ABSTRACT

AERATION AND OPERATION OF AN IMMOBILIZED CELL OXIDATIVE BIOREACTOR

by
James Joseph Woods

The primary purpose of this work is to help define the optimum window of operations for an immobilized cell oxidative bioreactor. The analytical technique employed requires no outside verification (such as G.C. analysis) and is independent of liquid flow rate. Method of aeration has been determined to be an important parameter for optimizing bioreactor efficiency, and optimization of the quantity of hydrogen peroxide added to provide oxygen during bio-oxidation has been investigated. Ammonium hydroxide as a fixed nitrogen source can be used to restore the vitality of the bioreactor under certain conditions.

The effects of several different methods of providing oxygen on bio-oxidation were analyzed. These methods included aeration at the center of the reservoir (18" from the pump inlet leading to the biosupport), aeration near the pump inlet (3" away), and providing oxygen by means of injection of hydrogen peroxide into the reservoir. Generally, aeration nearer to the cylinder led to faster initial rates of biodegradation of the phenol. With hydrogen peroxide, an injection of 0.5 ml of 30% H_2O_2 (3.8 ppm H_2O_2) best facilitated the bio-oxidation of 0.5 g phenol, whereas higher amounts caused inhibition.

The use of ammonium hydroxide to speed up slow reaction rates has been demonstrated, with the minimum effective injection determined to be approximately 10 ml concentrated NH_4OH (21 ppm). Quantitation using the dissolved oxygen reaction patterns has been briefly discussed. The effect of both dilution and of changing liquid flow rate on baseline dissolved oxygen levels has been analyzed. Also, a guide has been prepared for the recognition of some abnormal dissolved oxygen level patterns for troubleshooting and assessing systemic causes and solutions and general bioreactor operation observations and suggestions have been provided.

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by
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This thesis is dedicated to my family.

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CHAPTER 1

INTRODUCTION

1.1 Bio-Oxidation and Waste Water Treatment

Waste water is treated through primary, secondary, and sometimes tertiary treatment. In primary treatment, simple physical processes such as screening and settling are employed to remove insoluble matter such as grit, grease, and scum from water. Secondary treatment is used to lower the B.O.D. of water, and usually employs biological processes to oxidize waste. In bio-oxidation, heterotrophic microorganisms oxidize waste through aerobic respiration, using oxygen as a co-substrate. Methods of bio-oxidation have included the rotating biological disk and the trickling filter. Using activated sludge for waste water treatment is probably the most effective of all water treatment processes [1].

Utilizing activated sludge for waste water treatment has traditionally involved free microorganisms in an aeration tank which convert organic material in the waste water into microbial biomass and carbon dioxide. But where, traditionally, free microorganisms are used in the activated sludge process, immobilized microorganisms may instead be used, providing drastically improved and more efficient waste water treatment.

1.2 Immobilization

Utilizing immobilized microorganisms for waste water treatment has many advantages over traditional methods using free microorganisms. One important advantage of immobilization is that biomass may easily be recovered and reused. When using free as opposed to immobilized microorganisms, much biomass is lost through washout at high flow rates. Another advantage is that immobilization improves mass transfer and leads to increased biological activity as compared with methods utilizing free microorganisms.

Immobilization may be achieved by entrapment of biomass in a gel or polymer, or by attachment of the biomass to inert supports, such as a polymer membrane, as was used in this study. Entrapment affords some protection of the biomass from destructively high concentrations of the substrate. However, the entrapment matrix - calcium alginate, for example - causes decreased oxygen availability and high diffusional resistance. For these reasons, entrapment can be useful for higher concentrations of the substrate, whereas attachment may be better for lower concentrations to be degraded [2,16].

One problem with entrapment is that growing biomass eventually breaks up the gel matrix. Limiting fixed nitrogen can help prevent this growth but can also interfere with biodegradation. Polymer membrane attachment, in contrast, is largely self-regulating regarding biomass growth. Excess biomass is kept to a minimum through the process of sloughing, and also due to insufficient oxygen diffusivity with excess biomass.

1.3 Batch vs. Continuous Reactors, Spiral Wound Membrane

Batch reactors are spatially uniform, small reactors with liquid phase reactions. Batch reactors, as compared with continuous units, provide greater flexibility and control but can be more costly to operate. While the initial instrumentation cost is lower for a batch reactor, operating costs, including labor, are generally higher.

The polymeric membrane used in this study was a grooved sheet wound spirally into a cylinder. Liquid circulated through this cylinder enters through an opening at the top and spirals outward through a groove until emerging and reentering the reservoir. This configuration has several advantages. The spiral winding causes better mass transfer across the biofilm, thus enhancing biodegradation. Furthermore, the spiral winding forces the liquid to move through a long path across the membrane and prevents channeling, further increasing biological activity.

1.4 On-Line Oxygen Measurement

Since oxygen is a co-substrate during aerobic respiration, monitoring dissolved oxygen levels in the reactor liquid can provide patterns of oxygen use which indicate bio-oxidation of the substrate. This has been proven to be a reliable method of assessing phenol biodegradation.

On-line oxygen uptake measurements provide an extremely important means of assessing biodegradation. Since oxygen usage, and therefore biodegradation, is being monitored continuously instead of at a few selected points in time, the effect of changing operating parameters can easily be assessed. Also, on-line dissolved oxygen measurements allow the establishment of B.O.D. quickly with short period dissolved oxygen measurements.

1.5 Methods of Providing Air

The most important concern with immobilization technology is oxygen transfer across the biofilm. Because of the high cell density present with immobilization, a high dissolved oxygen level in liquid passing through the biosupport is required to support the bio-oxidation.

Three common methods of providing oxygen are air bubbling, pure oxygen bubbling, and injection of hydrogen peroxide. Oxygen is supplied by hydrogen peroxide when it is converted to water and oxygen in the presence of the enzyme Catalase.

Since providing oxygen is critical to immobilization technology, the effectiveness of various means of providing oxygen for the bioreactor used for this study have been examined. Oxygen has been provided by means of air bubbling in several locations and by injection of hydrogen peroxide. The effect of changing this parameter on bio-oxidation rates and patterns has been observed.

1.6 The Basic Dissolved Oxygen Pattern

Figure 1 demonstrates a typical pattern of dissolved oxygen measured over time which indicates a complete biodegradation reaction. The oscillating effect occurs because dissolved oxygen is being measured alternately between the reservoir liquid and the liquid coming out of the biosupport cylinder. Since the liquid emerging from the cylinder has just passed over the biomass, which has used some dissolved oxygen for respiration, the level of dissolved oxygen is generally lower than in the reservoir. The pattern begins with what is known as the baseline dissolved oxygen pattern. It is the steady pattern which occurs when no introduced substrate has been injected for bio-oxidation. The sudden dip in the pattern, indicating lower levels of dissolved oxygen, occurs after the injection of a substrate, and indicates that bio-oxidation of the substrate is underway. Eventually, the pattern returns to baseline, indicating the complete biodegradation of the substrate and all biodegradable intermediates. Intermediates are chemicals produced by the partial biodegradation of the substrate which can be further degraded. As the pattern returns to baseline, the concentration of the substrate itself may actually be zero, and the lowered dissolved oxygen levels the result of bio-oxidation of intermediates - the last stage in the reaction pattern.

With greater initial concentrations of substrate, the initial reaction rate generally increases. If initial reaction rates begin to decrease with greater initial concentrations of the substrate, substrate inhibition is probably occurring. This means that the ability of the biomass to degrade the substrate is being inhibited due to the toxic affect of that same substrate on the biomass.

The dissolved oxygen pattern can be related to the amount of substrate biodegraded. Borkowski [3] discusses this topic in depth, and experiments used in this study support his work.

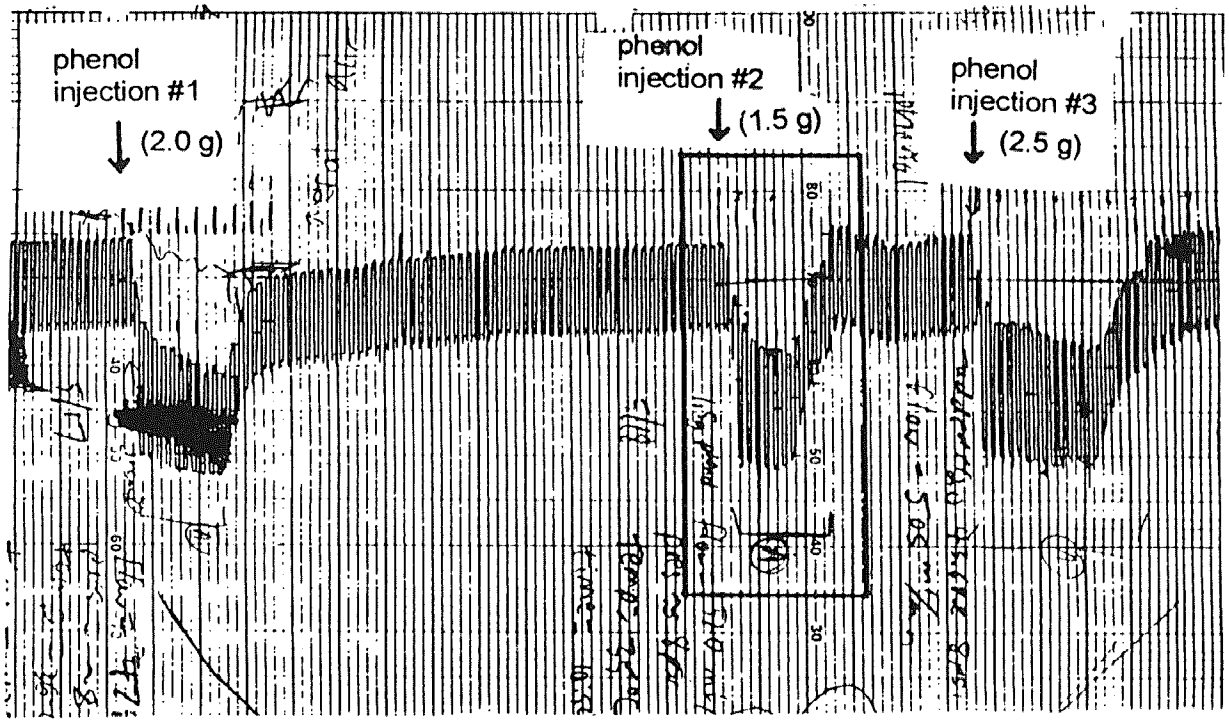

Figure 1 Typical Raw Data

These dissolved oxygen patterns show typical phenol reaction patterns with aeration near the pump inlet. Phenol injection #2 has been selected as an example pattern indicating phenol bio-oxidation, and an analysis of this pattern is provided in Appendix A.

CHAPTER 2

LITERATURE SURVEY

Lakhwala [2], of this laboratory, and others [16,17] performed work comparing two bioreactors treating phenol: one using calcium alginate entrapped microorganisms and the other using polymer membrane attached microorganisms. Lakhwala points out that immobilized microorganisms have great advantages over free microorganisms. These include easy recovery and reuse of biomass, high biomass density, ease in changing modes of operation and increased biological activity due to better mass transfer.

Lakhwala also notes the advantages and disadvantages of each method of immobilization - attachment and entrapment. Attachment to a polymer membrane, he concludes, offers less protection from high substrate concentrations, but better mass transfer properties and better performance at lower concentrations (less then 250 ppm phenol). Lakhwala also notes that attachment has the advantage of self-regulated microbial growth, while the entrapment matrix eventually breaks up due to excess microbial growth. Lakhwala notes that the spiral wound membrane used for attachment in his study (which was very similar to the biocatalyst used in this study) reduces loss and improves reactor performance.

Also of this laboratory, Jung [4] performed a study using immobilized microorganisms in a batch recirculation bioreactor to biodegrade several organic substrates. Jung points out that batch reactors, while being more costly to operate than continuous units, provide better flexibility and control. Jung notes that oxygen demand increases with cell density. Therefore, systems using immobilized microorganisms, because of the very high cell densities, have great dissolved oxygen requirements. For this reason, the major concern with immobilization technology is oxygen transfer across the biofilm.

Saghafi [5] further supports the importance of control and flexibility in a bioreactor. He concludes that control of a wide range of operating parameters is very important for an efficient biodegradation unit. Further, he states that such control is greater in a batch reactor than in a continuous unit.

Desai [6] used activated sludge in an aerated fill-and-draw reactor. He showed that the use of acclimated sludge did in fact reduce the lag time for the biodegradation of both phenol and 2,4-dichlorophenol. Also, the loss of substrate due to air-stripping was shown to be negligible for both compounds at room temperature.

Naik [7] shows that biodegradation of several compounds can give significantly faster biodegradation rates than biodegradation of one compound alone, due to the presence of multiple carbon sources.

Gonnabathula [8] discusses biodegradation of phenol in waste water in an aerated 5 liter batch bioreactor simultaneous with other phenolic substances (2-chlorophenol, 2-6 dichlorophenol and nitrobenzene). In these experiments biodegradation was the primary means of removal. Gonnabathula showed that the rate of biodegradation of phenol and phenolic substances was inhibited by the presence of 2,6-dichlorophenol and nitrobenzene, while the presence of phenol improved biodegradation of nitrobenzene. The work of Gonnabathula and Naik therefore shows that the rates of biodegradation of phenolic substances is dependent upon other such substances which may be present. This means that when attempting to identify or quantify substances being biodegraded by dissolved oxygen patterns, the presence of other biodegradable substances should be carefully considered.

Shah [9] investigated the performance of immobilized cells in the biological treatment of hazardous waste using a batch recirculation reactor. He notes that the large variety of microorganisms present in the activated sludge reactor and their interaction may hold the key to the effective treatment and removal of organic simple ring compounds such as benzene, toluene, xylene, styrene, etc. In his study, Shah used aeration and

periodic addition of hydrogen peroxide to provide oxygen. Shah preferred injection of hydrogen peroxide as the aeration mechanism because providing oxygen by means of air bubbling led to air stripping of the substrate due to the slow biodegradation rate. Catalase, a terminal respiratory enzyme present in practically all living aerobic cells, breaks the hydrogen peroxide into water plus oxygen. Chemical oxidation of the substrate could safely be assumed to not be present. This is because of the fleeting presence of H_2O_2 due to the very small amount injected and the large quantities of catalase present in activated sludge.

