	 63
 REFERENCES CITED	 65
 APPENDICES.....	 70
Appendix A: Spectrophotometry and Centrifugation	71
Photometric Linearity Check	72
Glucose Concentration Calibration Curve	73
Centrifugation Test.....	75
Appendix B: STR Batch Operation Data	76
Appendix C: RAR Batch Operation Data	86
Appendix D: STR Continuous Operation Data.....	102
Appendix E: RAR Continuous Operation Data	120
Appendix F: Parameters in Batch Operation.....	144
Appendix G: Statistical Analysis	157

LIST OF TABLES

Table

1. STR Batch Operation: Experiment – 1(a)	49
2. STR Batch Operation: Experiment – 1(b)	50
3. RAR Batch Operation, Bulk Phase Experiment –1(a)	51
4. RAR Batch Operation, Bulk Phase Experiment –1(b)	52
5. RAR Batch Operation, Biofilm, Experiment –1(c)	52
6. STR Continuous Operation Data Analysis	55
7. RAR Continuous Operation Data Analysis	55
8. Summary of STR and RAR Batch Operation Data	60
9. Summary of STR and RAR Continuous Operation Data	61
10. Photometric Linearity Check	72
11. Glucose Calibration	73
12. STR Batch Operation: Experiment – 2(a)	80
13. STR Batch Operation: Experiment – 2(b)	81
14. STR Batch Operation: Experiment – 3(a)	83
15. STR Batch Operation: Experiment – 3(b)	84
16. RAR Batch Operation, Bulk Phase, Experiment – 2(a)	92
17. RAR Batch Operation, Bulk Phase, Experiment – 2(b)	93
18. RAR Batch Operation, Biofilm, Experiment – 2(c)	94
19. RAR Batch Operation, Bulk Phase, Experiment – 3(a)	97
20. RAR Batch Operation, Bulk Phase, Experiment – 3(b)	98
21. RAR Batch Operation, Biofilm, Experiment – 3(c)	99
22. STR Continuous Operation, $D=0.05/h^{-1}$	103
23. STR Continuous Operation Raw Data, $D=0.05h^{-1}$	104
24. STR Continuous Operation, $D=0.10h^{-1}$	107
25. STR Continuous Operation, Raw Data, $D=0.10h^{-1}$	108
26. STR Continuous Operation Data, $D=0.15h^{-1}$	111
27. STR Continuous Operation, Raw Data, $D=0.15h^{-1}$	112
28. RAR Continuous Operation, $D=0.05/h^{-1}$	121
29. RAR Continuous Operation, Raw Data, $D=0.05h^{-1}$	122
30. RAR Continuous Operation Data, $D=0.10h^{-1}$	127
31. RAR Continuous Operation, Raw Data, $D=0.10h^{-1}$	128
32. RAR Continuous Operation Data, $D=0.15h^{-1}$	133
33. RAR Continuous Operation, Raw Data, $D=0.15h^{-1}$	134

LIST OF FIGURES

Figure

1. Schematic Diagram of Stirred Tank Reactor (STR)	18
2. Schematic Diagram of Rotating Annular Reactor (RAR).....	21
3. Schematic Diagram of STR Batch Operation	25
4. Schematic Diagram of STR Continuous Operation	25
5. Schematic Diagram of RAR Batch Operation	27
6. Schematic Diagram of RAR Continuous Operation	27
7. STR Batch Operation, Experiment - 1	50
8. RAR Batch Operation, In Bulk Phase, Experiment – 1(a).....	53
9. RAR Batch Operation, In Biofilm, Experiment – 1(b)	53
10. Glucose Calibration Curve	74
11. STR Batch Data, Experiment - 2.....	82
12. STR Batch, Experimen - 3	85
13. RAR Batch Data, in Bulk Phase, Experiment – 2(a)	95
14. RAR Batch Operation, Experiment – 2(b)	96
15. RAR Batch Operation, Bulk Phase, Experiment – 3(a).....	100
16. RAR Batch Operation, in Biofilm, Experiment – 3(b)	101
17. STR Continuous Operation, $D=0.05h^{-1}$, Experiment - 1.....	105
18. STR Continuous Operation, $D=0.05h^{-1}$, Experiment- 2.....	106
19. STR Continuous Operation, $D=0.10h^{-1}$, Experiment - 1.....	109
20. STR Continuous Operation, $D=0.10h^{-1}$, Experiment - 2.....	110
21. STR Continuous Operation, $D=0.15h^{-1}$, Experiment - 1.....	113
22. STR Continuous Operation, $D=0.15h^{-1}$, Experiment - 2.....	114
23. STR Continuous Data Analysis, S' vs D	116
24. STR Continuous Data Analysis, For μ_{max} and K_s	117
25. STR Continuous Data Analysis, For K and K'	118
26. STR Continuous Data Analysis, For Y_p and Y_x	119
27. RAR Continuous Operation, $D=0.05h^{-1}$, Experiment – 1(a).....	120
28. RAR Continuous Operation, $D=0.05h^{-1}$, Experiment – 1(b)	124
29. RAR Continuous Operation, $D=0.05h^{-1}$, Experiment – 2(a).....	125
30. RAR Continuous Operation, $D=0.05h^{-1}$, Experiment – 2(b)	126
31. RAR Continuous Operation, $D=0.10h^{-1}$, Experiment – 1(a).....	129
32. RAR Continuous Operation, $D=0.10h^{-1}$, Experiment – 1(b)	130
33. RAR Continuous Operation, $D=0.10h^{-1}$, Experiment – 2(a).....	131
34. RAR Continuous Operation, $D=0.10h^{-1}$, Experiment – 2(b)	132
35. RAR Continuous Operation, $D=0.15h^{-1}$, Experiment – 1(a).....	135
36. RAR Continuous Operation, $D=0.15h^{-1}$, Experiment – 1(b)	136
37. RAR Continuous Operation, $D=0.15h^{-1}$, Experiment – 2(a).....	137
38. RAR Continuous Operation, $D=0.15h^{-1}$, Experiment – 2(b)	138
39. RAR Continuous Data Analysis, S' vs D	140
40. RAR Continuous Data Analysis, For μ_{max} and K_s	141

41. RAR Continuous Data Analysis, For K and K'	142
42. RAR Continuous Data Analysis, For Y_p and Y_x	143
43. STR Batch Operation, For Mu and K, Experiment - 1	145
44. STR Batch Operation, For Alpha, Experiment - 1	146
45. STR Batch Operation, For Mu and K, Experiment - 2	147
46. STR Batch Operation, For Alpha, Experiment - 2	148
47. STR Batch Operation, For Mu and K, Experiment - 3	149
48. STR Batch Operation, For Alpha, Experiment - 3	150
49. RAR Batch Operation, For Mu and K, Experiment - 1	151
50. RAR Batch Operation, For Alpha, Experiment - 1	152
51. RAR Batch Operation, For Mu and K, Experiment - 2	153
52. RAR Batch Operation, For Alpha, Experiment - 2	154
53. RAR Batch Operation, For Mu and K, Experiment - 3	155
54. RAR Batch Operation, For Alpha, Experiment - 3	156

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 rotatorque, 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. In 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.

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 $2 - 50 \times 10^6$ 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 work⁴ 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 reports¹² 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 research¹³ 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 researchers²⁰ 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 literature²² 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 surfaces³. 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 time³. Concentration of nutrients on the substratum due to adsorption enhances the microbial growth. However, adsorbed molecules can inhibit microbial adsorption³. 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.

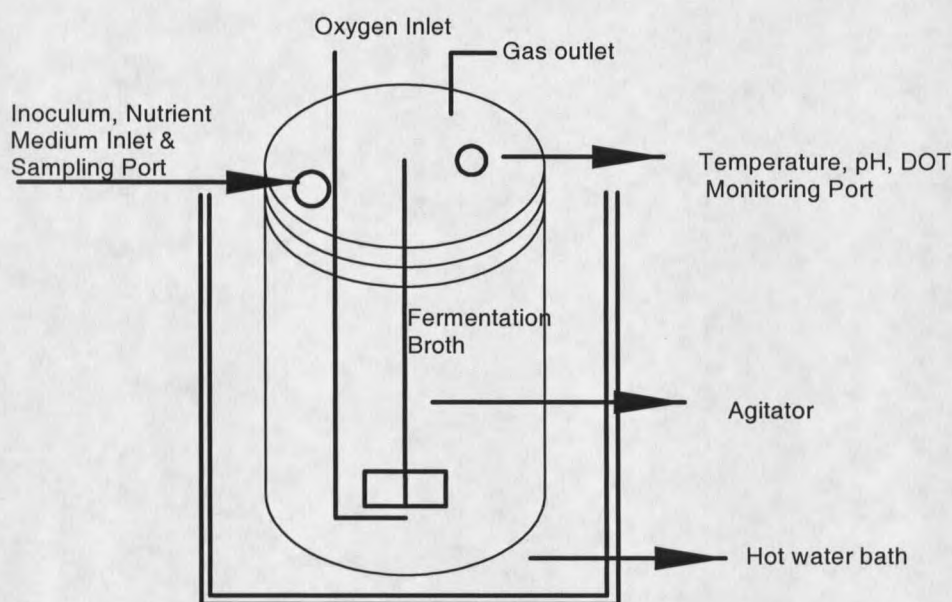


Figure 1 – Schematic Diagram of Stirred Tank Reactor

Rotating Annular Reactor

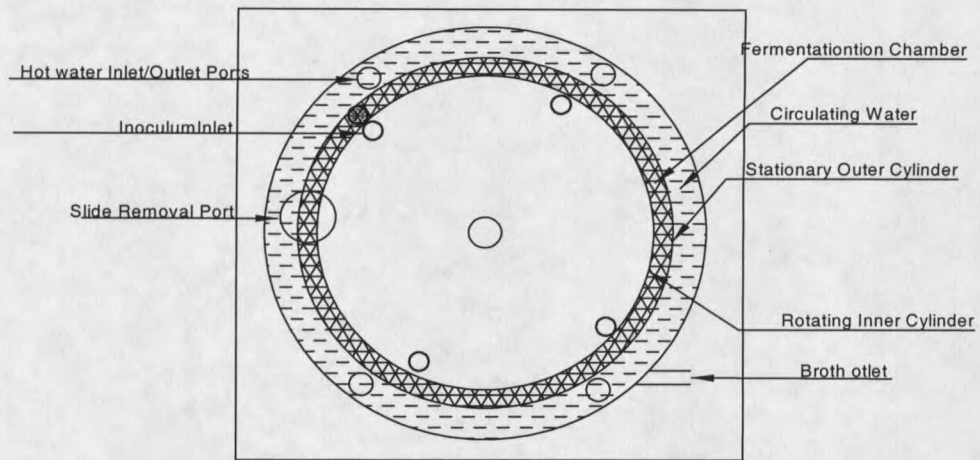
Construction

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 cells².

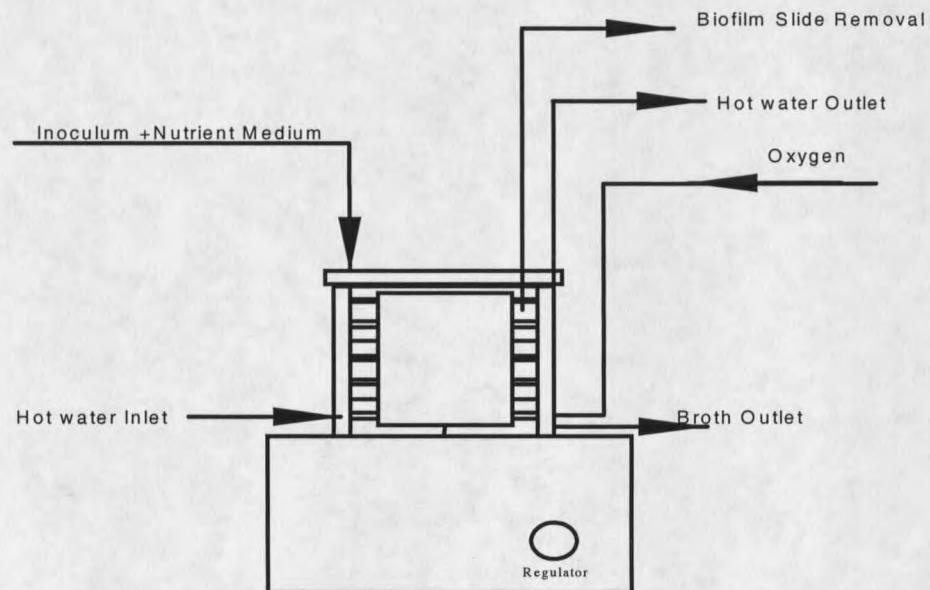
The inner wall of the stationary cylinder and the surface of the rotating cylinder are the areas considered for biofilm formation. Biofilm also grows on the top and bottom of the inner cylinder and the bottom (or floor) of the reactor, but have been neglected in the experimental analysis due to lack 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 change³².

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 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 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 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 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.

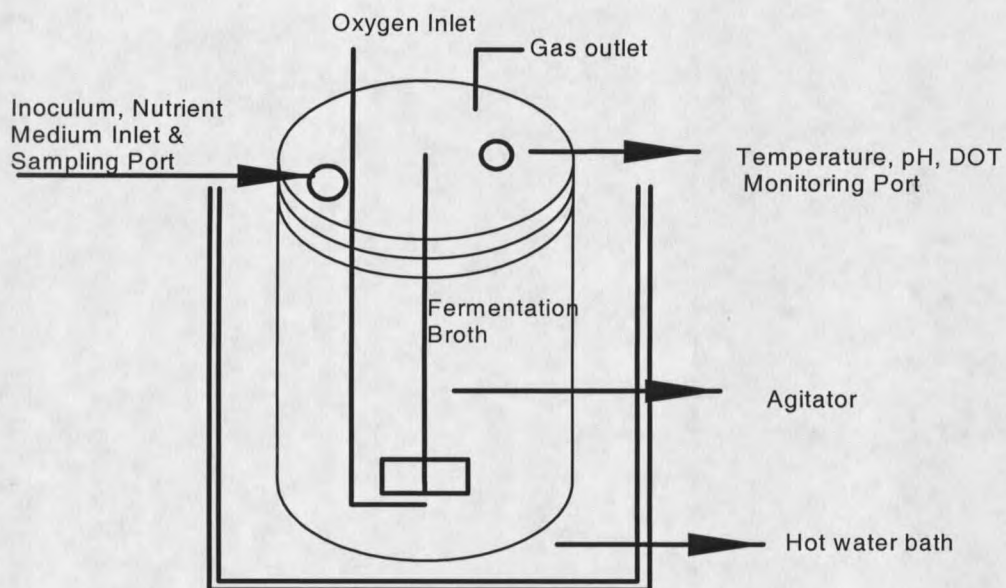


Figure – 3 Schematic Diagram of STR Batch Operation

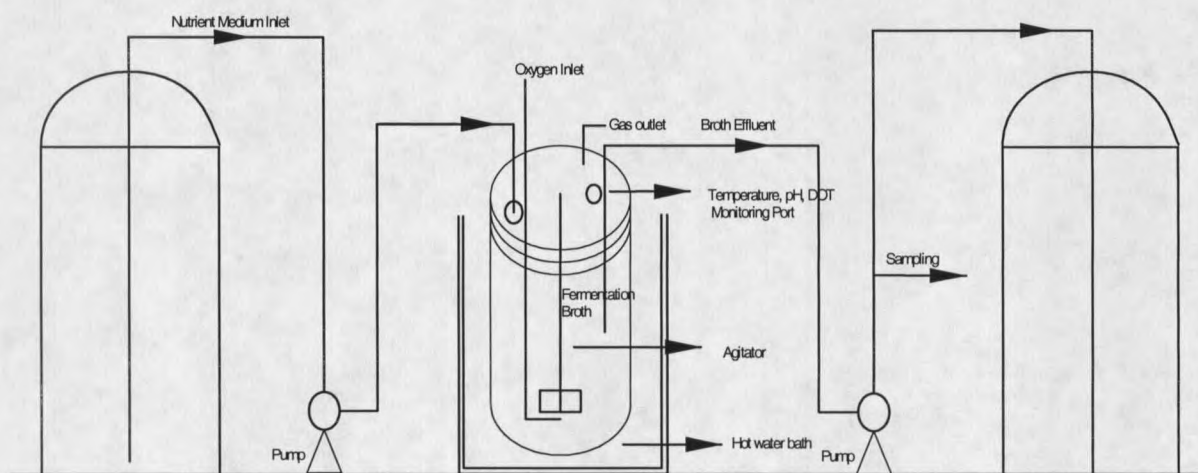


Figure – 4 Schematic Diagram of STR Continuous Operation

Batch Operation - Rotating annular reactor

The clean, washed reactor is first tested for leaks by running it for 8 h with water. The leaks, if any, are fixed and retested until there are no leaks. The reactor, along with tubing, is then autoclaved and cooled. Sterile medium is then aseptically added to the reactor, inoculated and O₂ is supplied. The fermentation is performed for a fixed amount of time, during which samples are taken through the effluent port at regular intervals for analysis.

Continuous Operation - rotating annular reactor

The continuous process is first run as batch process as detailed above until the stationary growth phase is reached, and then operation is switched over to continuous mode. The feed, sterile nutrient medium saturated with oxygen, is supplied from a carboy by a peristaltic pump and the fermentation broth is pumped out at the same rate, thus keeping the volume of the culture and pressure in the reactor constant. It is a single-stage, single-feed chemostat with no recycle of effluent. The effluent is collected in a carboy and is tapped from the effluent tube at regular intervals of time for analysis. At the same time a slide is pulled out to analyze biofilm. The experiments were performed until steady state of cell growth, xanthan production, and glucose consumption was achieved. Since *X. campestris* is a plant pathogen, the carboys containing cells from effluent were sterilized by steam heat before discarding.

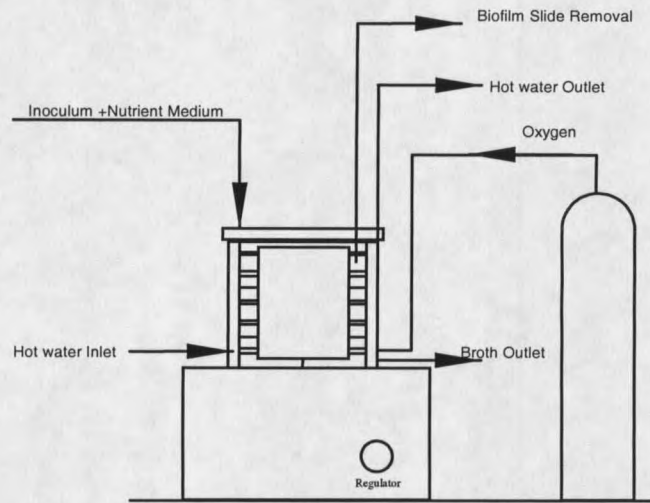


Figure – 5, Schematic Diagram of RAR Batch Operation

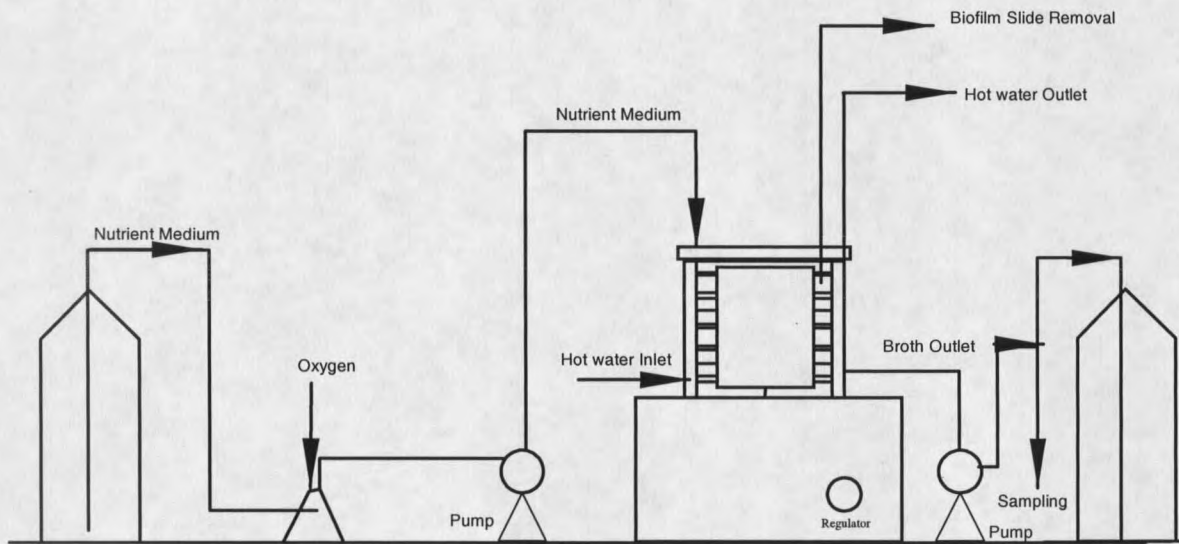


Figure – 6, Schematic Diagram of RAR Continuous Operation

Analytical Techniques

Cell Dry Weight

Weight was determined in duplicate (for continuous mode) and triplicate (for batch mode) by a gravimetric method. The culture broth was diluted 2:3 in 1 wt.% KCl solution, to reduce the viscosity and then freed from cells by centrifugation. The centrifugation was done for 45 min. at 13,000 rpm and 4 °C. After washing with water and recentrifugation for 45 min at 13,000 rpm, the deposit of cells was dried for 12 h at 100 °C. The dry cell weight was then determined for the sample, which was used to determine the cell concentration in the fermentation broth. The same procedure is employed to find cell density in biofilm, only in the case of biofilm the centrifugation is done in a microcentrifuge.

Glucose Assay by Glucose Oxidase Method

The Glucose oxidase kit (Sigma Diagnostics, Procedure No. 510) comes with PGO Enzymes, o-dianisidine dihydrochloride, glucose std. solution, enzyme solution and zinc sulfate solution.

Enzyme solution was prepared by adding contents of a capsule of PGO Enzymes to 100 ml distilled water and gently shaken till it dissolves. Color reagent solution is prepared by adding 20 ml distilled water to the vial of o-dianisidine dihydrochloride. Color reagent solution (1.6 ml) was added to 100 ml enzyme solution to make the combined enzyme-color reagent solution. The solutions were stored at appropriate temperatures.

The glucose assay was performed by first labeling the cuvettes as Test 1, Test 2, etc., Blank, and Standard. Each of the test samples was diluted 1:100, and the glucose standard solution was diluted 1:20. One half (0.5) ml of each test sample and 0.5 ml of standard solution was pipetted into respective cuvettes. To the Blank 0.5 ml water was added. To each of the cuvettes 5 ml combined enzyme-color reagent solution was added. The tubes were incubated at room temperature for 45 min. and the absorbance was compared with a calibration curve to get the concentration of glucose in the samples (Figure 10).

Xanthan Concentration

Xanthan Conc. in the fermentation broth was estimated from the supernatant of centrifuged fermentation broth. About 90 ml of isopropyl alcohol (suggested by Galindo et al.³⁴) was added to 30 ml cell free supernatant. Also, 5% of the total volume of saturated KCl solution was added to the solution to precipitate the gum. KCl reduces the viscosity and helps the polysaccharide to precipitate effectively, and KCl has good solubility in alcohol, which is used to precipitate xanthan gum in later stages. The solution was centrifuged at 13,000 rpm for 45 min. at 4°C. The precipitate was washed with water and recentrifuged for 45 min. at 13,000 rpm. The precipitate was dried at 110°C for 24 h. The measured dry wt. was then used to estimate the xanthan concentration in the fermentation broth. The same procedure is used to for estimating the xanthan concentration in biofilm except that in this case centrifugation is done in microcentrifuge.

Frozen Bacterial Stock

A 200 ml agar solution of following composition was prepared, 10 g/l glucose, 3 g/l beef extract, 5 g/l peptone and 15 g/l agar. The solution was initially boiled and then autoclaved for 15 min. at 121 °C and 15 psig. This agar solution was poured in to 6 petri dishes and let it solidify. A transfer loop was sterilized by inserting it into the blue area of bunsen flame. A visible amount of bacterial culture was pulled out with the loop and inoculated on to the agar. Care was taken to avoid aerosol formation by sputtering bacteria. The inoculum (original stock) was spread onto the prepared agar plates. The plates were labeled and sealed with parafilm. The plates were incubated at 27 °C for 24 h.

In the meantime, 100 ml of cryofreeze medium was prepared of following composition, 2% Peptone, 20% Glycerol in water. The cryofreeze medium was autoclaved at 121 °C and 15 psig for 10 min. and let cool. Approximately 6-7 ml of the sterile medium was aseptically pipetted onto the confluent bacteria and the culture was gently teased up using a sterile loop, to loosen it up from the agar. The plates were tilted, and aliquots were pipetted using sterile tip. 1ml was placed in each of the cryovials and the vials were capped tightly and stored at -40 °C.

Scraping of Biofilm

The lab bench was disinfected with 95% ethanol. Using a sterile hook a slide from the RAR is removed quickly and the reactor is tightly sealed. A sterile applicator stick (policeman) is then used to scrape the biofilm from the slide into 9 ml of sterile buffered water. After sufficient scraping, the slide is rinsed with 1ml of buffered water.

This solution is microcentrifuged at 13,000 rpm for 45 min. and supernatant is preserved and the vials are dried at 100 °C for 24 h. and weighed. Weight of the empty vials (determined prior to the centrifugation) was subtracted to find weight of cells formed.

The supernatant is diluted with isopropanol (3 volumes) to precipitate xanthan gum, and 30% KCl is added to reduce the viscosity. The solution is shaken thoroughly, and 4ml are pipetted into four 1 ml vials and microcentrifuged at 13,000 rpm for 45 min. The vials are dried at 100 °C for 24 h and weight of the empty vials is deducted from the cumulative weight to give weight of xanthan produced.

CHAPTER 4

MATHEMATICAL ANALYSIS

Assumptions in the Mathematical Models

Each individual cell is a complicated multi-component system, which is frequently not homogeneous even at the cell level¹¹. Within the cell there are many complex chemical reactions, depending on the environment. The cells might undergo spontaneous mutation, which produces cells that may then differ physiologically, morphologically and even genetically. Sometimes the reactor itself might influence the drift in genetic makeup. In addition, the different cells at any given point of time and region might exhibit heterogeneity in size, age, and chemical activity. Different types of metabolic functions and activities characterize cells of different age. These complex sets of features make it generally impossible or impractical to come up with a complete kinetic model incorporating those parameters. Thus it is necessary to make reasonable assumptions and simplify cell population kinetics.

In biochemical engineering it is common practice to prepare a nutrient medium that is rich in all nutrients but one, the rate limiting nutrient. All other nutrients are supplied in enough quantities that changes in their concentrations do not significantly affect the overall rates. This simplifies the analysis, since only the rate-limiting nutrient has to be considered in analyzing the effect of nutrient medium composition. In the present research the rate-limiting nutrient was carbon, in the form of glucose.

Life is segregated into structurally and functionally discrete units i.e. cells³. Thus it is imperative to know the number of individual cells present in the culture. At the same time, the amount of biomass is necessary for application of conservation equations, specifically conservation of mass. The number of cells in a culture is not directly related to biomass since the culture might be heterogeneous. Despite these facts, it is common practice in the engineering field to assume that biomass is distributed uniformly throughout the culture and segregation of life into discrete units can be ignored. Models based on this assumption are called "distributed" or "non-segregated model". This assumption is usually justified by assuming that biomass is directly related to the number of cells. In other words growth is proportional to proliferation, a situation referred to as "balanced growth". A non-segregated model has been adopted in this research.

Two organisms, exhibiting the same biomass and inhabiting the same environment, may have widely different properties and activities³. A segregated model recognizes this distribution of states while the non-segregated model does not. However, the non-segregated model recognizes the average states and regards the population structureless. In other words, a heterogeneous collection of cells is viewed as an average cell that represents the whole culture, like a component in a solution. This kind of representation is called "unstructured model" and is used in the present research. To use a non-segregated model, one has to assume the following: as it is impossible to predict the behavior of individual cells with certainty³, stochastic phenomena can be neglected and the growth can be treated as deterministic process when there is large number of cells per unit volume. This is called a "deterministic model" and is adopted here, so all the above

simplifying assumptions make the cells behave in a similar manner under similar conditions.

In the growth phase of a batch reactor system it is assumed that numerous factors, including agitation, viscosity and substrate diffusibility, which can influence growth through physical aspects are not limiting in determining kinetic parameters.

Cells

Bacterial cells reproduce by binary fission. The rate of reproduction depends on a number of factors including nutrient medium, pH, temperature, etc. The medium here has an excess of all nutrients except for glucose as a growth-limiting concentration. The specific growth rate will at first be maximal, but upon depletion of the limiting nutrient the kinetics will rapidly decline. Monod's equation describes the relation between specific growth rate and the limiting substrate concentration.

$$\mu = \mu_{\max} * S/(K_s + S) \text{ ----- (1)}$$

where, μ = Cellular specific growth rate (1/t)

$\mu_{\max}$ = Maximum cellular growth rate (1/t) at which cells can reproduce at a saturated condition of limiting nutrient

S = Limiting nutrient concentration (mass/volume)

K_s = Saturation coefficient (mass/volume)

Eq. (1) can be linearized to give,

$$S/\mu = S/\mu_{\max} + K_s/\mu_{\max} \text{ ----- (1b)}$$

Cell Growth

The concentration of cells in a reactor varies with time and can be divided into four phases, lag phase, exponential phase, stationary phase, and death phase.

Lag phase is the phase during which the cells are dormant, or are not multiplying. During this phase the cells adjust themselves to the new environment. An inoculum is a small size batch culture. When it is added to the fermentor, the cells in the inoculum encounter a new and different environment, which might lack some enzymes, vitamins, and ions, like cofactors. These highly permeable molecules and ions diffuse through the cell membrane into the medium. This causes deficiency of the requisites and the cells stop growing until those requisites are regenerated. This is just one of the several mechanisms which can lead to a lag phase. Other examples include the shock that cells experience when the inoculum is added to a fermentor with nutrient concentration, pH, temperature, etc., different from its native environment.

To reduce the length of lag phase in this research the culture medium used to grow the inoculum was the same as that used for full-scale fermentation. To avoid undue loss of required intermediates or activators, a large inoculum (10% of nutrient medium volume) was used.

At the end of lag phase the cell population becomes adapted to the new environment and starts multiplying exponentially. This phase is called an exponential phase. The growth is balanced in this stage of batch cultivation¹¹. This phase continues until the limiting nutrient gets exhausted or the toxin accumulation is high enough to reduce

growth. As the cells multiply exponentially, the nutrient is depleted sharply and EPS production increases very sharply.

The exponential growth starts to wane as the limiting nutrient drops closer to the limiting concentration. At some point of concentration of toxins the death rate equal growth rate, and remains that way for some time. This stage is called stationary phase. During this phase the living cells prey upon the components freed from the lysed cells. This helps maintain the population size until the nutrient concentration is so low or toxin concentration is so high that the population cannot sustain itself, and the death phase begins. Most industrial processes are terminated before the death phase is reached, and hence is not relevant to this research.

Batch Operation

Batch operation is usually preferred when producing small quantities of material. This kind of operation is very useful when rapid contamination of fermentation cultures is to be avoided, because it facilitates easy cleaning and sanitation procedures. The disadvantage of batch reactors is that the sometimes the sum emptying, cleaning and filling time is comparable to time necessary to carry out the process. In such situations continuous operation is used.

Mathematical Model for Batch Cultivation

Several mathematical models have been proposed by various researchers to describe batch kinetics of xanthan gum production. Constantinides et al.²⁷ proposed a logistic equation for cell growth and three different models for product formation. The first equation proposed did not fit the data of Moraine and Rogovin,²⁸ and the second and third

equations integrate to give relations that are not convenient to evaluate without knowing the constants a priori. Edwards et al.²⁹ and Kono³⁰ proposed models, but could not accurately determine the fermentation process.

The author of this report chose the model developed by Weiss et al.³¹ for its simplicity, convenience and ease by which the parameters could be evaluated. They have utilized an autonomous (logistic) biomass rate equation to evaluate the model parameters in sequential manner rather than simultaneous manner, which would require extensive computer techniques for parameter estimation.

The model employs rate equations for biomass (X), product (P) and substrate (S) to describe the fermentation process. For biomass the logistic rate equation is used³:

$$dX/dt = f(X) = \mu_{\max} X (1 - X/X_{\max}) \text{ ----- (2)}$$

Xanthan formation has been proven to be both growth related and non-growth related (GANG). In these kinds of processes, the main product appears as a result of primary energy metabolism and may even result from direct oxidation of carbohydrate substrate such as glucose. Also in these processes metabolic rate (e.g. cell synthesis, product formation, etc.) exhibits similar dependence to other rates, which explains the proportionality between product formation rate (R_p) and specific growth rate, μ , in the Luedeking-Piret equation,

$$R_p = dP/dt = K'X + K dX/dt \text{ ----- (3)}$$

where the first term on right hand side of the equation is a nongrowth-associated term and second term is a growth-associated term. K (Growth-associated coefficient) and K' (Nongrowth-associated coefficient) may depend upon fermentation process conditions (pH, temperature, etc) as well as microbial strains.

