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ABSTRACT

QUANTITATIVE STUDIES OF AN IMMOBILIZED CELL OXIDATIVE BIOREACTOR

by

Timothy Lance Borkowski

The purpose of this work was to determine a suitable method of utilizing dissolved oxygen in reservoir solution data to quantitatively measure the amount of oxygen and substrate consumed for an immobilized cell batch bioreactor in a recirculation configuration. Statistical analysis was performed on multiple phenol injections to determine the mathematical relationship between dissolved oxygen readout and the amount of phenol degraded.

Factors considered in the mathematical analysis include amount of substrate degraded, reaction time, recirculation flow rate, measured dissolved oxygen concentration and oxygen consumption, oxygen conversion per pass through the bioreactor during steady state, and vitality of the microorganisms. The recommended method of quantitation was found to be independent of recirculation rate, steady state oxygen conversion and vitality of the microorganisms, and was found to have a predictability error of 20.2%.

Additionally, four popular pesticides used in New Jersey: Acephate, Carbaryl, Chlorpyrifos, and Diazinon were tested to determine the capability of the bioreactor to degrade them. These pesticides were injected into the bioreactor to determine the bioreactor's oxidative response.
QUANTITATIVE STUDIES OF AN IMMOBILIZED CELL OXIDATIVE BIOREACTOR

by

Timothy Lance Borkowski

A Thesis
Submitted to the Faculty of
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in Partial Fulfillment of the Requirements for the Degree of
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Department of Chemical Engineering, Chemistry and Environmental Science

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APPROVAL PAGE

QUANTITATIVE STUDIES OF AN IMMOBILIZED CELL OXIDATIVE BIOREACTOR

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CHAPTER 1

INTRODUCTION

1.1 Bio-Oxidation of Wastewater Pollutants

Wastewater treatment applications provide methods for protecting the environment and human health from residential, commercial, and industrial pollutants. The water is cleaned of organics, metals, microorganisms and other pollutants and returned to the environment. The principles of wastewater treatment also apply to groundwater treatment, surface water treatment and drinking water treatment.

Biological treatment, also known as secondary treatment, involves the removal of organic matter by mineralization, and also by converting them into flocculant settleable solids. The four major biological processes are 1) activated sludge processes, 2) aerated lagoons, 3) trickling filters, and 4) stabilization ponds. The activated sludge process involves the biodegradation and oxidation of organics in the wastestream by microorganisms. Biological treatment is widely used in high volume, regional water treatment plants [17,10].

The development of immobilized bioreactors has led to a wider variety of biological treatment applications, including the removal of raw materials before processing, pre-discharge pipeline treatment, effluent stream treatment, and decontamination of soils, surface and groundwater. Biological treatment can be implemented at less cost than excavation, transportation and incineration of environmental contaminants. It has been shown, in certain applications, that immobilized cell biological
treatment can optimize and reduce the cost of pre-treatment, as compared to steam stripping, carbon adsorption and traditional biological treatment [10]. Immobilized bioreactors have also been used for the removal of VOC's in air phase [3] and the removal of heavy metals from aqueous streams [5].

1.2 Advantages of Immobilization

Several immobilization techniques have been developed in the past few decades to broaden the scope of activated sludge usage. Immobilization provides a support surface to which microorganisms adhere, as compared to traditional free microorganism reactors in which the biomass is suspended in a liquid medium. Methods of immobilization include entrapment of the biomass into the gel beads or a polymer matrix (alginate, carageenan or polyurethane) which provides a permeable membrane through which substrate and oxygen pass. The biomass can also attach itself to the surface of inert supports, including diatomaceous earth, silica beads, and polymeric membranes.