Yang [10], of this laboratory, studied the performance of immobilized cells in the treatment of 2-chlorophenol in a batch recirculation reactor. The batch reactor allows the determination of a regime of operation with respect to the liquid flow in order to minimize mass transfer resistance. With the recirculation reactor, an accurate operating window may be determined while optimizing with respect to the reaction rate. The configuration of the batch reactor enables continuous measurement of the rate of dissolved oxygen consumption.

Shim [11] discusses the advantages of activated sludge, immobilization and attachment. He notes that activated sludge contains a great variety of microorganisms which are able to degrade a broad range of organic compounds. The aerobic degradation of these compounds occurs in the biofilm. With attachment, the biofilm eventually reaches a point beyond which additional growth cannot be sustained, and excess biomass is removed through sloughing. This biomass self-regulation allows reactors utilizing membrane attachment not to be inhibited by excess biomass and to be operational for a long time.

Lodaya [12] and others [18] studied the aerobic biodegradation of benzene using activated sludge immobilized by entrapment in calcium alginate, and also by attachment to a silica-based catalyst support. He determined that about 67% of the isolates consisted of members of the genus Bacillus, Microbacterium, Plesiomonas, Kurthia, Lactobacillus, and

Pseudomonas. Lodaya notes that attachment on the surface of the solid support offers an advantage because the support is stronger than a gel matrix. He notes, however, that microorganisms are exposed to the bulk liquid, and therefore resistance to shock loads of toxics will not be as good as that of entrapped microorganisms.

CHAPTER 3

OBJECTIVES

The primary objective of this study has been to observe the effect of various means of providing oxygen in an immobilized cell oxidative bioreactor as well as describing several aspects of the operation of the bioreactor. The specific objectives are:

1. To observe and compare the effect of several methods of providing oxygen on the rate of biodegradation of phenol,
2. To establish recognizable abnormal dissolved oxygen patterns and their associated systemic causes and corrective measures,
3. To demonstrate the use of ammonium hydroxide to speed slow reaction rates under certain circumstances,
4. To observe the effect of changing liquid flow rates and of dilution on baseline dissolved oxygen patterns,
5. To provide general suggestions for the operation of the bioreactor based on experiments done during this study, and
6. To support the work of Borkowski [3] in optimization and quantization parameters in an immobilized cell oxidative bioreactor.

CHAPTER 4

MATERIALS AND EXPERIMENTAL METHODS

4.1 Microorganisms and Immobilization

For this study, activated sludge was obtained from the Parsippany Troy Hills Water Pollution Control Plant in New Jersey. The sludge was sieved through a 297 um opening screen and washed with 0.25% saline solution. The sludge was then acclimated with 50 ml of oily industrial waste and 5 ml ethanol with constant air bubbling.

11.15 G of dry biomass was initially attached to the polymeric membrane. This was determined by drying five 10 ml samples of washed activated sludge in a 120 degrees Celsius oven for 24 hours. 23.73 Liters of activated sludge was recirculated through the biocatalyst overnight for attachment using a water pump at 1.5 psi water pressure.

4.2 Polymeric Membrane for Bio-Oxidation

The method of immobilization utilized for this study is attachment to a U.S. patented spirally wound polymeric membrane. The membrane is hydrophilic and consists of 55% silica, 45% PVC and carbon (trace). The following physical characteristics are attributed to the membrane: porosity = 60% - 65%; pore size = 0.4 - 0.6 um; spacing = 1.2 mm; surface area = 50 square feet.

The polymeric membrane sheet used is 2.5 feet wide and 20 feet long. The surface of the membrane contains protective ribs that allow a spacing of 1.2 mm between the layers after it is spirally wrapped. The total surface area of the sheet is 50 square feet, but because both sides are utilized for immobilization by the bacteria, a potential of 100 square feet of polymeric sheet is obtained. The actual amount available for biomass attachment is 96.5 square feet because the outer surface of the membrane is essentially unavailable for use. After the membrane is wrapped in the spiral configuration, two plastic discs with a

diameter of 6.5 inches are attached to seal each end and to provide mechanical strength and rigidity.

The reactor water is pumped to the top of the biosupport and enters a cylindrical space inside the reactor. The water exits the support by flowing through the 1.2 mm spacing between wrappings and flowing down the biosupport when it has reached the end. Readings indicated the water temperature to range from 25 - 30 degrees Celsius.

4.3 Experimental Set Up

Figure 2 shows a schematic of the reactor pump configuration. The reactor stands upright in the 6.5 inch high reservoir. The capacity of the reservoir is 2.03 cubic feet, and is filled to a maximum of 43.3 liters. The pump recirculates the reservoir liquid into the top of the biosupport, and a small plastic container collects the output from the surface of the support. This container is added to facilitate flow rate and temperature measurements. Two bypass valves are added to the pump output to control flow rate and water pressure, and to aid in mixing the reservoir liquid. A pressure gauge measures the water pressure into the biosupport. A steady flow of air bubbling is provided in the reservoir, and oxygenation also occurs while the output liquid of the biosupport trickles down the surface. In experiments where hydrogen peroxide was used to provide oxygen, the 30% H_2O_2 solution was first mixed in a beaker with reservoir liquid, then poured throughout the reservoir.

Flow rate through the biosupport is measured before each substrate injection by collecting the biosupport output liquid in a beaker and measuring the time and volume. Temperature of the biosupport output is also measured in the plastic container before each injection.

Substrate injections are prepared by dissolving a weighed amount of substrate with about 500 ml of reservoir liquid in a beaker. The beaker solution is then poured throughout the reservoir. All experiments are done at room temperature under a

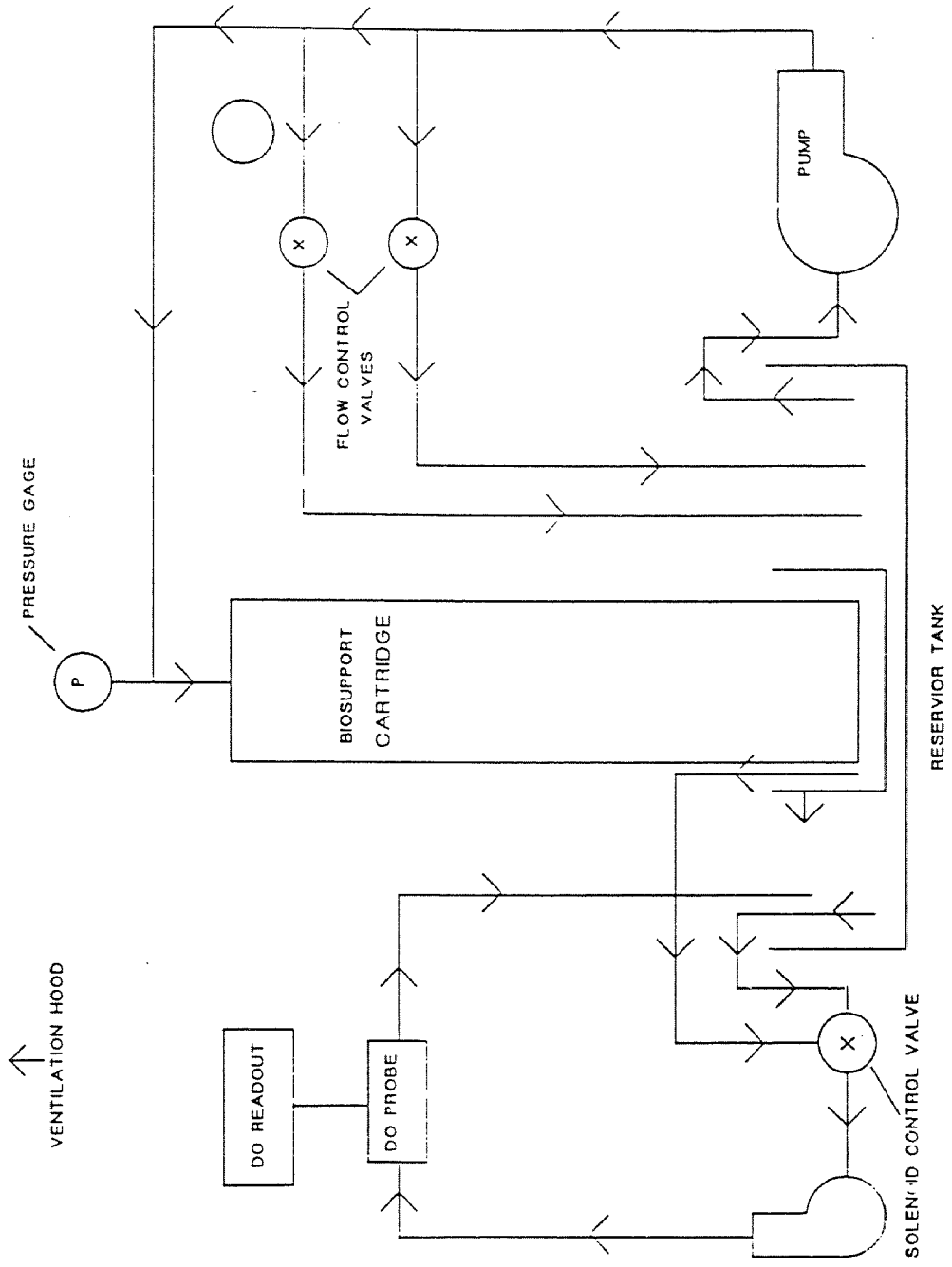

Figure 2 Schematic of Bioreactor Set Up

ventilating hood. Nitrogen is provided throughout the experimental process by adding 2.5 to 10 ml of 29% aqueous ammonia to stimulate bioactivity.

4.4 Dissolved Oxygen Measurements

The method of monitoring the oxidation of the substrate material involves the use of the dissolved oxygen probe and chart recorder. Flow across the DO probe is provided by a small water pump. The inputs to the DO probe are located 1) in the reservoir (Catalyst input DO concentration) and 2) in the plastic container which collects the catalyst output (Catalyst output DO concentration). An oscillating control valve with a 10 minute timer switches periodically from input DO concentration to output DO concentration. On the chart recorder, the higher DO concentration indicates cartridge input DO concentration. The input and output measurements are constantly recorded on the chart recorder during the reaction. Within the range of liquid flow rates through the biosupport used in this study (from about 300 ml/min. to about 1200 ml/min), no obvious relationship between flow rate and oxidation rate has been observed for this type of cartridge.

First, a steady state baseline is achieved, which indicates no substrate oxidation reaction. Then, upon addition of substrate, the DO (input and output) decreases, indicating bio-oxidation of the substrate. The developed DO pattern then returns to steady state baseline upon completion of the biodegradation reaction. A sample chart recorder output for a phenol bio-oxidation and a more in-depth discussion of the basic pattern of phenol biodegradation are provided in the Introduction.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Effect of Aeration Location

For most of this study, aeration was provided by bubbling approximately 4.5 l/min. of air into the reservoir at a location about 3" away from the inlet to the pump leading to the biosupport cylinder. In previous experiments, the location of aeration had been near the center of the reservoir, about 18" from the pump inlet. Generally, locating the aeration closer to the pump inlet led to faster initial biodegradation rates. This is reasonable, since providing oxygen is extremely important to the efficiency of reactors utilizing immobilized biomass, and since aeration nearer to the pump inlet led to more air per unit time entering the biosupport cylinder.

Figure 3 contrasts a typical central-aeration reaction with two typical reactions with aeration near the pump inlet. The reactions with aeration near the pump inlet rise steeply to a maximum rate of biodegradation and remain at about this level until the reaction is nearly complete. The central aeration reaction, by contrast, rises more slowly to a peak biodegradation rate. This indicates that aeration near the pump inlet allows greater initial rates of biodegradation due to increased oxygen availability to the biomass.

5.2 Quantitation

As discussed by Borkowski (3), the data obtained from the analysis of phenol reactions can be used to estimate the amount of dissolved oxygen used during the biodegradation process as well as the amount of phenol biodegraded. A set of eight 2.5 gram analyzed phenol reactions have been quantized using two methods presented by Borkowski, and the results presented in Tables i and ii.

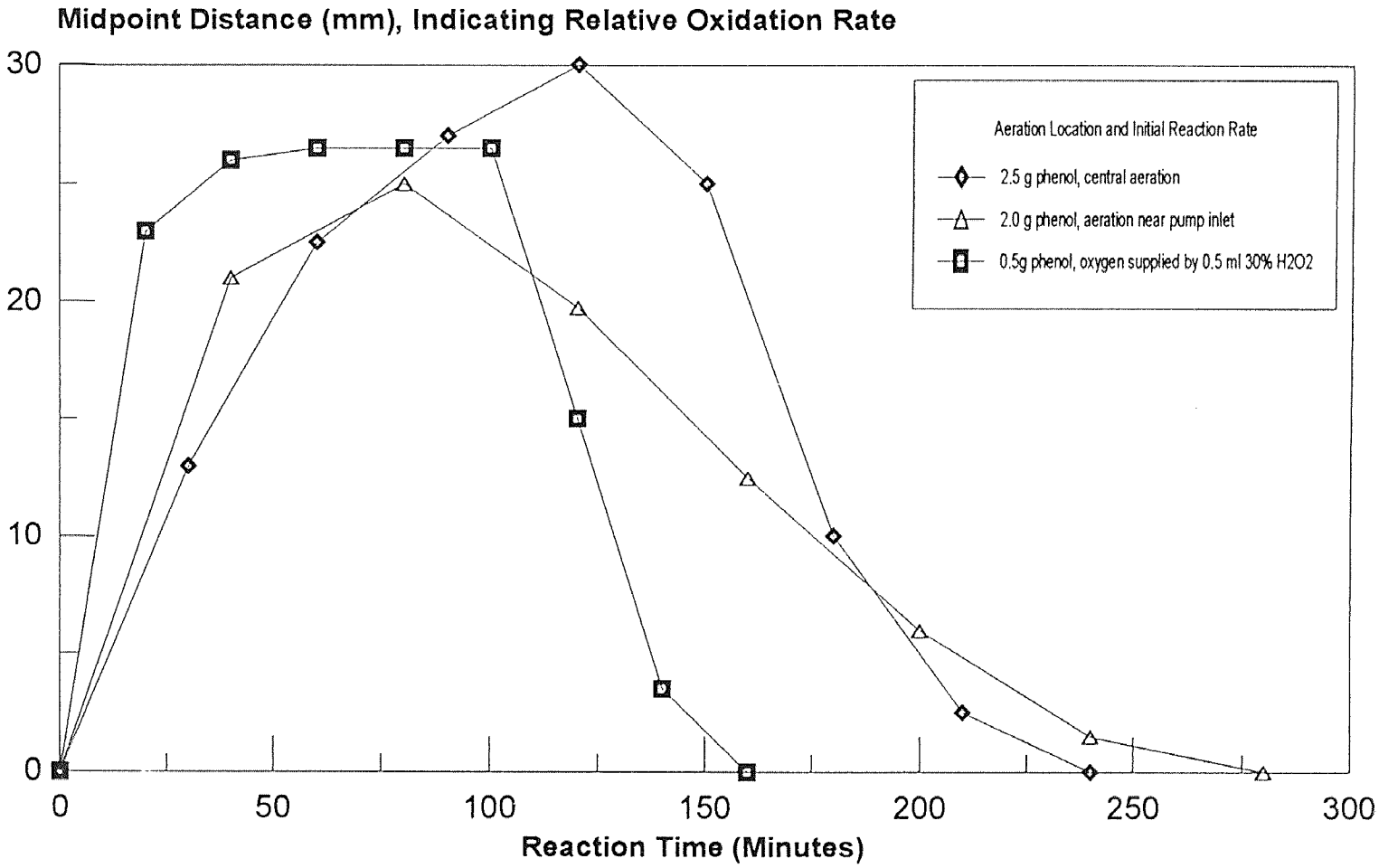

Figure 3 Aeration Location and Initial Reaction Rate

These reactions show the effect of aeration method on typical initial reaction rates. Initial rates can be improved with aeration close to the pump inlet or by injection of the correct amount of hydrogen peroxide (injected 20 minutes after the phenol), as compared with central aeration.