The substrate consumption equation is shown below to be a modified form of the Luedeking-Piret equation. The substrate consumption equation is taken to depend on the magnitudes of three sink terms: the instantaneous biomass growth, product formation and a biomass maintenance function. The assumed kinetic form is a linear combination of these sinks:

$$dS/dt = -1/Y_x dX/dt - 1/Y_p dP/dt - K_m X \text{ ----- (4)}$$

Using equation (3), the previous equation becomes,

$$dS/dt = -(1/Y_x + K/Y_p)dX/dt - (K'/Y_p + K_m)X \text{ ----- (5)}$$

Or,

$$dS/dt = \alpha dX/dt - \beta X \text{ ----- (5a)}$$

$$\text{where, } \alpha = -(1/Y_x + K/Y_p) \text{ ----- (5b)}$$

$$\text{and } \beta = K'/Y_p + K_m \text{ ----- (5c)}$$

The parameters listed in equations (2) – (5) are evaluated sequentially as follows:

Integrate Eq. (2) with initial cell concentration as X_0 and final cell concentration as $X_{\max}$ (in stationary phase). This results in a sigmoidal variation of X that represents both the exponential and stationary phases, over which most xanthan gum is produced.

$$X = X_o e^{\mu t} / (1 - X_o/X_{\max} (1 - e^{\mu t})) \text{ ----- (6)}$$

To evaluate μ an undesirable graphical differentiation of batch data could be required. Instead the previous equation can be rearranged into a convenient form to produce

$$\mu t = \ln(X_{\max}/X_o - 1) + \ln(X / (X_{\max} - X)) \text{ ----- (7)}$$

The value $X_{\max}$ is evident from a completed fermentation. A plot of $\ln(X / (X_{\max} - X))$ against time, t , will yield a straight line of slope μ and y-intercept equal to $-\ln((X_{\max}/X_o) - 1)$, from which the initial viable inoculum size X_o can be found.

For product formation (Eq.(3)), the parameter K' is evaluated from dP/dt data in the stationary phase, where $dX/dt = 0$ and $X = X_{\max}$, and from Eq. (3), $K' = (dP/dt)_{\text{stat}}/X_{\max}$. Integration of Eq. (3) (with known K') yields the evolution of P in time:

$$P = P_o + KX_o (e^{\mu t}/(1 - (X_o/X_{\max})(1 - e^{\mu t})) - 1) + K' X_{\max}/\mu \ln(1 - X_o/X_{\max}(1 - e^{\mu t})) \text{ ----- (8)}$$

where P_o = Initial product concentration

Rearrangement of Eq.(8) gives

$$P - P_o - K' X_{\max}/\mu \ln(1 - X_o/X_{\max}(1 - e^{\mu t})) = K X_o (e^{\mu t}/(1 - (X_o/X_{\max})(1 - e^{\mu t})) - 1) \text{ ----- (8a)}$$

$$\text{i.e. } g(P, P_o, K', X_{\max}, X_o, \mu, t) = K f(X_{\max}, X_o, \mu, t) \text{ ----- (8b)}$$

Given all the values in the parenthesis on both sides of the equation, K can be found by plotting the function 'g' against the function 'f' and finding the slope of the straight line.

From the substrate rate equation, Eq. (5), the last parameters to be evaluated are α and β . In the stationary phase we again evaluate one parameter, β , from an experimental slope of the plot between $(dS/dt)_{\text{stat}}$ and X:

$$\beta = (dS/dt)_{\text{stat}}/X_{\text{max}} \text{ ----- (9)}$$

Integration of Eq. (5) gives,

$$S_0 - S - \alpha(X - X_0) = \beta X_{\text{max}}/\mu \ln(1 - X_0/X_{\text{max}} (1 - e^{\mu t})) \text{ ----- (10)}$$

or

$$S_0 - S - \beta X_{\text{max}}/\mu \ln(1 - X_0/X_{\text{max}} (1 - e^{\mu t})) = \alpha(X - X_0) \text{ ----- (10a)}$$

where S_0 is the initial substrate concentration.

The right hand side of the above equation signifies that substrate may continue to diminish even when cell growth plateaus, due to product formation and to cell maintenance.

Equation (10) can be restated as,

$$g'(X_{\text{max}}, \mu, X_0, \beta, S_0, S, t) = \alpha f'(X, X_0) \text{ ----- (10b)}$$

so that a plot of f' vs. g' from the batch data yields the slope α .

This method of sequential evaluation helps find seven parameters viz. X_o , X_{max} , μ , K , K' , α , and β in Eq. (2) – (5) from the batch fermentation data. This procedure helped avoid the cumbersome iterative techniques.

Continuous Operation

It's theoretically possible to devise a continuous pipe reactor such that the whole process from primary inoculation to final stationary phase takes place within its length. In view of the time most normal fermentations take, the pipe would, however, be inconveniently long. Continuous stirred tank reactors solve this problem. Continuous stirred tank reactors or chemostats are open systems that can maintain cells growing at a specific growth rate under steady state condition. Mathematical analysis of such a continuous fermentor is easier if the following assumptions are made: The growth rate of the microorganism can be held constant and usually directly controlled by the feed rate of a major nutrient. Only a single strain of microorganism is present, which maintains its growth characteristics, particularly rate and cell size, throughout time.

Due to the supply of fresh medium, growth does not stop completely, but continues at a rate determined by flow rate (F) in a constant volume (V) reactor, or dilution rate D , defined as ratio of influent (or effluent) flow rate to volume of the reactor (F/V). In continuous cultivation of microorganisms at steady state there is a balance between growth based on in-flow of fresh medium and broth washout. Cell and product formation are controlled by dilution rate, which determines limiting nutrient concentration. The continuous cultures are usually expressed by productivity curves, where the rates of

formation are plotted against dilution rates (D). The profiles typically rise to a peak, and then any increase in D reduces the productivity.

Mathematical Model for Continuous Cultivation

Cell growth

A mass balance across the chemostat for cell mass is:

$$\begin{aligned} \text{(Rate of cell mass accumulation)} = & \text{(Rate of cell mass in influent)} \\ & - \text{(Rate of cell mass in effluent)} \\ & + \text{(Rate of cell growth)} \end{aligned}$$

This then can be written as,

$$V \frac{dX}{dt} = F(X_i - X) + \mu XV \text{ ----- (11)}$$

At steady state cell accumulation equals zero, and since the influent feed is cell-free, X_i equals zero. Therefore the above equation becomes,

$$FX' = \mu XV, \text{ ----- (12)}$$

where X' = Steady state cell density

Dividing both sides by V and noting F/V equals the dilution rate, D , the above equation yields,

$$D = \mu \text{ ----- (13)}$$

This means at steady state the cell growth equals the dilution rate. Therefore, the combined effect of growth and dilution leads to a steady state in which $D = \mu$.

Using this result from Eq. (13) in the linearized form of Monod's equation one obtains

$$S/D = S/\mu_{\max} + K_s/\mu_{\max} \text{ ----- (14)}$$

A plot of S/D vs. S results in a straight line of slope equal to $1/\mu_{\max}$ and y-intercept equal to $K_s/\mu_{\max}$.

Xanthan gum formation

A mass balance of product (xanthan gum) over the chemostat is given as,

$$\begin{aligned} \text{(Rate of product accumulated)} &= \text{(Rate of product input)} \\ &\quad - \text{(Rate of product output)} \\ &\quad + \text{(Rate of product formed)} \end{aligned}$$

$$V \, dP/dt = F(P_i - P) + V R_p \text{ ----- (15)}$$

Since the influent feed has no product in it, P_i equals zero and at steady state the rate of product accumulation is zero. Considering these things and using the definition of dilution rate the above equation can be written as,

$$DP' = R_p \text{ ----- (16)}$$

where P' = steady state product concentration

R_p = rate of product formation

Here R_p is again given by Luedeking-Piret equation, Eq. (3), with steady state cell concentration, X' . Using Eq. (3) in the above equation results in

$$DP' = K\mu X' + K'X' \text{ ----- (17)}$$

Using Eq. (13) in the above equation and dividing both sides by X' , it can be rewritten as,

$$DP'/X' = KD + K' \text{ ----- (18)}$$

Plotting DP'/X' against D should result in straight line with slope equal to K and y-intercept equal to K' .

Glucose Consumption

The glucose, as mentioned earlier, is utilized by cells for growth and product formation. Mathematically the balance is written as,

$$V \, dS/dt = F(S_i - S) - \mu XV/Y_x - (K\mu X + K'X) V/Y_p \text{ ----- (19)}$$

In the above equation Y_x represents the cell yield coefficient and Y_p represents the product yield coefficient. At steady state glucose accumulation equals zero, and F/V equals D . Using these results in the above equation,

$$D(S_i - S') = \mu X'/Y_x + (K\mu X' + K'X')/Y_p \text{ ----- (20)}$$

Linearizing this equation and noting $D=\mu$, Eq. (20) is rewritten as,

$$D(S_i - S')/X' = D(1/Y_x + K/Y_p) + K'/Y_p \text{ ----- (21)}$$

A plot of $D(S_i - S')/X'$ vs. D must give a straight line of slope equal to $(1/Y_x + K/Y_p)$ and y-intercept equal to K'/Y_p . K and K' are already known from Eq.6. Using that information Y_x and Y_p can be found.

CHAPTER 5

RESULTS

Batch Operation

The experiments in the STR and RAR in batch operation yielded very consistent results from one run to the next. A detailed list of data for STR batch operation is presented in Table 1 and Table 2 and for RAR batch operation is presented in Table 3, Table 4 and Table 5. An average cell density of 2.92 g/l in the STR and 3.89 g/l in the RAR in bulk phase were achieved. The cell growth followed the logistic equation with sigmoidal shaped profile with respect to time. In both types of reactors the lag phase was less than 6 h, followed by an exponential growth until around 36 h. The stationary phase lasted until about 60 h. For analysis, however, the stationary phase was assumed to extend to 80 h, at which point the glucose was almost exhausted, but the culture was still producing xanthan gum.

The glucose oxidase method, used to determine glucose concentration is accurate down to 3 g/l. Concentrations less than this value results in small absorbance readings, and results are subject to greater error. It was noticed that the glucose consumption curve had an elongated tail in the stationary phase, indicating consumption of glucose for xanthan production. After 60 h the culture appeared to enter into a death phase in that the cell density slightly decreased. It is interesting to note that product yield in both reactors was greater than unity during the stationary phase. Due to lack of a comprehensive chemical analysis of the broth samples, the reasons for the high product yields could not

be determined. An average product concentration of 15.33 g/l in the STR and 18.23 g/l in the RAR was obtained after 80hr. However, during the exponential phase the product yield was 0.57 g/g glucose in the STR and in the RAR it was 0.82 g/g, which were comparable to other reports.^{1, 9, 13, 14, 16, 31, 37, 38, 39, 40, 41} The RAR had a higher Y_p than STR in all the experiments.

The cell density and xanthan gum concentration in the biofilm were determined in terms of aerial density, which provides a good estimate of the biofilm growth. The cell growth in the biofilm apparently had a slightly longer lag phase than the bulk phase. The cell growth in the exponential phase in the biofilm was slower in one of the experiments, while higher in other replicates, and this phase sustained for a longer duration than in the bulk phase. The biofilm reached the stationary phase at different times for different replicates, varying from 54 h to 68 h. The xanthan gum production in the biofilm, however, was invariably slower than in the bulk phase. The glucose consumption in the biofilm could not be determined due to inapplicability of glucose oxidase method for small concentrations of glucose and inadequate resources to other procedures.

The average cell yield in the STR was 0.47 g/g, while in the RAR it was 0.39 g/g. The cell growth during the exponential phase in the RAR was more rapid than in the STR. During the same time the glucose consumption was also higher. But the overall glucose consumption rate in the STR and RAR were almost the same. Specific growth rate (μ) in the STR was 0.22 h^{-1} , as opposed to 0.19 h^{-1} in the RAR. Specific growth rate changes with time but for curve-fitting analysis it is assumed constant.

In STR α was found to be 2.96 g glucose/g cell, and in the RAR it was about 20% higher at 3.52 g glucose/g cell. The product yield used to calculate α was the

instantaneous yield (xanthan formed/substrate consumed between the 2 sampling periods) to facilitate analysis. However, the "quasi-stoichiometric yield coefficient" ($Y_{p,growth} = \text{Product formed} / \text{Substrate consumed in the growth phase}$) was assumed constant, as has been often used in bioprocessing modeling to relate the increase in cell or metabolite concentration to depletion of limiting nutrient.^{31, 37, 43} This was necessary due to the lack of respiration data to determine the maintenance coefficient (K_m), which in turn would be used to find actual product yield using the equations Eq. (5c) and Eq. (9). The author has used $Y_{p,growth}$ to find K_m and it was found to be small (0.05 g glucose/g cell.h in STR and 0.005 g glucose/g cell.h in RAR). Thus the quasi-stoichiometric yield and actual product yield are almost equal. Overall yield ($Y_{p,overall}$) is defined as the ratio of total product formed to total substrate consumed. This value is not used in any curve-fitting analysis but it is an indicator of the process efficiency. Overall product yield was found to be close to unity in both the STR ($Y_{p,overall} = 0.96\text{g/g}$) and RAR ($Y_{p,overall} = 1.08\text{g/g}$).

The growth-associated coefficient (K) was 0.44 g xanthan/g cell in the STR and in the RAR it was about 70% higher at 0.73 g xanthan/g cell, which indicates the higher ability of cells in the RAR to produce xanthan gum. The nongrowth-associated coefficient (K') was about 20% less in the RAR (0.06 g xanthan/g cell.h) than in the STR (0.08 g xanthan/g cell.h). These values are low as compared to values reported by Weiss et al.³¹ ($K = 0.254\text{ g/g}$ and $K' = 0.155\text{ g/g.h}$). They had a ratio between K and K' of about 1.8, while in this research it was about 12.

$X_{\max}$ (g/l) =	3.1
X_o (g/l) =	0.14
μ (1/h) =	0.18
β =	0.04
α =	2.65

K' (g/g.cell.h) =	0.08
K (g/g.cell) =	0.19
K_m (g/g.cell) =	0.005
$Y_{p, \text{overall}}$ =	1.06
$Y_{p, \text{growth}}$ =	0.61
Y_x =	0.43
$Y_{p, \text{stat}}$ =	2.41

Time, h	X , g/l	dX/dt	S , g/l	dS/dt	P , g/l	dP/dt	$\ln(X/(X_{\max}-X))$
0	0.10	0.000	17.5	0.000	0.00	0.000	-3.4012
6	0.37	0.045	16.5	0.167	0.20	0.033	-1.9986
12	1.28	0.152	15.0	0.250	0.60	0.067	-0.3520
18	1.90	0.103	12.5	0.417	1.65	0.175	0.4595
24	2.60	0.117	11.0	0.250	2.70	0.175	1.6487
28	2.80	0.050	9.5	0.375	3.50	0.200	2.2336
36	3.00	0.025	7.5	0.250	5.30	0.225	3.4012
43	3.10	0.014	6.5	0.143	6.70	0.200	
49	3.10	0.000	5.8	0.117	8.10	0.233	
55	3.10	0.000	5.2	0.100	9.40	0.217	
60	3.10	0.000	4.5	0.140	10.80	0.280	
73	3.10	0.000	3.2	0.100	13.90	0.238	
80	3.10	0.000	2.8	0.057	15.60	0.243	
							$\mu=0.18$

Table 1: STR Batch Operation: Experiment – 1(a)

K' , g/g Cell .hr	K, X-axis	K, Y-axis	β	For α , X-axis	For α , Y-axis
	0.000	0.000		0.00	0.00
	0.252	0.086		0.23	0.43
	0.803	0.193		1.14	0.99
	1.621	0.630		1.76	2.57
	2.332	0.703		2.46	3.65
	2.621	0.704		2.66	4.51
	2.872	0.734		2.86	4.93
0.0645	2.932	0.513	0.0461	2.96	6.58
0.0753	2.948	0.508	0.0376	2.96	7.90
0.0699	2.953	0.399	0.0323	2.96	8.15
0.0903	2.955	0.623	0.0452	2.96	8.31
0.0769	2.956	0.666	0.0323	2.96	8.20
0.0783	2.956	0.719	0.0184	2.96	7.84
0.0759		0.191	0.0353		2.647

Table 2: STR Batch Operation: Experiment – 1(b)

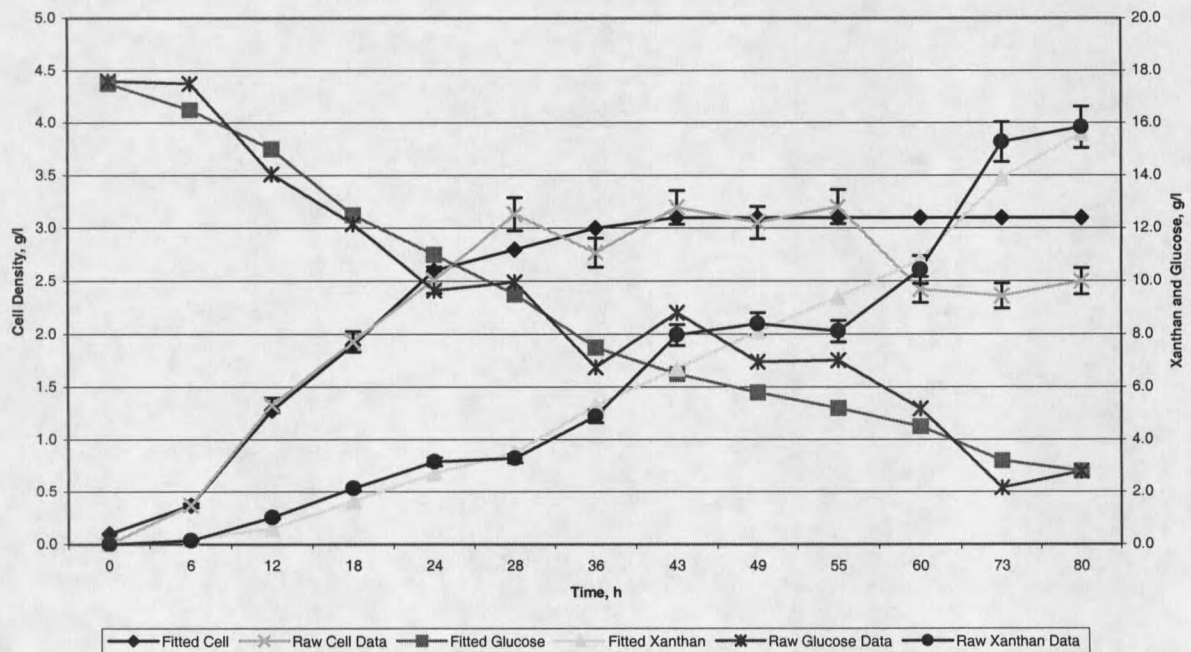


Figure 7: STR Batch Operation, Experiment - 1

$X_{\max}$ (g/l) =	4.40
X_0 (g/l) =	0.16
μ (1/h) =	0.22
β =	0.020
α =	2.770

K' (g/g cell.h) =	0.059
K (g/g cell) =	0.349
K_m (g/g cell) =	-
$Y_{p, \text{overall}}$ =	1.12
$Y_{p, \text{growth}}$ =	0.85
Y_x =	0.42
$Y_{p, \text{stat}}$ =	3.54

Time, h	pH	X, g/l	dX/dt	S, g/l	dS/dt	P, g/l	dP/dt
0	7.00	0.10	0.00	17.60	0.00	0.00	0
6	6.30	0.65	0.09	17.20	0.07	0.24	0.040
12	6.10	1.70	0.18	15.00	0.37	0.91	0.112
18	5.80	3.05	0.23	10.80	0.70	2.16	0.208
24	5.60	3.95	0.15	7.60	0.53	3.71	0.258
28	5.40	4.20	0.06	6.00	0.40	4.76	0.263
36	5.40	4.35	0.02	4.80	0.15	6.84	0.260
43	5.30	4.40	0.01	4.00	0.11	8.65	0.259
49	5.00	4.40	0.00	3.30	0.12	10.20	0.258
55	4.80	4.40	0.00	2.70	0.10	11.75	0.258
60	5.00	4.40	0.00	2.20	0.10	13.04	0.258
73	4.90	4.40	0.00	1.80	0.03	16.40	0.258
80	5.20	4.40	0.00	1.30	0.07	18.21	0.259

Table 3: RAR Batch Operation, Bulk Phase Experiment -1(a)

$\ln(X/X_{\max} - X)$	$K', \text{g/g.h}$	$K, \text{X-axis}$	$K, \text{Y-axis}$	β	For $\alpha, \text{X-axis}$	For $\alpha, \text{Y-axis}$	Y_p
-3.76		0.00	0.00		0.00	0.00	0.00
-1.75		0.37	0.13		0.49	0.22	0.60
-0.46		1.33	0.47		1.54	1.15	0.30
0.82		2.73	0.95		2.89	2.74	0.30
2.17		3.70	1.29		3.79	5.01	0.48
3.04		4.00	1.40		4.04	6.48	0.66
4.47		4.20	1.47		4.19	9.70	1.73
	0.059	4.24	1.48	0.026	4.24	10.43	2.26
	0.059	4.24	1.48	0.027	4.24	10.36	2.21
	0.059	4.24	1.48	0.023	4.24	10.92	2.58
	0.059	4.24	1.48	0.023	4.24	10.92	2.58
	0.059	4.24	1.48	0.007	4.24	14.02	8.40
	0.059	4.24	1.48	0.016	4.24	11.68	3.62
$\mu = 0.2194$	0.059		0.349	0.020		2.770	

Table 4: RAR Batch Operation, Bulk Phase Experiment –1(b)

Biofilm X, mg/sq.m	dX/dt	Biofilm P, mg/sq.m	dP/dt
0	0	0	0.00
6.00	1.00	38.00	6.33
19.00	2.17	72.00	5.67
35.00	2.67	116.00	7.33
55.00	3.33	165.00	8.17
73.00	4.50	232.00	16.75
90.00	2.13	305.00	9.13
100.00	1.43	375.00	10.00
105.00	0.83	445.00	11.67
109.00	0.67	510.00	10.83
110.00	0.20	590.00	16.00
110.00	0.00	670.00	6.15
110.00	0.00	755.00	12.14

Table 5: RAR Batch Operation, Biofilm, Experiment –1(c)

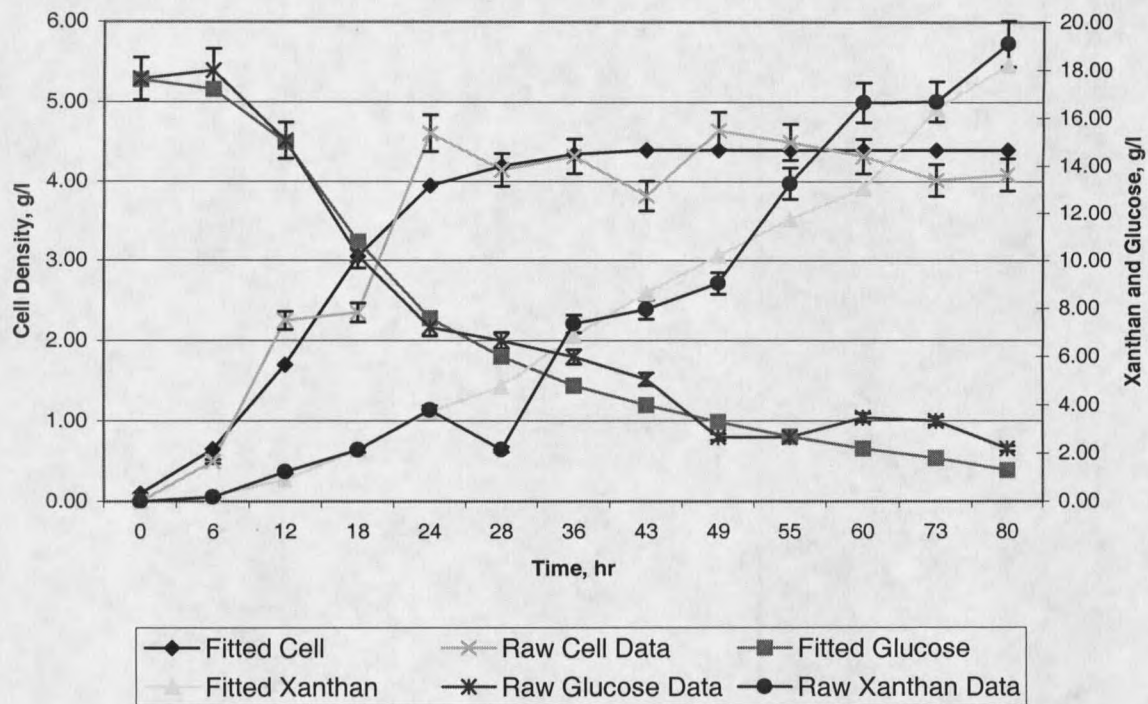


Figure-8, RAR Batch Data, in Bulk Phase, Experiment - 1(a)

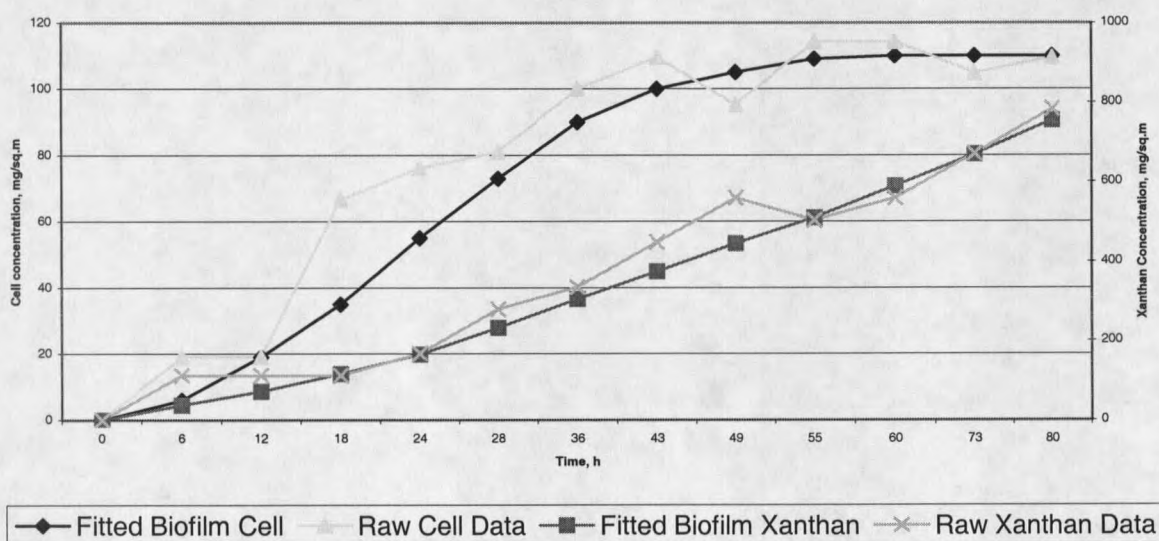


Figure-9, RAR Batch Data, in Biofilm, Experiment - 1(b)

Continuous Operation

In the chemostat the cell density was highest (2.41 g/l) for $D = 0.05 \text{ h}^{-1}$ and decreased to 2.35 g/l for $D = 0.10 \text{ h}^{-1}$ and further decreased to 2.27 g/l for $D = 0.15 \text{ h}^{-1}$. A similar trend was observed in xanthan gum production and glucose consumption. Xanthan gum production decreased from 17.6 g/l at $D = 0.05 \text{ h}^{-1}$, to 11.8 g/l at $D = 0.10 \text{ h}^{-1}$ to 7.6 g/l at $D = 0.15 \text{ h}^{-1}$, while glucose consumption decreased from 22.6 g/l to 20.95 g/l to 11.1 g/l for the same dilution rates. The cell density in the RAR, however, was highest at 2.71 g/l for $D = 0.1 \text{ h}^{-1}$, lowest at 1.98 g/l for $D = 0.15 \text{ h}^{-1}$ and for $D = 0.05 \text{ h}^{-1}$ it was 2.26 g/l. As in the chemostat, in the RAR the xanthan gum production and glucose consumption decreased with increasing dilution rates. The quasi-stoichiometric yield coefficient in the chemostat was highest at 0.78 g/g for $D = 0.05 \text{ h}^{-1}$ and lowest at 0.56 g/g for $D = 0.10 \text{ h}^{-1}$, and for $D = 0.15 \text{ h}^{-1}$, it was 0.68 g/g. In the RAR, it was 0.82 g/g, 0.72 g/g and 0.86 g/g for the same dilution rates. The K_s value for both the chemostat and the RAR was high as compared to the literature. It was 8.4 g/l in the chemostat and 9.52 g/l in the RAR. Though this value was high, the steady state glucose concentration in both types of reactors was around this value. The growth-associated coefficient (K) in both the reactors increased by 2- to 3- fold from batch operation. The nongrowth-associated coefficient (K') showed a 4-fold increase from batch operation in the chemostat, whereas in the RAR it increased by about 5 times. Both these parameters were greater in the RAR than in the chemostat. K increased by about 25% and K' increased by about 7%. The biofilm formation was well established and cell growth in the biofilm reached stationary phase considerably later than in the bulk phase. The stationary phase was reached around 65 h,

and in some experiments it was around 85 h. The xanthan formation in the biofilm did not reach steady state even after 96 h of fermentation.

D, 1/h	S', g/l	X', g/l	P', g/l	S'/D	D*P'/X'	R _g = D(S _i -S')/X'
0.05	1.39	2.41	17.60	27.80	0.37	0.49
0.10	4.05	2.35	11.80	40.50	0.50	0.89
0.15	6.41	2.27	7.60	42.73	0.50	0.90

$\mu_{\max} =$	0.34	K =	1.321	$Y_p =$	0.88
$K_s =$	8.42	$K' =$	0.318	$Y_x =$	0.40

Table 6: STR Continuous Operation Data Analysis

D, 1/h	X'	S'	P'	S'/D	D*P'/X'	R _g = D(S _i -S')/X'
0.05	2.26	1.71	19.00	34.14	0.42	0.52
0.10	2.71	4.39	14.90	43.94	0.55	0.76
0.15	1.98	8.51	7.76	56.74	0.59	0.87

$\mu_{\max} =$	0.31	K =	1.67	$Y_p =$	0.97
$K_s =$	9.52	$K' =$	0.35	$Y_x =$	0.55

Table 7: RAR Continuous Operation Data Analysis

CHAPTER 6

DISCUSSION AND CONCLUSIONS

Batch Operation

A detailed numerical comparison between STR and RAR is presented in Table 8 and statistical analysis for the same is presented in Appendix - G. The growth rate in the RAR was 12% lower than the STR, but statistically it was not a significant difference (by two-sample T-Test, P-Value = 0.795). Glucose consumption (and glucose consumption rate) was not significantly different in the RAR than in the STR (P-Value=0.180). Therefore, glucose concentration played no role in affecting cell growth or xanthan production. Weiss et al.³¹ recommend the use of respiration data to calculate K_m , but due to lack of respiration data Y_x and Y_p could not analytically be found simultaneously. For this reason Y_p has been assumed to be equal to the ratio of total product formed to total substrate consumed during cell growth phase. This might result in slightly erroneous yield, but for this research, which mainly aims at qualitative analysis, the above assumption is justified. It was found that in two of the three STR experiments, the Y_p in stationary phase was 1 g/g and 2 g/g, a result similar to those obtained by other researchers.^{12, 22, 43} In the case of a RAR, it could be due to sloughing. However, a significant increase in product yield, Y_p , in a RAR compared to a STR was found (P-Value = 0.001) during the exponential phase. It was higher by 43% in the RAR than in the STR, and cell yield, Y_x , was lower by about 18% in the RAR, which is statistically not significantly different (P-Value = 0.135). Higher Y_p appears to be due to the ability of cells to produce xanthan during the growth phase, as is evident from the higher values of

K. The value of K' , on the other hand, has slightly decreased by about 9%, which indicates that cells in the RAR take slightly longer to ferment, producing more product per unit amount of cell growth.

Biofilm in the RAR was well established, and cell density and polymer concentration were relatively high, but their amounts were negligible compared to the bulk liquid phase. Moreover due to the weighing instrument's limitations, an accurate biofilm cell density and product concentration could not be found. The low cell and xanthan gum concentration in the biofilm could be due to the detachment/erosion of biofilm and continuous dissolution of xanthan gum into the bulk liquid phase from the biofilm. This might have also caused a slight increase in Y_p in the bulk phase. The other possible reasons include the difference in hydrodynamics between the RAR and STR.