The primary advantage of immobilizing microbes is that they become more resistant to higher toxic chemical concentrations. Immobilization also provides easy recovery and reuse of biomass and there are no washout problems which occur with free microbes. Biomass attached to the surface of a support provides reduction of external mass transfer resistance and increases degradation rates. Immobilization also eliminates the problem of rising and bulking sludge [17].
1.3 Advantages of Spiral Biosupport

Immobilization on a polymeric membrane surface allows the bio-support to be designed in a more efficient spiral configuration. This design restricts the flow to the inner spacing of the membrane and eliminates the channeling effect. The biomass directly contacts the water flow (as compared to gel beads) which provides less diffusional resistance. The spiral configuration provides high surface area and therefore more biomass per unit volume of the reactor. The configuration also provides a structural resistance to water pressure drop.

1.4 Batch Recirculating Reactor

The batch recirculating reactor is a closed system with no inflow or outflow of material. The liquid contents are completely mixed and pass through the biosupport until the reaction is complete. Batch reactors are generally more expensive than continuous systems because laboratory and operational costs are higher. However, batch systems provide more flexibility and control, and can be scheduled and monitored for reaction completion.

1.5 Oxygen Measurements and Quantitation of the Bioreactor

Biological Oxygen Demand (BOD) is the amount of dissolved oxygen consumed by microbes while oxidizing contaminants in water. The Dissolved Oxygen (DO) concentration in water decreases while microorganisms degrade pollutants. Electrolytic respirometers monitor the oxygen concentration in water and indicate the amount of oxygen used by microbes. Analysis of continuous output from DO measurement during
degradation yields information concerning the nature of degradation. The total degradation rate is easily determined by analyzing DO measurement if oxygen consumption ceases at mineralization. It has been determined that different compounds produce different types of oxidative behavior and different types of DO patterns [1]. Therefore, certain qualitative conclusions of the substrate can be reached by analyzing the DO pattern of degradation. The DO pattern of the reactor provides a tool for measuring degradation rate and for distinguishing between types of compounds being degraded.

Further analysis of the DO output reveals the total amount of oxygen consumed by the microorganisms. However, due to the physical set up of the reactor, this is not an accurate reading. Oxygen is replenished into the reactor solution while the solution flows down the outer surface of the catalyst and also by constant air bubbling. Therefore, the DO measurements indicate a relative oxygen consumption specific to the reactor configuration. Mathematical analysis of the relative oxygen concentration throughout the reaction offers the possibility of quantizing the bioreactor. The DO output can be used to determine the amount of substrate oxidized.
CHAPTER 2

LITERATURE SURVEY

Lakwala [2] has performed a study which compares two reactors with different types of immobilization for degrading phenol. One reactor utilized calcium alginate gel entrapment of bacteria, and the other utilized polymeric membrane attachment. He concluded that the gel bead method of immobilization is more efficient at high concentrations of phenol, while the polymeric membrane method of immobilization is more efficient at lower concentrations (<150 ppm). Lakwala stated that substrate inhibition occurred at lower concentrations of phenol with the polymeric membrane attachment because the substrate is in direct contact with the microorganisms. Lakwala also concluded that polymeric membrane sheets offer an additional advantage over gel bead entrapment. The entrapped biomass in the gel beads can grow and break the beads. Polymeric membrane sheets provide an environment where the biomass growth is regulated by the polymer area and sloughing.

P. J. Allsop et al. [11] stated that the most probable site of substrate inhibitory action of phenol degradation by *Pseudomonas putida* occurs at phenol permease or phenol hydroxylase. Allsop has also shown an increase of Dissolved Organic Carbon in the reactor solution after the addition of phenol. He concluded that this increase was due to 1) loss of cell membrane integrity, 2) excretion of extracellular anabolites, or 3) production of intermediate metabolites. According to Tang [4], the introduction of phenol to an immobilized cell reactor produces extracellular phenol oxidase in the reactor solution. Tang also indicated that the optimal conditions of the activated sludge phenol oxidase are: phenol concentration of 100 ppm; pH = 6.0; temperature = 37 degrees Celsius. This enzyme was also shown to oxidize 2-chlorophenol, styrene and methylene chloride.
Broholm et al. [6] has demonstrated the effects of biodegradation by different mixed cultures. Mixed cultures of bacteria obtained from different water work sources have shown varying abilities to degrade contaminants. The abilities of eight mixed cultures of methane oxidizing bacteria to degrade TCE were compared. Three of the eight cultures indicated the ability to degrade TCE in the thirty day experimental time, while the other cultures showed little or no ability to degrade TCE.