First, curves representing phenol reactions were cut out and weighed. By using the weight of a known area (in units of minutes*grams), these weights were used to determine the area under each reaction curve in minute*grams. From this, the amount of oxygen used for biodegradation and the amount of phenol biodegraded can be estimated. Borkowski has determined that 0.478 g oxygen are used for each 1 min.*g of area under the reaction curve, and that bio-oxidation of 1 g of phenol requires 2.408 g oxygen. These relationships have been used in this analysis.

Table i shows analysis of 8 phenol reactions using Quantitation Method #1 of Borkowski. Method #1 simply applies the relationships noted above to relate area of the curve to the amount of oxygen used during biodegradation and amount of phenol biodegraded. After averaging the results of the eight analyzed 2.5 g phenol reactions, Method #1 predicted 2.88 g phenol biodegraded per reaction and 6.93 g oxygen used per reaction, with a standard deviation of 0.72 g phenol. These results show a 13% error.

Table i 2.5 g Phenol Reactions, Analyzed Using Borkowski (3) Quantitation Method #1

Reaction	Aeration Location	Liquid Flow Rate (ml/min.)	Area Under the Curve (min.*g)	Oxygen, Calculated (grams)	Phenol, calculated (grams)
1	near pump inlet (3")	340	19.7	9.42	3.92
2	3"	420	9.60	4.59	1.91
3	3"	460	19.0	9.08	3.77
4	3"	480	14.6	6.98	2.90
5	3"	500	12.5	5.98	2.48
6	3"	505	11.9	5.69	2.36
7	3"	730	17.7	8.46	3.51
8	central (18")	420	11.0	5.26	2.18
Average			14.5	6.93	2.88

Table ii shows the eight 2.5 g phenol reactions analyzed by method #3 of Borkowski. This method proceeds exactly as method #1, except that an adjustment is made based on the initial slope of the reaction curve (the initial reaction rate). This adjustment is intended to compensate for the slightly lower area under the curve for reactions which proceed quickly at first. The logic is that faster reactions cause a larger deviation from the baseline. Operation at lower oxygen concentrations causes a larger transfer of oxygen to the reaction mixture, requiring a correction. A factor of 1.3 has been applied to steep initial slopes, 1.0 to average initial slopes, and 0.8 to slight initial slopes. Method #3 resulted in a predicted 2.66 g phenol biodegraded per reaction, with a standard deviation of 0.58 g phenol. These results show a 6% error.

Table ii 2.5 g Phenol Reactions, Analyzed Using Borkowski (3) Method #3

Reaction Number	Aeration	Liquid Flow Rate (ml/min.)	Area Under Curve (min.*g)	Initial Slope Adjust Factor	Adjusted Area Under Curve (min.*g)	Oxygen, Calc. (g)	Phenol Calc. (g)
1	3"	340	19.7	0.8	15.8	7.55	3.14
2	3"	420	9.60	1.3	12.5	5.98	2.48
3	3"	460	19.0	0.8	15.2	7.27	3.02
4	3"	480	14.6	0.8	11.7	5.59	2.32
5	3"	500	12.5	0.8	10.0	4.78	1.99
6	3"	505	11.9	1.3	15.5	7.41	3.08
7	3"	730	17.7	1.0	17.7	8.46	3.51
8	18"	420	11.0	0.8	8.8	4.21	1.75
Average					13.4	6.41	2.66

5.3 Providing Oxygen by Hydrogen Peroxide Injection

As part of this study, several phenol injections were made using a single injection of hydrogen peroxide as the means of providing oxygen (beyond that which occurs as the result of aeration due to the trickling of the reservoir liquid down the surface of the biosupport cylinder). During these reactions, aeration was not provided. In each reaction, the H_2O_2 was injected 20 minutes after the phenol injection. Since injection of H_2O_2 quickly generates a large amount of dissolved oxygen, dips can sometimes be seen in the patterns of dissolved oxygen uptake which characterize the phenol biodegradation reaction. Figure 4 summarizes four H_2O_2 assisted phenol injections. Amounts of 30% H_2O_2 solution of between 0.5 ml and 6.0 ml were used to provide oxygen. As Figure 4 shows, the most successful phenol injection involved the use of only 0.5 ml 30% H_2O_2, and is indicated by darkened squares. In all the other phenol injections, a dip in the pattern due to the large amount of oxygen liberated can be seen. In this injection, as with the others, a dip indicating increased dissolved oxygen did occur before the second point, but it was quickly overcome by the decrease in D.O. due to bio-oxidation, and therefore does not appear on the pattern for this reaction. In summary, injection of 0.5 ml 30% H_2O_2 best facilitated the biodegradation of 0.5 g phenol. This amounts to 3.8 ppm H_2O_2.

Figure 5 shows a phenol reaction which followed an H_2O_2 assisted reaction. Although no aeration or H_2O_2 was provided, the reaction proceeded very quickly. It is possible that this fast reaction rate was due to stored oxygen in the biosupport cylinder caused by the injection of 2 ml H_2O_2 during the previous reaction.

Table iii presents the results of a quantitative analysis performed on the dissolved oxygen data regarding injection of H_2O_2 each of four 0.5 g phenol reactions. From Borkowski (3) the relationship between DO peak height (as compared with baseline) and actual DO has been determined to be 0.0281 g oxygen per cm peak height. This relationship has been used to determine the calculated DO increase. For comparison, the theoretical increase in DO which would be expected from the breakdown of the H_2O_2 is

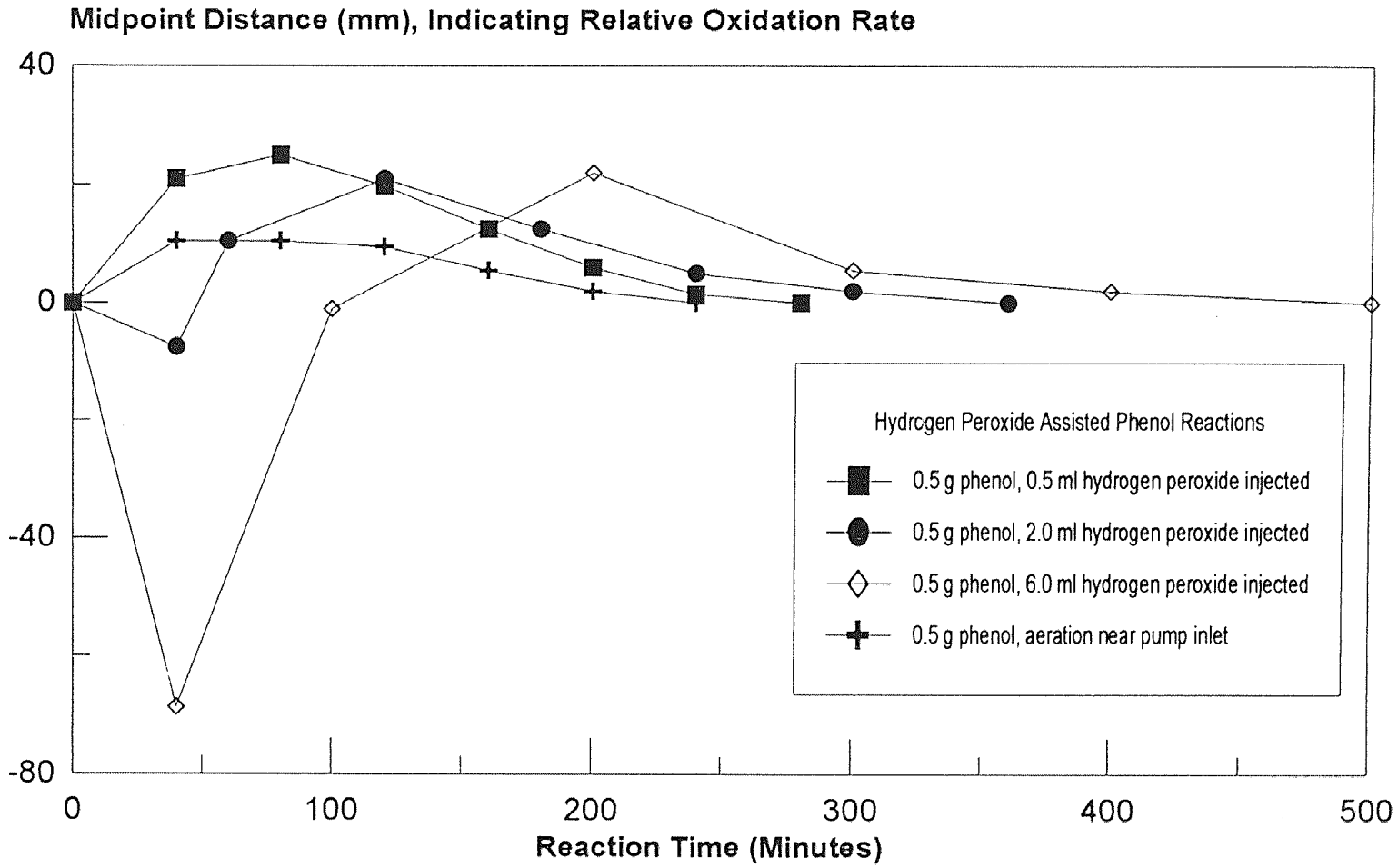

Figure 4 Phenol Reactions with Oxygen Provided by Hydrogen Peroxide Injection

Hydrogen peroxide at low concentrations helps, at high concentrations inhibits. The peroxide was injected 20 minutes after phenol injection in each case.

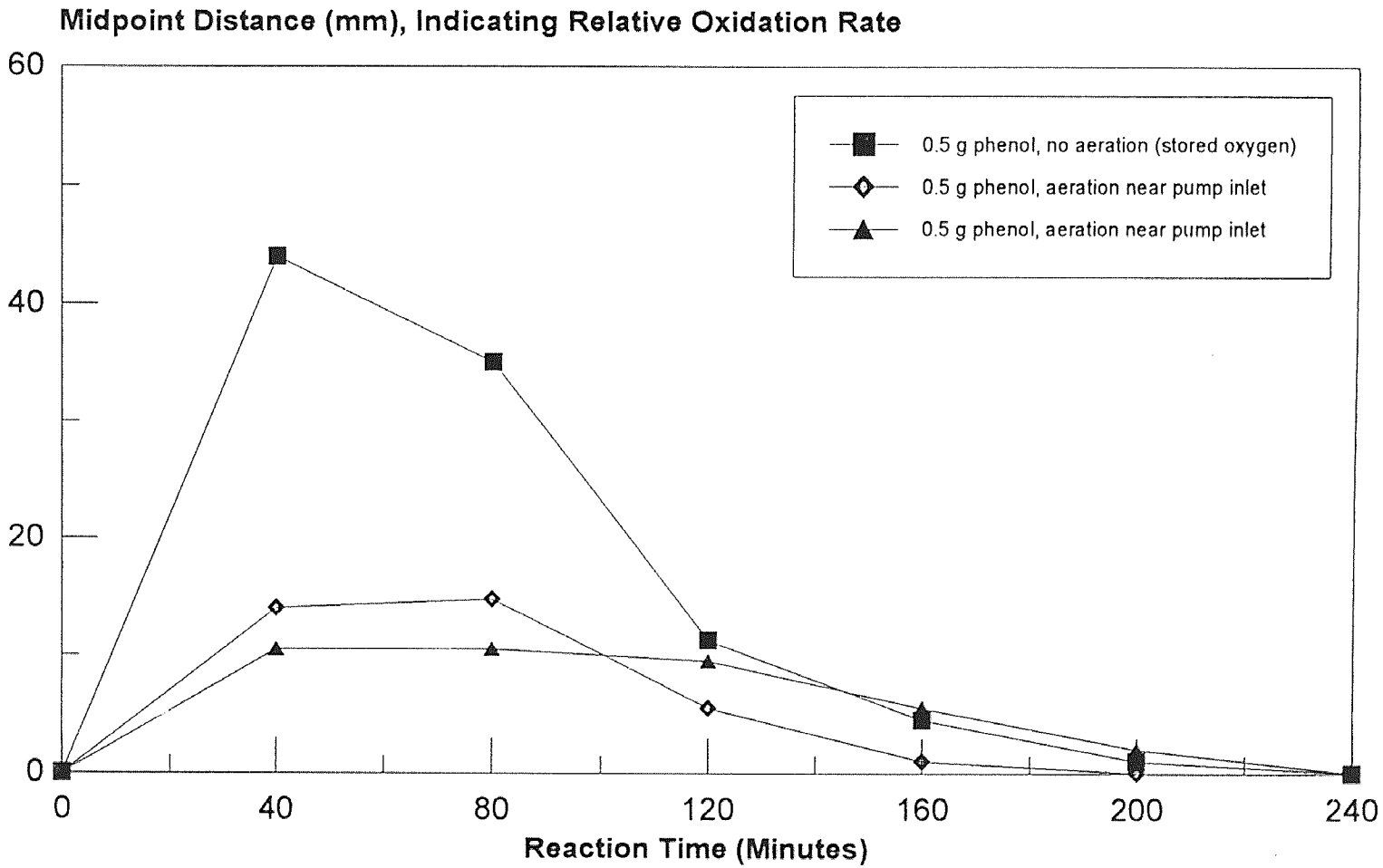

Figure 5 Post-Hydrogen Peroxide Injection Reaction

The high initial rate of the reaction with no aeration is probably due to oxygen stored by the bacteria. The non-aerated reaction followed injections of a total of 11 ml 30% hydrogen peroxide over the previous 48 hours which supplied the oxygen stored by the bacteria.

also presented. It must be noted, however, that the action of the bacteria significantly affects the measured DO and causes a discrepency between measured and theoretically liberated H_2O_2.