It can be observed from the RAR and STR batch data that xanthan production rate is highest during the later part of exponential growth stage and in the stationary phase. During the same period the culture broth gets extremely viscous, making the mixing less effective in the outer region (away from agitator in STR and from rotating inner cylinder in the RAR) of the reactor. This might result in making the cells essentially stationary with respect to the substrate. This in reality should be counter-productive, as the nutrients in the immediate surroundings of the cells would be depleted faster, causing a deficiency for the cells. The reason for high xanthan production rate is not very clear in the case of STR, but in RAR this could be due to the formation of established biofilm on the walls.

The parameter, α indicates the amount of substrate consumed per unit amount of cell growth, which increased marginally from a STR to a RAR (P-value = 0.14). But, the other parameter, β (specific glucose consumption rate) has decreased significantly (P-

value = 0.0001) indicating a longer fermentation time in the RAR to consume the same amount of glucose as the STR to produce a unit amount of cells. From the values of K' and β , it could be inferred that RAR has slightly longer fermentation time.

Continuous Operation

A comparison of STR and RAR data is presented Table 9. In the continuous operation the cell density in RAR increased from 2.26 g/l at dilution rate, D , of 0.05 h^{-1} to 2.7 g/l at $D = 0.1 \text{ h}^{-1}$ and then decreased to 1.98 g/l at $D = 0.15 \text{ h}^{-1}$. In the case of the CSTR the trend is different. The cell density was maximum at 2.41 g/l for $D = 0.05 \text{ h}^{-1}$, decreased to 2.35 g/l at $D=0.1 \text{ h}^{-1}$, and further decreased to 2.27 g/l at 0.15 h^{-1} . This does not lead to any optimal dilution rates. Comparing the CSTR and the RAR at each dilution rate, it is observed in RAR that the cell density is higher by 15% at $D = 0.1 \text{ h}^{-1}$, than in the CSTR, and the cell density is lower for other dilution rates. There is a speculation that the cells undergo certain phenotype changes in the RAR due to the formation of biofilm. This might have been the reason for increased production of xanthan gum. In fact, at $D = 0.1 \text{ h}^{-1}$, the polysaccharide produced in the RAR was about 25% higher than in the CSTR. Due to the lack of sufficient data, a statistical analysis could not be performed. It was also noticed that at each dilution rate the substrate consumption was almost the same in the RAR as in the CSTR, which has resulted in a higher Y_p , especially at $D = 0.1 \text{ h}^{-1}$. Y_p in the RAR was higher by 28% than in the CSTR and Y_x was higher by 17%. It is quite clear that the RAR, due to the formation of biofilm, has outperformed the STR in batch mode and more so in the continuous operation at $D = 0.1 \text{ h}^{-1}$.

The other possible explanations for better performance of the RAR over the STR in terms of product yield, cell yield, product concentration and cell concentration than STR

are: It is believed that some cells can experience reversible attachment to the substratum and that these cells may display different physiology than suspended cells.³⁵ It was suggested that the cells upon immobilization on a surfaces switch the substrate uptake mechanism. The reasons or mechanisms for this switch are not known. Davies has experimentally shown that attached cells are more active in their uptake of substrate than suspended cells³⁵ and showed that the immobilized cells synthesized DNA and RNA at a higher rate than suspended cells. Davies³⁶ has studied the alginate biosynthesis in *P. aeruginosa* biofilms and reported the activation of a specific gene for the production of EPS as the result of bacterial attachment to the surfaces. This activation was detected in individual cells attached to a surface, as well as in the whole culture.³⁶ The method of activation is not known yet. Yu⁸ has shown that the initially invading non-mucoid organism quickly changes into a mucoid form, producing large amounts of EPS alginate. Overproduction of alginate facilitates the formation of exopolysaccharide-embedded colonies or biofilms. The alginates in a micro colony are also thought to trap or mask released toxins in the remission stage rendering protection to the cells.

It is also possible, in the RAR, that the shear stress exerted by the rotating cylinder and the shear between the fluid layers in viscous bulk phase induce the cells to produce more EPS. This might not apply to the STR due to the relatively small shear zone around the agitator.

STR Batch

	Time, h	Xmax, g/l	Xo, g/l	S, g/l	P, g/l	μ , 1/h
Expt.1	80	3.10	0.14	14.70	15.60	0.18
Expt.2	76	2.85	0.56	16.00	14.50	0.26
Expt.3	81	2.80	0.10	17.50	15.90	0.22
Avg.		2.92	0.27	16.07	15.33	0.22

RAR Batch

	Time, h	Xmax, g/l	Xo, g/l	S, g/l	P, g/l	μ , 1/h
Expt.1	80	4.40	0.16	16.30	18.21	0.22
Expt.2	82	3.56	0.09	17.26	18.40	0.20
Expt.3	81	3.70	0.14	17.36	18.09	0.16
Avg.		3.89	0.13	16.97	18.23	0.19

STR Batch

Yp, g/g	Yp,overall, g/g	Yx, g/g	Km	K	K'	α	β
0.61	1.06	0.43	0.01	0.19	0.08	2.65	0.04
0.52	0.91	0.59	0.01	0.55	0.07	2.76	0.04
0.58	0.91	0.40	0.07	0.57	0.08	3.46	0.05
0.57	0.96	0.47	0.03	0.44	0.08	2.96	0.04

RAR Batch

Yp, g/g	Yp,overall, g/g	Yx, g/g	Km	K	K'	α	β
0.85	1.12	0.42	-	0.35	0.06	2.77	0.02
0.81	1.07	0.32	-	0.56	0.07	3.83	0.015
0.79	1.04	0.42	-	1.29	0.06	3.99	0.014
0.82	1.08	0.39	-	0.73	0.06	3.53	0.016

Percentage Difference Between STR and RAR

Xo =	-52.05		μ =	-11.85		K' =	-18.26
Xmax =	33.26		Yp =	43.27		K =	68.02
S =	5.64		Yx =	-18.31		α =	19.37
P =	18.91		Yp,overall =	12.14		β =	-62.31

Table 8: Summary of STR and RAR Batch Operation Data

STR Continuous

D, 1/h	X', g/l	S', g/l	P', g/l	Y _p , g/g	Y _x , g/g	SXP, g/g.h	SGC, g/g.h
0.05	2.41	22.60	17.60	0.78	0.11	0.365	0.489
0.10	2.35	20.95	11.80	0.56	0.11	0.502	0.890
0.15	2.27	11.10	7.60	0.68	0.20	0.502	0.900

$$\mu_{\max} = 0.34$$

$$Y_p = 0.88$$

$$K = 1.32$$

$$K_s = 8.42$$

$$Y_x = 0.40$$

$$K' = 0.318$$

RAR Continuous

D, 1/h	X', g/l	S', g/l	P', g/l	Y _p , g/g	Y _x , g/g	SXP, g/g.h	SGC, g/g.h
0.05	2.26	23.30	19.00	0.82	0.10	0.420	0.515
0.10	2.71	20.60	14.90	0.72	0.13	0.550	0.760
0.15	1.98	9.00	7.70	0.86	0.22	0.580	0.870

$$\mu_{\max} = 0.31$$

$$Y_p = 0.97$$

$$K = 1.67$$

$$K_s = 9.52$$

$$Y_x = 0.55$$

$$K' = 0.35$$

Percentage Difference Between STR and RAR

D	X'	S'	P'	Y _p	Y _x	SXP	SGC
0.05	-6.22	3.10	7.95	4.71	-9.04	15.07	5.32
0.10	15.32	-1.67	26.27	28.42	17.28	9.56	-14.61
0.15	-12.78	-18.92	1.32	24.96	7.58	15.54	-3.33

Table 9: Summary of STR and RAR Continuous Operation Data

RECOMMENDATIONS FOR FUTURE RESEARCH

These results are a promising new approach to a higher xanthan gum yield. However, this exploratory work in the next step must emphasize verification of implied mechanisms

- Detailed microscopic and microbiological analysis should be accomplished on bacterial samples to verify that physiological phenotype changes occurred, and led to increased gum formation.
- Molecular weight and molecular weight distributions of xanthan gum by HPLC need to be accomplished to ascertain if the increased yield changed the gum properties. Analysis should also include pyruvate analysis for determination of gum quality
- Detachment of immobile film into the bulk appears to have slightly increased xanthan gum yield. Can this increase be obtained in other reactor configurations by shear, scraping, or other procedures?

NOMENCLATURE

D = Dilution rate, h^{-1}

F = Influent or effluent flow rate, h^{-1}

K_s = Saturation coefficient, g/l

K_m = Maintenance coefficient, $\text{g glucose/g cell.h}^{-1}$

K = Growth-associated coefficient, g xanthan/g cell

K' = Nongrowth-associated coefficient, $\text{g xanthan/g cell.h}^{-1}$

P = Product (xanthan gum) concentration, g/l

P_o = Initial product concentration, g/l

P_i = Influent xanthan concentration, g/l

P' = Steady state xanthan concentration, g/l

S = Substrate (Glucose) concentration, g/l

S_o = Initial substrate concentration, g/l

S_i = Influent glucose concentration, g/l

S' = Steady state glucose concentration, g/l

V = Volume of reactor, l

X = Biomass or cell concentration, g/l

X_0 = Initial cell concentration, g/l

X_i = Influent cell concentration, g/l

X' = Steady state cell concentration, g/l

$X_{\max}$ = Cell concentration in stationary phase, g/l

Y_x = Cell yield coefficient, g cell/g glucose

Y_p = Product yield coefficient, g xanthan/g glucose

$Y_{p,\text{growth}}$ = Quasi-stoichiometric yield coefficient, g xanthan/g glucose

$Y_{p,\text{overall}}$ = Overall yield coefficient, g xanthan/ g glucose

$Y_{p,\text{stat}}$ = Yield coefficient in the stationary phase, g xanthan/ g glucose

$\alpha = 1/Y_x + K/Y_p$, g glucose/ g cell

β = Specific glucose consumption rate, g glucose/g cell.h⁻¹

μ = Cellular specific growth rate, h⁻¹

$\mu_{\max}$ = Maximum cellular specific growth rate, h⁻¹

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APPENDICES

APPENDIX – A

SPECTROSCOPY AND CENTRIFUGATION

Photometric Linearity Check

0.01N H_2SO_4 , 0.025g/l and 0.05g/l Potassium dichromate in 0.01N H_2SO_4 solutions were prepared. Absorbance of the potassium dichromate solutions was read at 350nm wavelength against 0.01N H_2SO_4 as reference solution. The absorbance varied linearly with concentration.

Concentration, g/l	Absorbance
0.025	0.254
0.05	0.521

Table 10: Photometric Linearity Check

Glucose Concentration Calibration Curve

Glucose conc. in the cell-free supernatant is determined by the Glucose oxidase method (Sigma Chemicals, Catalog No. 510). After the enzymatic reactions, the color intensity of the solution is measured at 450 nm (OD_{450}) on a spectrophotometer. The glucose concentration in the samples was then determined by comparing the OD_{450} reading with a calibration curve of standard glucose solution. A 300mg/dl glucose solution was prepared and serially diluted to various concentrations and the absorbance was read against tap water as blank.

Conc, g/l	Run 1	Run 2	Avg.
1	0.165	0.198	0.1815
2.5	0.275	0.359	0.317
5	0.376	0.472	0.424
7.5	0.525	0.597	0.561
10	0.638	0.71	0.674
12.5	0.773	0.85	0.8115
15	0.94	0.908	0.924
17.5	1.06	1.07	1.065
20	1.17	1.17	1.17

Table 11: Glucose Calibration

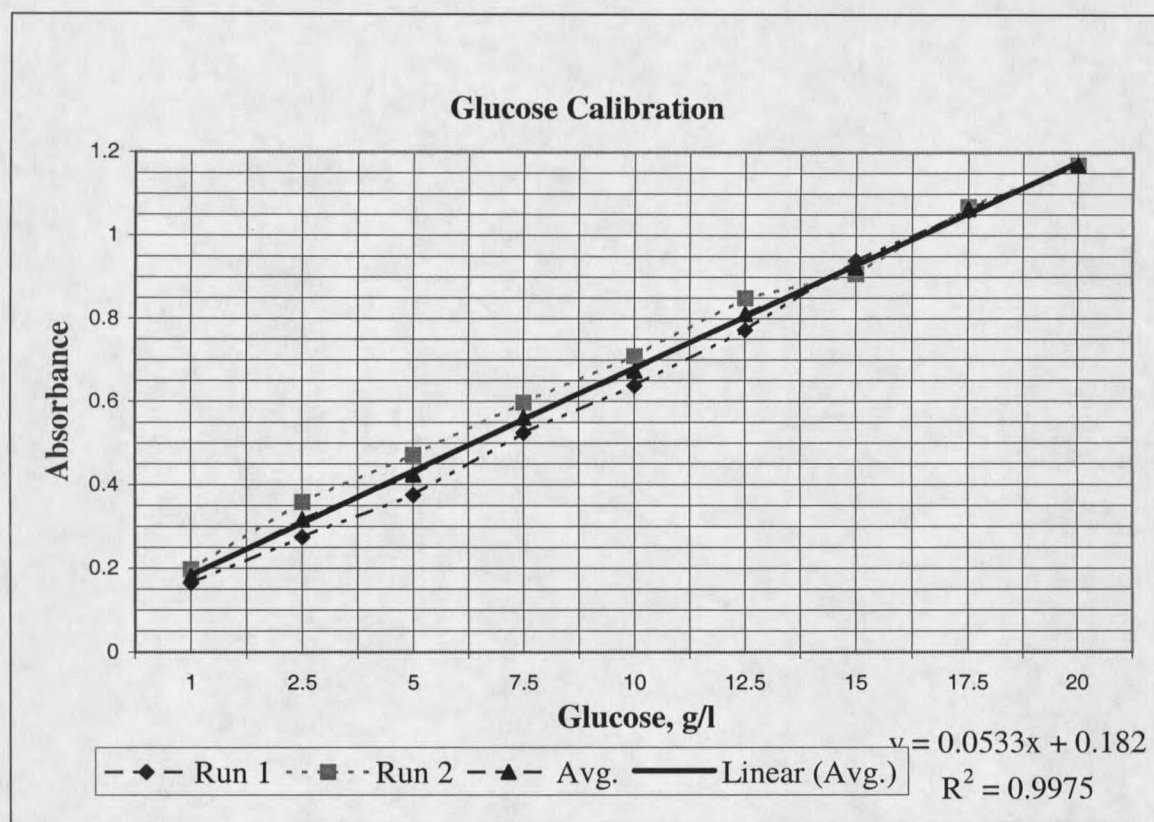


Figure 10. Glucose Calibration Curve

The calibration curve shows a linear relationship between glucose concentration and absorbance to about 25g/l.

Centrifugation Test

To find if any of the nutrients in the medium are centrifuged along with the cells, a test was performed. Twenty ml of freshly prepared, sterile nutrient solution was taken, and to simulate the same conditions as for fermentation broth, 10ml 1 wt.% KCl solution was added and centrifuged at 12,000 rpm at 4°C for 45 min. There was absolutely no sediment. This test was performed in duplicate.

Also, a test was performed to see if all the xanthan gum in the broth was centrifuged. For this purpose, solutions of varying concentrations of commercially available xanthan gum were prepared. Twenty ml of each solution was taken and 10ml of 1% wt. KCl and 60 ml isopropanol were added and centrifuged at 12,000 rpm at 4°C for 45 min. At the end of centrifugation, the supernatant was disposed and the sediment was dried overnight at 110°C. The dry weight of xanthan gum was found and it was same as the amount put in initially. This test was performed in duplicate.

APPENDIX – B

STR BATCH OPERATION DATA

$X_{\max}$ (g/l) =	3.1
X_o (g/l) =	0.14
μ (1/h) =	0.18
β =	0.04
α =	2.65

K' (g/g.cell.h) =	0.08
K (g/g.cell) =	0.19
K_m (g/g.cell) =	0.005
$Y_{p, \text{overall}}$ =	1.06
$Y_{p, \text{growth}}$ =	0.61
Y_x =	0.43
$Y_{p, \text{stat}}$ =	2.41

Time, h	X, g/l	dX/dt	S, g/l	dS/dt	P, g/l	dP/dt	$\ln(X/X_{\max}-X)$
0	0.10	0.000	17.5	0.000	0.00	0.000	-3.4012
6	0.37	0.045	16.5	0.167	0.20	0.033	-1.9986
12	1.28	0.152	15.0	0.250	0.60	0.067	-0.3520
18	1.90	0.103	12.5	0.417	1.65	0.175	0.4595
24	2.60	0.117	11.0	0.250	2.70	0.175	1.6487
28	2.80	0.050	9.5	0.375	3.50	0.200	2.2336
36	3.00	0.025	7.5	0.250	5.30	0.225	3.4012
43	3.10	0.014	6.5	0.143	6.70	0.200	
49	3.10	0.000	5.8	0.117	8.10	0.233	
55	3.10	0.000	5.2	0.100	9.40	0.217	
60	3.10	0.000	4.5	0.140	10.80	0.280	
73	3.10	0.000	3.2	0.100	13.90	0.238	
80	3.10	0.000	2.8	0.057	15.60	0.243	
							$\mu = 0.18$

Table 1: STR Batch Operation: Experiment 1(a)

K', g/g Cell .hr	K, X-axis	K, Y-axis	β	For α , X-axis	For α , Y-axis
	0.000	0.000		0.00	0.00
	0.252	0.086		0.23	0.43
	0.803	0.193		1.14	0.99
	1.621	0.630		1.76	2.57
	2.332	0.703		2.46	3.65
	2.621	0.704		2.66	4.51
	2.872	0.734		2.86	4.93
0.0645	2.932	0.513	0.0461	2.96	6.58
0.0753	2.948	0.508	0.0376	2.96	7.90
0.0699	2.953	0.399	0.0323	2.96	8.15
0.0903	2.955	0.623	0.0452	2.96	8.31
0.0769	2.956	0.666	0.0323	2.96	8.20
0.0783	2.956	0.719	0.0184	2.96	7.84
0.0759		0.191	0.0353		2.647

Table 2: STR Batch Operation: Experiment – 1(b)

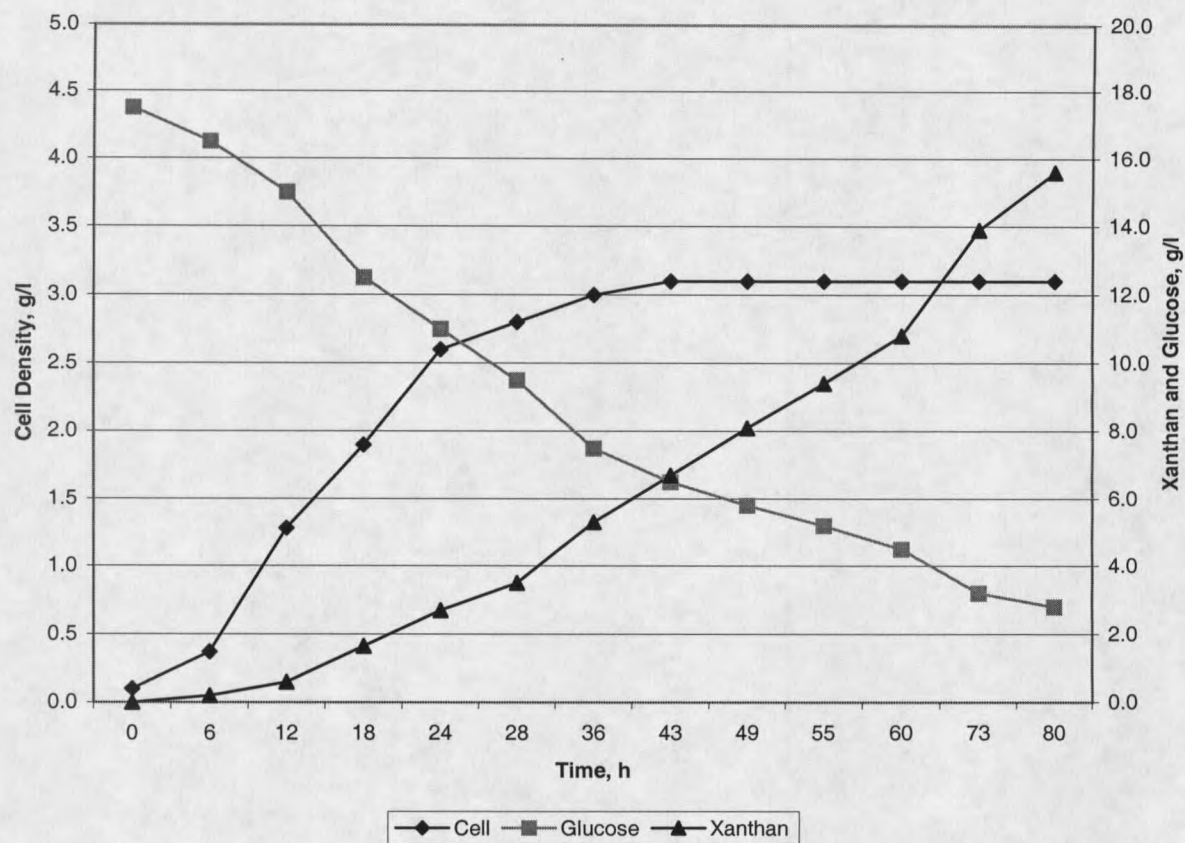


Figure 7. STR Batch Data, Experiment - 1

$X_{\max}$ (g/l) =	2.85
X_o (g/l) =	0.06
μ (1/h) =	0.26
β =	0.04
α =	2.76

K' (g/g.cell. h) =	0.07
K (g/g.cell) =	0.55
K_m (g/g.cell) =	0.015
$Y_{p,overall}$ =	0.91
$Y_{p,growth}$ =	0.52
Y_x =	0.59
$Y_{p,stat}$ =	1.93

Time, h	X, g/l	dX/dt	S, g/l	dS/dt	P, g/l	dP/dt	For μ , 1/h
0	0.07	0.000	17.6	0.000	0.00	0	-3.6817
7	0.20	0.019	15.5	0.300	0.22	0.031	-2.5840
13	0.90	0.117	13.5	0.333	0.90	0.113	-0.7732
19	1.95	0.175	10.5	0.500	2.10	0.200	0.7732
26	2.70	0.107	8.5	0.286	3.60	0.214	2.8904
31	2.80	0.020	7.0	0.300	5.00	0.280	4.0254
36	2.85	0.010	6.0	0.200	6.00	0.200	
42	2.85	0.000	5.0	0.167	7.10	0.183	
48	2.85	0.000	4.2	0.133	8.30	0.200	
54	2.85	0.000	3.5	0.117	9.50	0.200	
62	2.85	0.000	2.9	0.075	11.30	0.225	
69	2.85	0.000	2.1	0.114	12.80	0.214	
76	2.85	0.000	1.6	0.071	14.50	0.243	
							$\mu=0.25806$

Table 12: STR Batch Operation: Experiment – 2(a)

K', g/g Cell.h	K, X-axis	K, Y-axis	β	For α , X-axis	For α , Y-axis
	0.000	0.000		0.00	0.000
	0.256	0.142		0.14	1.320
	0.989	0.545		0.84	3.057
	2.029	1.049		1.89	4.473
	2.632	1.290		2.64	6.019
	2.747	1.677		2.74	7.027
0.0702	2.781	1.640	0.0702	2.79	7.240
0.0643	2.791	1.487	0.0585	2.79	7.497
0.0702	2.793	1.431	0.0468	2.79	8.821
0.0702	2.793	1.375	0.0409	2.79	9.349
0.0789	2.793	1.500	0.0263	2.79	8.969
0.0752	2.793	1.535	0.0401	2.79	8.912
0.0852	2.793	1.769	0.0251	2.79	8.555
0.0735		0.551	0.0440		2.756

Table 13: STR Batch Operation: Experiment – 2(b)

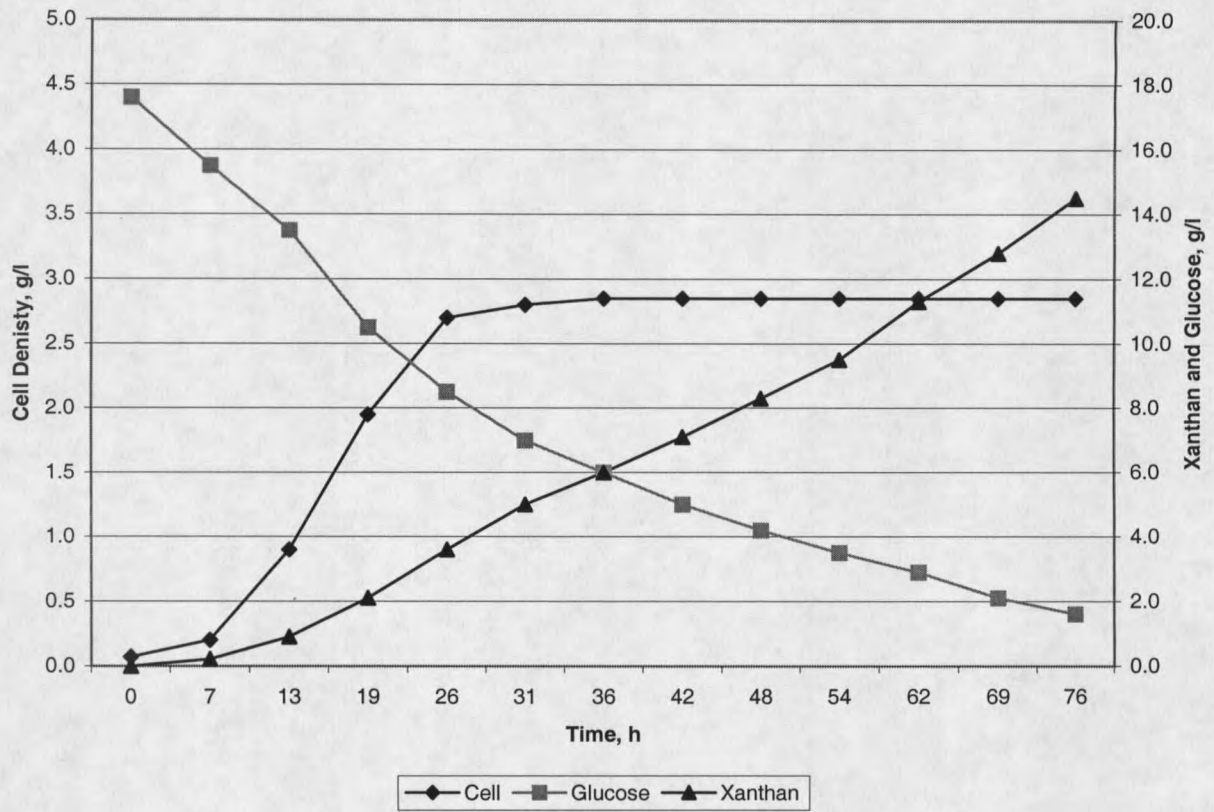


Figure 11. STR Batch Data, Experiment - 2

$X_{\max}$ (g/l) =	2.8
X_0 (g/l) =	0.10
μ (1/h) =	0.22
β =	0.05
α =	3.46

K' (g/g.cell.h) =	0.08
K (g/g.cell) =	0.57
K_m (g/g.cell) =	0.07
$Y_{p, \text{overall}}$ =	0.90
$Y_{p, \text{growth}}$ =	0.58
Y_x =	0.40
$Y_{p, \text{stat}}$ =	1.77

Time, h	X, g/l	dX/dt	S, g/l	dS/dt	P, g/l	dP/dt	For μ , 1/h
0	0.10	0.000	17.6	0.000	0.00	0	-3.2958
5	0.30	0.040	16.5	0.220	0.40	0.080	-2.1203
11	0.95	0.108	15.0	0.250	1.00	0.100	-0.6665
17	1.80	0.142	11.5	0.583	2.00	0.167	0.5878
23	2.40	0.100	8.0	0.583	3.30	0.217	1.7918
29	2.70	0.050	6.9	0.183	4.90	0.267	3.2958
35	2.75	0.008	5.8	0.183	6.00	0.183	4.0073
42	2.80	0.007	4.8	0.143	7.40	0.200	
49	2.80	0.000	3.8	0.143	8.90	0.214	
55	2.80	0.000	3.0	0.133	10.10	0.200	
61	2.80	0.000	2.2	0.133	11.50	0.233	
68	2.80	0.000	1.5	0.100	13.00	0.214	
75	2.80	0.000	1.0	0.071	14.50	0.214	
81	2.80	0.000	0.0	0.167	15.90	0.233	
							$\mu=0.2171$

Table 14: STR Batch Operation: Experiment – 3(a)

K', g/g Cell.h	K, X-axis	K, Y-axis	β	For α , X-axis	For α , Y-axis
	0.000	0.000		0.00	0.000
	0.176	0.333		0.20	0.916
	0.702	0.701		0.85	1.852
	1.569	1.135		1.70	3.073
	2.265	1.487		2.30	4.720
	2.567	1.913		2.60	6.433
	2.663	1.754		2.65	7.554
0.0714	2.692	1.655	0.0510	2.70	8.697
0.0765	2.699	1.648	0.0510	2.70	8.965
0.0714	2.700	1.555	0.0476	2.70	8.903
0.0833	2.700	1.661	0.0476	2.70	9.778
0.0765	2.700	1.651	0.0357	2.70	10.804
0.0765	2.700	1.642	0.0255	2.70	12.314
0.0833	2.700	1.748	0.0595	2.70	7.491
0.0770		0.566	0.0454		3.463

Table 15: STR Batch Operation: Experiment – 3(b)

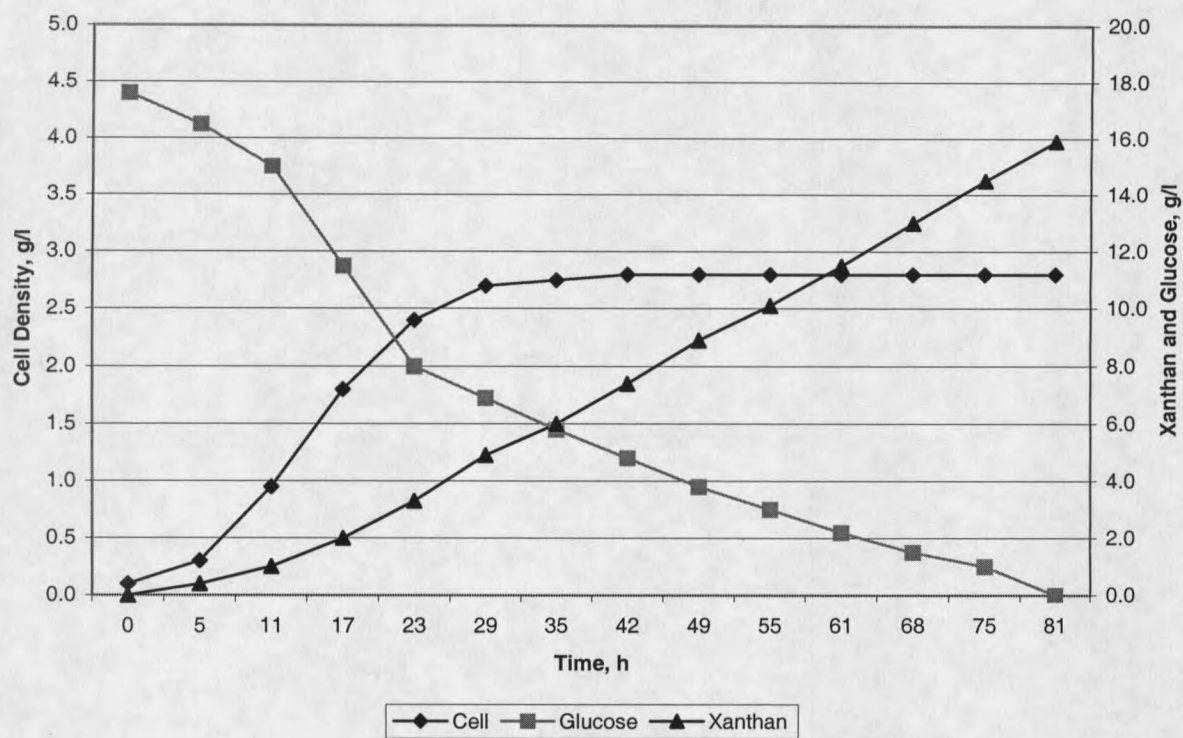


Figure 12. STR Batch, Experiment - 3

APPENDIX – C

ROTOTORQUE BATCH OPERATION DATA

$X_{\max}$ (g/l) =	4.40
X_0 (g/l) =	0.16
μ (1/h) =	0.22
β =	0.020
α =	2.770

K' (g/g cell.h) =	0.059
K (g/g cell) =	0.349
K_m (g/g cell) =	-
$Y_{p, \text{overall}}$ =	1.12
$Y_{p, \text{growth}}$ =	0.85
Y_x =	0.42
$Y_{p, \text{stat}}$ =	3.54