It has been demonstrated by Buitron [14] that the characteristics of a mixed culture change after repeated additions of phenol, and according to Jung [1], in an immobilized gel bead reactor, the reaction rates increase as the number of phenol additions increase. In relation, Naik [20] has shown the importance of acclimation to reduce lag time and increase degradation rate in a suspended microorganism batch reactor.

Gonnabathula [19] utilized a suspended microorganism batch reactor to analyze the effects of multiple substrates on the degradation of phenol. Multiple substrates were introduced to the bioreactor simultaneously. It was found that 2,6-dichlorophenol or nitrobenzene inhibited phenol reduction, and nitrobenzene degradation was enhanced in the presence of phenol.

M. G. Roig et al. [10] has stated that the greatest problem regarding biological treatability of wastewater is when heavy metals in wastestreams delay or abolish biological degradation. Rus [5] has demonstrated the biosorbent ability of gel entrapped immobilized microorganisms to remove heavy metals (lead, chromium, cadmium and copper) from wastestreams. Rus indicated that until metabolic activity is inhibited, the biomass can adsorb the heavy metals, while continuing to degrade organic contaminants. Rus has also indicated that proper adjustment of biosorption conditions (pH), allows specific metal ions to be selectively removed from the wastestream.

Chien [13] has shown the effects of flow rate on the degradative capabilities of immobilized yeast reactors. As flow rate increased, the productivity also increased due to
the improved mass transfer properties. Chien indicated that a maximum reaction rate was achieved despite further increases in flow rate.

Shim [3] has demonstrated the use of a spirally wound polymeric membrane immobilized bioreactor to degrade a model VOC, ethanol, in air. A mixed culture was attached to the biocatalyst and air mixed with ethanol was fed through the batch reactor. Shim concluded that the bioreactor was capable of degrading 99 percent of ethanol within the 6 hour experimental time. The operating parameters flow rate and ethanol inlet concentration were examined and optimized. Shim determined that as air flow rate through the reactor increased, reaction rate increased. However, further increases in flow rate produced a decrease in reaction rate due to sloughing of biomass from the polymeric sheet. Shim also determined that reaction rate increased with ethanol concentration. After a maximum was reached, the increase in ethanol concentration decreased the reaction rate due to substrate inhibition.

Suschka et al. [7] has demonstrated the use of using a direct oxygen probe to monitor the oxygen consumption of microorganisms during substrate biooxidation. He determined that respirometric data of activated sludge can provide valuable information regarding the kinetics of biooxidation, including the biomass yield coefficient. Suschka demonstrated that the bacteria consume more after injection because of the increase in substrate concentration. The bioactivity can be monitored by the respirometric data.

Larson et al. [8] used respirometric data to measure biodegradation of surfactants at ppm levels. He showed at low substrate concentrations, an estimate of biodegradation could be obtained from respirometric data, and he concluded that in the absence of specific analytical methods, the respirometer can be used to measure biodegradation.

Jung [1] utilized an online direct oxygen probe to monitor the biodegradation of substrates by an immobilized gel reactor. At the point of injection of a substrate into the reactor, the oxygen consumption by the microbes increases and the oxygen concentration in the water decrease. The oxygen concentration in the water is analyzed over the course
of biodegradation. The oxygen concentration in the water returns to normal, or baseline, when biodegradation ceases. Jung showed that complete mineralization of phenol occurs on the basis of oxygen measurement. Jung has also shown that different substrates produce different oxygen patterns, thus indicating the use of respirometric data for qualitative purposes.
CHAPTER 3

OBJECTIVES

The primary objective of this work was to develop a feasible quantitation method to determine the relationship between oxygen consumption data and amount of substrate degraded in the bioreactor.