As shown in Table iii, the "peak" which occurred 20 minutes after the the 0.5 ml 30% H_2O_2 actually indicated a decrease in DO. This is because the rise in DO caused by the relatively small H_2O_2 injection was smaller than the decrease in DO resulting from the use of oxygen by the bacteria.

Table iii Oxygen liberated by H_2O_2 Injections

Amount Phenol Injected (g)	Amount 30% H_2O_2 Injected (ml)	Theoretical Oxygen Increase (g)	Peak Midpoint Distance (cm)	Calculated Oxygen Increase (g)	Calculated Oxygen/ Theoretical Oxygen (%)
0.5	0.5	0.084	2.1	-0.059	-7.02
1.5	1.0	0.167	-1.1	0.031	18.6
0.5	2.0	0.334	-0.75	0.021	6.3
0.5	6.0	1.002	-6.88	0.193	19.3

5.4 Effect of Ammonium Hydroxide

In our method of operations, fixed nitrogen is not routinely added and sometimes becomes a limiting nutrient in the growth and health of the biomass. Occasionally, injections of phenol will be biodegraded very slowly as the result of a lack of fixed nitrogen. Such reactions typically reach a low rate of biodegradation and remain there for a very long period of time before the reaction is complete. Figure 6 shows two such reactions, which were finally brought quickly to completion with injection of ammonium hydroxide. The increased biodegradation rate due to the NH_4OH injection can be seen in these two

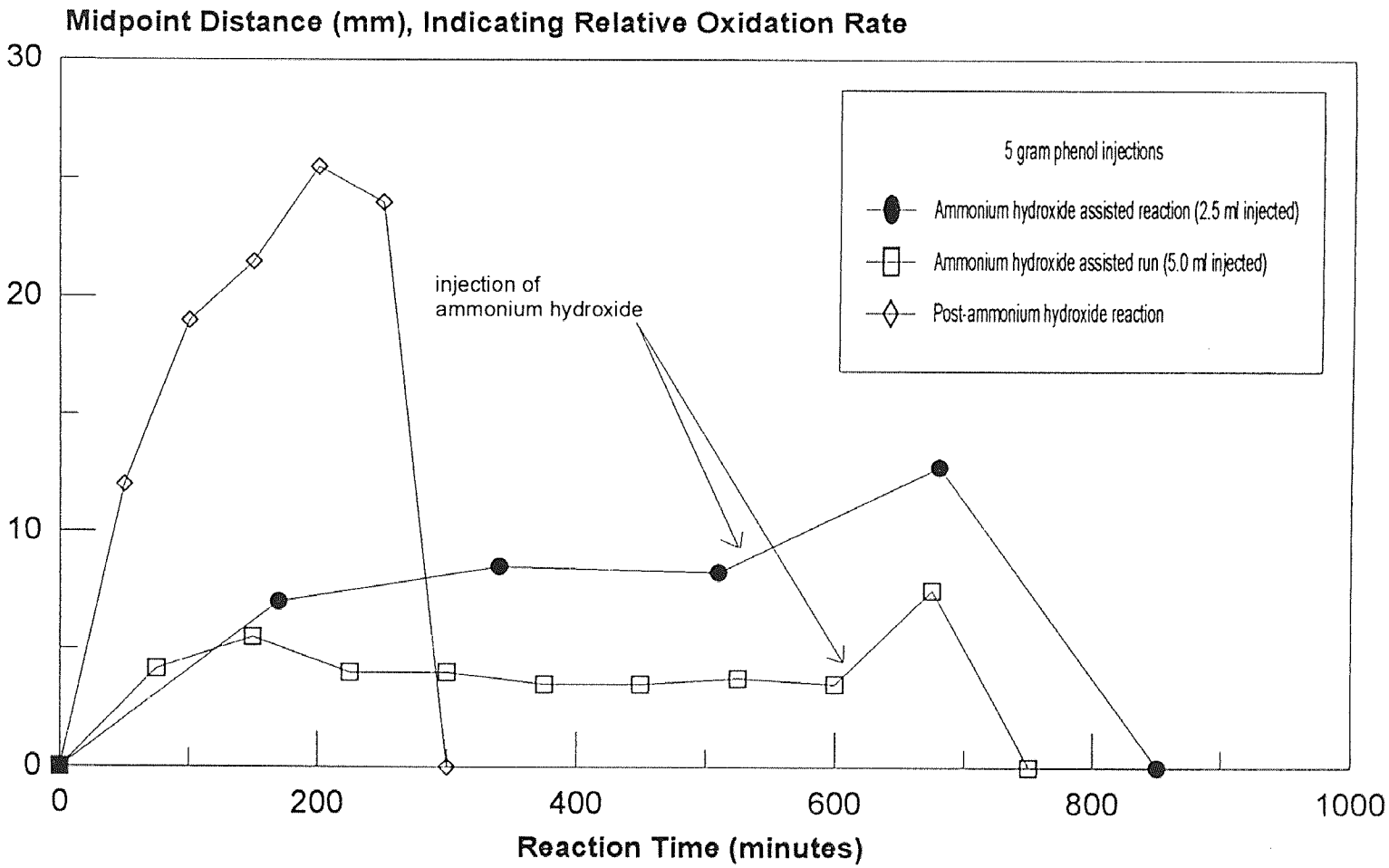

Figure 6 Effect of Ammonium Hydroxide

This series of reactions depicts the the effect of NH3 addition. Addition of NH3 facilitated the completion of lingering reactions (NH3 assisted reactions). Further addition of NH3 led to the fastest reaction, during which no NH3 was needed (post-NH3 reaction).

reactions as a brief rise in the pattern of oxygen uptake before the reaction finishes. The very fast reaction, indicated by hollow diamond shapes and appearing as a single steep curve, occurred shortly after the first two reactions, and after two injections of 5 ml 29% NH_4OH. It demonstrates the effect of NH_4OH in re-establishing the health and activity of the biomass. From these reactions as well as experiments performed by Borkowski [3], it is estimated that an injection of 10 ml NH_4OH should restore biomass health and efficiency when fixed nitrogen is lacking in the bioreactor used in this study. This situation is characterized by poor reactions of the pattern shown in Figure 6 which occur for no obvious reason. If too much NH_4OH is used, excess biomass growth may occur and liquid flow through the biosupport may be inhibited.

5.5 Effect of Dilution on Baseline Dissolved Oxygen Pattern

As part of this study, the effect of dilution of the reservoir liquid with regular tap water on the baseline conversion per pass (cpp) when no added substrate is being biodegraded has been analyzed. The cpp is the difference between the dissolved oxygen level at the input to the pump leading to the biosupport and the DO level of liquid emerging from the biosupport cylinder (generally lower as the result of aerobic biodegradation). As the result of dilution, the baseline cpp immediately shrinks, and then eventually (after about one hour) expands slightly, though not back to the original width, and levels off there.

Three cases of dilution have been quantitatively analyzed, and the results averaged. Based on this analysis, it has been determined that, on average, with each 1% liquid dilution, the baseline cpp immediately decreased by 0.33%, and eventually (after about one hour) leveled off with a decrease of 0.14%. These results can be used to predict the change in the baseline DO pattern which may be expected as the result of dilution for the bioreactor used in this study.

5.6 Effect of Liquid Flow Rate on Baseline Dissolved Oxygen Pattern

As with liquid dilution, changing the liquid flow rate has also been observed to effect the baseline dissolved oxygen pattern. In two separate instances, the flow rate of liquid through the biosupport cylinder was increased for a period of several hours and then lowered again to approximately the original flow rate. The result on the baseline DO pattern was that the increased liquid flow rate immediately caused a shrinkage in the baseline conversion per pass. This baseline DO pattern remained steady and essentially unchanged until the original liquid flow rate was restored, at which time the baseline cpp also expanded to about its original size. The decrease in baseline cpp (expressed as ppm oxygen) with increased liquid flow rate through the biosupport cylinder is shown in Figure 17, Appendix C. Although the slopes of these two lines are quite different, it can generally be observed that increasing liquid flow rate causes a shrinkage in baseline cpp, and that restoring the original flow causes the baseline cpp to expand to its original size.

5.7 Dissolved Oxygen Pattern Troubleshooting Guide

The purpose of this section is to outline some characteristic abnormal dissolved oxygen patterns which indicate specific systemic problems, and identify these systemic causes as well as their solutions. Figures 7 through 10 show example read-outs demonstrating each type of pattern.

The pattern in Figure 7 indicated trouble with the membrane covering the dissolved oxygen probe. This pattern was characterized by the following: expanded conversion per pass (about 1.5 to 2 times), confused, wildly varying DO readings at peaks, and an overall elevation in the DO readings at both locations. Often, a simple, thorough rinsing of the DO probe membrane with deionized water solved this problem. Sometimes, however, the membrane had to be changed. On one occasion, the membrane had come loose from its stretched, secure position over the probe and had to be resecured.

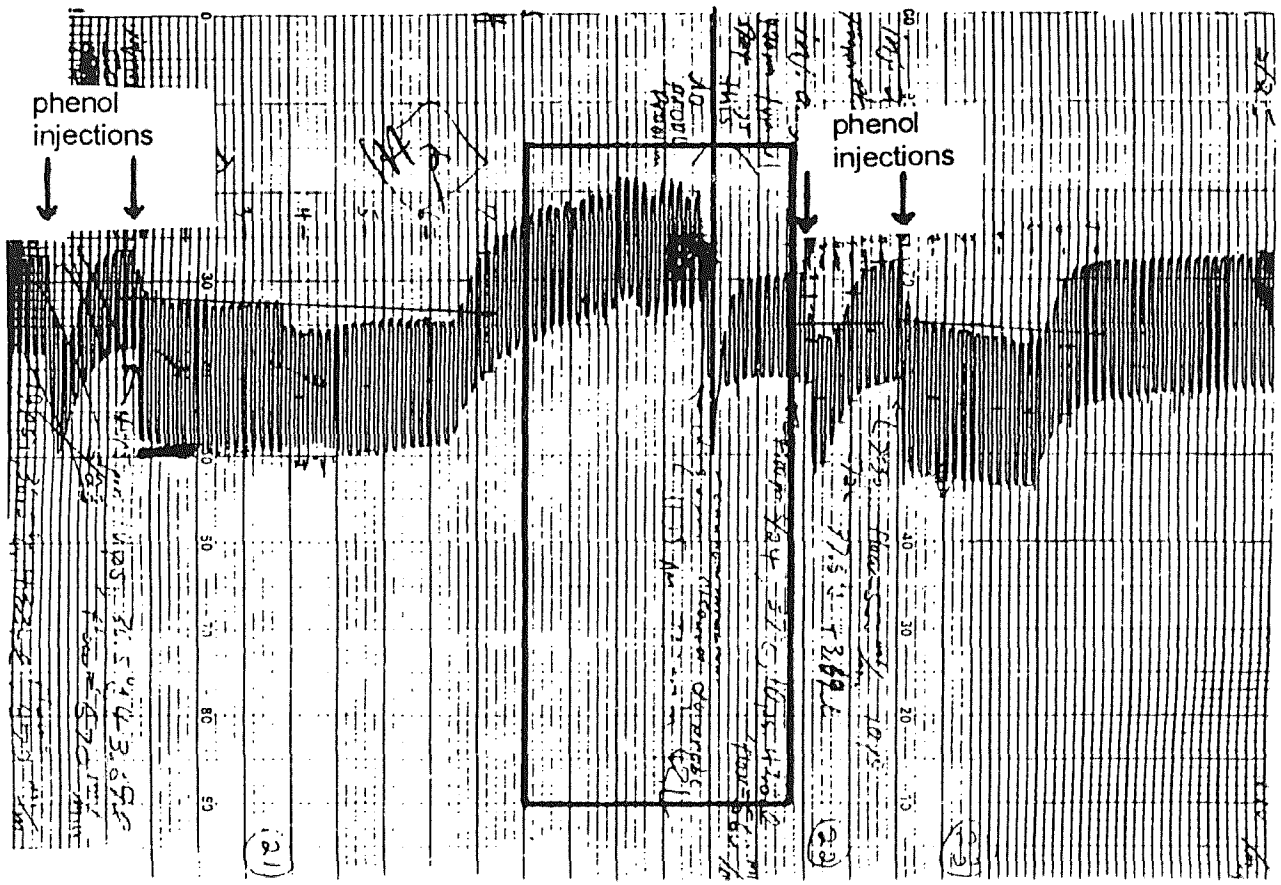

Figure 7 D.O. Troubleshooting Guide - Typical Membrane Trouble

Trouble with the membrane covering the D.O. probe commonly caused erroneous D.O. patterns. The boxed region shows such a pattern, which occurred during a long phenol reaction. The problem was corrected by changing the membrane, though simply rinsing the probe and membrane was often enough to restore normal D.O. measurement when this problem arose.

Figure 8 shows a pattern which occurred many times consecutively before the cause was determined. This problem resulted from exposure of the DO probe set up to sunlight. It is characterized by a sudden sharp drop off in DO and a shrinking conversion per pass which tails off slowly, and sometimes confused DO readings. The problem was temporarily solved by rinsing the DO membrane with deionized water, as described above. However, the pattern kept recurring until the probe set up was protected from sunlight coming through the window by simply replacing the cardboard over it, which had been accidentally removed. It is thought that sunlight may interfere with DO readings by stimulating the growth of microorganisms in the vicinity of the probe membrane which use DO for respiration, thereby causing lower apparent DO readings.