Time, h	pH	X, g/l	dX/dt	S, g/l	dS/dt	P, g/l	dP/dt
0	7.00	0.10	0.00	17.60	0.00	0.00	0
6	6.30	0.65	0.09	17.20	0.07	0.24	0.040
12	6.10	1.70	0.18	15.00	0.37	0.91	0.112
18	5.80	3.05	0.23	10.80	0.70	2.16	0.208
24	5.60	3.95	0.15	7.60	0.53	3.71	0.258
28	5.40	4.20	0.06	6.00	0.40	4.76	0.263
36	5.40	4.35	0.02	4.80	0.15	6.84	0.260
43	5.30	4.40	0.01	4.00	0.11	8.65	0.259
49	5.00	4.40	0.00	3.30	0.12	10.20	0.258
55	4.80	4.40	0.00	2.70	0.10	11.75	0.258
60	5.00	4.40	0.00	2.20	0.10	13.04	0.258
73	4.90	4.40	0.00	1.80	0.03	16.40	0.258
80	5.20	4.40	0.00	1.30	0.07	18.21	0.259

Table 3: Rotatorque Batch Operation, Bulk Phase, Experiment – 1(a)

For μ , 1/h	K, g/g.h	K, X-axis	K, Y-axis	β	For α , X-axis	For α , Y-axis	Yp
-3.76		0.00	0.00		0.00	0.00	0.00
-1.75		0.37	0.13		0.49	0.22	0.60
-0.46		1.33	0.47		1.54	1.15	0.30
0.82		2.73	0.95		2.89	2.74	0.30
2.17		3.70	1.29		3.79	5.01	0.48
3.04		4.00	1.40		4.04	6.48	0.66
4.47		4.20	1.47		4.19	9.70	1.73
	0.059	4.24	1.48	0.026	4.24	10.43	2.26
	0.059	4.24	1.48	0.027	4.24	10.36	2.21
	0.059	4.24	1.48	0.023	4.24	10.92	2.58
	0.059	4.24	1.48	0.023	4.24	10.92	2.58
	0.059	4.24	1.48	0.007	4.24	14.02	8.40
	0.059	4.24	1.48	0.016	4.24	11.68	3.62
$\mu=0.2194$	0.059		0.349	0.020		2.770	

Table 4: Rototorque Batch Operation, Bulk Phase, Experiment – 1(b)

Biofilm X, mg/sq.m	dX/dt	Biofilm P, mg/sq.m	dP/dt
0	0	0	0.00
6.00	1.00	38.00	6.33
19.00	2.17	72.00	5.67
35.00	2.67	116.00	7.33
55.00	3.33	165.00	8.17
73.00	4.50	232.00	16.75
90.00	2.13	305.00	9.13
100.00	1.43	375.00	10.00
105.00	0.83	445.00	11.67
109.00	0.67	510.00	10.83
110.00	0.20	590.00	16.00
110.00	0.00	670.00	6.15
110.00	0.00	755.00	12.14

Table 5: Rotatorque Batch Operation, Biofilm, Experiment – 1(c)

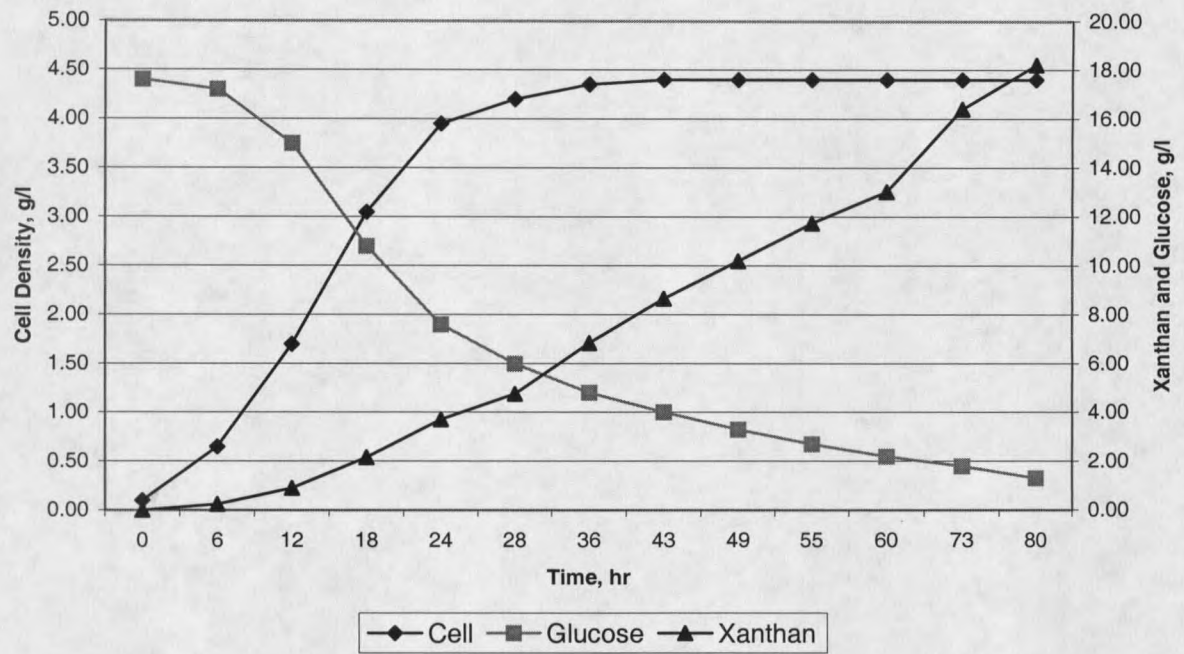


Figure 8. Rotator Batch Data, in Bulk Phase, Experiment – 1(a)

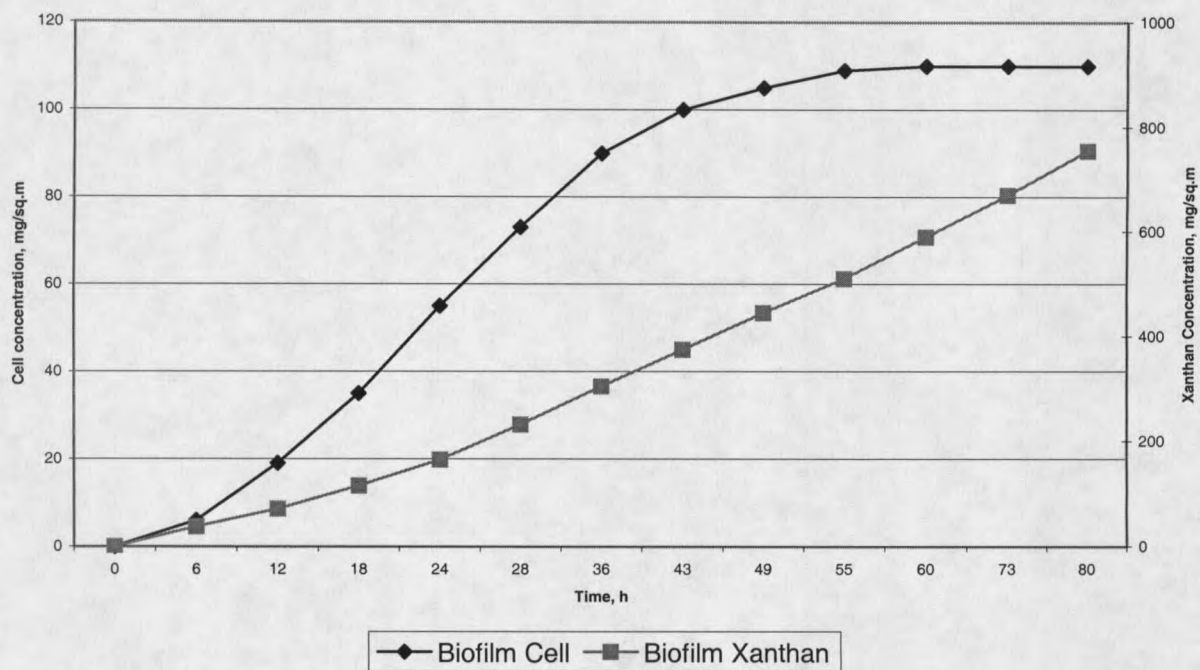


Figure 9. Rototorque Batch Data, in Biofilm, Experiment – 1(b)

X_{max} (g/l) =	3.56
X_o (g/l) =	0.09
μ (1/h) =	0.20
β =	0.015
α =	3.825

K' (g/g cell.h) =	0.074
K (g/g cell) =	0.558
K_m (g/g cell) =	-
$Y_{p,overall}$ =	1.07
$Y_{p,growth}$ =	0.81
Y_x =	0.32
$Y_{p,stat}$ =	4.77

Time, h	pH	X , g/l	dX/dt	S , g/l	dS/dt	P , g/l	dP/dt
0	7.00	0.10	0.00	17.56	0.00	0.00	0.00
7	6.30	0.30	0.03	16.50	0.15	0.29	0.041
13	6.30	1.08	0.13	11.30	0.87	1.01	0.120
19	6.10	1.84	0.13	8.00	0.55	2.38	0.228
26	5.80	2.65	0.12	5.69	0.33	4.11	0.247
31	5.60	3.20	0.11	4.27	0.28	5.77	0.332
36	5.40	3.45	0.05	3.20	0.21	7.07	0.260
42	5.10	3.54	0.02	2.50	0.12	8.57	0.250
48	5.00	3.56	0.00	2.05	0.08	10.05	0.247
54	5.10	3.56	0.00	1.60	0.08	11.53	0.247
62	5.00	3.56	0.00	1.00	0.08	13.49	0.245
69	5.00	3.56	0.00	0.80	0.03	15.21	0.245
76	4.80	3.56	0.00	0.50	0.04	16.92	0.245
82	4.70	3.56	0.00	0.30	0.03	18.40	0.245

Table 16: Rototorque Batch Operation, Bulk Phase, Experiment – 2(a)

For μ , 1/h	K', g/g cell.h	K, X-axis	K, Y-axis	β	For α , X-axis	For α , Y-axis	Yp
-3.54		0.000	0.000		0.00	0.000	0.00
-2.39		0.261	0.187		0.21	0.684	0.274
-0.84		0.866	0.632		0.98	3.531	0.138
0.06		1.869	1.361		1.74	7.106	0.415
1.07		2.872	1.790		2.56	9.204	0.870
2.18		3.222	2.279		3.11	9.698	0.972
3.45		3.372	2.320		3.36	10.451	1.215
5.18		3.437	2.264		3.45	12.117	2.143
	0.069	3.457	2.171	0.021	3.47	13.114	3.289
	0.069	3.463	2.072	0.021	3.47	13.084	3.289
	0.069	3.465	1.926	0.021	3.47	13.020	3.267
	0.069	3.47	1.799	0.008	3.47	15.201	8.600
	0.069	3.47	1.672	0.012	3.47	14.384	5.700
	0.069	3.47	1.563	0.009	3.47	14.985	7.400
$\mu=0.2004$	0.069		0.558	0.015		3.82	

Table 17: Rototorque Batch Operation, Bulk Phase, Experiment – 2(b)

Biofilm X, mg/sq.m	dX/dt	Biofilm P, mg/sq.m	dP/dt
0.00	0.00	0	0.00
6.00	0.86	25	3.57
21.00	2.50	60	5.83
51.00	5.00	90	5.00
71.00	2.86	142	7.43
83.00	2.40	190	9.60
95.00	2.40	255	13.00
105.00	1.67	320	10.83
110.00	0.83	400	13.33
112.00	0.33	505	17.50
112.00	0.00	600	11.88
112.00	0.00	698	14.00
112.00	0.00	800	14.57
112.00	0.00	895	15.83

Table 18: Rototorque Batch Operation, Biofilm, Experiment – 2(c)

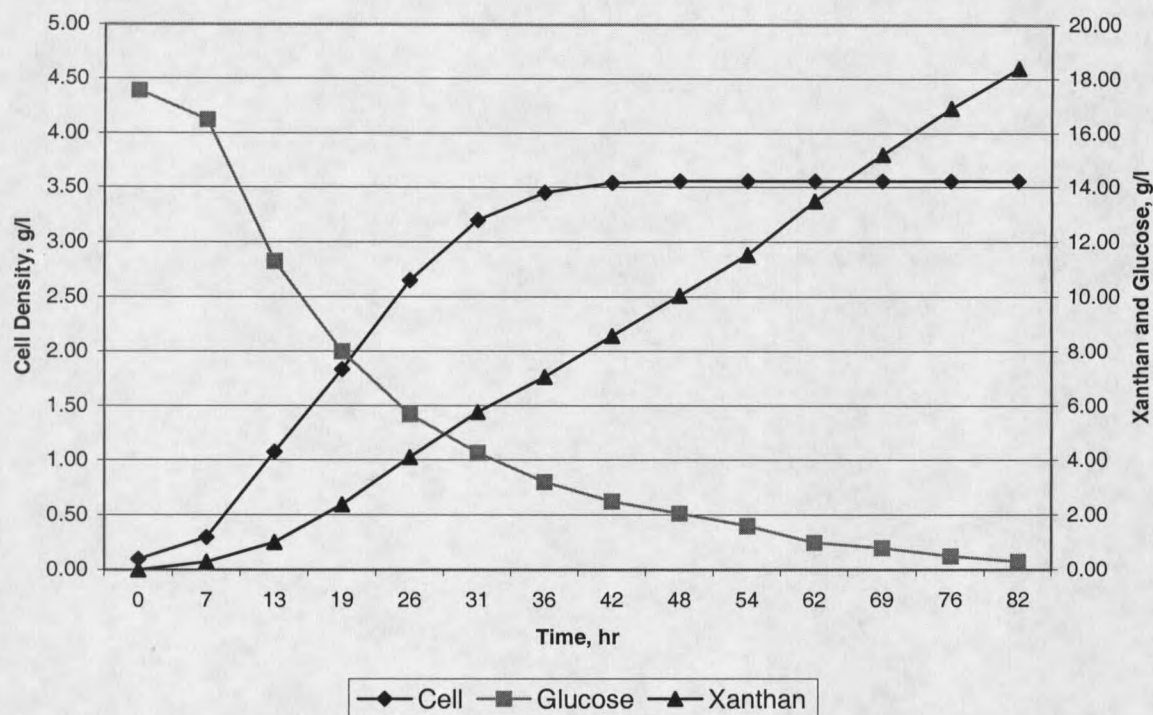


Figure 13. Rotator Batch Operation, Cell, Glucose, and Xanthan in Bulk Phase,

Experiment – 2(a)

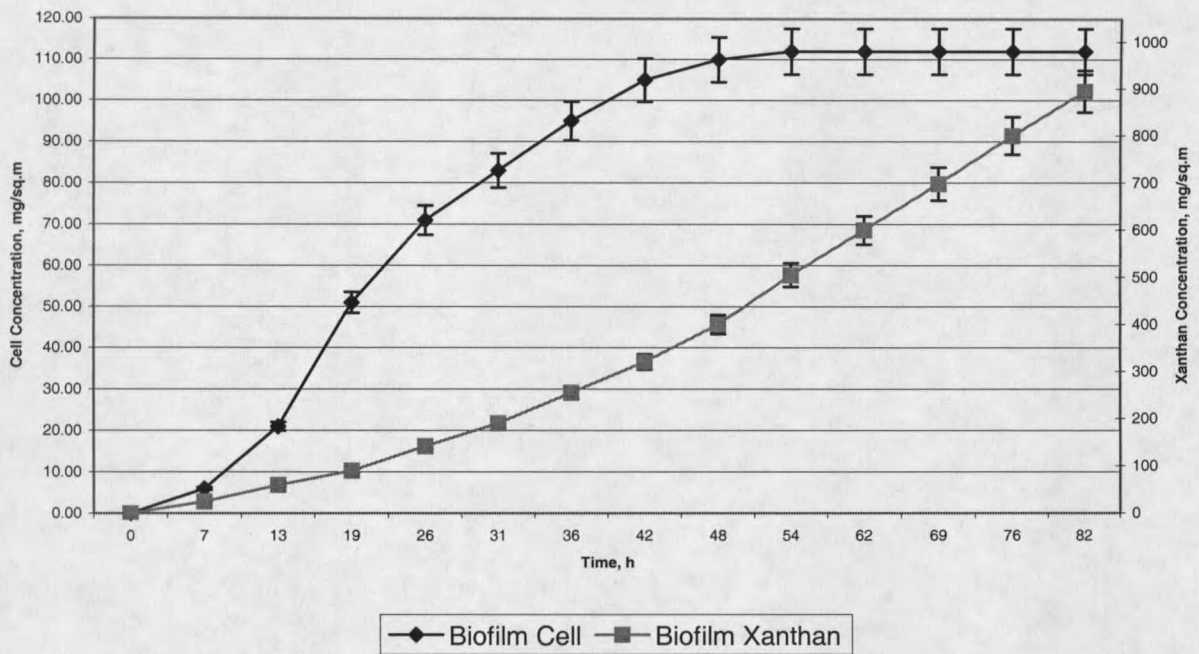


Figure 14. Rototorque Batch Operation, Cell and Xanthan in Biofilm, Experiment – 2(b)

$X_{\max} \text{ (g/l)} =$	3.70
$X_0 \text{ (g/l)} =$	0.14
$\mu \text{ (1/h)} =$	0.16
$\beta =$	0.014
$\alpha =$	3.993

$K' \text{ (g P/g X hr)} =$	0.060
$K \text{ (g P/g X)} =$	1.294
$K_m \text{ (g S/g X)} =$	-
$Y_{p, \text{overall}} =$	1.04
$Y_{p, \text{growth}} =$	0.80
$Y_x =$	0.42
$Y_{p, \text{stat}} =$	5.04

Time, h	pH	X, g/l	dX/dt	S, g/l	dS/dt	P, g/l	dP/dt
0	7.00	0.10	0.00	17.56	0.00	0.00	0
5	6.40	0.28	0.04	16.30	0.25	0.28	0.056
11	6.20	0.99	0.12	11.35	0.83	0.97	0.115
17	6.00	2.00	0.17	7.75	0.60	2.27	0.217
23	5.70	2.67	0.11	5.35	0.40	4.09	0.303
29	5.40	3.05	0.06	3.90	0.24	5.99	0.317
35	5.50	3.37	0.05	3.00	0.15	7.69	0.283
42	5.20	3.60	0.03	2.14	0.12	9.42	0.247
49	5.10	3.70	0.01	1.60	0.08	11.03	0.230
55	4.90	3.70	0.00	1.00	0.10	12.37	0.223
61	5.00	3.70	0.00	0.60	0.07	13.69	0.220
68	4.70	3.70	0.00	0.40	0.03	15.23	0.221
75	4.90	3.70	0.00	0.30	0.01	16.77	0.220
81	5.10	3.70	0.00	0.20	0.02	18.09	0.219

Table 19: Rotatorque Batch Operation, Bulk Phase, Experiment – 3(a)

For μ , 1/h	K, g/g cell/h	K, X-axis	K, Y-axis	β	For α , X-axis	For α , Y-axis	Y_p
-3.58		0.000	0.000		0	0.000	0.00
-2.49		0.161	0.217		0.14	0.976	0.222
-1.01		0.565	0.734		0.85	4.520	0.139
0.16		1.285	1.660		1.86	8.121	0.361
0.95		2.173	2.807		2.53	10.518	0.758
1.55		2.879	3.737		2.91	11.941	1.310
2.32		3.271	4.271		3.23	12.750	1.889
3.58		3.463	4.520		3.46	12.984	2.012
	0.062	3.529	4.600	0.021	3.56	13.803	2.981
	0.060	3.549	4.614	0.027	3.56	13.087	2.233
	0.059	3.556	4.604	0.018	3.56	14.207	3.300
	0.060	3.559	4.594	0.008	3.56	15.778	7.700
	0.059	3.560	4.576	0.004	3.56	16.468	15.400
	0.059	3.561	4.560	0.005	3.56	16.335	13.200
$\mu=0.1631$	0.060		1.294	0.014		3.9926	

Table 20: Rototorque Batch Operation, Bulk Phase, Experiment – 3(b)

Biofilm X, mg/sq.m	dX/dt	Biofilm P, mg/sq.m	dP/dt
0	0	0	0.00
2.0	0.40	38	7.60
15.1	2.18	83	7.50
30.0	2.48	130	7.83
49.0	3.17	176	7.67
67.0	3.00	230	9.00
85.0	3.00	280	8.33
98.6	1.94	335	7.86
110.0	1.63	390	7.86
115.0	0.83	440	8.33
118.0	0.50	520	13.33
120.0	0.29	620	14.29
120.0	0.00	730	15.71
120.0	0.00	840	18.33

Table 21: Rototorque Batch Operation, Biofilm, Experiment – 3(c)

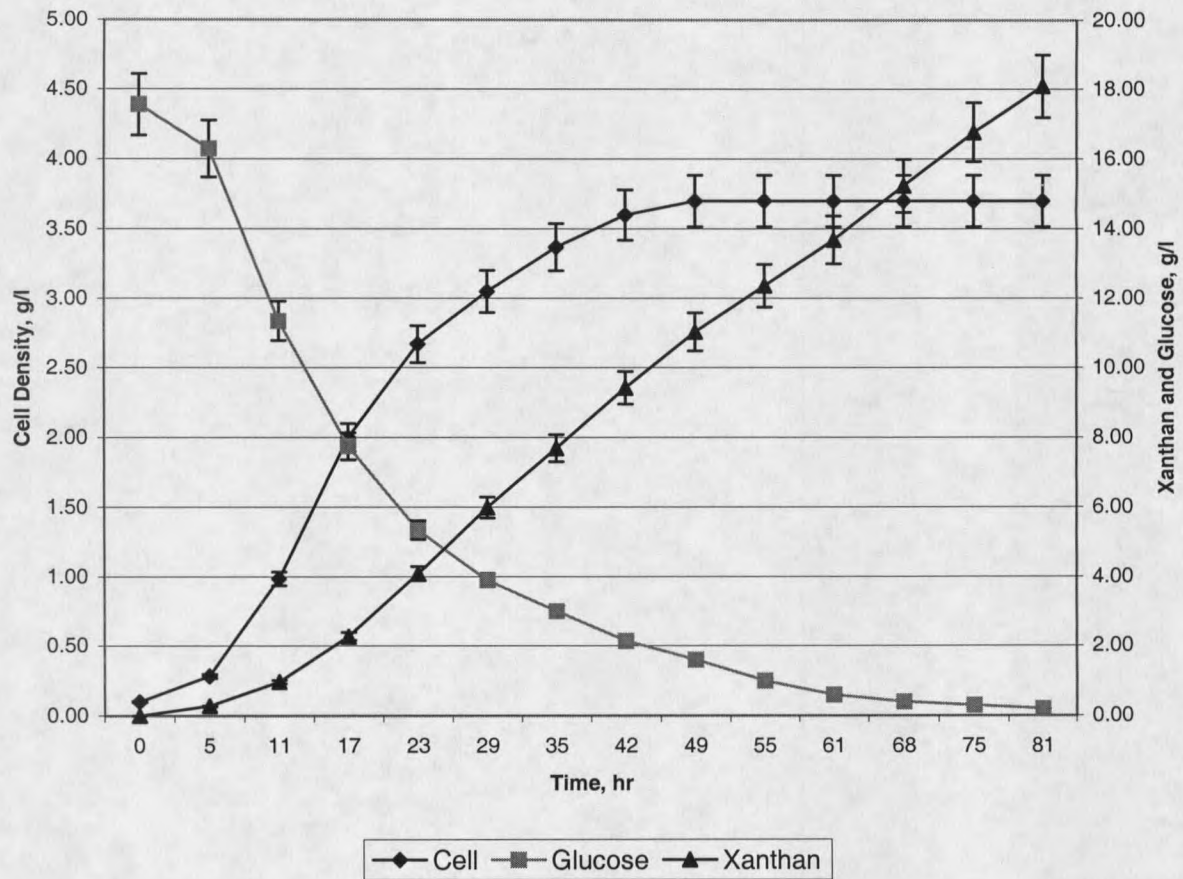


Figure 15. Rotator Batch Operation, Cell, Glucose, and Xanthan in Bulk Phase,
Experiment – 3(a)

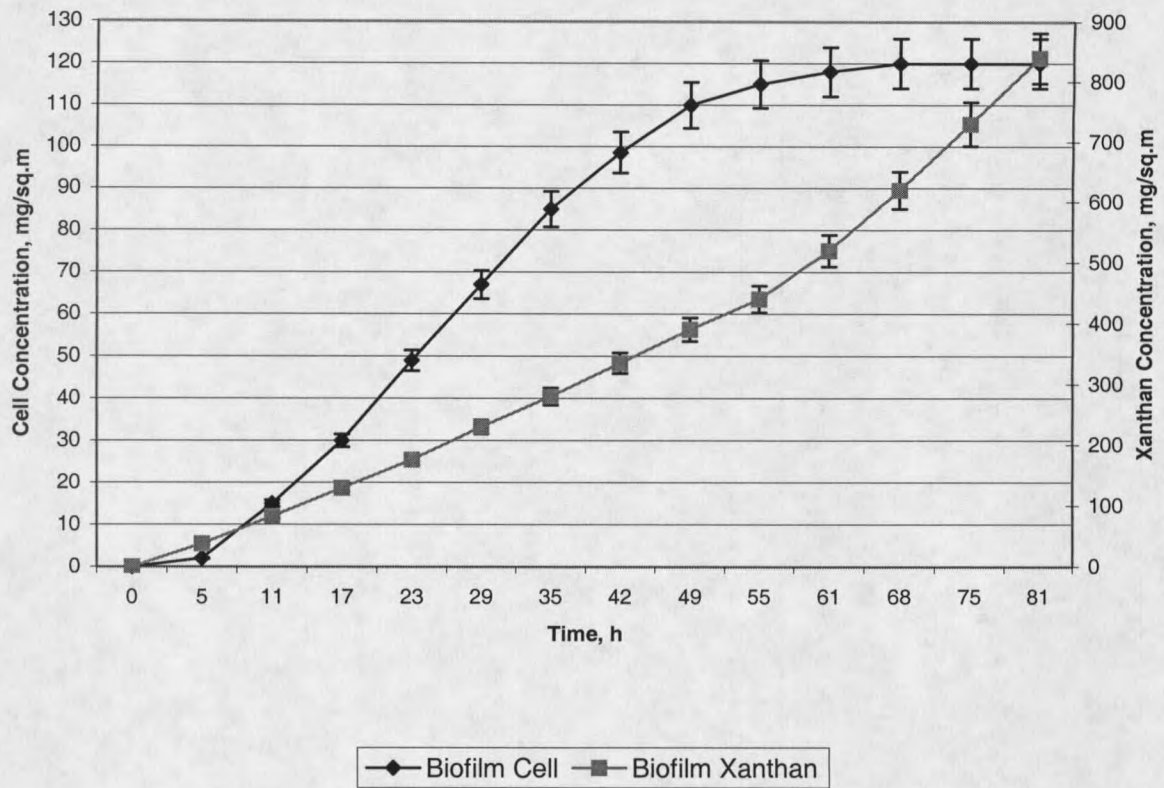


Figure 16. Rototorque Batch Operation, Cell and Xanthan in Biofilm, Experiment – 3(b)

APPENDIX – D

STR CONTINUOUS OPERATION DATA

Expt - 1

Time, h	pH	X, g/l	S, g/l	P, g/l
0	7	0.00	25	0.00
6	6.5	0.25	24	0.50
13	6.3	1.00	23	1.00
19	6.3	1.70	21	1.50
25	6	2.29	19	2.40
31	5.7	2.51	16	3.60
38	5.5	2.55	12	5.20
44	5.5	2.55	8	7.60
51	5.3	2.55	4	9.80
58	5.4	2.55	3	13.00
64	5.1	2.55	2	15.20
70	5.2	2.55	2	17.00
77	5.4	2.55	2	17.60
84	5.2	2.55	2	17.60
91	5.3	2.55	2	17.60
98	5.4	2.55	2	17.60

Expt-2

Time, h	pH	X, g/l	S, g/l	P, g/l
0	7	0.00	25	0.00
6	6.6	0.20	24	0.50
13	6.4	1.00	22	1.00
19	6.2	1.75	18	2.00
25	6.2	2.07	14	2.80
31	5.9	2.21	10	4.00
39	5.6	2.27	7	5.20
45	5.4	2.27	5	6.80
51	5.5	2.27	4	8.80
58	5.2	2.27	3	10.40
64	5.1	2.27	2	13.00
70	5.3	2.27	1	14.94
76	5.2	2.27	1	16.71
83	5.1	2.27	1	17.60
91	5.2	2.27	1	17.60
98	5.3	2.27	1	17.60

Table 22: STR Continuous Operation, $D=0.05/h$

Expt - 1

Time, h	pH	X, g/l	S, Abs	S, g/l	P/20ml	P, g/l
0	7	0.00	0.836	24.54	0.00	0.00
6	6.5	0.24	1.448	23.76	0.01	0.50
13	6.3	0.99	1.374	22.37	0.02	1.00
19	6.3	1.70	1.133	17.84	0.03	1.50
25	6	2.15	1.117	17.54	0.04	2.00
31	5.7	2.55	1.129	17.77	0.08	4.00
38	5.5	2.60	0.431	4.67	0.10	5.00
44	5.5	2.54	0.720	10.10	0.16	8.00
51	5.3	2.60	0.320	2.58	0.18	9.00
58	5.4	2.53	0.299	2.20	0.26	13.00
64	5.1	2.50	0.363	3.40	0.29	14.50
70	5.2	2.49	0.292	2.06	0.34	17.00
77	5.4	2.57	0.238	1.06	0.36	18.00
84	5.2	2.60	0.289	2.00	0.35	17.50
91	5.3	2.47	0.307	2.35	0.35	17.50
98	5.4	2.44	0.231	0.92	0.35	17.50

Expt-2

Time, h	pH	X, g/l	S, Abs	S, g/l	P/20ml	P, g/l
0	7	0.00	0.841	24.73	0.00	0.00
6	6.6	0.20	1.456	23.90	0.01	0.50
13	6.4	0.98	1.389	22.65	0.02	1.00
19	6.2	1.74	1.150	18.17	0.04	2.00
25	6.2	2.18	0.904	13.54	0.05	2.50
31	5.9	2.11	0.814	11.85	0.07	3.50
39	5.6	2.36	0.478	5.55	0.13	6.50
45	5.4	2.29	0.477	5.54	0.17	8.50
51	5.5	2.20	0.388	3.87	0.16	8.00
58	5.2	2.39	0.342	3.00	0.20	10.00
64	5.1	2.30	0.223	0.76	0.26	13.00
70	5.3	2.32	0.275	1.75	0.27	13.50
76	5.2	2.21	0.267	1.59	0.34	17.00
83	5.1	2.25	0.218	0.68	0.34	17.00
91	5.2	2.38	0.267	1.59	0.35	17.50
98	5.3	2.30	0.227	0.84	0.36	18.00

Table 23: STR Continuous Operation Raw Data, $D=0.05h^{-1}$

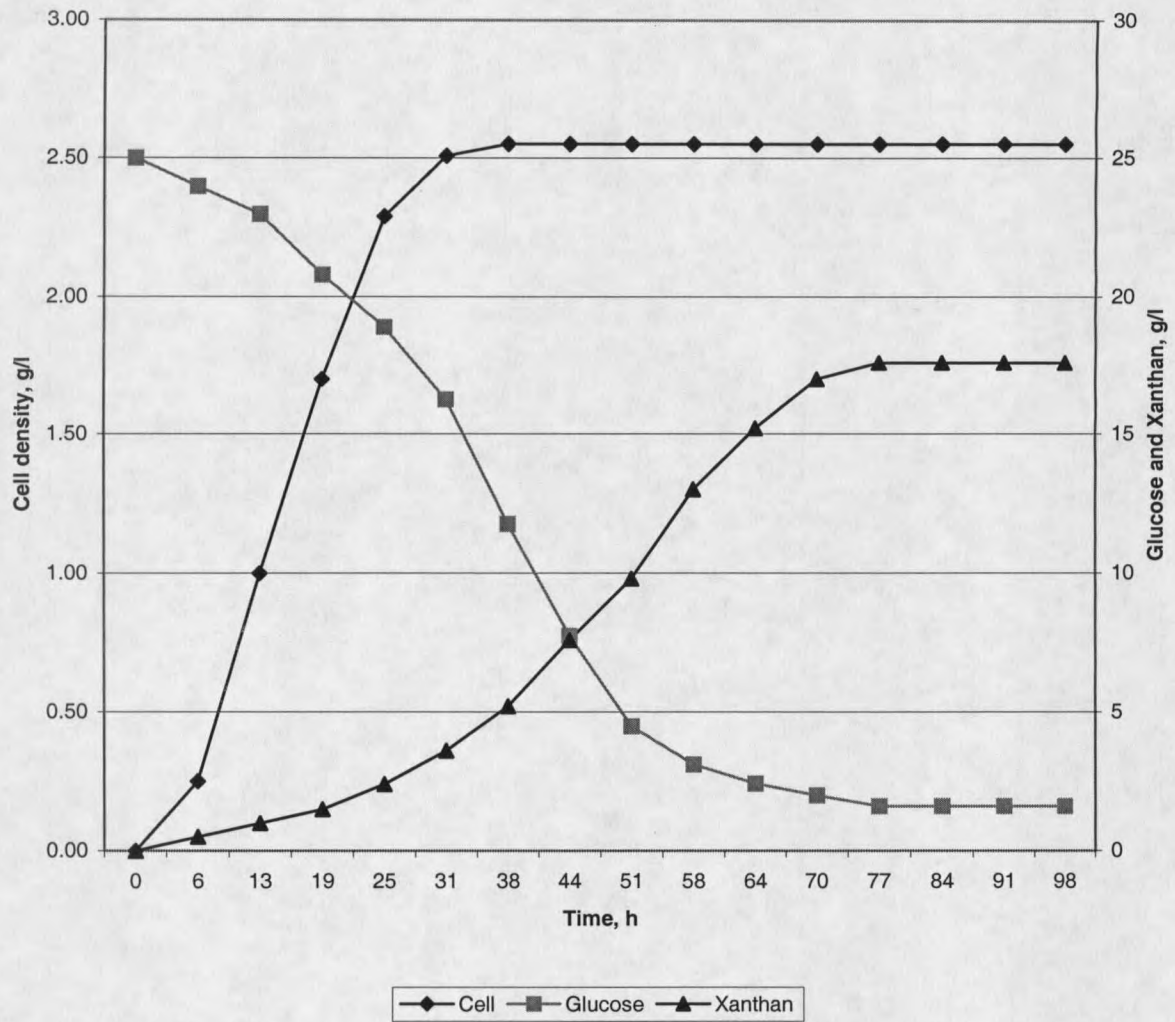


Figure 17. STR Continuous Operation, Cell, Glucose and Xanthan Profiles, $D=0.05h^{-1}$,
Experiment - 1

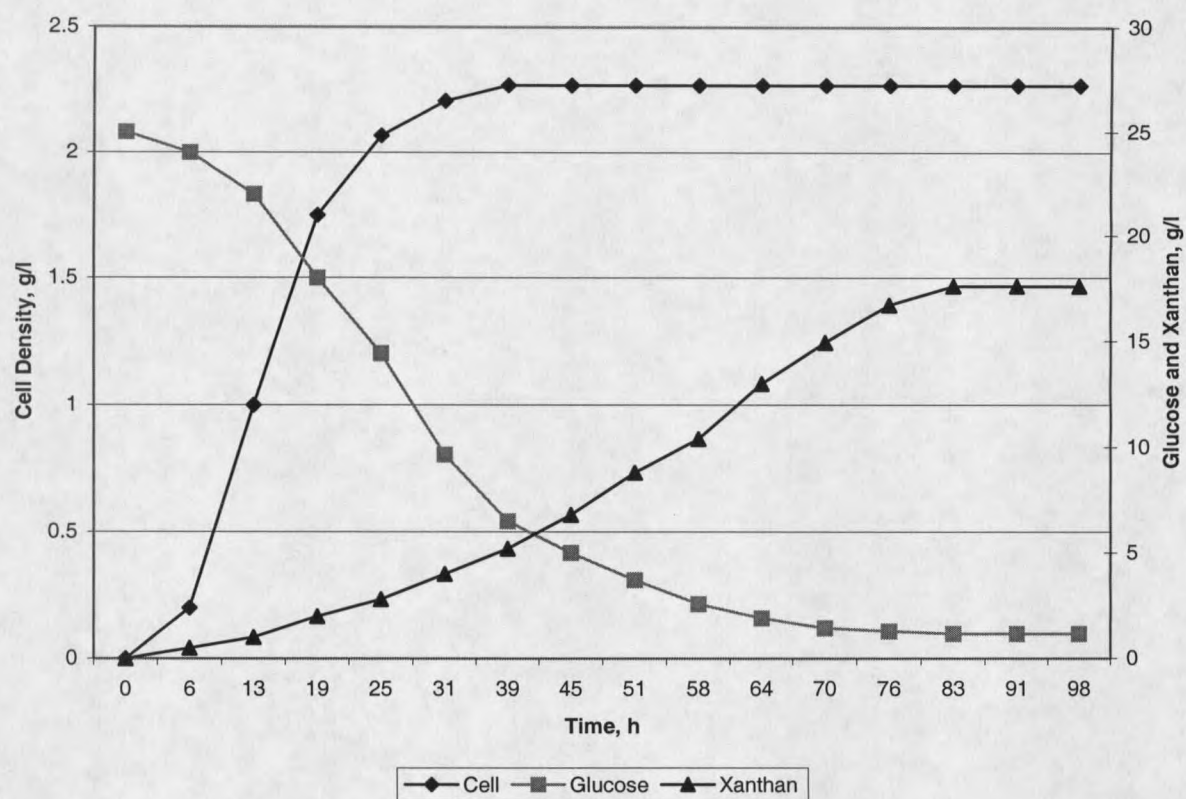


Figure 18. STR Continuous Operation, Cell, Glucose and Xanthan Profiles, $D=0.05h^{-1}$,

Experiment- 2

Expt - 1

Time, h	pH	X, g/l	S, g/l	P, g/l
0	7	0.00	25.00	0.00
7	6.5	0.20	23.50	0.40
13	6.3	0.80	21.50	1.10
20	6.3	1.40	18.20	2.00
26	6	1.90	14.00	3.00
32	5.7	2.20	10.00	4.40
38	5.5	2.40	7.42	6.18
45	5.5	2.45	6.00	8.80
52	5.3	2.45	5.00	10.62
59	5.4	2.45	4.33	11.48
65	5.1	2.45	4.10	12.03
71	5.2	2.45	3.88	12.18
78	5.4	2.45	3.88	12.18
84	5.2	2.45	3.88	12.18
91	5.3	2.45	3.88	12.18
98	5.4	2.45	3.88	12.18

Expt - 2

Time, h	pH	X, g/l	S, g/l	P, g/l
0	7	0.00	25.00	0
6	6.6	0.10	24.10	0.4
12	6.4	0.65	23.00	0.8
18	6.2	1.10	19.70	1.3
25	6.2	1.58	16.00	2.19
32	5.9	1.98	11.50	4
39	5.6	2.12	8.00	6.4
45	5.4	2.23	4.92	8.57
50	5.5	2.25	4.30	10.03
57	5.2	2.25	4.30	10.94
64	5.1	2.25	4.30	11.35
70	5.3	2.25	4.30	11.42
76	5.2	2.25	4.30	11.42
83	5.1	2.25	4.30	11.42
90	5.2	2.25	4.30	11.42
97	5.3	2.25	4.30	11.42

Table 24: STR Continuous Operation, $D=0.10h^{-1}$

Expt - 1

Time, h	pH	X, g/l	S Abs.	S, g/l	P/20ml	P, g/l
0	7	0.00	0.824	24.09	0.00	0
7	6.5	0.17	1.431	23.43	0.01	0.4
13	6.3	0.79	1.330	21.54	0.02	1.11
20	6.3	1.42	1.207	19.23	0.04	1.91
26	6	1.92	0.923	13.90	0.05	2.37
32	5.7	2.11	0.704	9.79	0.11	5.42
38	5.5	2.42	0.517	6.29	0.12	5.95
45	5.5	2.51	0.500	5.97	0.18	9.09
52	5.3	2.42	0.469	5.38	0.20	10.21
59	5.4	2.51	0.356	3.26	0.25	12.35
65	5.1	2.45	0.433	4.71	0.23	11.39
71	5.2	2.31	0.433	4.71	0.26	12.94
78	5.4	2.42	0.458	5.18	0.25	12.67
84	5.2	2.58	0.340	2.96	0.25	12.49
91	5.3	2.52	0.364	3.41	0.23	11.53
98	5.4	2.49	0.417	4.41	0.24	11.8

Expt - 2

Time, h	pH	X, g/l	S Abs.	S, g/l	P/20ml	P, g/l
0	7	0.00	0.830	24.32	0.00	0.00
6	6.6	0.12	1.489	24.53	0.01	0.37
12	6.4	0.64	1.380	22.48	0.03	1.29
18	6.2	1.22	1.192	18.95	0.03	1.33
25	6.2	1.49	1.085	16.94	0.05	2.66
32	5.9	1.92	0.838	12.30	0.06	2.90
39	5.6	2.18	0.472	5.44	0.15	7.67
45	5.4	2.19	0.466	5.33	0.18	9.02
50	5.5	2.18	0.498	5.93	0.19	9.66
57	5.2	2.27	0.377	3.66	0.21	10.44
64	5.1	2.30	0.449	5.01	0.23	11.73
70	5.3	2.19	0.433	4.71	0.23	11.48
76	5.2	2.35	0.377	3.66	0.22	11.12
83	5.1	2.22	0.393	3.96	0.24	11.80
90	5.2	2.32	0.441	4.86	0.24	11.91
97	5.3	2.26	0.340	2.96	0.22	11.03

Table 25: STR Continuous Operation, Raw Data, $D=0.10h^{-1}$

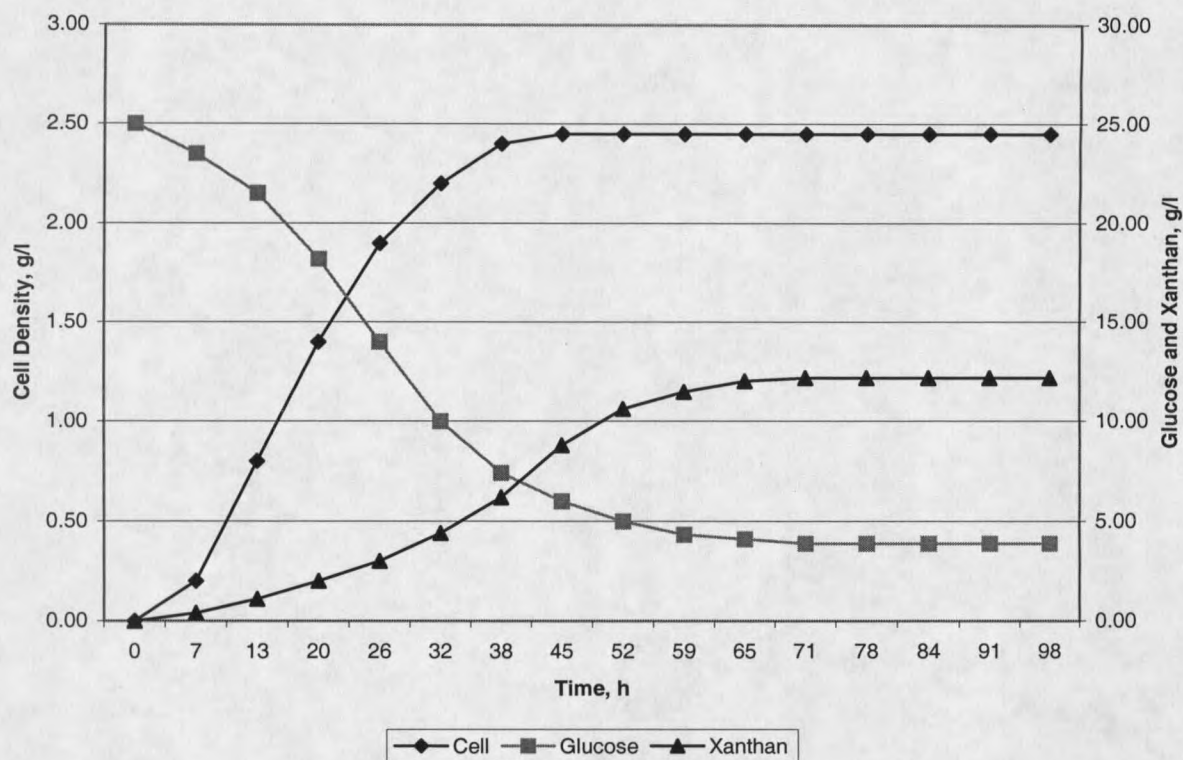


Figure 19. STR Continuous Operation, Cell, Glucose and Xanthan Profiles, $D=0.10\text{h}^{-1}$,

Experiment - 1

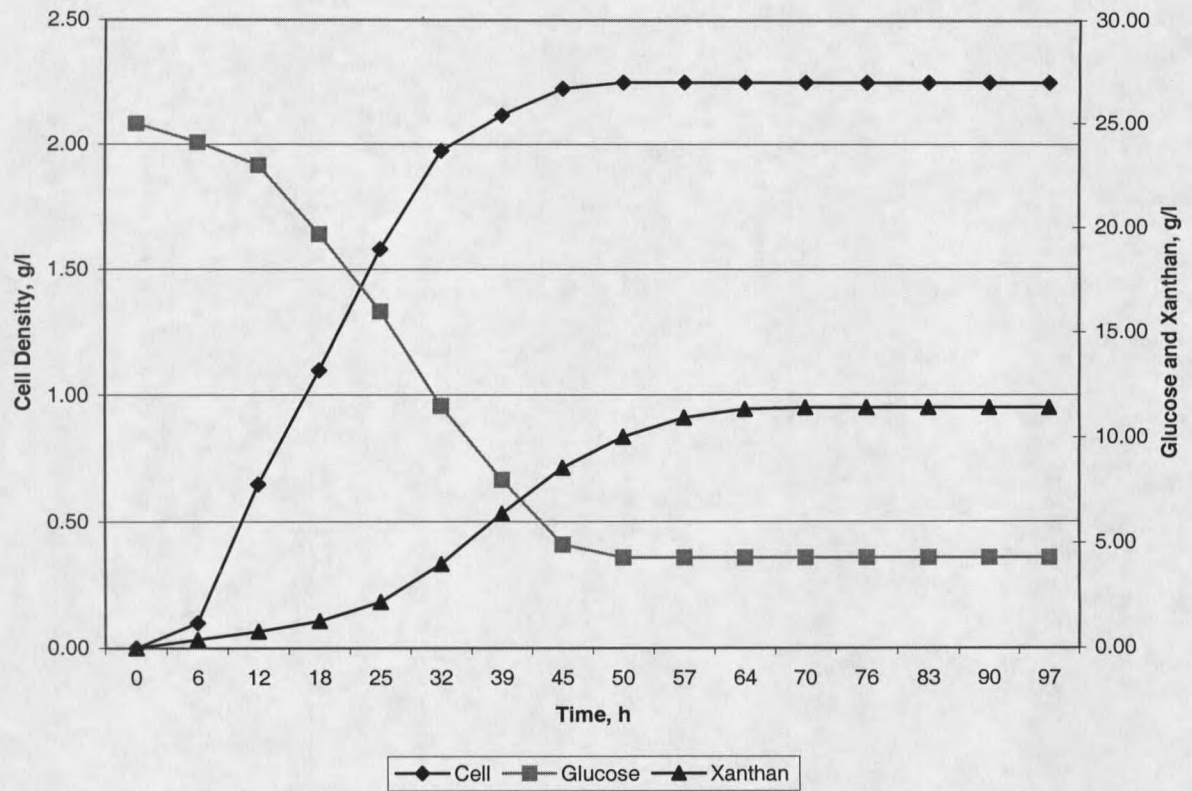


Figure 20. STR Continuous Operation, Cell, Glucose and Xanthan Profiles, $D=0.10\text{h}^{-1}$,

Experiment - 2

Expt-1

Time, h	pH	X, g/l	S, g/l	P, g/l
0	7	0.00	17.5	0.0
7	6.9	0.40	17.0	0.5
13	6.4	1.26	15.0	1.3
18	6.5	1.86	13.0	2.2
24	6.3	2.18	11.8	3.0
29	5.8	2.33	10.5	3.9
36	5.7	2.38	9.9	4.6
42	5.5	2.38	9.2	5.2
49	5.4	2.38	8.7	5.8
55	5.5	2.38	7.9	6.4
61	5.5	2.38	7.0	6.9
69	5.6	2.38	6.3	7.5
76	5.6	2.38	5.8	7.9
83	5.7	2.38	5.8	8.1
90	5.6	2.38	5.8	8.1
97	5.7	2.38	5.8	8.1

Expt-2

Time, h	pH	X, g/l	S, g/l	P, g/l
0	7	0	17.5	0.0
7	6.8	0.5	17.0	0.4
13	6.8	1.28	16.2	1.0
20	6.2	1.94	14.8	1.6
26	6.6	2.22	12.5	2.4
32	6	2.34	11.0	2.9
39	5.5	2.42	9.3	3.7
45	5.6	2.42	8.7	4.6
50	5.2	2.42	8.4	5.4
56	5.5	2.42	8.1	6.0
62	5.4	2.42	7.9	6.3
69	5.3	2.42	7.6	6.8
75	5.5	2.42	7.3	7.0
81	5.4	2.42	7.0	7.2
89	5.4	2.42	7.0	7.2
96	5.3	2.42	7.0	7.2

Table 26: STR Continuous Operation Data, $D=0.15h^{-1}$

Expt-1

Time, h	pH	X, g/l	S, Abs.	S, g/l	P/20ml	P, g/l
0	7	0.00	0.649	17.5235	0.00	0.00
7	6.9	0.34	1.200	19.0994	0.01	0.50
13	6.4	1.26	0.980	14.9719	0.03	1.50
18	6.5	2.01	0.870	12.9081	0.04	2.00
24	6.3	2.07	0.760	10.8443	0.05	2.50
29	5.8	2.36	0.765	10.9381	0.07	3.50
36	5.7	2.41	0.730	10.2814	0.11	5.28
42	5.5	2.45	0.690	9.53096	0.11	5.50
49	5.4	2.52	0.750	10.6567	0.11	5.50
55	5.5	2.47	0.710	9.90619	0.12	6.10
61	5.5	2.28	0.660	8.96811	0.14	7.23
69	5.6	2.31	0.520	6.34146	0.15	7.73
76	5.6	2.36	0.480	5.59099	0.15	7.60
83	5.7	2.43	0.469	5.39	0.17	8.33
90	5.6	2.54	0.532	6.56	0.17	8.27
97	5.7	2.44	0.512	6.2	0.16	7.87

Expt-2

Time, h	pH	X, g/l	S, Abs.	S, g/l	P/20ml	P, g/l
0	7	0	0.649	17.5235	0.00	0.00
7	6.8	0.43	1.186	18.829	0.02	1.00
13	6.8	1.27	1.069	16.6354	0.03	1.50
20	6.2	1.99	0.965	14.6987	0.02	1.00
26	6.6	2.26	0.817	11.9122	0.04	2.00
32	6	2.64	0.718	10.0545	0.07	3.38
39	5.5	2.55	0.615	8.12771	0.08	4.20
45	5.6	2.62	0.725	10.183	0.08	4.20
50	5.2	2.48	0.595	7.74235	0.12	5.84
56	5.5	2.19	0.542	6.75	0.11	5.63
62	5.4	2.53	0.644	8.67	0.12	6.12
69	5.3	2.64	0.628	8.36	0.15	7.39
75	5.5	2.53	0.539	6.69	0.10	4.80
81	5.4	2.55	0.525	6.44	0.15	7.67
89	5.4	1.99	0.581	7.49	0.15	7.46
96	5.3	2.1	0.568	7.24	0.14	7.04

Table 27: STR Continuous Operation, Raw Data, $D=0.15h^{-1}$

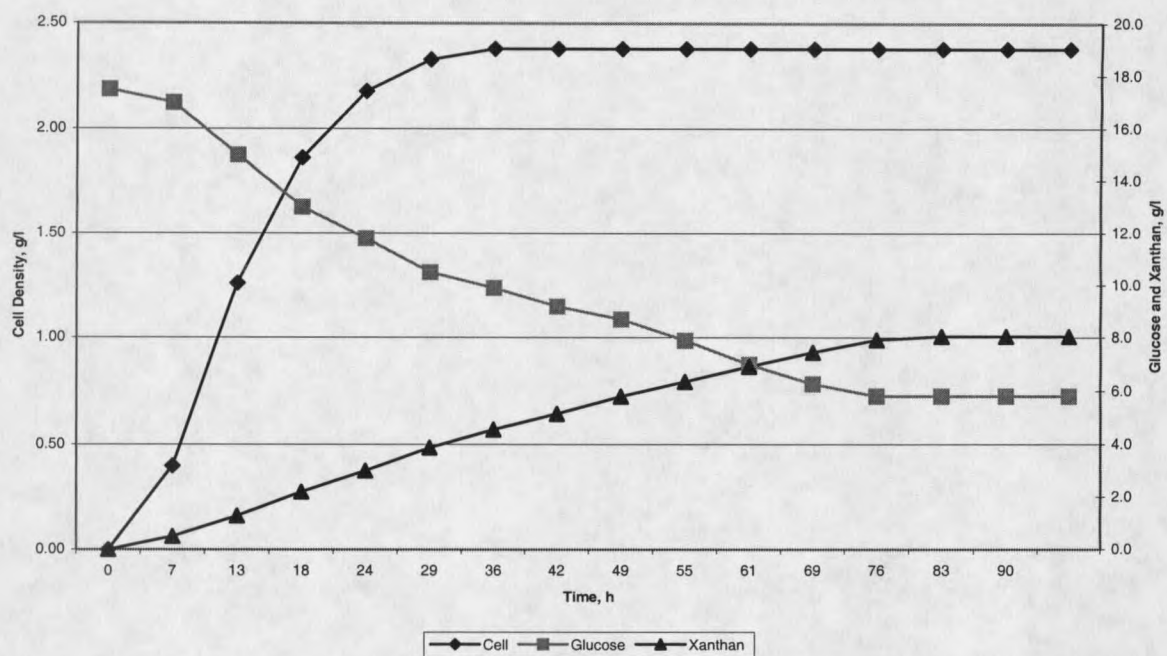


Figure 21. STR Continuous Operation, Cell, Glucose and Xanthan Profiles, $D=0.15\text{h}^{-1}$,
Experiment - 1

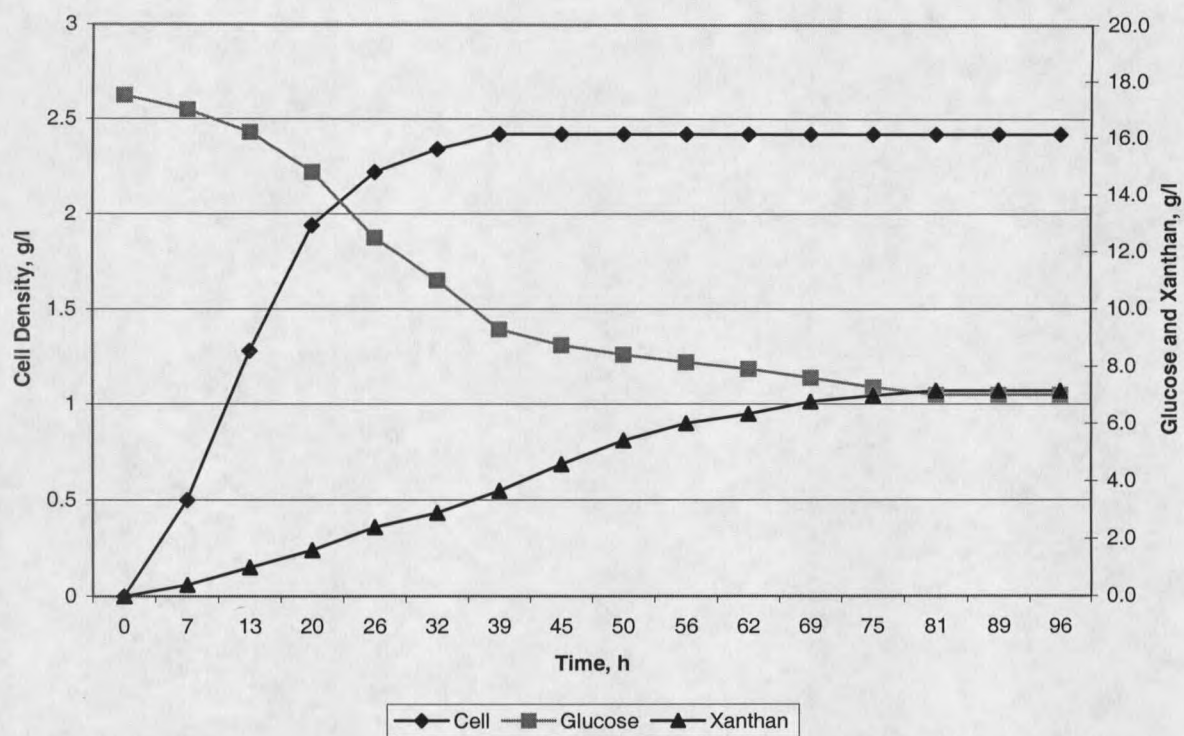


Figure 22. STR Continuous Operation, Cell, Glucose and Xanthan Profiles, $D=0.15h^{-1}$,
Experiment - 2

D, 1/h	S', g/l	X', g/l	P', g/l	S'/D	D*P'/X'	R _g = D(Si-S')/X'
0.05	1.39	2.41	17.60	27.80	0.37	0.49
0.10	4.05	2.35	11.80	40.50	0.50	0.89
0.15	6.41	2.27	7.60	42.73	0.50	0.90

$\mu_{\max} =$	0.34	K =	1.321	$Y_p =$	0.88
$K_s =$	8.42	$K' =$	0.318	$Y_x =$	0.40

Table 6: STR Continuous Operation Data Analysis

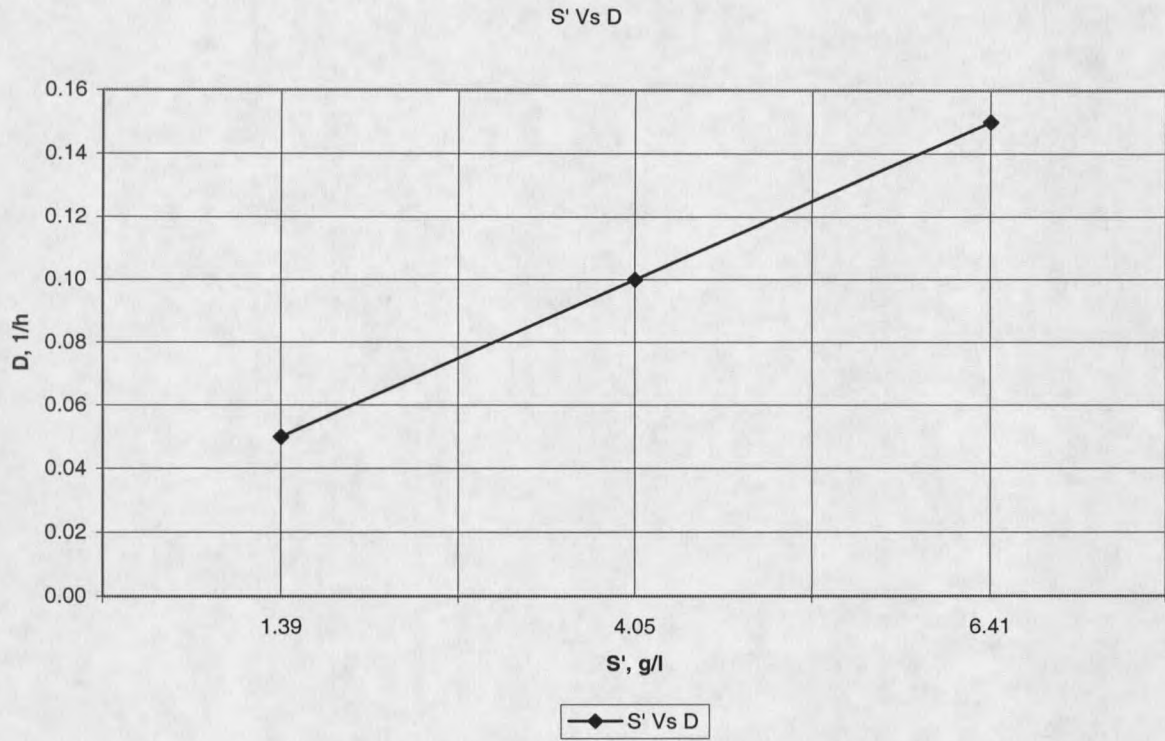


Figure 23. STR Continuous Data Analysis, S' vs D

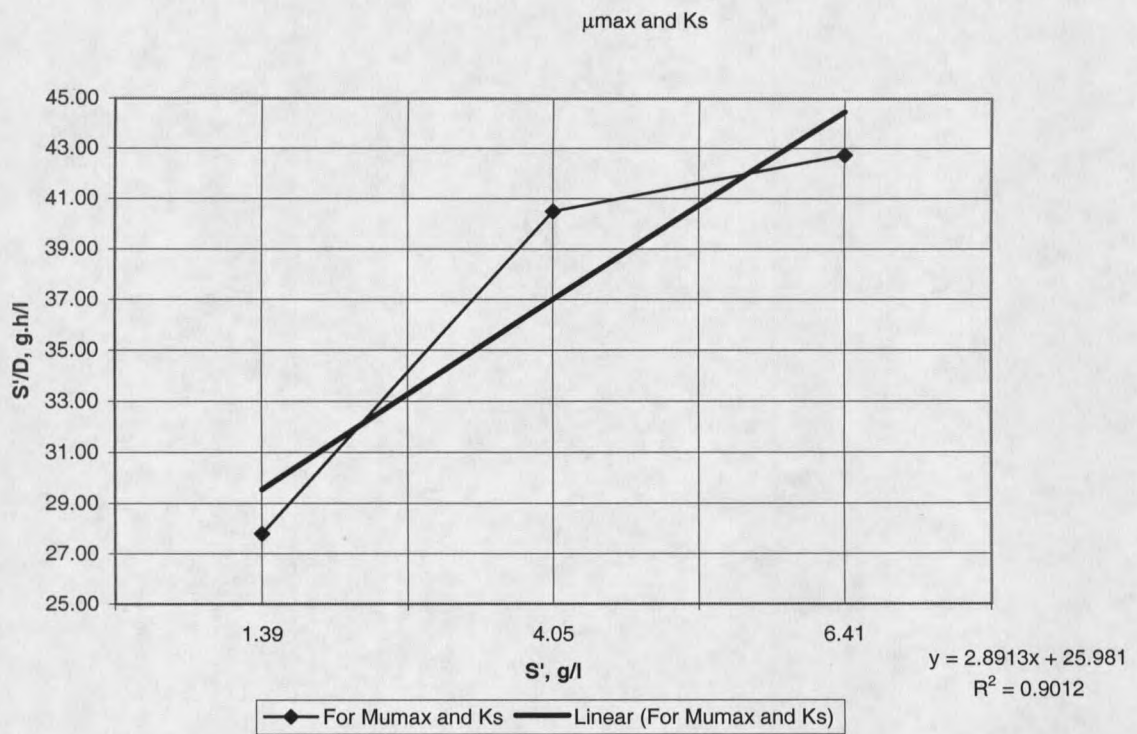


Figure 24. STR Continuous Data Analysis, For $\mu_{\max}$ and K_s

K and K'