Figure 9 shows a simple pattern characterized by a wiggly line at the top of the baseline D.O. pattern instead of the normal oscillating baseline DO pattern. This pattern turned out to have been the result of a loose electrical connection in the D.O. monitoring polarograph set up which caused system not to switch back and forth from the two sampling locations, but instead to continuously sample liquid from the reservoir and never from the biosupport cylinder output. This caused the almost linear pattern instead of the normal oscillating pattern. The problem was elusive, since the switching system indicated normal oscillation between the two liquid sampling locations. The problem was solved by simply checking and tightening all electrical connections in the system.

The pattern in Figure 10 shows another problem which was difficult to find. The pattern was characterized simply by a wildly varying and confused DO pattern. Upon dissembling the system, the liquid flow through the DO measuring set up was found to be mostly blocked at the very small inlet to the electric valve, where a small fly was lodged. Removing the fly solved the problem.

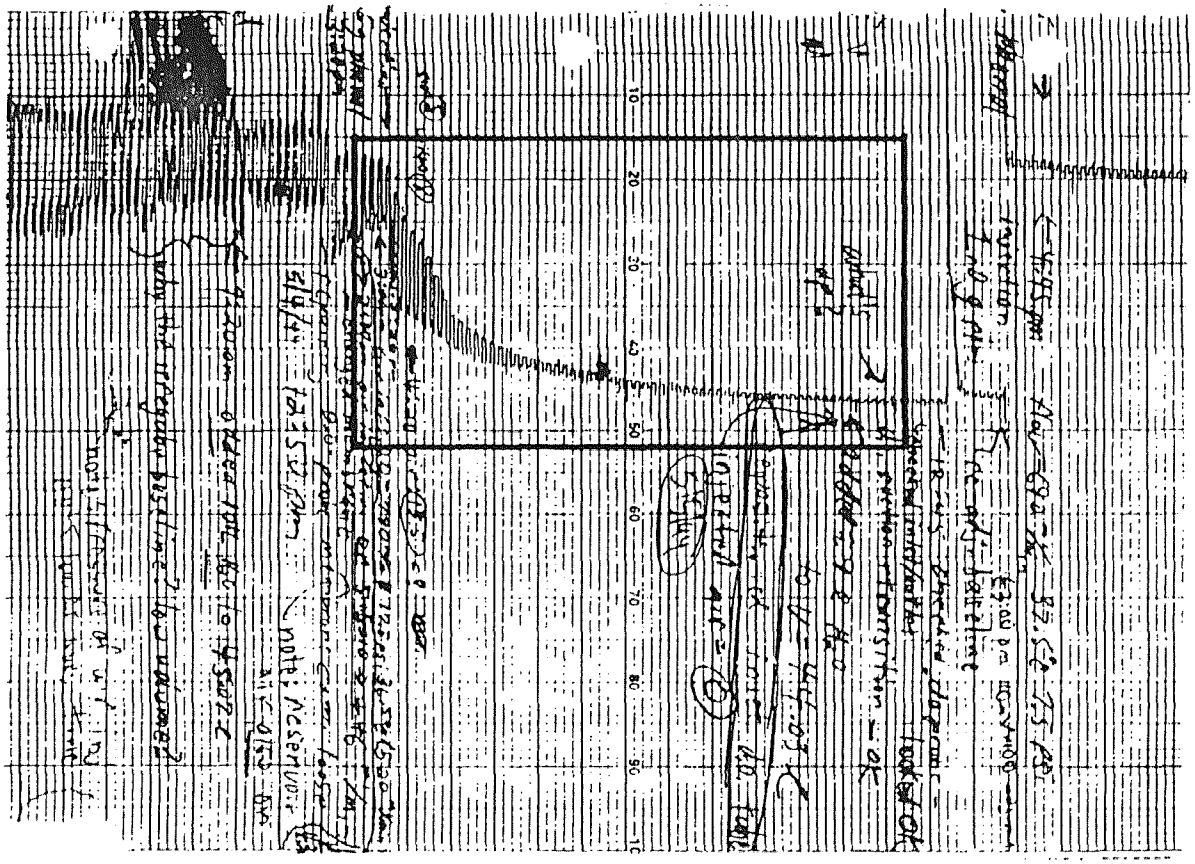

Figure 8 D.O. Troubleshooting Guide - Sunlight affecting the Membrane

This type of erroneous D.O. pattern was caused by exposure of the D.O. probe set up to sunlight. Cleaning the probe and membrane eliminated the problem, but shielding the probe set up from sunlight was necessary to prevent the problem from recurring.

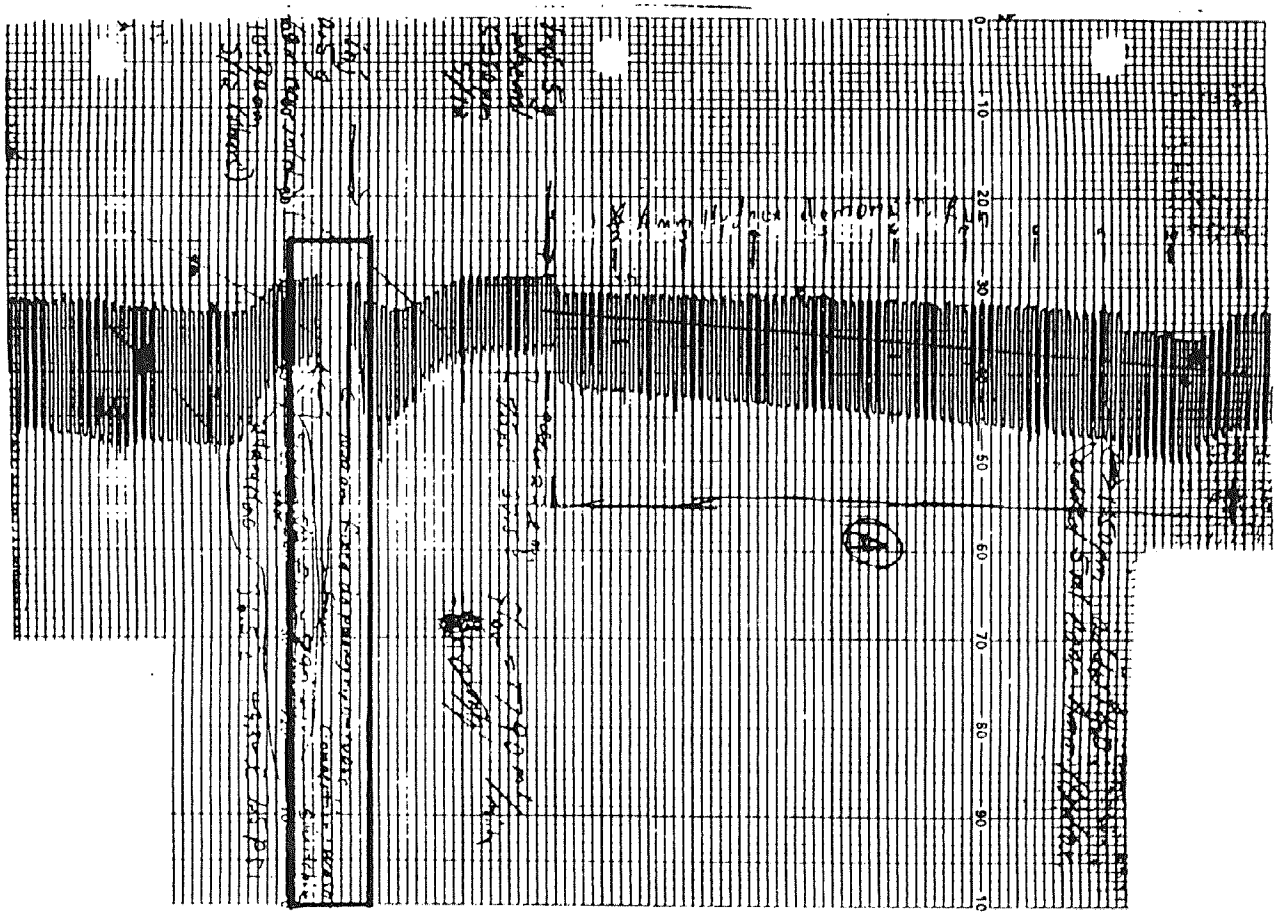

Figure 9 D.O. Troubleshooting Guide - Loose Electrical Connection

This pattern - a slightly wiggling line instead of the normal oscillating pattern, was found to be the result of a loose electrical connection to the polarogragh. This caused liquid to be sampled from the reservoir only, instead of correctly alternating between the reservoir liquid and the biosupport cylinder output liquid.

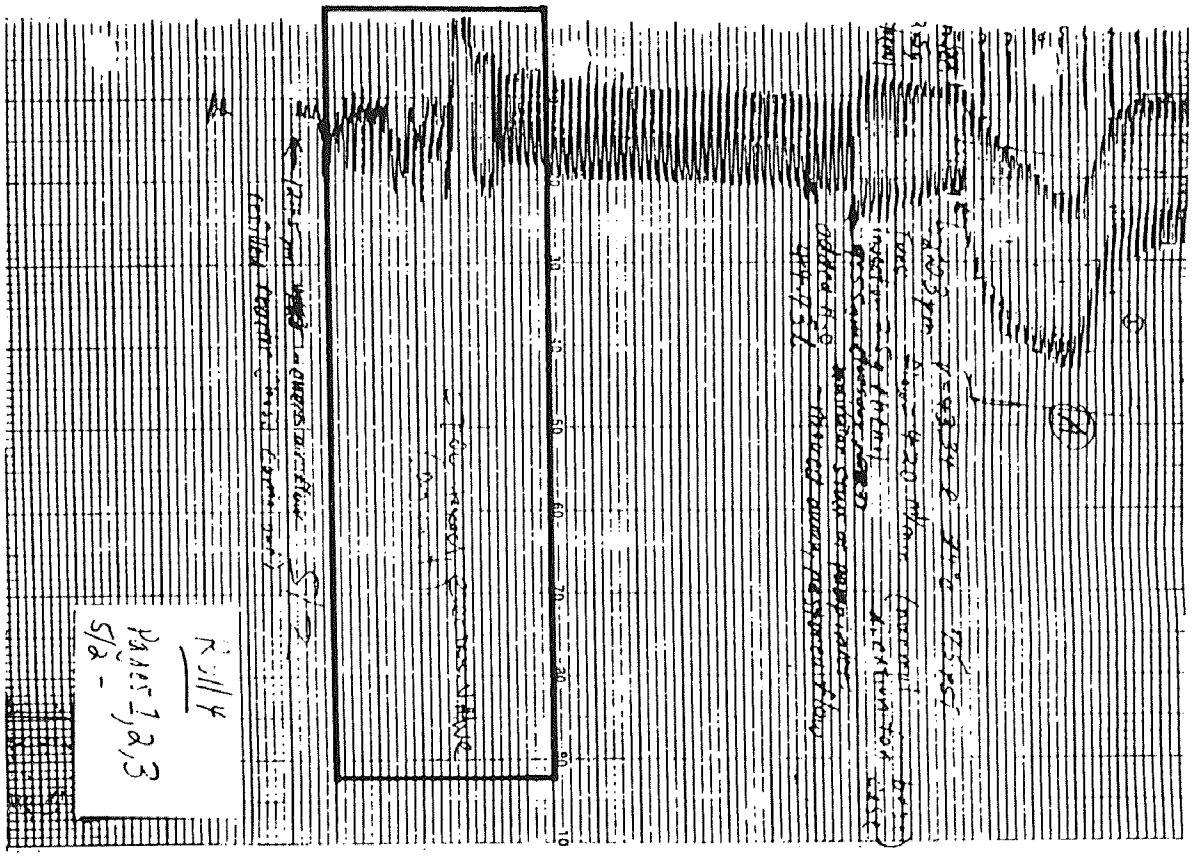

Figure 10 D.O. Troubleshooting Guide - Blocked Liquid Flow

The confused pattern shown here resulted from partially blocked liquid flow through the D.O. probe set up. The problem was found to be caused by a small fly which got stuck at the narrow inlet to the electric valve, which led to the probe set up.

5.8 General Observations Regarding the Operation of the Bioreactor

For the bioreactor used in this study, it was observed that the general appearance of the reservoir liquid and the surface of the biosupport cylinder provided a fairly reliable indication of the health of the biomass and the vitality of the bioreactor in general. During periods of highly efficient bioreactor operation, the reservoir liquid tended to be clear, not cloudy, and had a slight yellow hue, due to the presence of enzymes secreted by the healthy biomass. Cloudy liquid could indicate dead or unattached biomass, and generally was a sign of inefficient bioreactor operation.

Also, during periods of efficient bioreactor operation, the surface of the biosupport cylinder was largely covered with a thin layer of greenish slime - the appearance of moist, healthy biomass. If the surface biomass appeared dark green to black and dry, or if the surface was only sparsely covered with slime, the biomass was generally unhealthy and bioactivity was low. Sometimes the surface biomass appeared overgrown, fuzzy, and spotty, and this also meant unhealthy biomass and poor bioreactor efficiency.

It has been observed that liquid flow rates through the biosupport cylinder below about 225 ml/min. led to slow biodegradation rates for phenol injections of the range used in this study - between 0.5 and 5.0 grams.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Aeration close to the inlet of the pump leading to the biosupport cylinder led to faster initial rates of biodegradation than aeration in the center of the reservoir. Generally, locating the source of aeration near the inlet to the biosupport cylinder is recommended in order to provide attached biomass with maximum dissolved oxygen for bio-oxidation. In experiments performed in this study using a single injection of between 0.5 to 6 ml 30% hydrogen peroxide solution to provide dissolved oxygen, a single injection of 0.5 ml (3.8 ppm H_2O_2) best facilitated the biodegradation of 0.5 g phenol.

Quantitation of a set of eight 2.5 gram phenol reactions using Quantitation Method #1 and #3 as discussed in detail in Borkowski (3) were in reasonable agreement with the results of Borkowski (3). Results using Method #1 and Method #3 showed 13% and 6% error respectively.

When aqueous ammonia was injected to speed slow biodegradation rates by providing fixed nitrogen to the biomass, the best results were obtained by injecting 10 ml concentrated NH_4OH (21 ppm NH_4OH). Injections of less than 5 ml concentrated NH_4OH were less effective.

Both dilution of the reservoir liquid and higher liquid flow rates through the biosupport cylinder led to a smaller baseline DO pattern conversion per pass. With dilution, the baseline cpp immediately decreased an average of 0.33% per 1% dilution, but later leveled off with an average 0.14% decrease per 1% dilution.

Many abnormal DO patterns are characteristic of a specific systemic cause, and can therefore be used for troubleshooting purposes. Some such patterns and associated causes and solutions have been discussed in this Thesis.

Topics warranting further study include automatic continuous injection of H_2O_2 to provide dissolved oxygen for bio-oxidation. Also, optimization of liquid flow rate with respect to amount of substrate being biodegraded could prove useful.

Finally, since the DO pattern indicating the bio-oxidation of each different pollutant tends to be very distinct, it may be possible to assemble a catalogue of different DO patterns and each associated bio-oxidized chemical. This could ultimately lead to the use of the bioreactor as an analytical instrument for the identification of unknown pollutants in waste water. Concepts discussed in the work of Borkowski [3] in quantitation of the amount of substrate injected based on the DO pattern of its biodegradation could feasibly be integrated with such a project for quantitative as well as qualitative analysis.

APPENDIX A

Sample Calculations

1. The most important calculation used in this study has been the analysis of each phenol reaction DO read-out to obtain a curve which represents the biodegradation of the substrate from start to finish in terms of DO consumption. The following is an explanation of how each curve was determined. The basic DO pattern indicating a reaction is discussed in the Introduction Section of this Thesis. Figure 17 shows an example of an analyzed reaction.

The first step in the analysis is the drawing of a corrected baseline through the length of the reaction. The reaction begins when the phenol is injected, and ends when the baseline DO pattern returns. First, the midpoint of the baseline pattern at the start of the reaction is determined and marked. This midpoint is simply the center of the line on the read-out connecting the two alternating DO measurements (that is, one segment in the pattern). Next, the midpoint of the baseline pattern at the end of the reaction is similarly marked. Then, a line is drawn connecting the midpoints at the start and finish of the reaction. This is known as the corrected baseline, and represents the line going through the baseline DO pattern which would have existed if no substrate had been injected.

The next step is to divide the reaction into about 6 to 9 equal intervals of paper length, indicating equal lengths of time throughout the reaction. At each interval, spanning the length of the reaction as marked by the corrected baseline, the midpoint of that particular cpp is marked.

Finally, at each interval, the distance between the midpoint of the cpp and the adjusted baseline is measured and recorded. At the start and finish of each reaction, this distance is zero, since the midpoint of the cpp is on the corrected baseline itself. If, at any interval, the cpp midpoint is above the adjusted baseline, the measured distance is reported

as negative. Such a high DO level generally does not occur during a reaction, except when a large amount of DO is provided at one time, such as with injection of hydrogen peroxide into the reservoir. The recorded positive distances are proportional to the DO drop from baseline at that point in the reaction, and indicate the rate of the reaction in terms of DO usage at that time.

For the 1.5 g phenol reaction shown in Figure 18, the following data was obtained:

Table iv Example Phenol Reaction Analysis Data

Point #	mm of Paper	Midpoint Distance (mm)
1	0	0
2	2	22
3	4	26
4	6	29
5	8	27
6	10	12
7	12	0