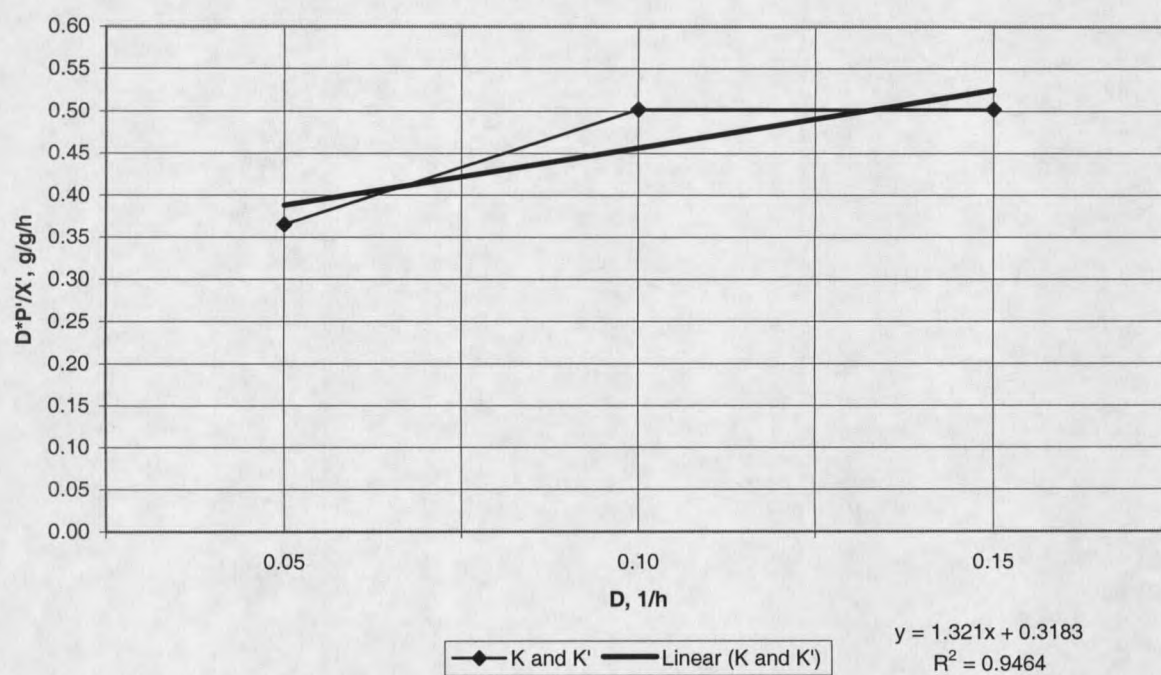
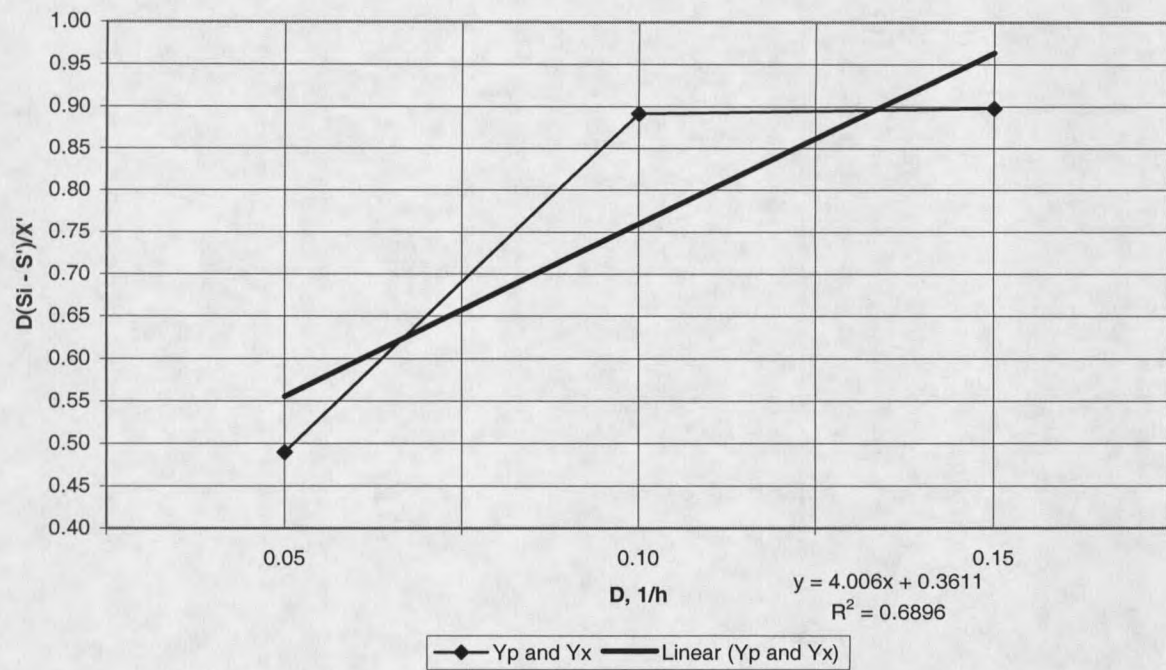


Figure 25. STR Continuous Data Analysis, For K and K'

Y_p and Y_xFigure 26. STR Continuous Data Analysis, For Y_P and Y_X

APPENDIX – E

ROTOTORQUE CONTINUOUS OPERATION DATA

Expt-1

Time	pH	X, g/l	S, g/l	P, g/l	Biofilm X, mg/sq.m	Biofilm P, mg/sq.m
0	7	0.00	25.00	0.00	0	0
6	6.5	0.25	24.50	0.35	4.50	45
13	6.1	0.92	23.50	0.75	12.00	90
19	5.4	1.60	22.28	1.50	24.00	130
25	5.3	1.94	20.33	3.00	52.00	170
31	5.1	2.14	16.50	5.50	81.00	235
38	5.1	2.27	12.08	8.00	90.00	314
44	5.2	2.32	7.43	10.36	95.00	408
51	4.8	2.32	3.10	12.87	98.00	522
58	4.4	2.32	1.83	15.00	99.40	651
64	4.5	2.32	1.83	17.00	101.00	773
70	4.3	2.32	1.83	17.78	101.00	927
77	4.2	2.32	1.83	18.17	101.00	1100
84	4.2	2.32	1.83	18.50	101.00	1180
91	4.1	2.32	1.83	18.50	101.00	1210
98	4.2	2.32	1.83	18.50	101.00	1210

Expt-2

Time	pH	X, g/l	S, g/l	P, g/l	Biofilm X, mg/sq.m	Biofilm P, mg/sq.m
0	7	0.00	25.00	0.00	0	0
6	6.4	0.25	24.74	0.30	4.00	50
13	6.2	0.95	23.31	0.85	15.50	105
19	5.6	1.54	21.81	1.45	29.00	251
25	5.5	1.90	19.96	2.15	45.70	390
31	5.2	2.06	17.64	4.00	71.00	503
39	4.8	2.15	15.17	6.66	82.00	578
45	4.9	2.20	12.44	10.50	87.50	645
51	4.6	2.20	7.66	13.80	91.50	708
58	4.3	2.20	4.24	17.00	94.10	773
64	4.3	2.20	3.14	19.00	96.00	851
70	4.1	2.20	2.46	20.28	97.50	927
76	4.3	2.20	1.98	21.00	98.00	1000
83	4.4	2.20	1.59	21.50	98.00	1060
91	4.2	2.20	1.59	21.50	98.00	1130
98	4.3	2.20	1.59	21.50	98.00	1190

Table 28: Rotator Continuous Operation, D=0.05/h

Expt-1

Time	pH	X, g/l	S, Abs.	S, g/l	P/20ml	P, g/l
0	7	0.00	0.840	24.70	0.00	0.00
6	6.5	0.16	1.490	24.54	0.01	0.50
13	6.1	1.09	1.416	23.15	0.02	1.00
19	5.4	1.59	1.293	20.85	0.02	1.00
25	5.3	1.95	1.317	21.30	0.04	2.00
31	5.1	2.07	0.982	15.00	0.11	5.50
38	5.1	1.89	0.918	13.80	0.16	8.00
44	5.2	2.39	0.402	4.13	0.16	9.63
51	4.8	2.21	0.335	2.87	0.26	13.00
58	4.4	2.39	0.310	2.40	0.29	14.50
64	4.5	2.39	0.261	1.48	0.33	16.50
70	4.3	2.50	0.306	2.33	0.37	18.29
77	4.2	2.22	0.298	2.18	0.35	17.66
84	4.2	2.41	0.267	1.60	0.37	18.35
91	4.1	2.37	0.302	2.26	0.38	19.18
98	4.2	2.26	0.257	1.40	0.38	18.86

Expt-2

Time	pH	X, g/l	S, Abs.	S, g/l	P/20ml	P, g/l
0	7	0.00	0.843	24.80	0.00	0.00
6	6.4	0.26	1.473	24.22	0.01	0.31
13	6.2	0.95	1.438	23.56	0.02	0.85
19	5.6	1.37	1.397	22.80	0.03	1.46
25	5.5	1.99	1.177	18.66	0.04	2.04
31	5.2	2.07	1.024	15.79	0.06	3.12
39	4.8	2.08	1.064	16.54	0.15	7.43
45	4.9	2.25	0.918	13.80	0.16	8.24
51	4.6	2.27	0.397	4.03	0.28	14.21
58	4.3	2.14	0.474	5.47	0.32	16.02
64	4.3	2.56	0.299	2.20	0.38	19.00
70	4.1	2.25	0.283	1.90	0.33	21.00
76	4.3	2.12	0.251	1.29	0.41	20.25
83	4.4	2.23	0.263	1.52	0.43	21.33
91	4.2	2.17	0.251	1.29	0.43	21.27
98	4.3	2.25	0.255	1.37	0.44	21.84

Table 29: Rotatorque Continuous Operation, Raw Data, $D=0.05h^{-1}$

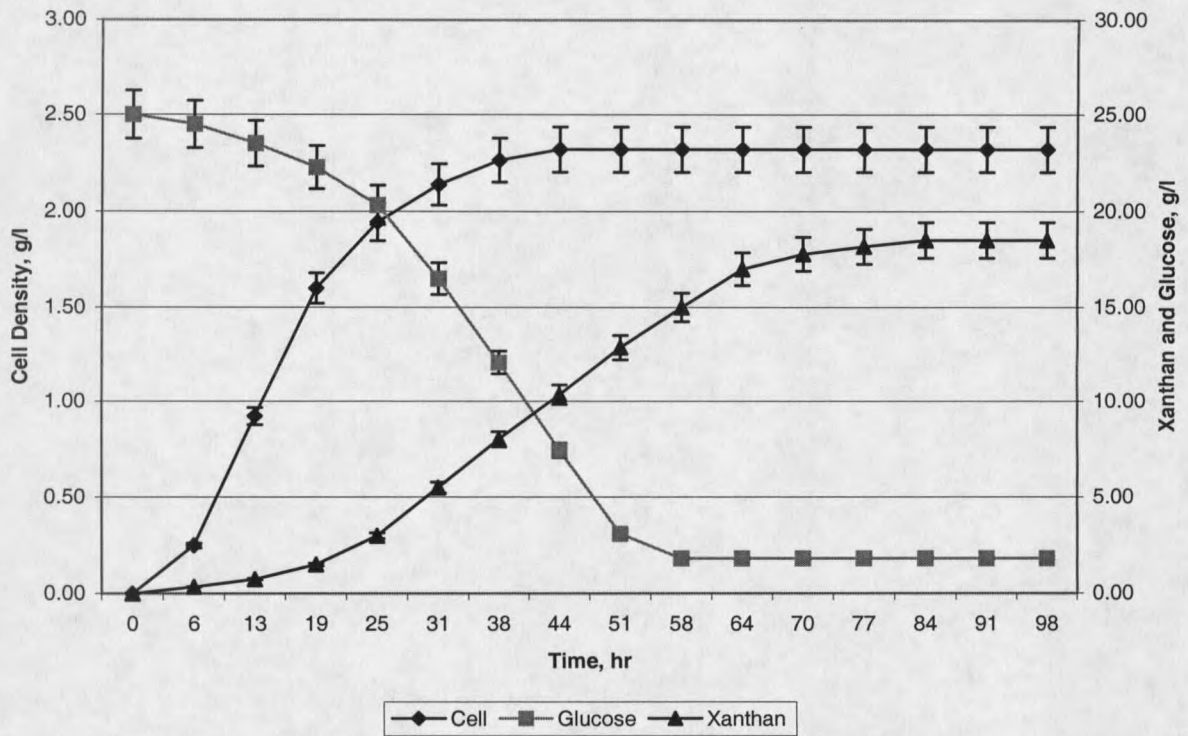


Figure 27. Rototorque Continuous Operation, Cell, Glucose and Xanthan Profiles in Bulk

Phase, $D=0.05\text{h}^{-1}$, Experiment – 1(a)

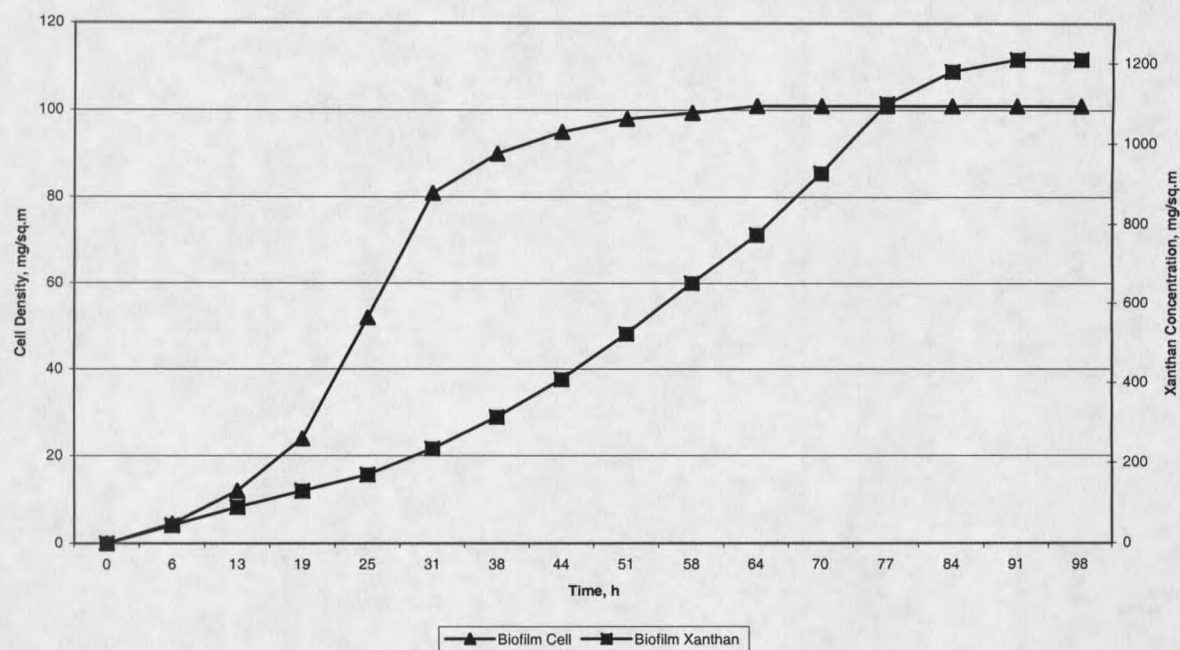


Figure 28. Rototorque Continuous Operation, Cell and Xanthan Profiles in Biofilm,

$D=0.05h^{-1}$, Experiment – 1(b)

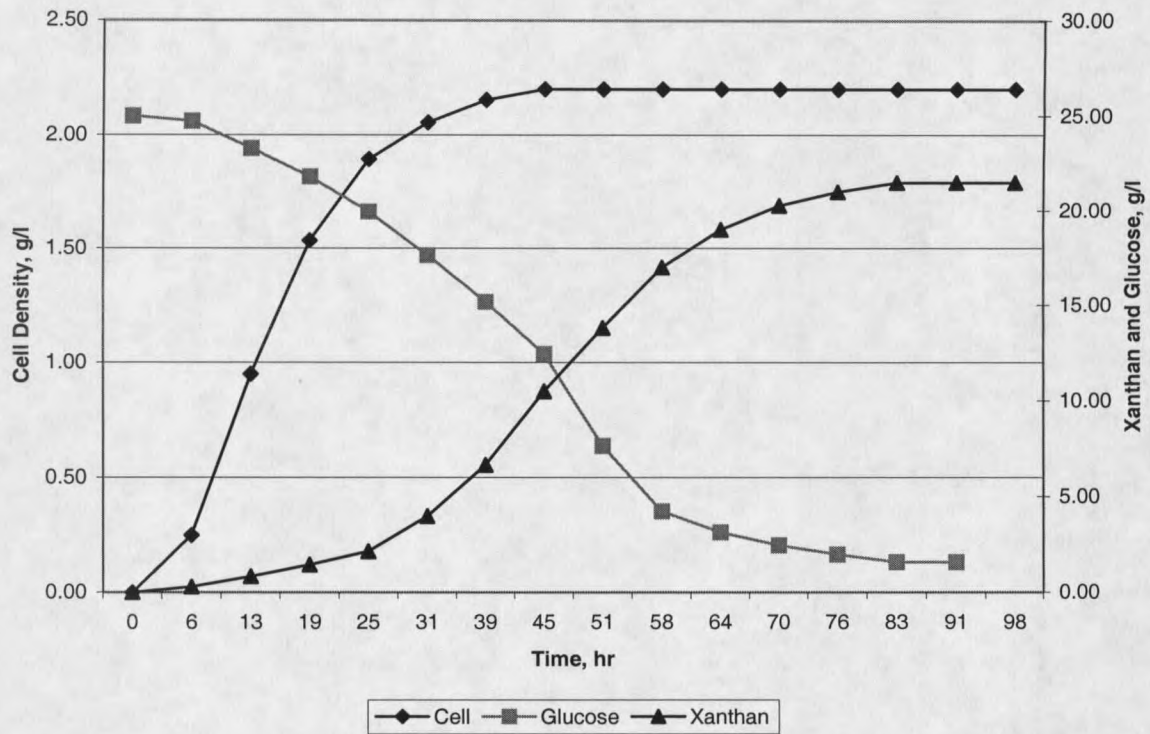


Figure 29. Rotator Continuous Operation, Cell, Glucose and Xanthan Profiles in Bulk

Phase, $D=0.05\text{h}^{-1}$, Experiment - 2(a)

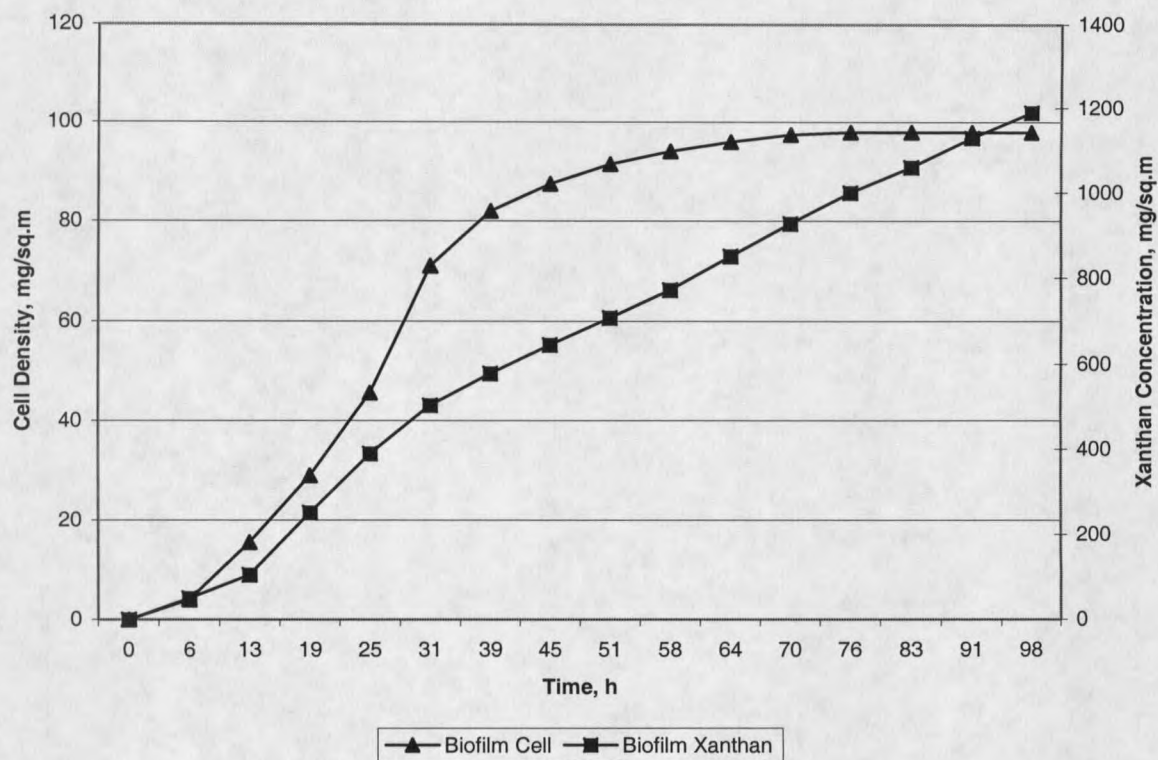


Figure 30. Rotator Continuous Operation, Cell and Xanthan Profiles in Biofilm,
 $D=0.05h^{-1}$, Experiment – 2(b)

Expt-1

Time	pH	X, g/l	S, g/l	P, g/l	Biofilm X, mg/sq.m	Biofilm P, mg/sq.m
0	7	0.00	25.00	0.00	0	0
7	6.6	0.50	24.37	0.50	4.00	33
13	5.8	1.40	23.24	1.00	20.00	56
20	5.3	2.11	21.48	1.80	36.00	100
26	5.6	2.46	19.30	2.80	65.00	150
32	4.9	2.64	17.39	3.80	78.00	200
38	5.2	2.77	14.65	4.95	88.00	250
45	5.2	2.86	11.83	6.70	96.00	313
52	5.1	2.93	8.59	9.15	100.00	390
59	4.5	2.93	6.41	11.45	101.00	480
65	4.6	2.93	5.42	13.56	102.00	575
71	4.4	2.93	4.65	14.17	102.00	676
78	4.5	2.93	4.18	14.20	102.00	776
84	4.6	2.93	4.18	14.20	102.00	840
91	4.3	2.93	4.18	14.20	102.00	880
98	4.2	2.93	4.18	14.20	102.00	920

Expt-2

Time	pH	X, g/l	S, g/l	P, g/l	Biofilm X, mg/sq.m	Biofilm P, mg/sq.m
0	7	0.00	25.00	0	0	0
6	6.4	0.35	24.72	0.5	6.00	20
12	6.2	1.05	23.54	0.9	20.00	50
18	5.9	1.73	22.43	1.5	36.00	75
25	5.5	2.13	20.90	2.5	51.20	120
32	5	2.29	19.24	4	63.70	160
39	4.9	2.42	17.00	5.5	75.30	220
45	4.5	2.49	14.38	7.5	86.00	280
50	4.8	2.49	9.86	10.06	94.00	357
57	4.6	2.49	7.08	11.94	100.00	440
64	4.2	2.49	5.69	13.72	102.00	547
70	4.5	2.49	5.07	14.94	104.00	670
76	4.5	2.49	4.79	15.39	105.00	762
83	4.6	2.49	4.60	15.6	106.00	840
90	4.2	2.49	4.60	15.6	106.00	920
97	4.5	2.49	4.60	15.6	106.00	1000

Table 30: Rotator Continuous Operation Data, $D=0.10h^{-1}$

Expt-1

Time	pH	X, g/l	S, Abs	S, g/l	P/20ml	P, g/l
0	7	0.00	0.840	24.69	0.00	0.00
7	6.6	0.27	1.492	24.58	0.01	0.50
13	5.8	1.37	1.383	22.54	0.02	1.04
20	5.3	2.22	1.357	22.04	0.03	1.50
26	5.6	2.41	1.113	17.46	0.05	2.50
32	4.9	2.72	1.088	17.00	0.10	5.05
38	5.2	2.84	1.038	16.06	0.09	4.30
45	5.2	2.79	0.906	13.59	0.16	6.20
52	5.1	2.92	0.462	5.25	0.20	10.10
59	4.5	2.97	0.561	7.11	0.24	12.00
65	4.6	2.67	0.467	5.35	0.27	13.31
71	4.4	3.04	0.452	5.07	0.33	14.85
78	4.5	2.98	0.362	3.38	0.31	15.25
84	4.6	2.85	0.367	3.47	0.25	12.51
91	4.3	2.94	0.392	3.94	0.30	15.05
98	4.2	2.98	0.390	3.90	0.31	15.45

Expt-2

Time	pH	X, g/l	S, Abs	S, g/l	P/20ml	P, g/l
0	7	0.00	0.848	25.00	0.00	0
6	6.4	0.36	1.448	23.75	0.01	0.5
12	6.2	1.01	1.396	22.78	0.02	1.25
18	5.9	1.81	1.193	18.96	0.02	1
25	5.5	2.04	1.259	20.21	0.05	2.5
32	5	2.24	1.274	20.49	0.07	3.5
39	4.9	2.54	1.041	16.11	0.13	6.5
45	4.5	2.44	0.926	13.96	0.16	7.65
50	4.8	2.52	0.856	12.64	0.20	9.83
57	4.6	2.47	0.552	6.94	0.23	11.5
64	4.2	2.56	0.409	4.26	0.28	14.17
70	4.5	2.57	0.441	4.86	0.33	14.84
76	4.5	2.53	0.417	4.41	0.29	14.51
83	4.6	2.45	0.449	5.00	0.30	14.78
90	4.2	2.53	0.449	5.00	0.33	16.38
97	4.5	2.53	0.315	2.50	0.33	16.58

Table 31: Rotatorque Continuous Operation, Raw Data, $D=0.10h^{-1}$

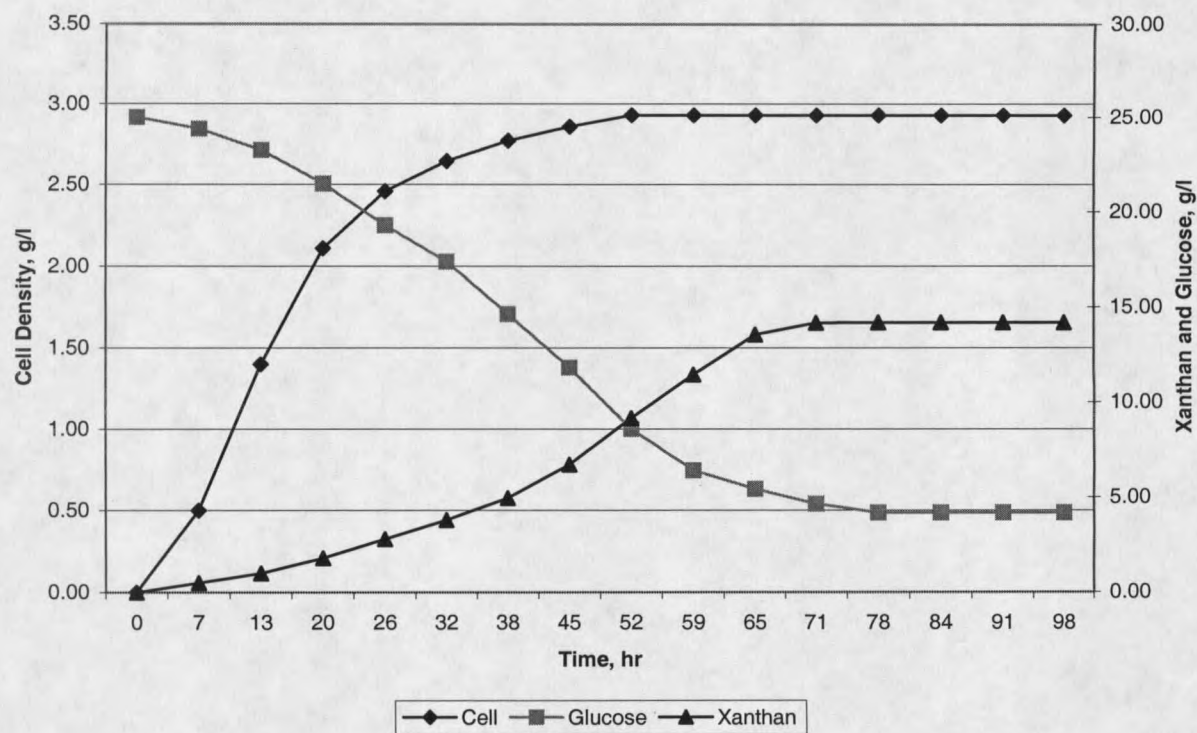


Figure 31. Rotator Continuous Operation, Cell, Glucose and Xanthan Profiles in Bulk
Phase, $D=0.10\text{h}^{-1}$, Experiment – 1(a)

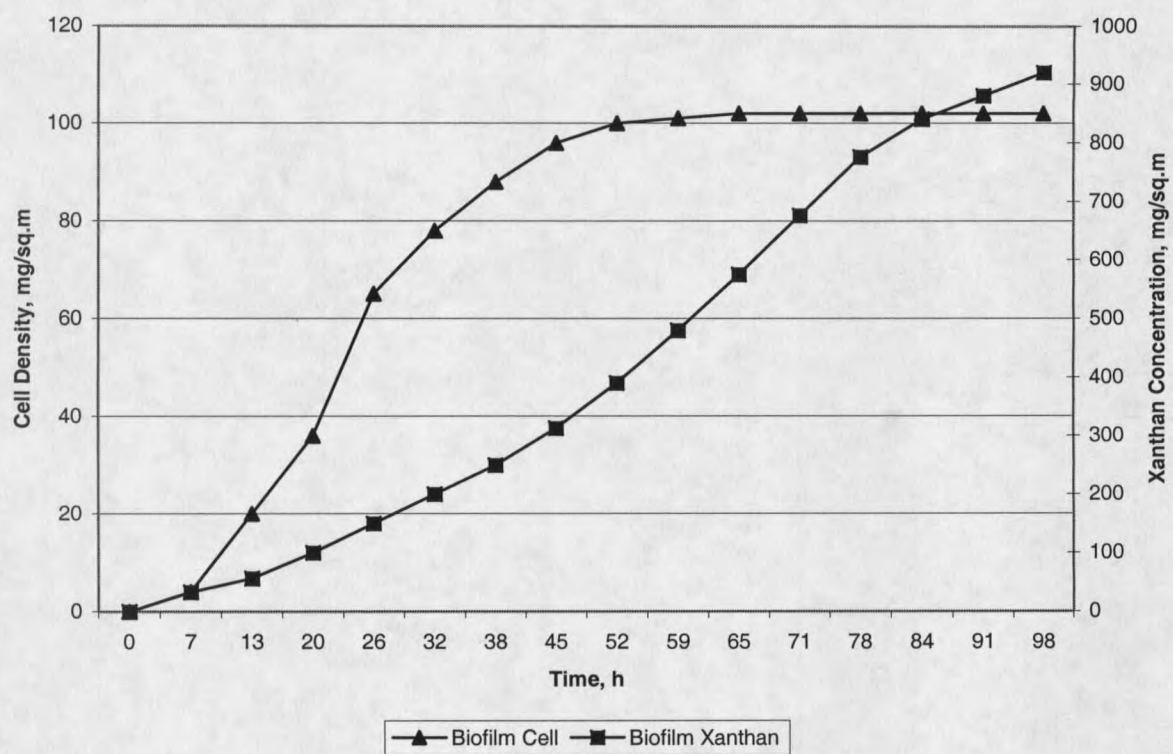


Figure 32. Rototorque Continuous Operation, Cell and Xanthan Profiles in Biofilm,

$D=0.10h^{-1}$, Experiment – 1(b)

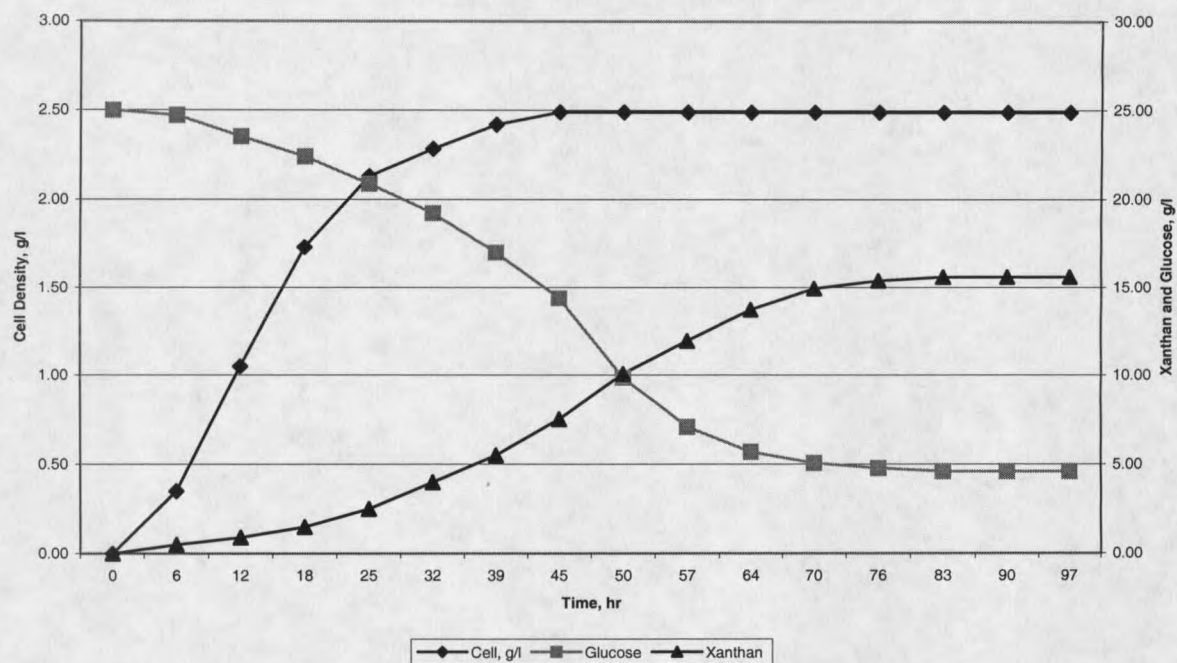


Figure 33. Rotator Continuous Operation, Cell, Glucose and Xanthan Profiles in Bulk

Phase, $D=0.10\text{h}^{-1}$, Experiment – 2(a)