APPENDIX B

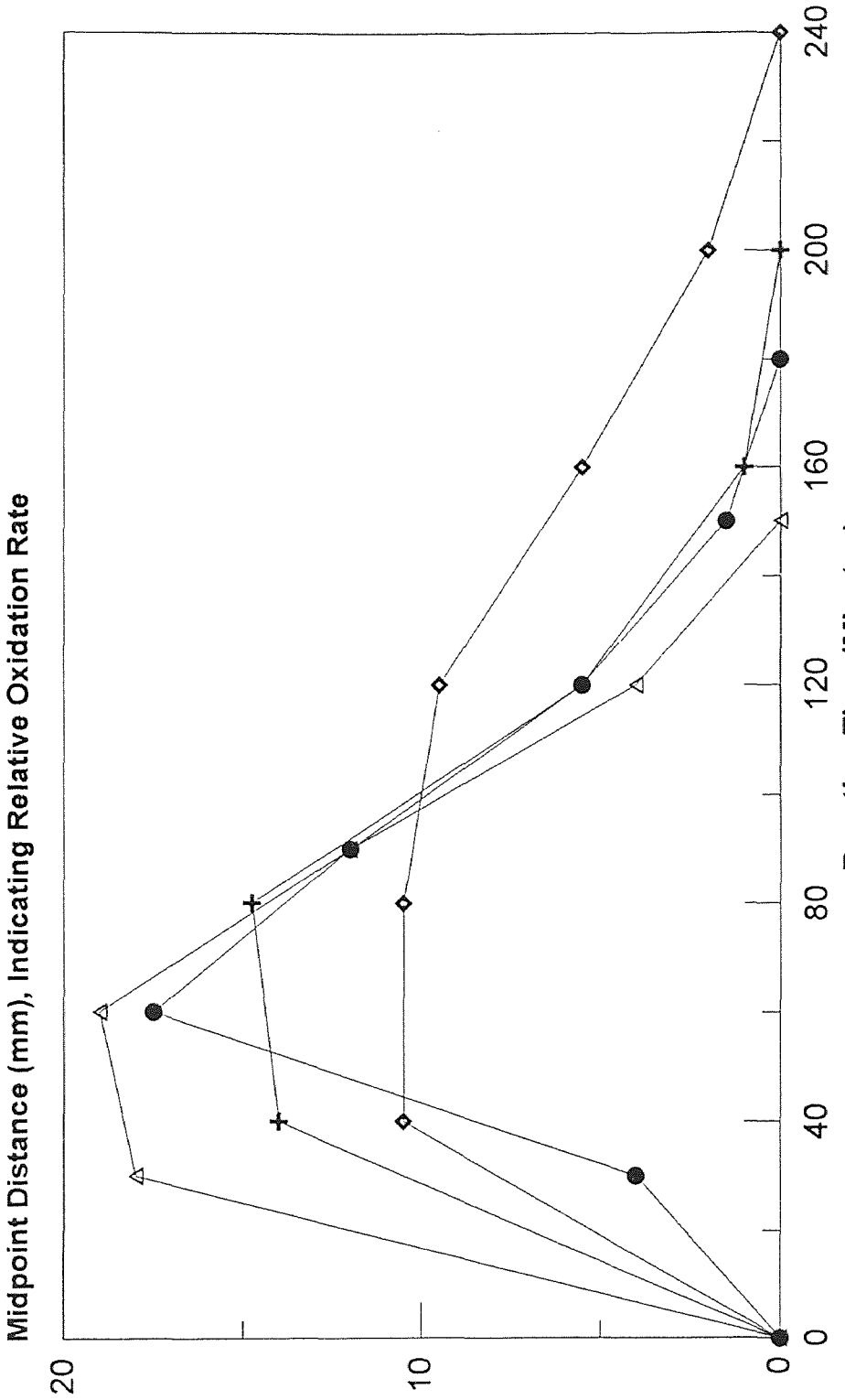

Figure 11 0.5 Gram Phenol Reactions, Aeration near Pump Inlet

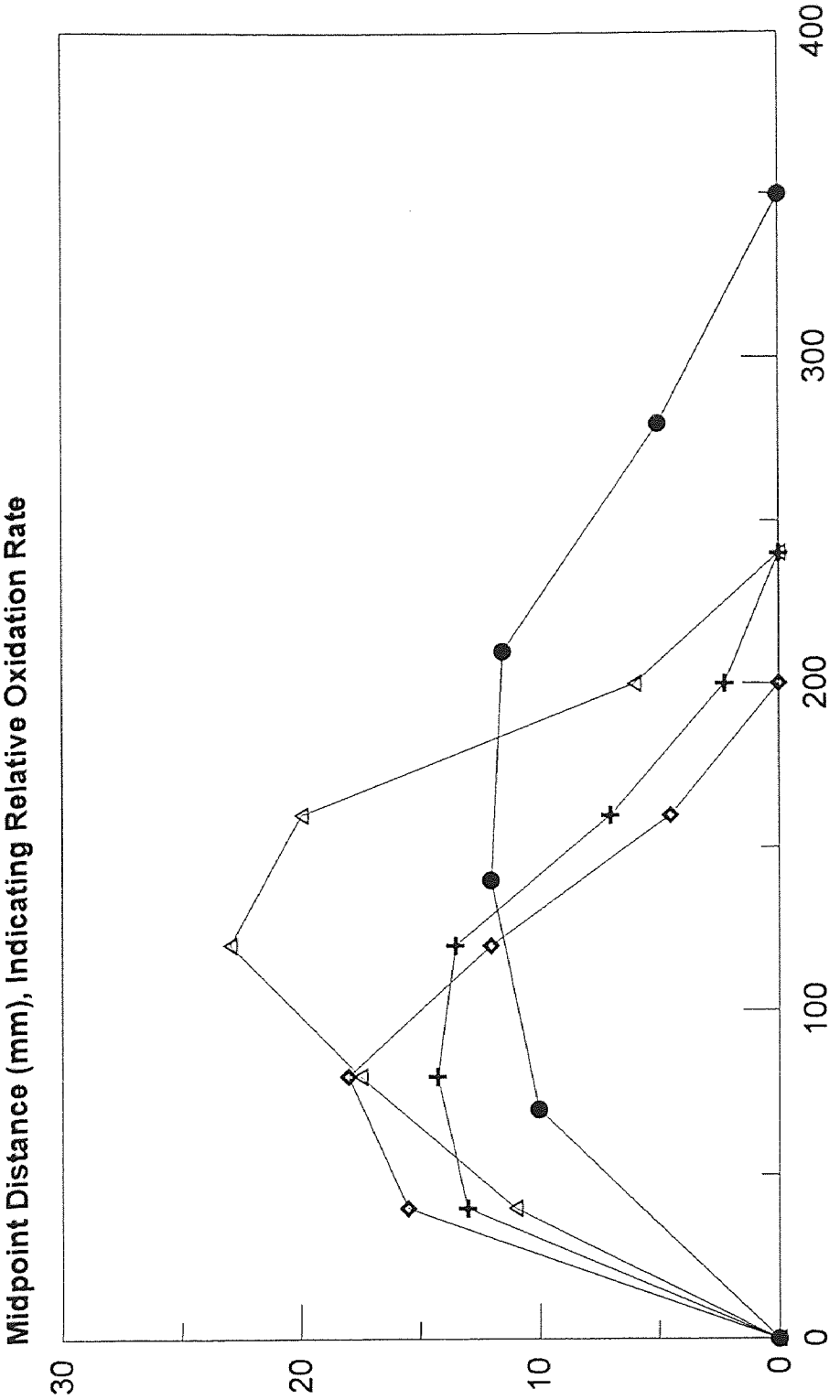

Figure 12 1.0 Gram Phenol Reactions, Aeration near Pump Inlet

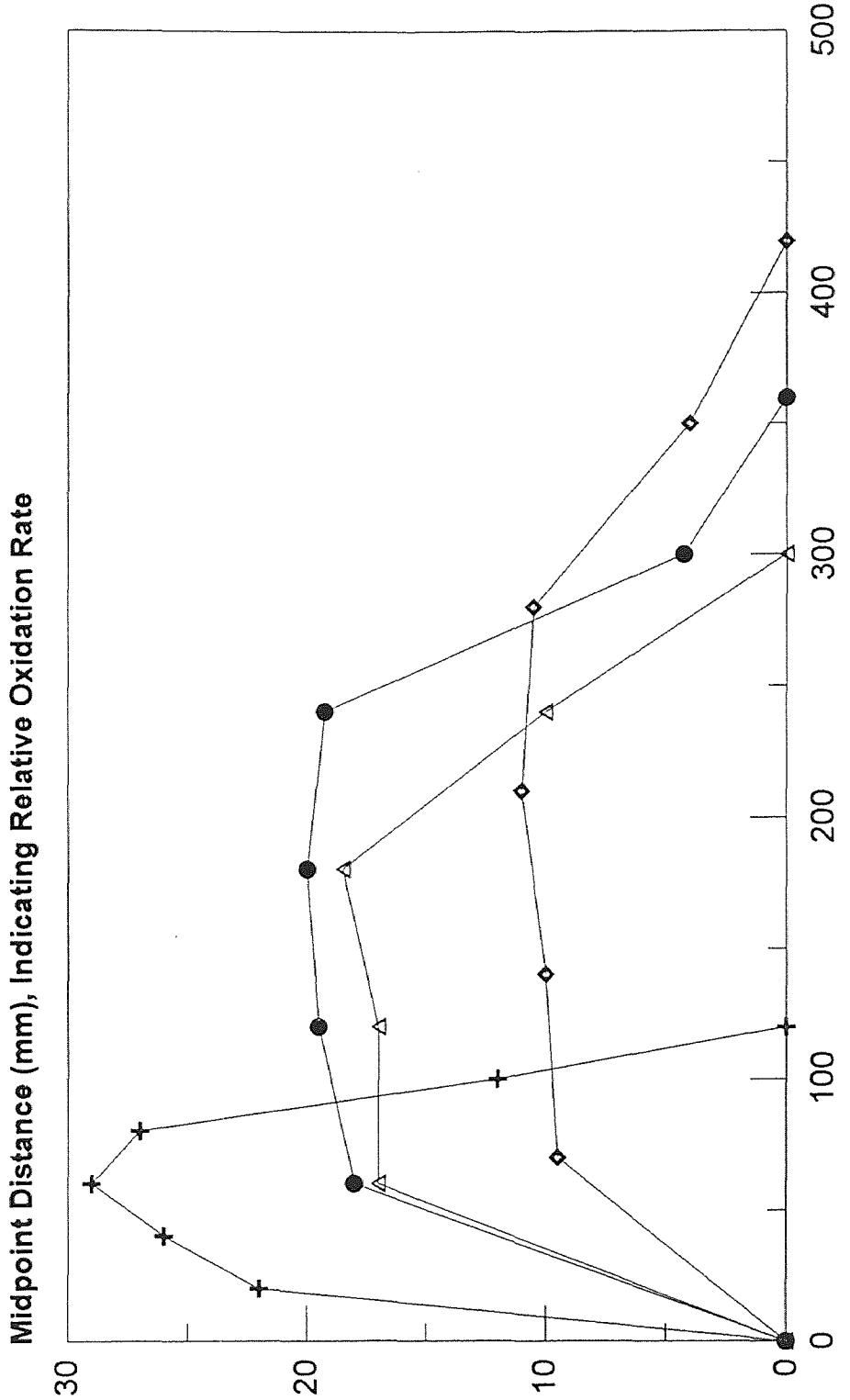

Figure 13 1.5 Gram Phenol Injections, Aeration near Pump Inlet

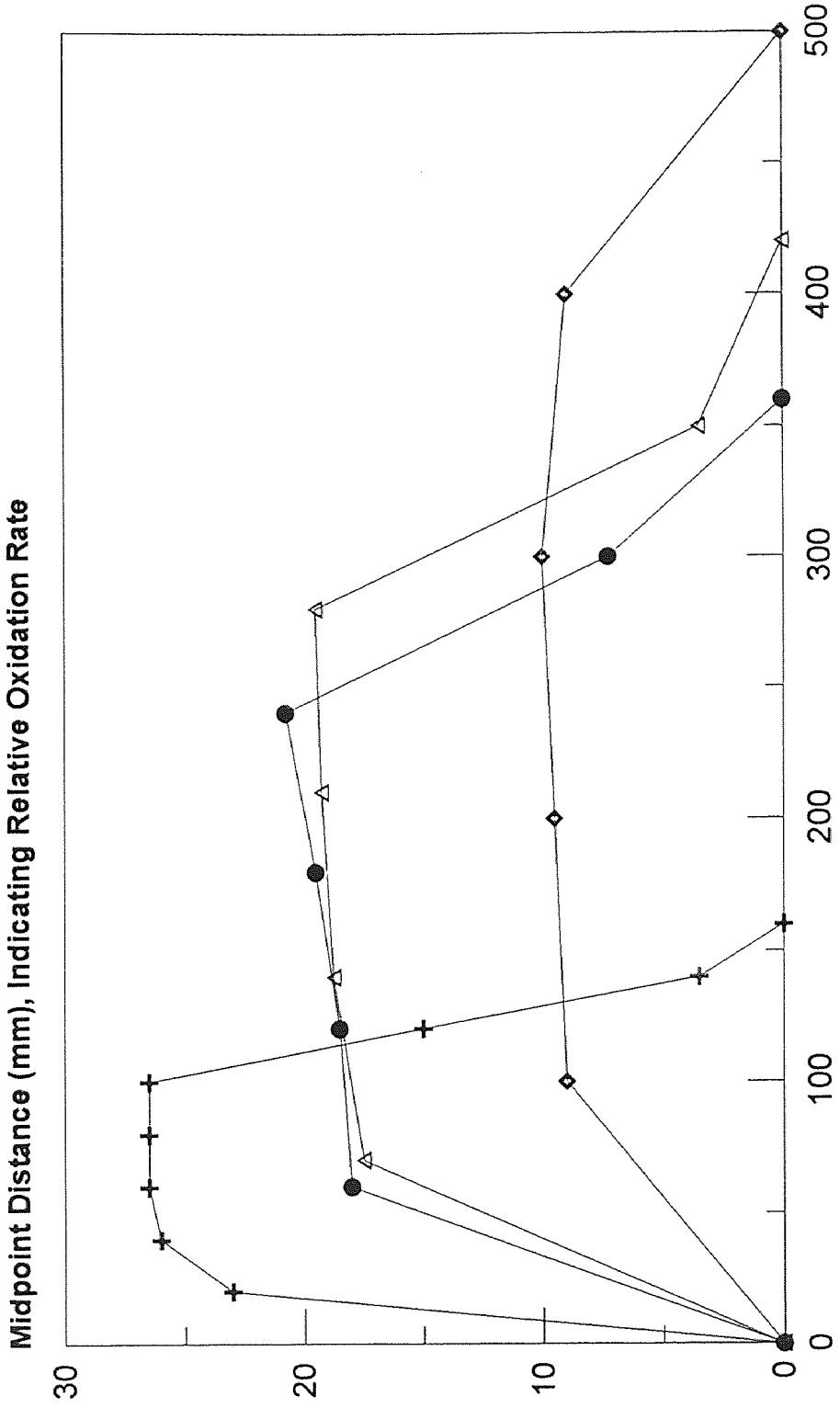

Figure 14 2.0 Gram Phenol Reactions, Aeration near Pump Inlet

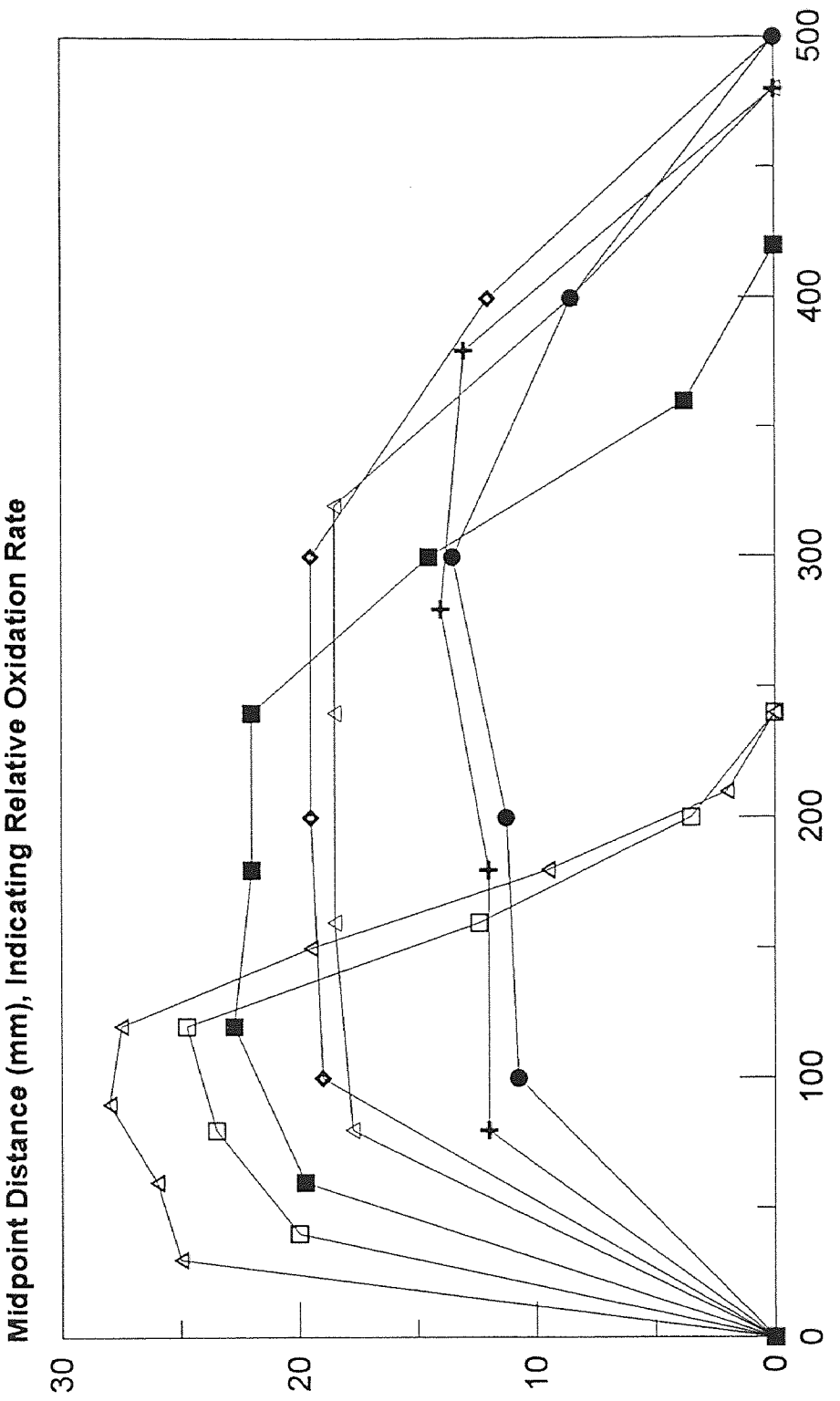

Figure 15 2.5 Gram Phenol Reactions, Aeration near Pump Inlet

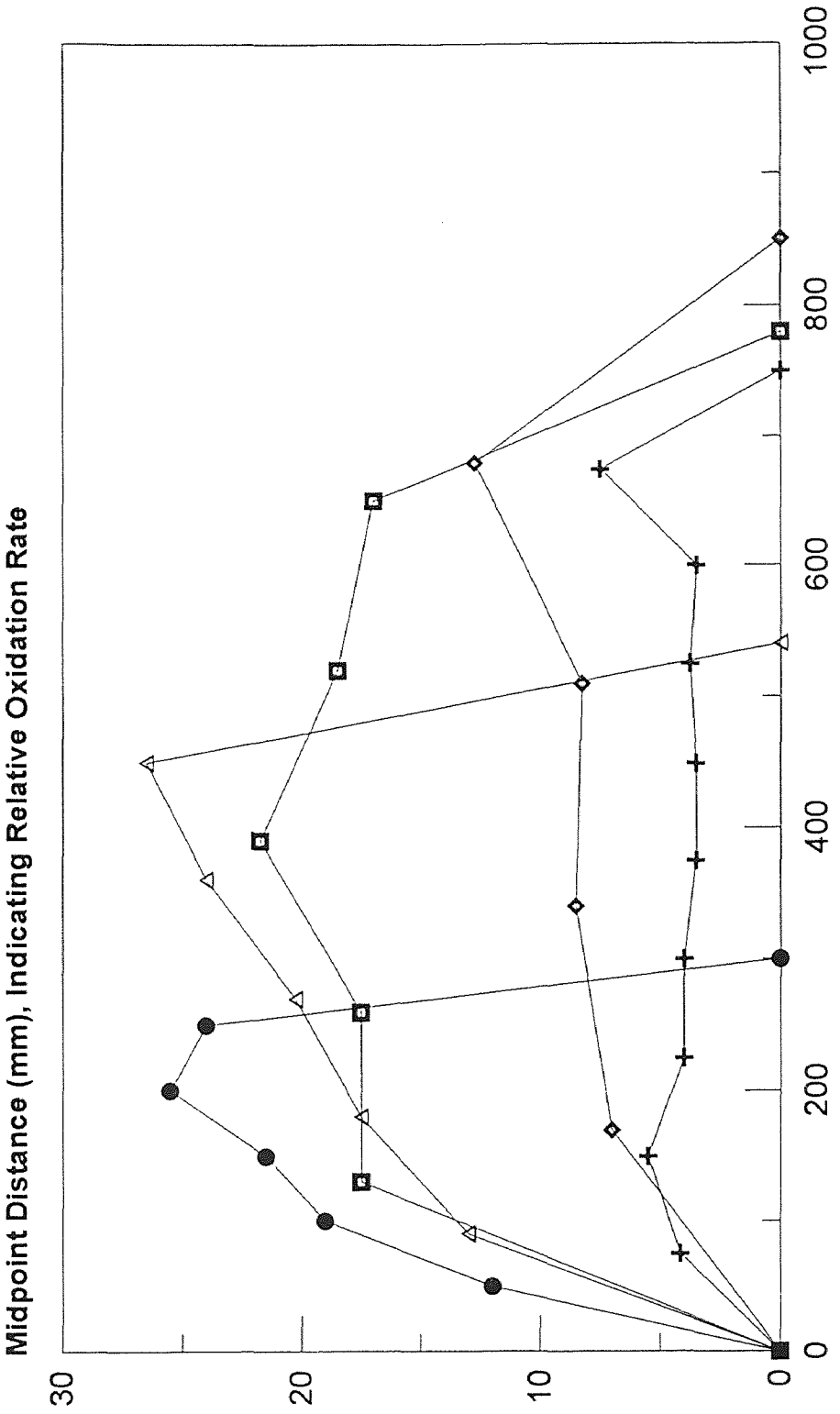

Figure 16 5.0 Gram Phenol Reactions, Aeration near Pump Inlet

APPENDIX C

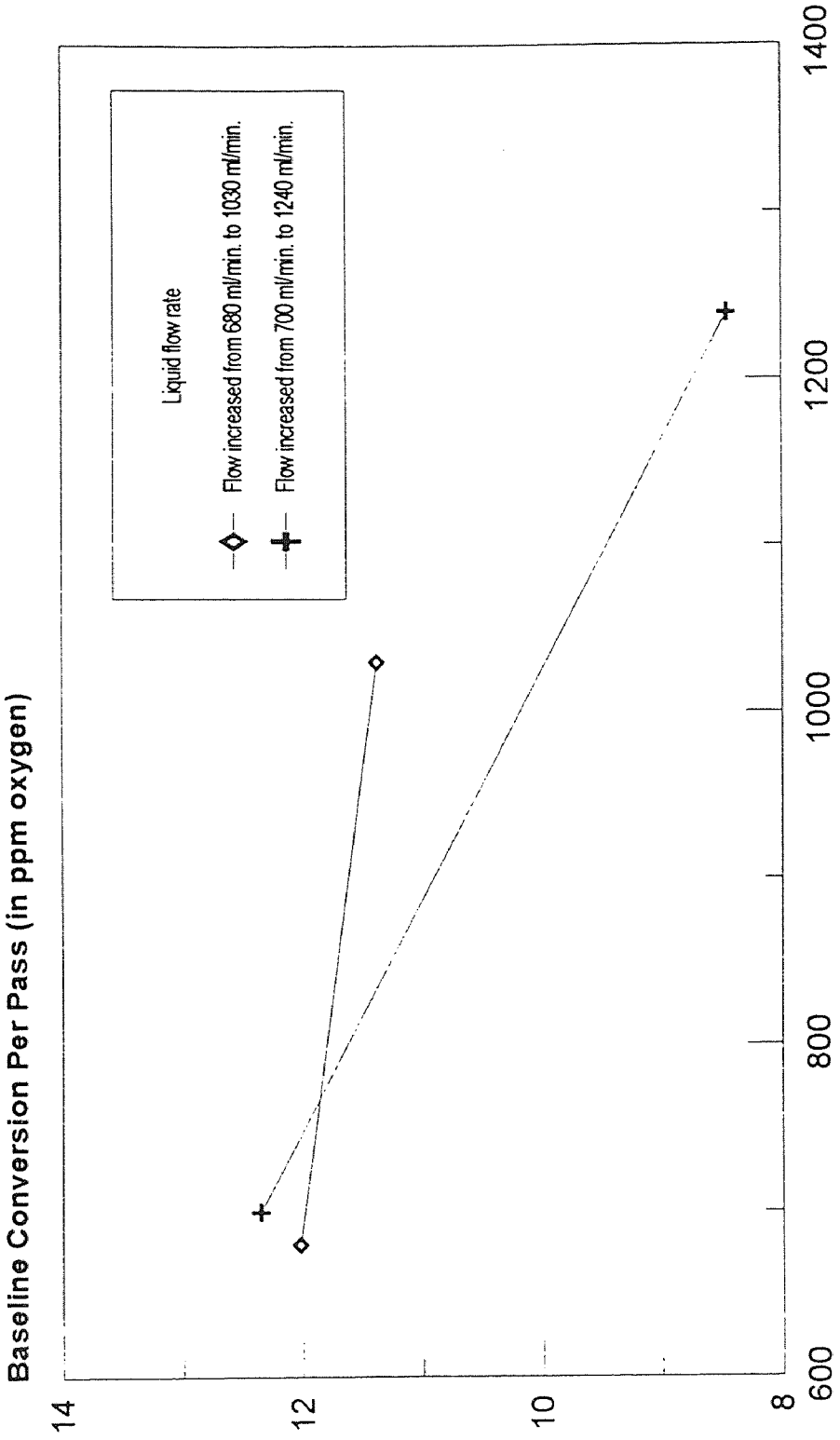

Figure 17 Effect of Changing Liquid Flow Rate on Baseline Dissolved Oxygen

Increasing liquid flow through the biosupport cylinder led to a decreased baseline conversion per pass, indicating that, at higher flow rates, the difference between main reservoir D.O. and cylinder output D.O. decreases slightly.

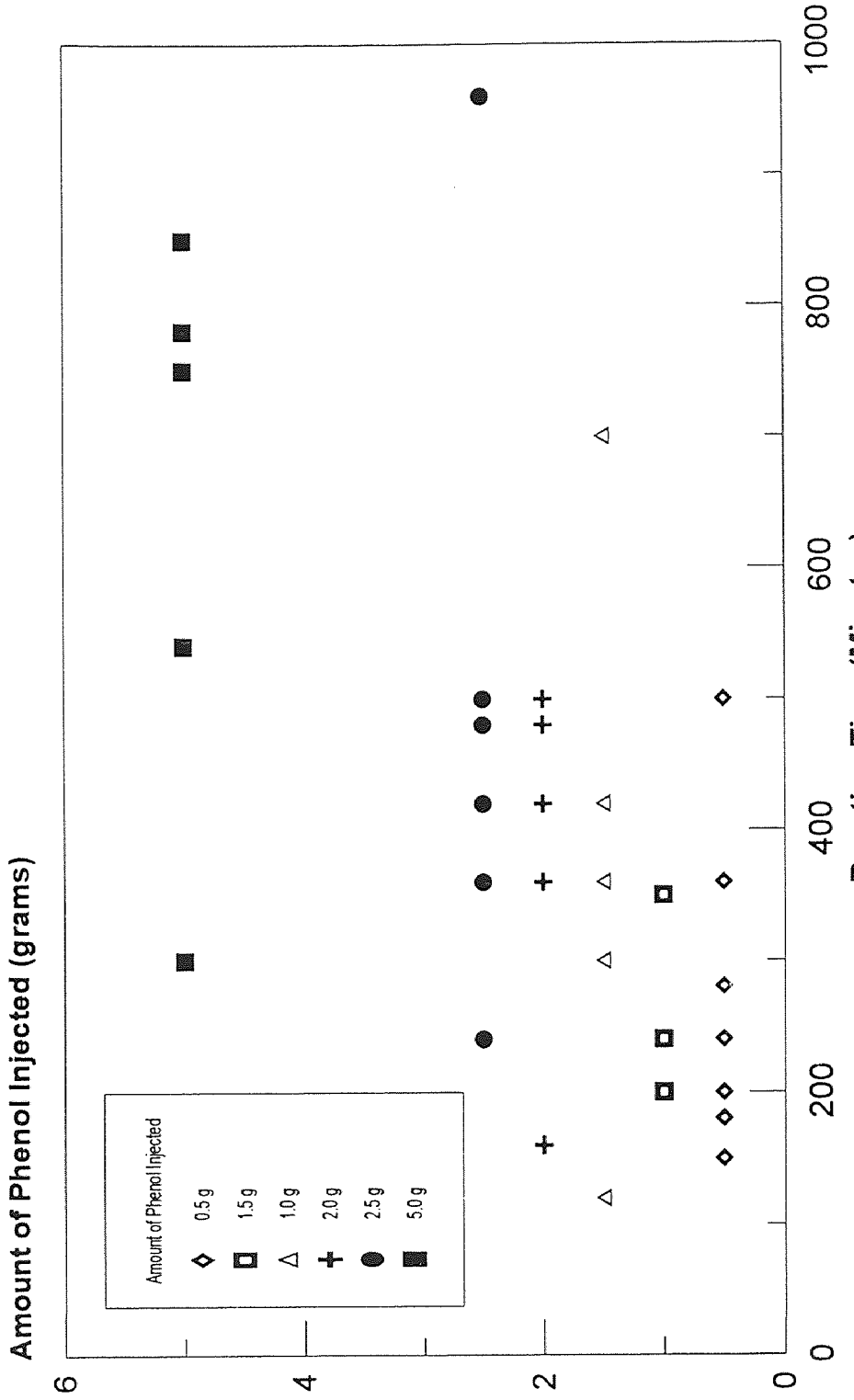

Figure 18 Amount Phenol Injected versus Time for Reaction Completion

Overall, reaction length varied directly with amount of phenol injected, as expected.

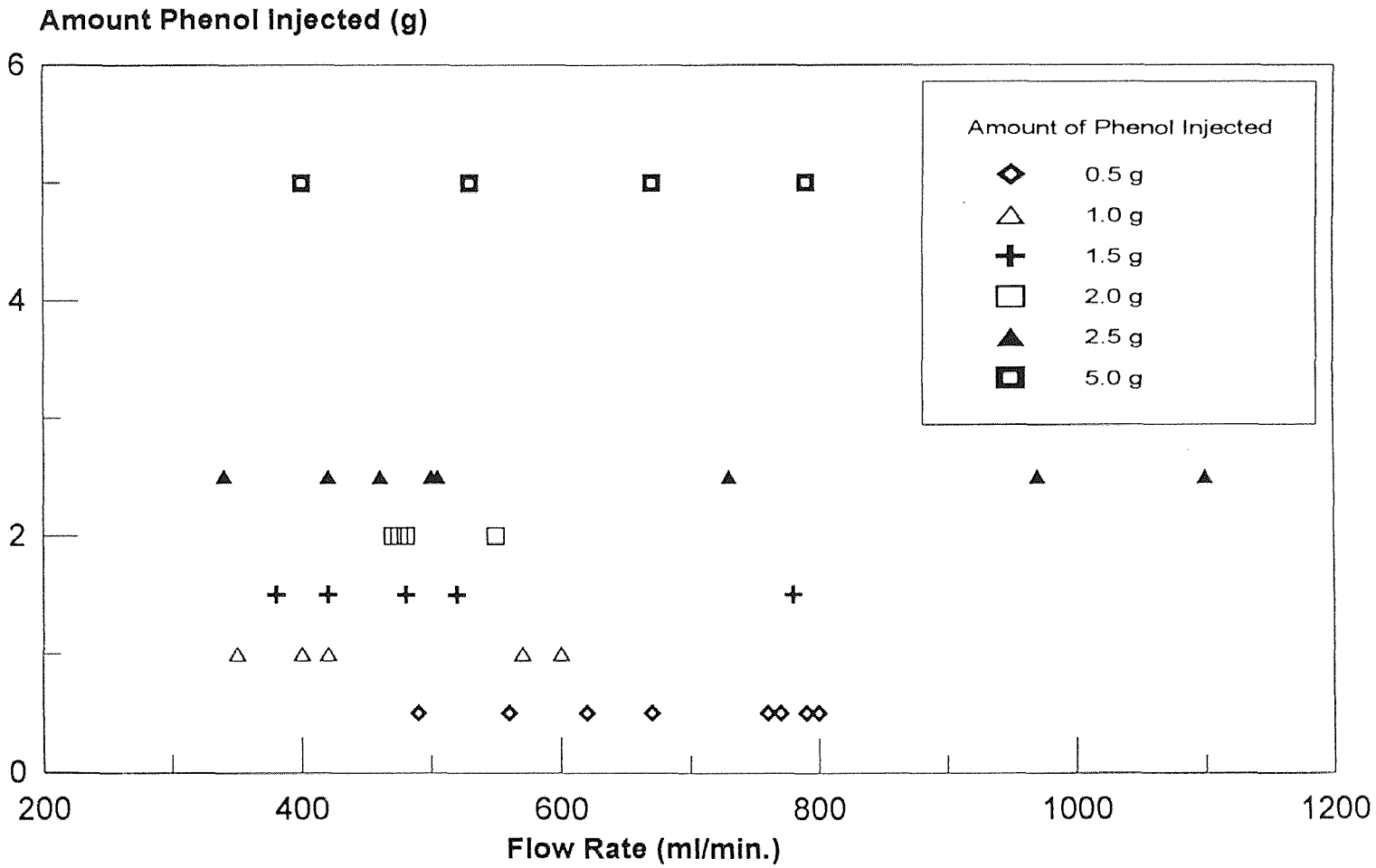

Figure 19 Amount Phenol Injected versus Flow Rate

This figure shows the flow rate during each phenol reaction, thus indicating the regime of operation with respect to flow.

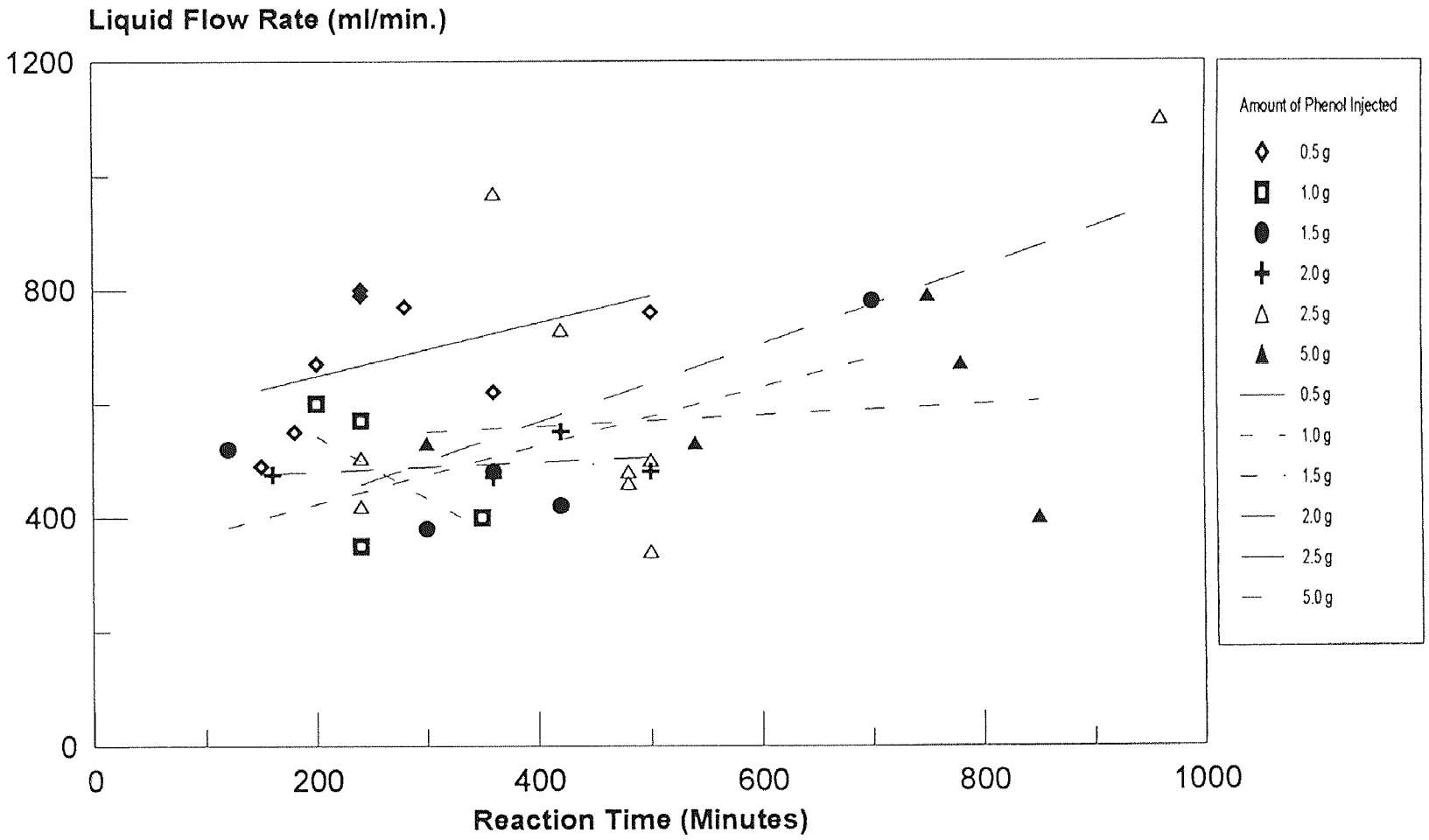

Figure 20 Liquid Flow Rate versus Reaction Time

Within the range of flow rates used, reaction rate appears to be independent of flow rate.

APPENDIX D

Table v Phenol Reaction Data

Amount Phenol (g)	Liquid Flow Rate (ml/min.)	Reaction Length (min.)	Amount Phenol (g)	Liquid Flow Rate (ml/min.)	Reaction Time (min.)
0.5	490	150	2.0	480	500
0.5	550	180	2.0	550	420
0.5	670	200	2.5	340	500
0.5	790	240	2.5	420	240
1.0	350	240	2.5	460	480
1.0	400	350	2.5	480	480
1.0	570	240	2.5	500	500
1.0	600	200	2.5	505	240
1.5	380	300	2.5	730	420
1.5	420	420	5.0	400	850
1.5	480	360	5.0	530	300
1.5	520	120	5.0	530	540
2.0	470	360	5.0	670	780
2.0	475	160	5.0	790	750

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