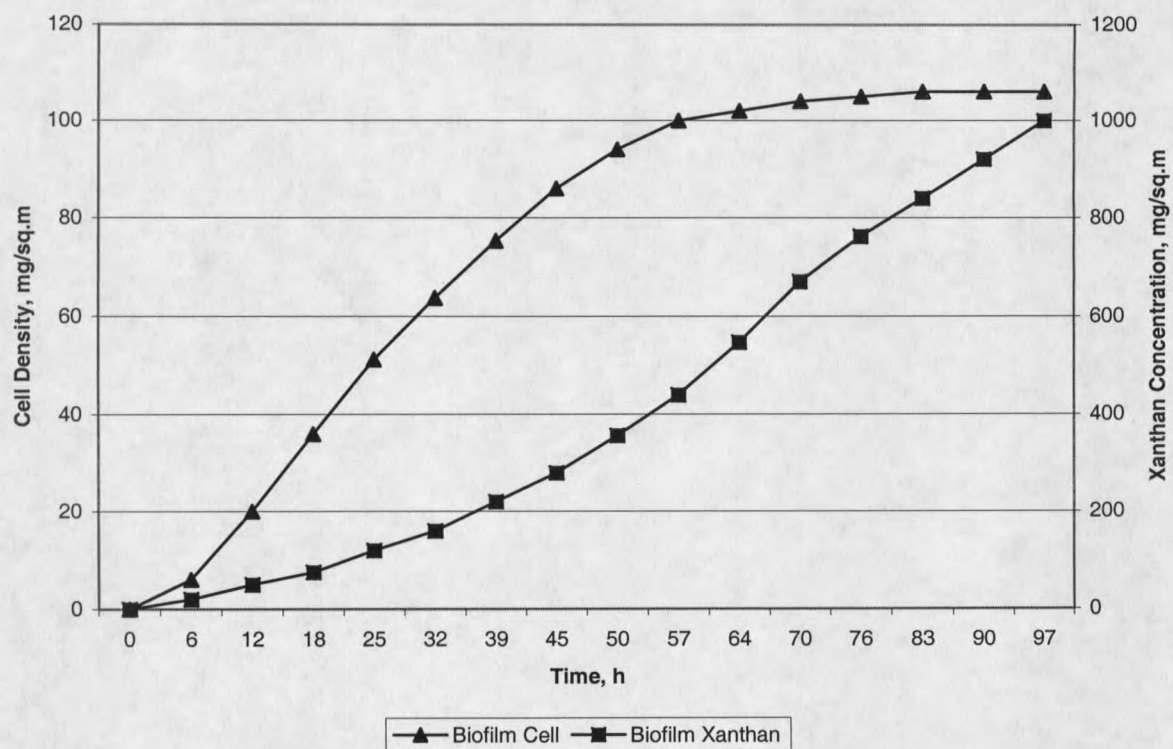


Figure 34. Rotator Continuous Operation, Cell and Xanthan Profiles in Biofilm,

$D=0.10h^{-1}$, Experiment – 2(b)

Expt-1

Time, h	pH	X, g/l	S, g/l	P, g/l	Biofilm X, mg/sq.m	Biofilm P, mg/sq.m
0	7	0	17.5	0.0	0	0
6	6.5	0.4	17.4	0.4	6.00	20
12	6.1	1.4	16.7	0.9	19.00	59
18	5.7	1.755	15.5	1.6	35.70	110
24	5.7	1.988	14.0	2.6	52.00	167
31	5.1	2.062	12.9	4.0	73.00	225
37	5.4	2.12	11.4	5.7	85.00	299
43	5.2	2.12	10.0	6.6	90.00	363
49	5.2	2.12	8.9	7.1	92.00	426
55	5.2	2.12	8.4	7.4	93.00	485
61	5.4	2.12	8.1	7.7	94.00	560
69	5.1	2.12	7.9	8.0	94.00	650
75	5.1	2.12	8.0	8.1	94.00	727
82	5.2	2.12	8.0	8.2	94.00	785
89	5.2	2.12	8.0	8.2	94.00	820
96	5.1	2.12	8.0	8.2	94.00	850

Expt-2

Time, h	pH	X, g/l	S, g/l	P, g/l	Biofilm X, mg/sq.m	Biofilm P, mg/sq.m
0	7	0	17.5	0	0	0
7	6.7	0.4	17.4	0.55	4.00	10
13	6.5	1.15	17.2	1	10.00	20
20	6.3	1.517	15.6	1.73	24.00	60
26	6.1	1.68	13.5	2.9	43.00	100
32	5.6	1.765	11.9	4	55.00	167
39	5.5	1.819	10.7	4.95	66.00	238
45	5.3	1.84	10.0	5.51	76.00	321
50	5.1	1.84	9.5	6.07	82.00	393
56	5.2	1.84	9.5	6.4	84.00	480
62	5.1	1.84	9.4	6.75	84.00	560
69	5	1.84	9.3	7.06	84.00	615
75	5.2	1.84	9.2	7.18	84.00	670
81	5	1.84	9.1	7.28	84.00	709
89	5.1	1.84	9.1	7.28	84.00	740
96	5.1	1.84	9.1	7.28	84.00	770

Table 32: Rotator Continuous Operation Data, $D=0.15h^{-1}$

Expt-1

Time, h	pH	X, g/l	S, Abs	S, g/l	P/20ml	P, g/l
0	7	0.00	0.647	17.45	0.00	0
6	6.5	0.27	1.210	19.29	0.01	0.419
12	6.1	1.50	1.140	17.97	0.02	0.84
18	5.7	1.75	0.970	14.78	0.03	1.63
24	5.7	1.91	0.930	14.03	0.04	2.21
31	5.1	2.16	0.870	12.91	0.08	4
37	5.4	2.26	0.790	11.41	0.13	6.27
43	5.2	2.17	0.710	9.91	0.16	5.65
49	5.2	2.03	0.650	8.78	0.13	6.42
55	5.2	2.08	0.662	9.00	0.15	7.66
61	5.4	2.10	0.649	8.77	0.17	8.44
69	5.1	2.22	0.622	8.25	0.33	8.13
75	5.1	2.16	0.573	7.34	0.16	8.05
82	5.2	2.04	0.582	7.50	0.15	7.59
89	5.2	2.18	0.590	7.66	0.16	7.89
96	5.1	2.13	0.632	8.45	0.17	8.45

Expt-2

Time, h	pH	X, g/l	S, Abs	S, g/l	P/20ml	P, g/l
0	7	0	0.647	17.45	0	0
7	6.7	0.25	1.171	18.56	0.011	0.55
13	6.5	1.15	1.139	17.96	0.019	0.95
20	6.3	1.263	1.051	16.30	0.045	2.25
26	6.1	1.765	0.902	13.50	0.058	2.9
32	5.6	1.923	0.746	10.58	0.079	3.95
39	5.5	1.848	0.783	11.27	0.09	4.5
45	5.3	1.83	0.723	10.15	0.161	7.14
50	5.1	1.904	0.667	9.10	0.1052	5.26
56	5.2	1.746	0.703	9.78	0.13	6.5
62	5.1	1.87	0.680	9.35	0.144	7.2
69	5	1.839	0.634	8.48	0.311	7.93
75	5.2	1.97	0.672	9.20	0.1338	6.69
81	5	1.876	0.843	12.40	0.1412	7.06
89	5.1	1.757	0.687	9.47	0.1498	7.49
96	5.1	1.757	0.654	8.85	0.151	7.55

Table 33: Rotatorque Continuous Operation, Raw Data, $D=0.15h^{-1}$

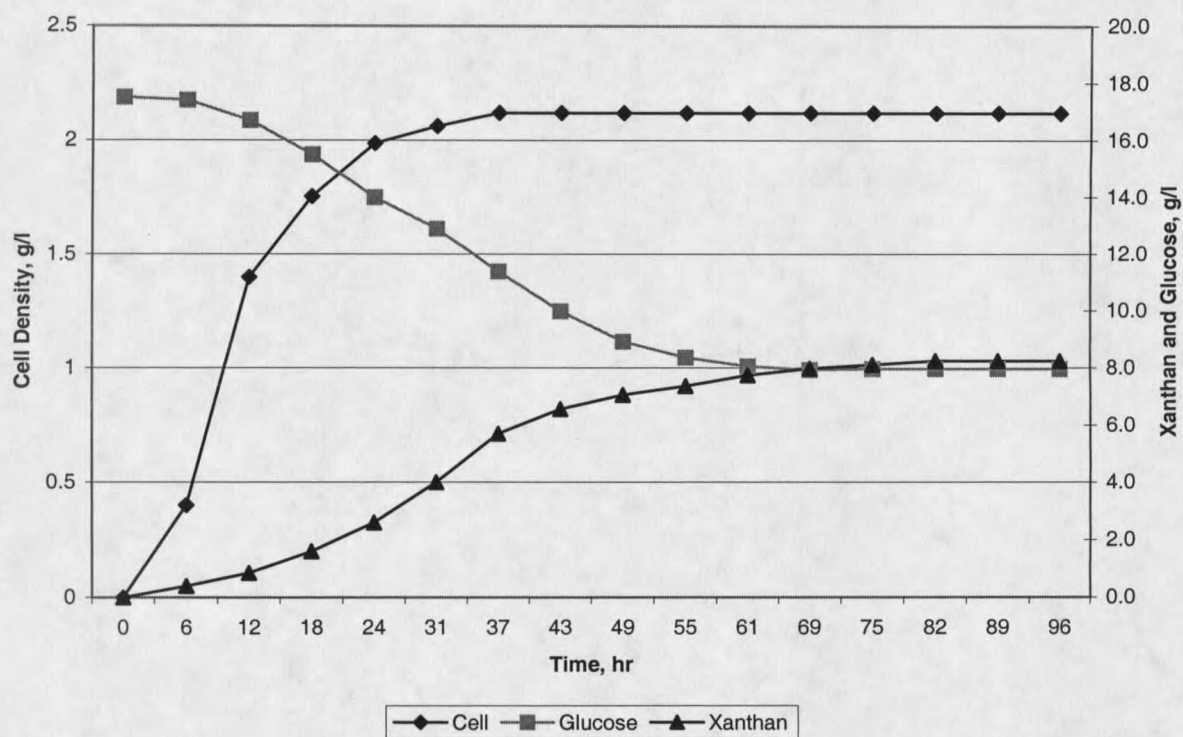


Figure 35. Rotator Continuous Operation, Cell, Glucose and Xanthan Profiles in Bulk

Phase, $D=0.15h^{-1}$, Experiment – 1(a)

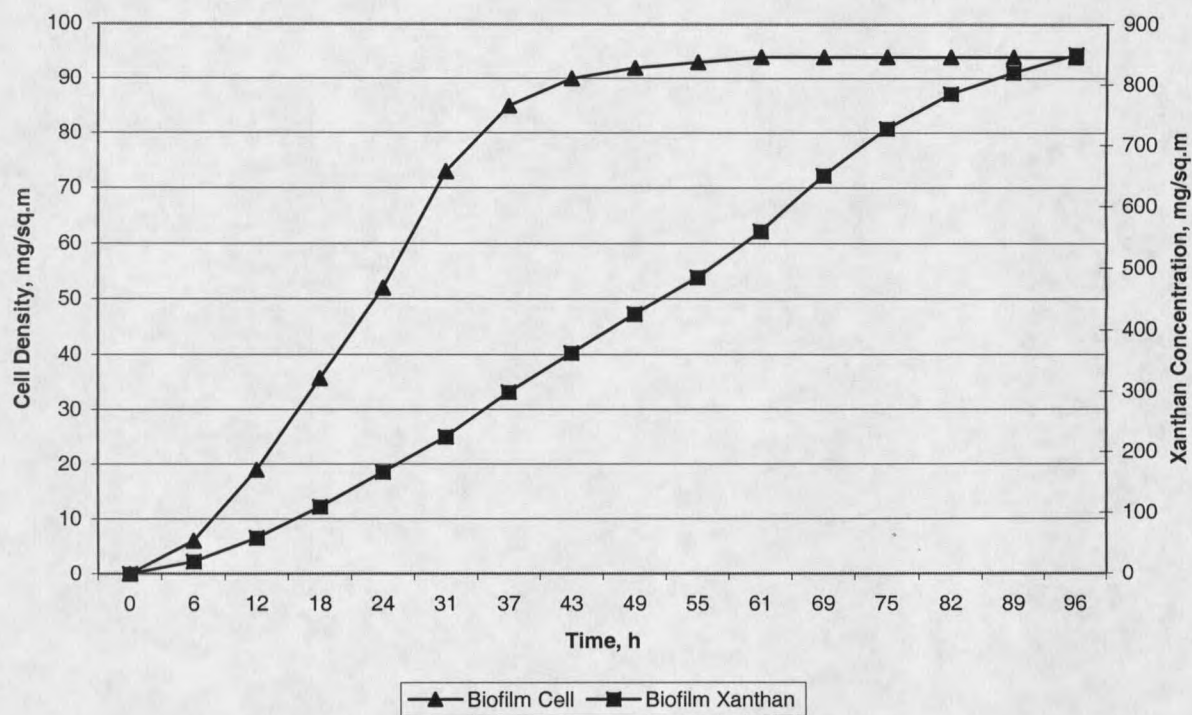


Figure 36. Rotator Continuous Operation, Cell and Xanthan Profiles in Biofilm,

$D=0.15h^{-1}$, Experiment – 1(b)

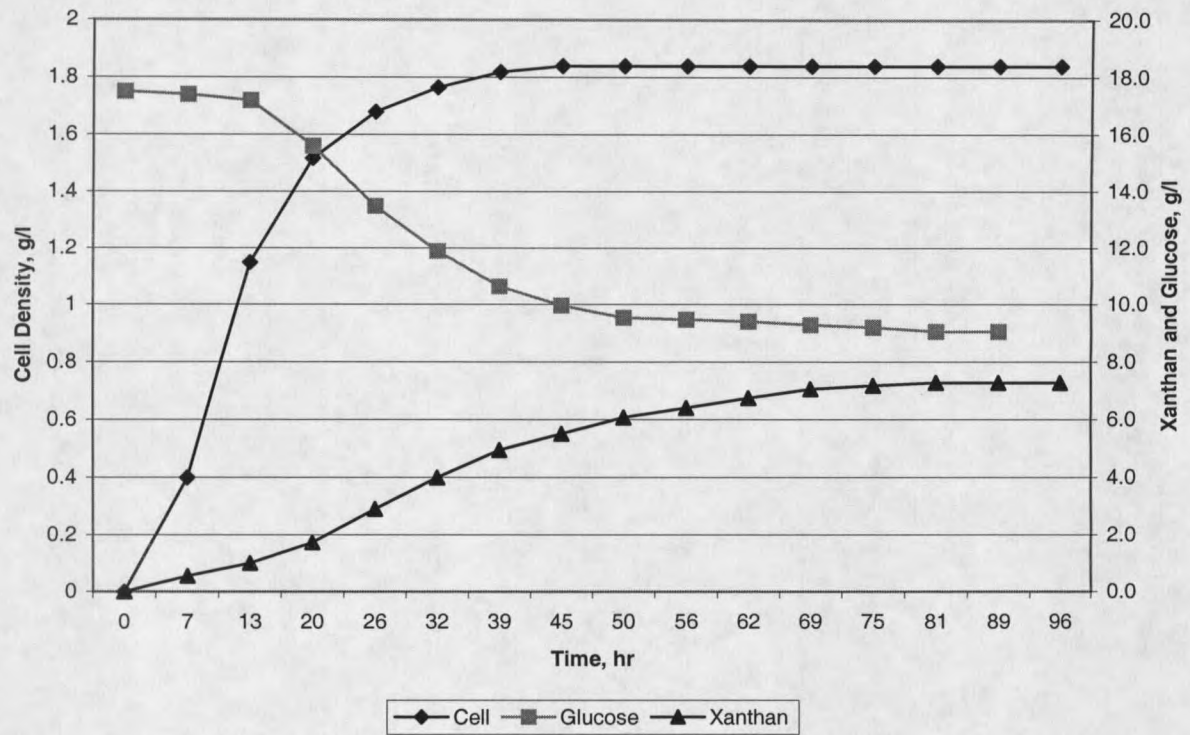


Figure 37. Rotator Continuous Operation, Cell, Glucose and Xanthan Profiles in Bulk Phase, $D=0.15h^{-1}$, Experiment - 2(a)

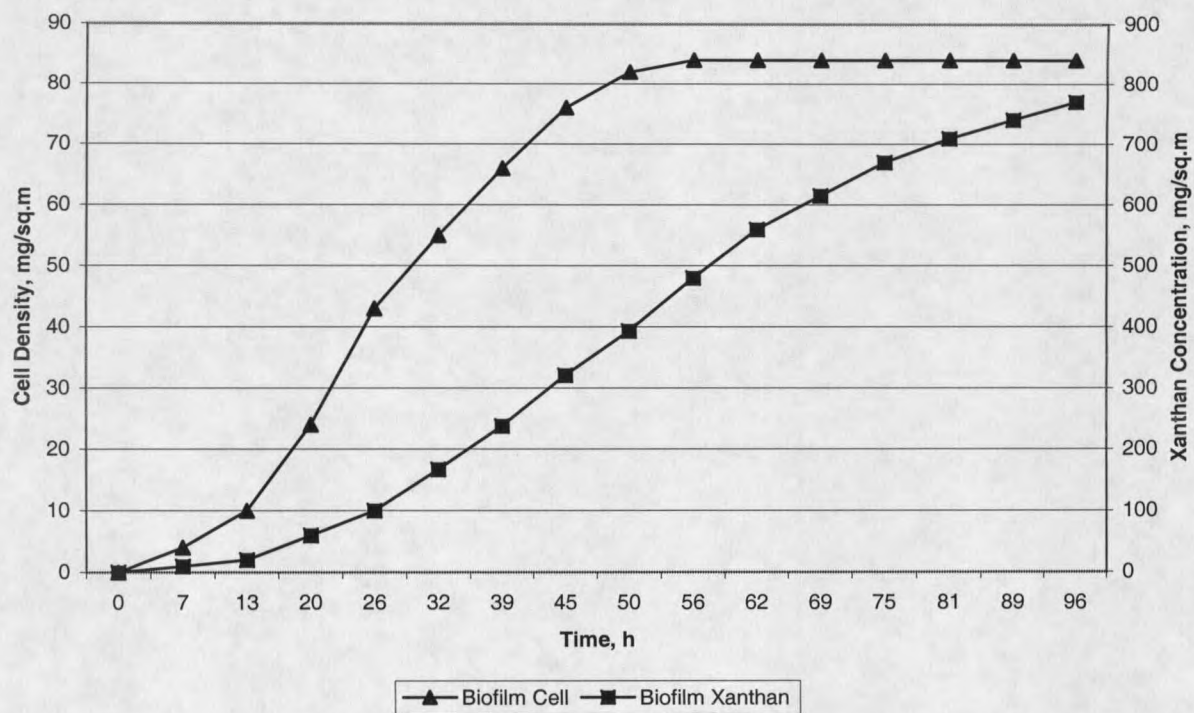


Figure 38. Rotator Continuous Operation, Cell and Xanthan Profiles in Biofilm,

$D=0.15h^{-1}$, Experiment – 2(b)

D, 1/h	X'	S'	P'	S'/D	D*P'/X'	Rg = D(Si-S')/X'
0.05	2.26	1.71	19.00	34.14	0.42	0.52
0.10	2.71	4.39	14.90	43.94	0.55	0.76
0.15	1.98	8.51	7.76	56.74	0.59	0.87

$\mu_{\max} =$	0.31	K=	1.67	$Y_p =$	0.97
$K_s =$	9.52	K'=	0.35	$Y_x =$	0.55

Table7: Rototorque Continuous Operation Data Analysis

S' Vs D

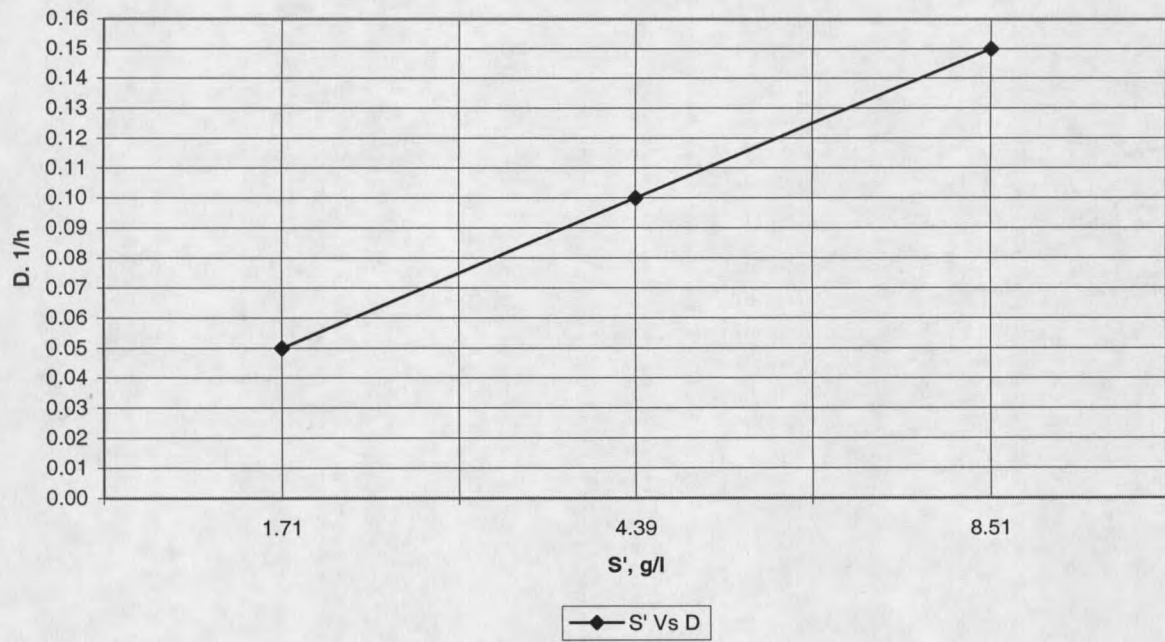


Figure 39. Rototorque Continuous Data Analysis, S' vs D

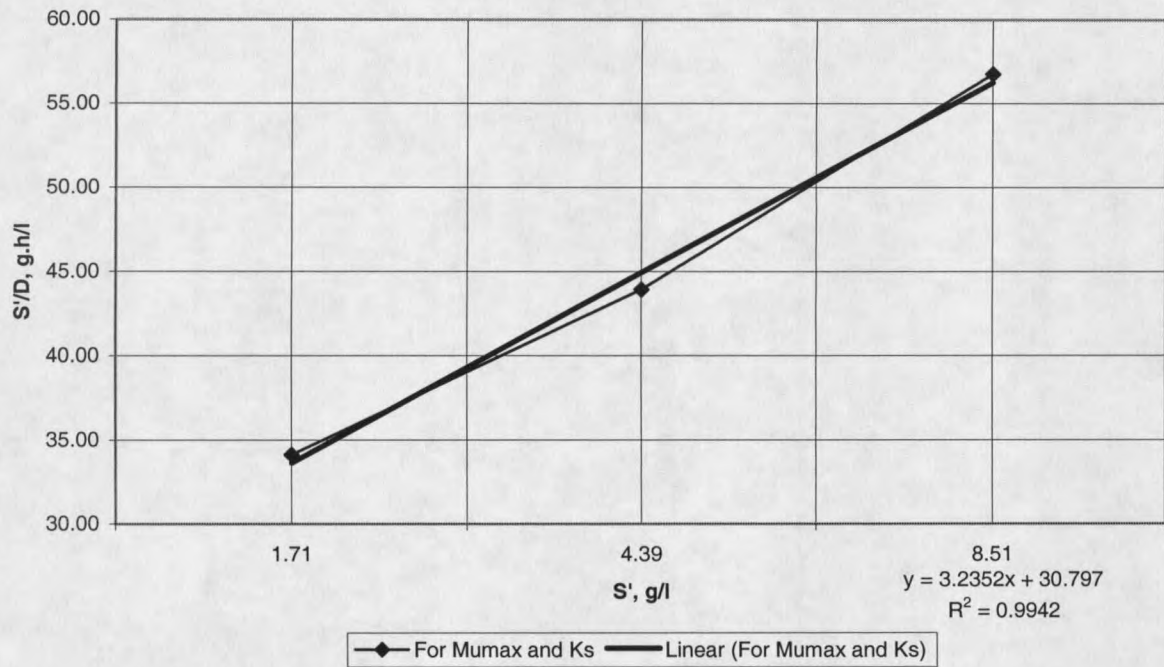
$\mu_{\max}$ and K_s 

Figure 40. Rototorque Continuous Data Analysis, For $\mu_{\max}$ and K_s

K and K'

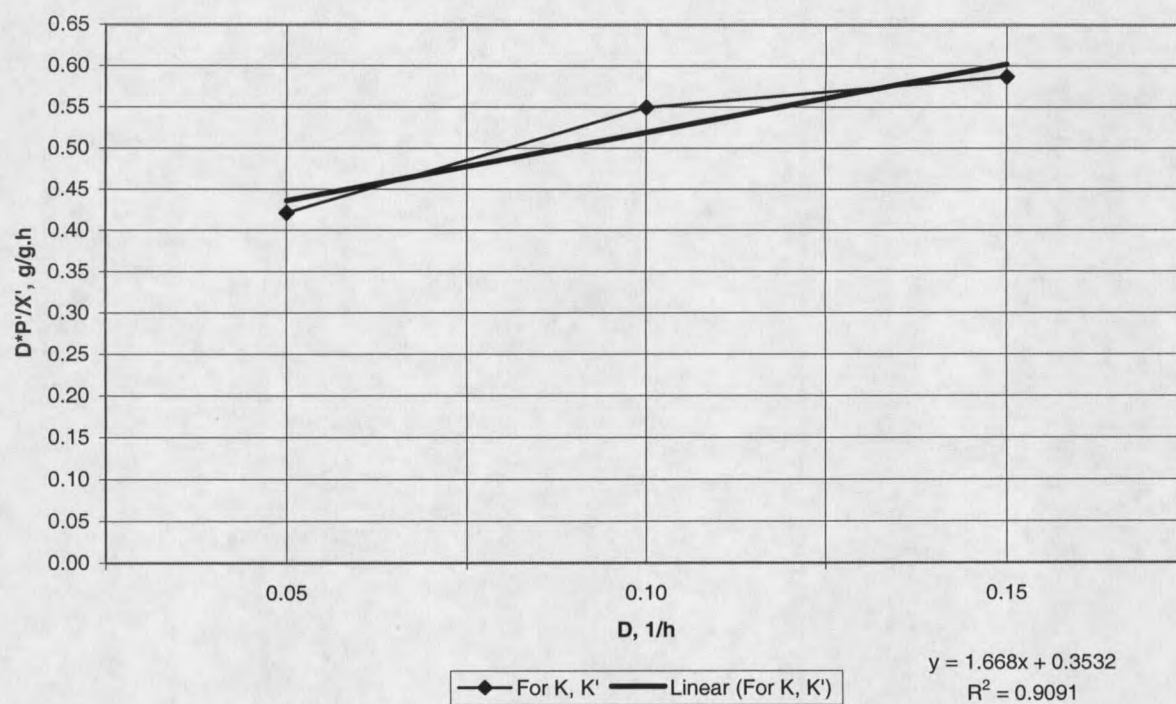


Figure 41. Rototorque Continuous Data Analysis, For K and K'

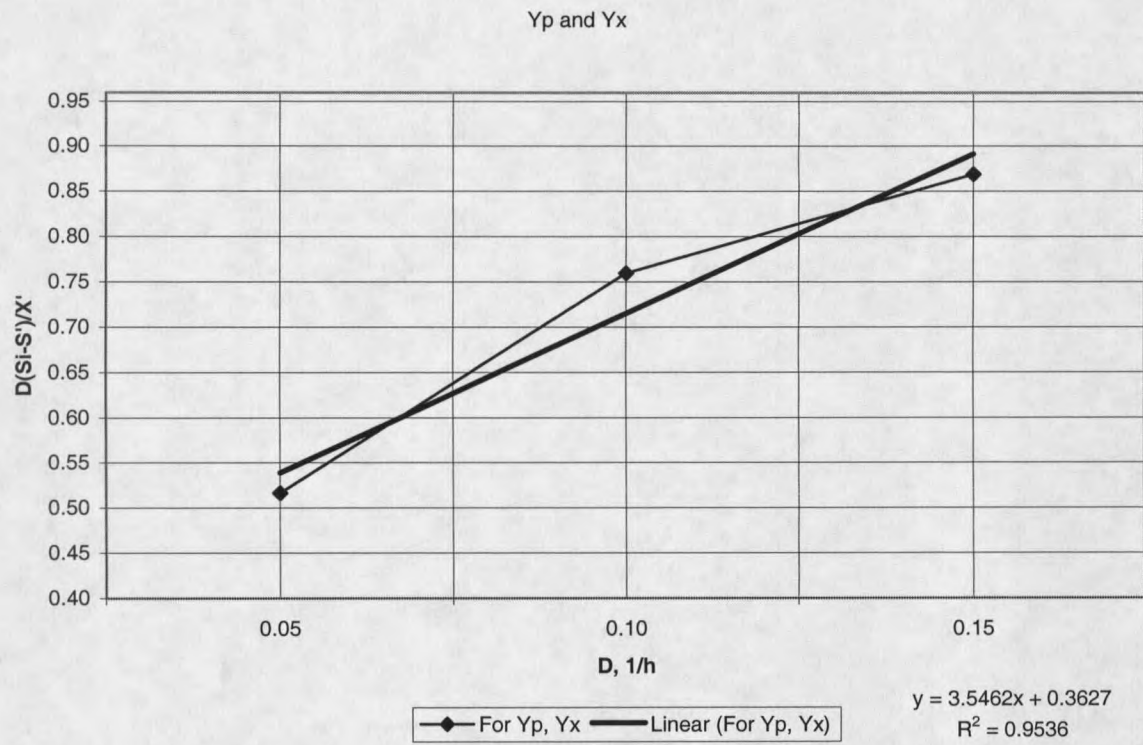


Figure 42. Rototorque Continuous Data Analysis, For Y_P and Y_X

APPENDIX – F

PARAMETERS FOR BATCH OPERATION

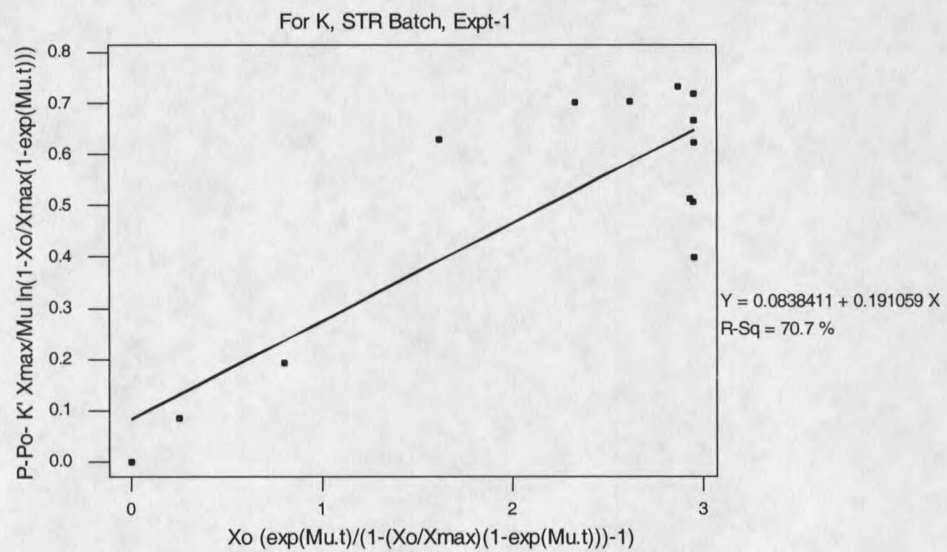
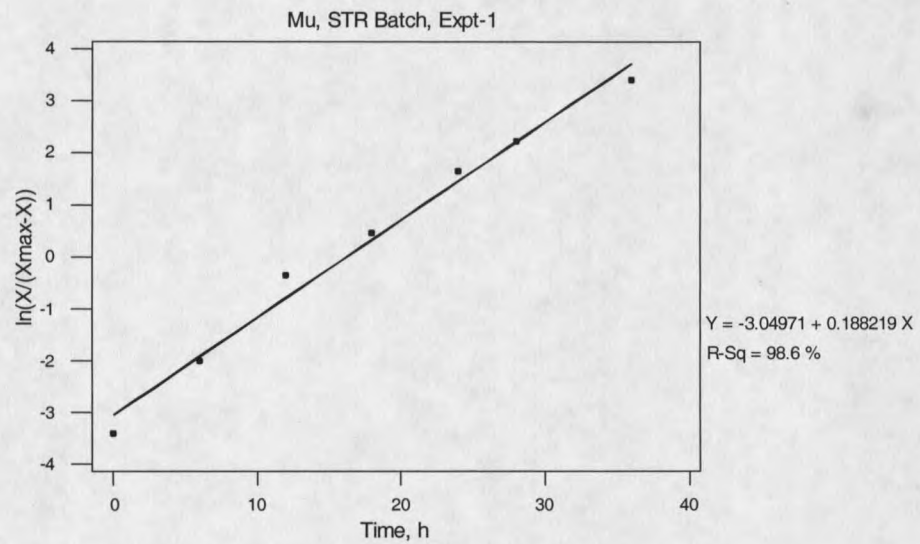


Figure 43. STR Batch Operation, For Mu and K, Experiment - 1

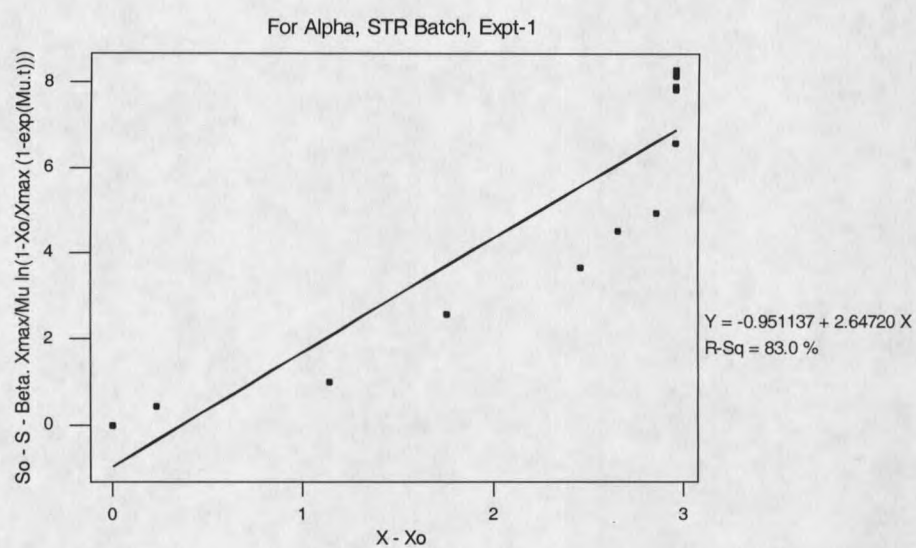


Figure 44. STR Batch Operation, For Alpha, Experiment - 1

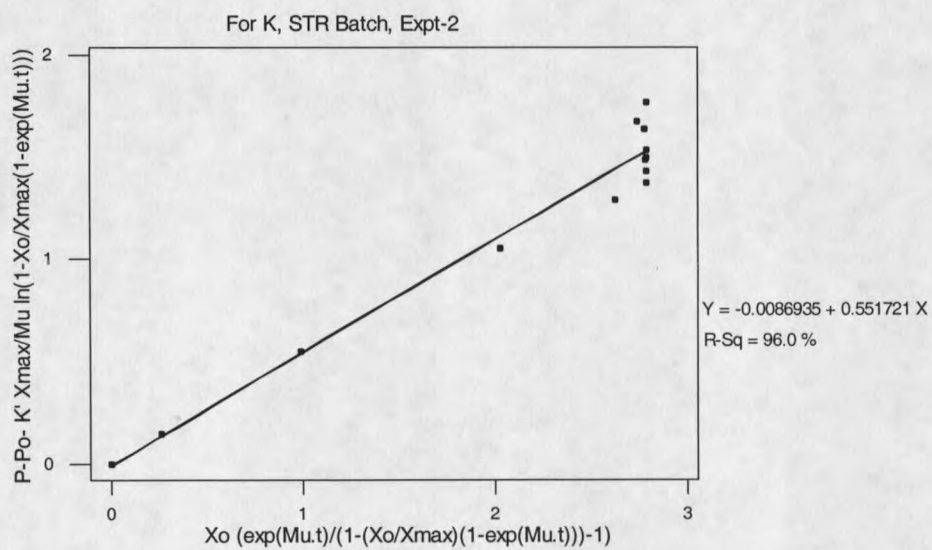
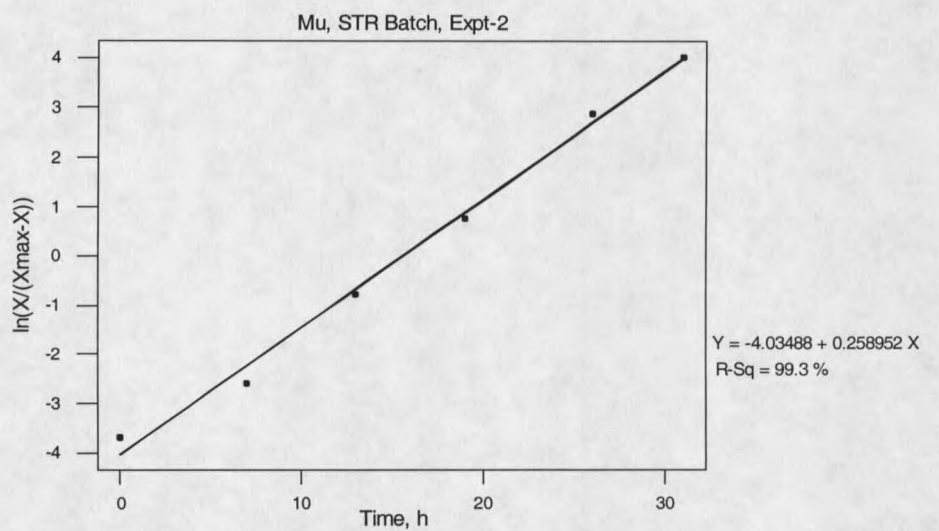


Figure 45. STR Batch Operation, For Mu and K, Experiment - 2

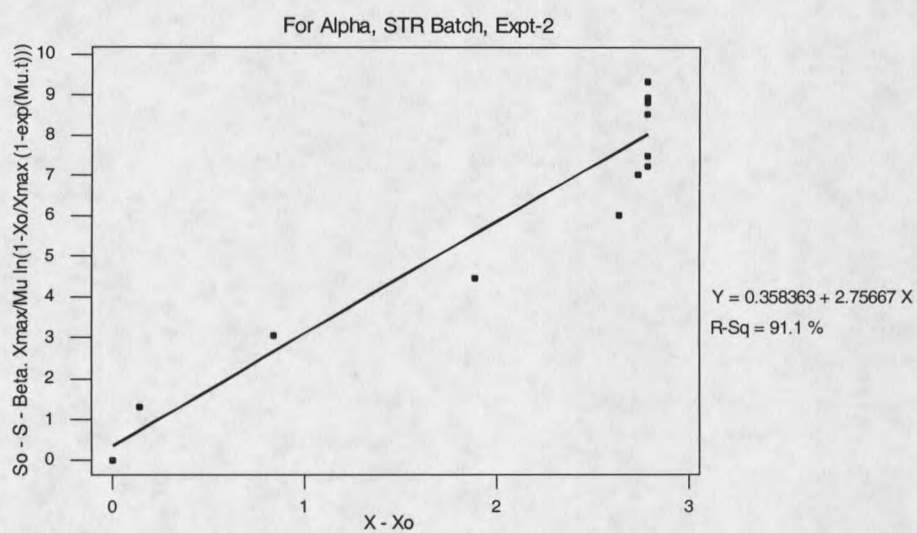


Figure 46. STR Batch Operation, For Alpha, Experiment - 2

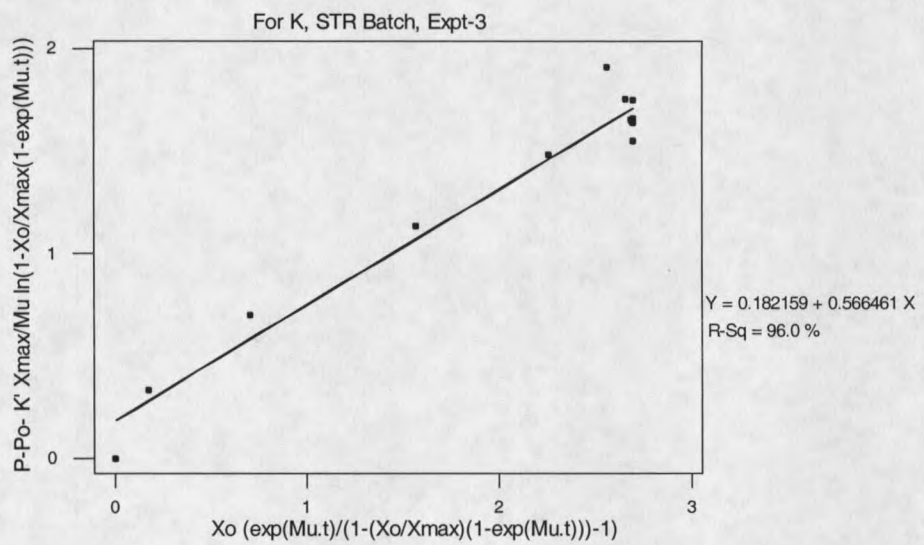
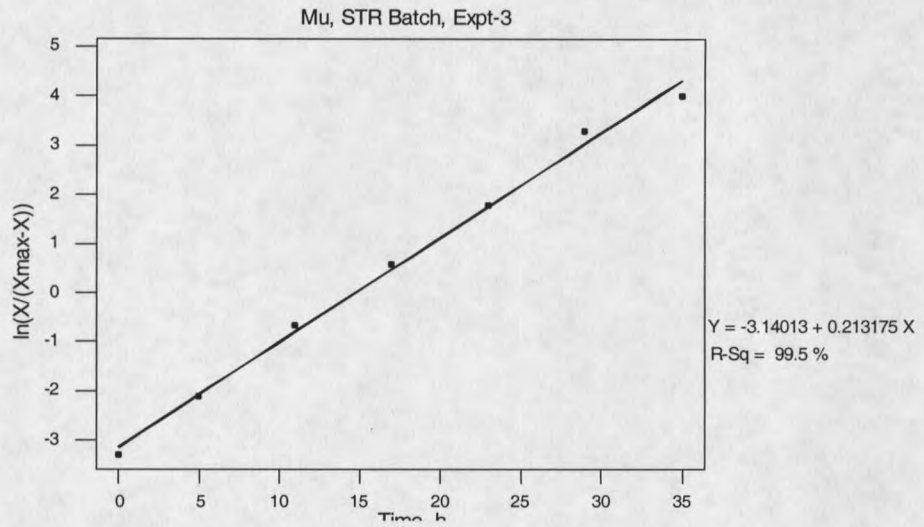


Figure 47. STR Batch Operation, For Mu and K, Experiment - 3

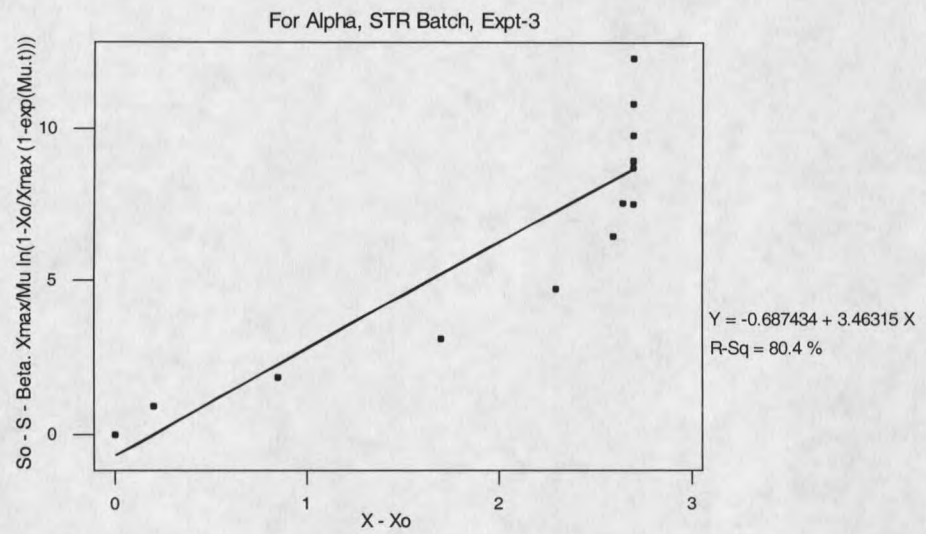


Figure 48. STR Batch Operation, For Alpha, Experiment - 3

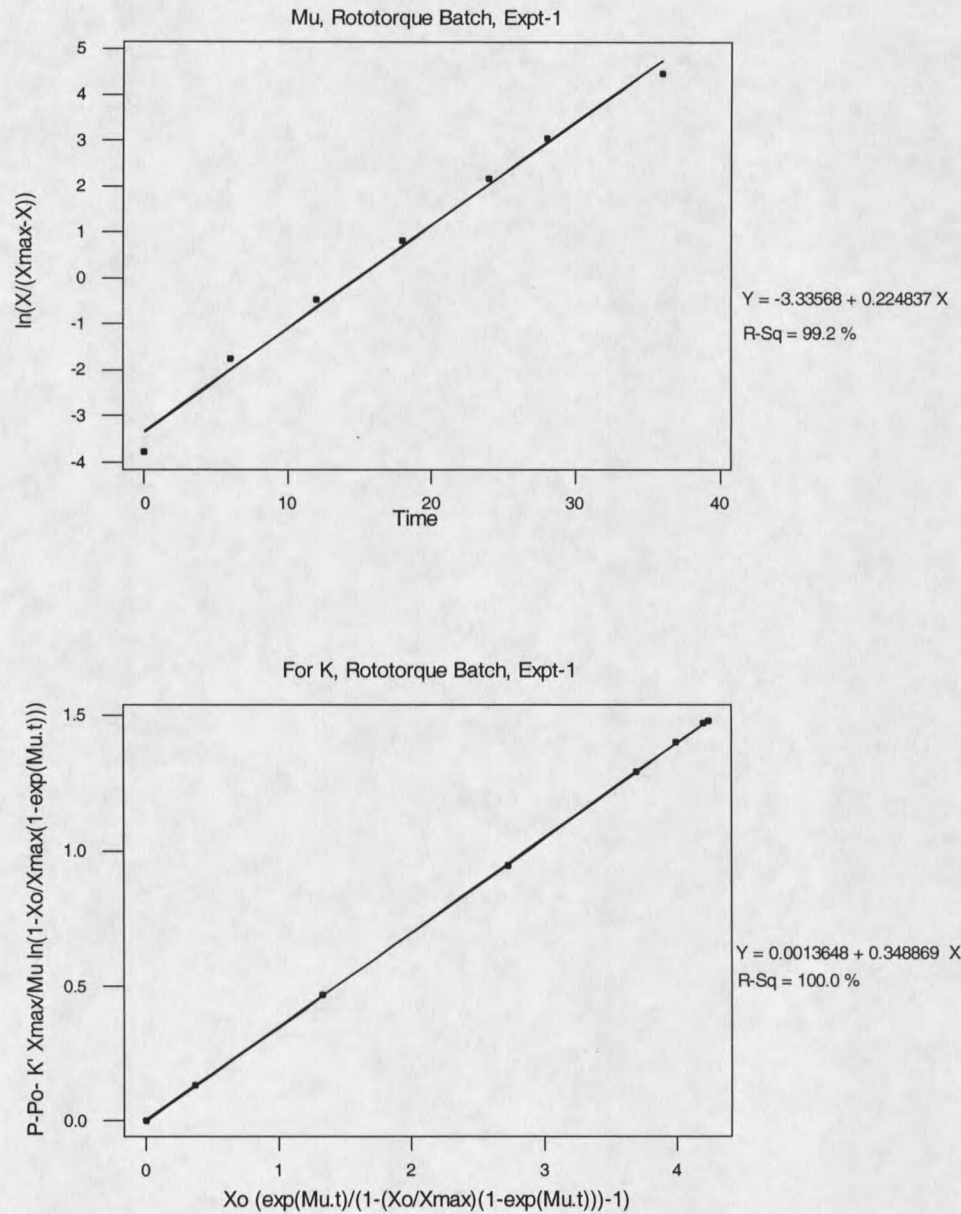


Figure 49. Rototorque Batch Operation, For Mu and K, Experiment - 1

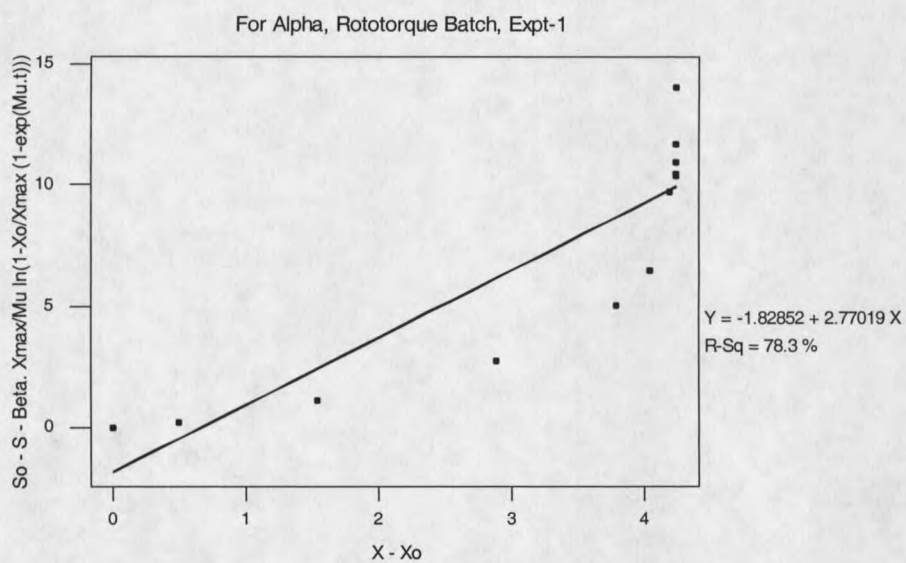


Figure 50. Rototorque Batch Operation, For Alpha, Experiment - 1

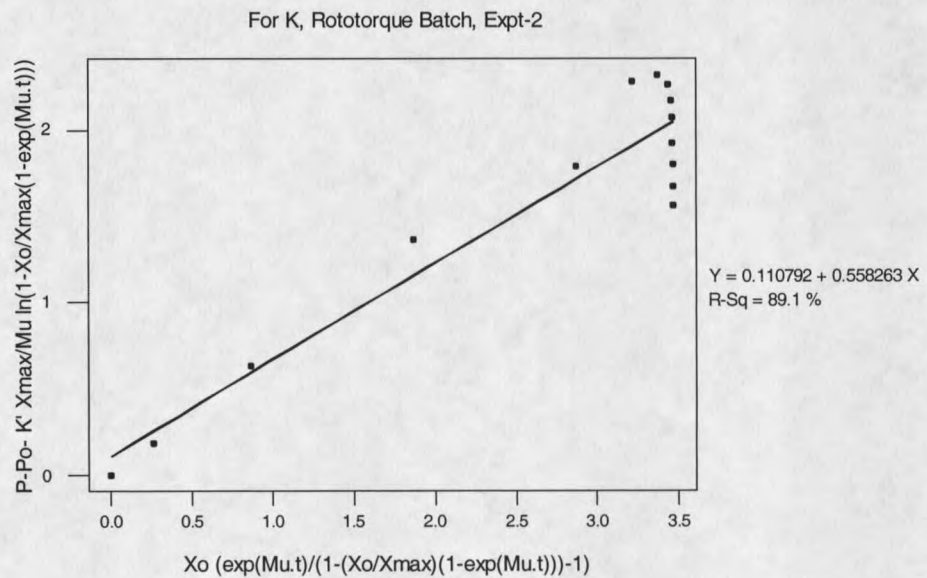
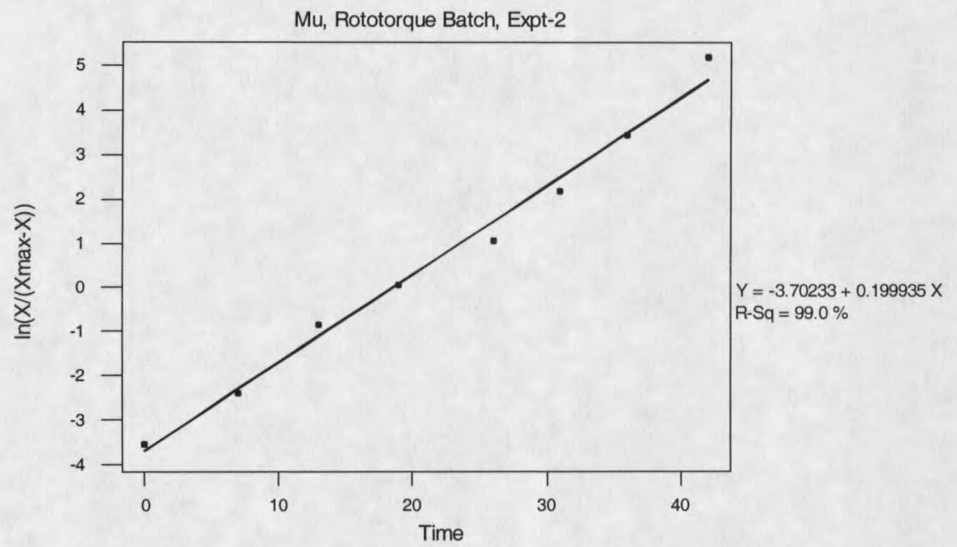


Figure 51. Rototorque Batch Operation, For Mu and K, Experiment - 2

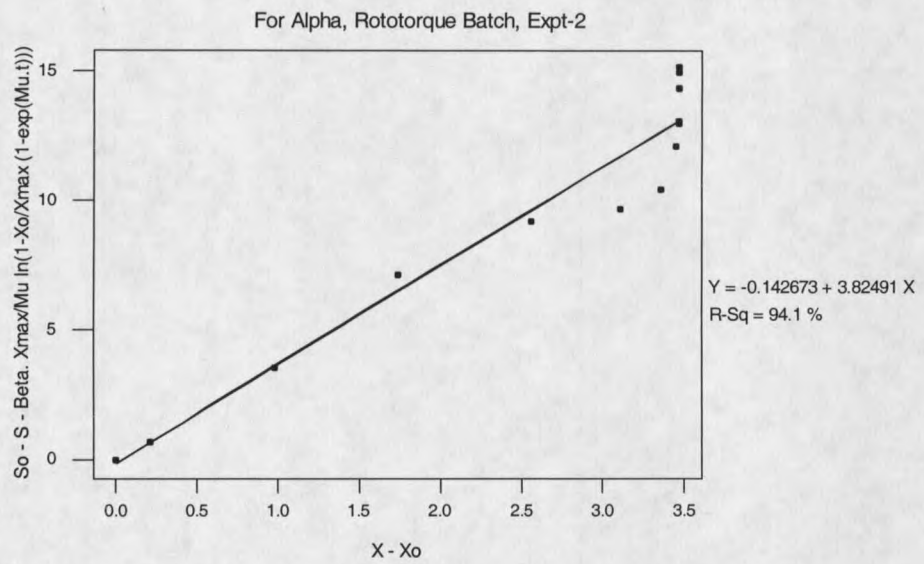


Figure 52. Rototorque Batch Operation, For Alpha, Experiment - 2

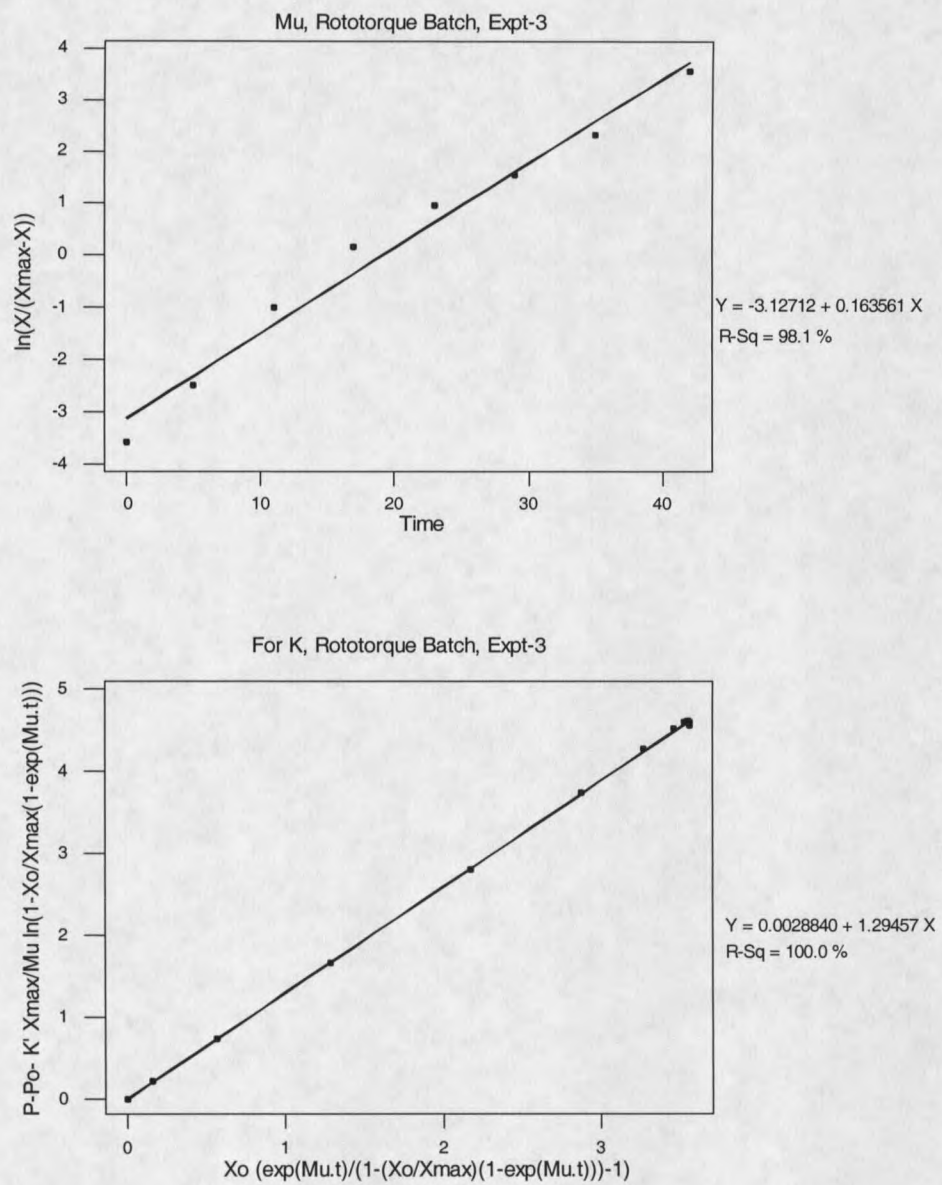


Figure 53. Rototorque Batch Operation, For Mu and K, Experiment - 3

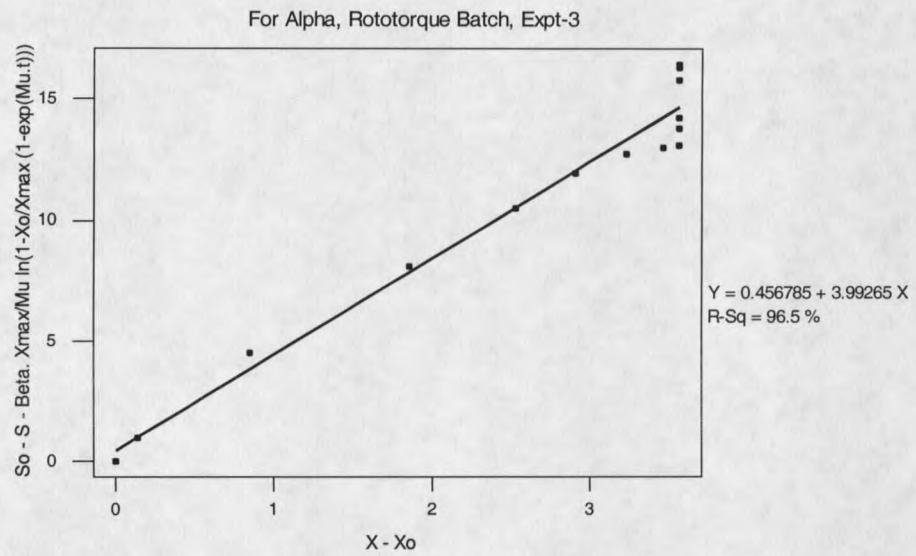


Figure 54. Rototorque Batch Operation, For Alpha, Experiment - 3

APPENDIX – G

STATISTICAL ANALYSIS

Two-Sample T-Test and CI: STR Batch, Xmax, g/l, Rototorque, Batch, Xmax, g/l

	N	Mean	StDev	SE Mean
STR Batch	3	2.917	0.161	0.093

Rototorque Batch	3	3.887	0.450	0.26
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Difference = μ STR Batch, Xmax, g/l - μ Rototorque, Batch, Xmax, g/l

Estimate for difference: -0.970

95% lower bound for difference: -1.558

T-Test of difference = 0 (vs >): T-Value = -3.52 P-Value = 0.988 DF = 4

Both use Pooled StDev = 0.338

Two-Sample T-Test and CI: Rototorque, Batch, S, g/l, STR Batch, S, g/l

	N	Mean	StDev	SE Mean
Rototorque Batch	3	16.973	0.585	0.34

STR Batch	3	16.07	1.40	0.81
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Difference = μ Rototorque, Batch, S, g/l - μ STR Batch, S, g/l

Estimate for difference: 0.907

95% lower bound for difference: -0.962

T-Test of difference = 0 (vs >): T-Value = 1.03 P-Value = 0.180 DF = 4

Both use Pooled StDev = 1.07

Two-Sample T-Test and CI: Rototorque, Batch, P, g/l, STR Batch, P, g/l

	N	Mean	StDev	SE Mean
Rototorque Batch	3	18.233	0.156	0.090
STR Batch	3	15.333	0.737	0.43

Difference = μ Rototorque, Batch, P, g/l - μ STR Batch, P, g/l

Estimate for difference: 2.900

95% lower bound for difference: 1.972

T-Test of difference = 0 (vs >): T-Value = 6.67 P-Value = 0.001 DF = 4

Both use Pooled StDev = 0.533

Two-Sample T-Test and CI: Rototorque, Batch, Mu, 1/h, STR Batch, Mu, 1/h

	N	Mean	StDev	SE Mean
Rototorque Batch	3	0.1933	0.0306	0.018
STR Batch	3	0.2200	0.0400	0.023

Difference = μ Rototorque, Batch, Mu, 1/h - μ STR Batch, Mu, 1/h

Estimate for difference: -0.0267

95% lower bound for difference: -0.0886

T-Test of difference = 0 (vs >): T-Value = -0.92 P-Value = 0.795 DF = 4

Both use Pooled StDev = 0.0356

Two-Sample T-Test and CI: Rototorque Batch, Yp, g/g, STR Batch, Yp, g/g

	N	Mean	StDev	SE Mean
Rototorque Batch	3	0.8167	0.0306	0.018
STR Batch	3	0.5700	0.0458	0.026

Difference = μ Rototorque Batch, Yp, g/g - μ STR Batch, Yp, g/g

Estimate for difference: 0.2467

95% lower bound for difference: 0.1789

T-Test of difference = 0 (vs >): T-Value = 7.76 P-Value = 0.001 DF = 4

Both use Pooled StDev = 0.0389

Two-Sample T-Test and CI: Rototorque Batch, Yx, g/g, STR Batch, Yx, g/g

	N	Mean	StDev	SE Mean
Rototorque Batch	3	0.3867	0.0577	0.033
STR Batch	3	0.473	0.102	0.059

Difference = μ Rototorque Batch, Yx, g/g - μ STR Batch, Yx, g/g

Estimate for difference: -0.0867

95% lower bound for difference: -0.2311

T-Test of difference = 0 (vs >): T-Value = -1.28 P-Value = 0.865 DF = 4

Both use Pooled StDev = 0.0830

Two-Sample T-Test and CI: Rototorque Batch K, STR Batch K

	N	Mean	StDev	SE Mean
Rototorque Batch	3	0.733	0.493	0.28
STR Batch	3	0.437	0.214	0.12

Difference = μ Rototorque Batch, K,growth - μ STR Batch, K,growth

Estimate for difference: 0.297

95% lower bound for difference: -0.365

T-Test of difference = 0 (vs >): T-Value = 0.96 P-Value = 0.197 DF = 4

Both use Pooled StDev = 0.380

Two-Sample T-Test and CI: Rototorque Batch K', STR Batch K'

	N	Mean	StDev	SE Mean
Rototorque Batch	3	0.06333	0.00577	0.0033
STR Batch	3	0.07667	0.00577	0.0033

Difference = μ Rototorque Batch, K,nongrowth - μ STR Batch, K,nongrowth

Estimate for difference: -0.01333

95% lower bound for difference: -0.02338

T-Test of difference = 0 (vs >): T-Value = -2.83 P-Value = 0.976 DF = 4

Both use Pooled StDev = 0.00577

Two-Sample T-Test and CI: Rototorque Batch, Alpha, STR Batch, Alpha

	N	Mean	StDev	SE Mean
Rototorque Batch	3	3.530	0.663	0.38
STR Batch	3	2.957	0.439	0.25

Difference = μ Rototorque Batch, Alpha - μ STR Batch, Alpha

Estimate for difference: 0.573

95% lower bound for difference: -0.406

T-Test of difference = 0 (vs >): T-Value = 1.25 P-Value = 0.140 DF = 4

Both use Pooled StDev = 0.562

Two-Sample T-Test and CI: Rototorque Batch, Beta, STR Batch, Beta

	N	Mean	StDev	SE Mean
Rototorque Batch	3	0.01633	0.00321	0.0019
STR Batch	3	0.04333	0.00577	0.0033

Difference = μ Rototorque Batch, Beta - μ STR Batch, Beta

Estimate for difference: -0.02700

95% lower bound for difference: -0.03513

T-Test of difference = 0 (vs >): T-Value = -7.08 P-Value = 0.999 DF = 4

Both use Pooled StDev = 0.00467

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