The specific objectives are:

1. To evaluate the capability of the immobilized cell bioreactor to degrade pollutants,
2. To demonstrate the effect of recirculation flow rate on degradation,
3. To demonstrate the effect of substrate (phenol) concentration on degradation rate and to determine an optimum operating range,
4. To derive a method of utilizing measured oxygen consumption data to qualitatively identify classes of compounds,
5. To derive a method of utilizing measure oxygen consumption data to quantitatively determine the amount of phenol injected, and
6. To evaluate the immobilized cell bioreactor's oxidative response to and rough quantitation of the pesticides Acephate, Carbaryl, Chlorpyrifos, and Diazinon.
CHAPTER 4

MATERIALS AND EXPERIMENTAL METHODS

4.1 Microorganisms and Immobilization
The 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. About 50 liters of sludge were then acclimated with 50 ml of oily industrial waste and 5 ml ethanol with constant air bubbling.

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

4.2 Polymeric Membrane for Bio-Oxidation
The organisms utilized during this experiment were immobilized on a spirally wound polymeric membrane catalyst. The membrane is hydrophilic and consists of 55% silica, 45% PVC and carbon (trace) [2]. 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 catalyst 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 biosupport volume is 11 liters.

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 spirally along the biosupport until it has reached the end. Temperature readings of the water indicate a range of 25 - 35 degrees Celsius.

4.3 Experimental Set Up

Figure 1 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 (57.49 liters), and the water volume is maintained at 42 liters. The pump recirculates the reservoir water into the top of the biosupport, and a small plastic container collects the output from the surface of the support. This container was added to facilitate flow rate and temperature measurements. Two bypass valves were added to the pump output to control flow rate and water pressure, and to aid in mixing the reservoir. A pressure gage measures the water pressure into the biosupport. A steady flow of constant air bubbling is provided in the reservoir. Biooxygenation also occurs while the output of the biosupport trickles down the outside of the cartridge.

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

Substrate injections were prepared by dissolving a weighed amount of substrate in a beaker filled with reservoir water. The beaker solution was then poured throughout the
Figure 1 Experimental Setup of the Immobilized Cell Batch Recirculating Bioreactor
reservoir. All experiments were done at room temperature inside a ventilating hood. Fixed nitrogen was provided from time to time by adding 10 ml of NH₄OH (29% W/W) in 42 liters of reservoir volume to stimulate bioactivity.

4.4 Dissolved Oxygen Measurements
Flow across the DO probe was provided by a small water pump. The inputs to the DO probe were located 1) in the reservoir (biocatalyst input DO concentration) and 2) in the plastic container which collected the catalyst output (biocatalyst output DO concentration). An oscillating control valve with a 10 minute timer switched the flow to the DO sensor periodically from input DO concentration to output DO concentration. On the chart recorder, the higher DO concentration indicates biocatalyst input DO concentration. The input and output measurements were constantly recorded on the chart recorder during a reaction.

For a typical substrate injection of phenol, first a steady state baseline was achieved, which indicates no reaction. Then, upon addition of substrate, the DO (input and output) decreased, indicating bio-oxidation. The developed DO pattern then returned to steady state baseline upon completion of biodegradation. It was shown by Lakwala [2] and Jung [1] that the completion of this pattern represents 100% mineralization of phenol. An example of chart recorder output for two injections of phenol is shown in Figure 5. The chart speed was set at 0.01 cm/min.

4.5 Substrate Characteristics
Phenol was chosen as the initial substrate to perform optimum operating parameters for degradation with the reactor. It has been shown that phenol degrades quickly and easily and is therefore suitable for determining the reactor characteristics [1,2].
Phenol, C₆H₅O, is a colorless acicular crystal or a white crystalline mass. It is obtained from coal tar, or by fusing sodium benzenesulfonate with sodium hydroxide. It can also be obtained by heating monochlorobenzene with aqueous sodium hydroxide under high pressure.

Phenol is reported as poisonous and caustic. Ingestion of small amounts cause nausea, vomiting, circulatory collapse, tachypnea, paralysis, convulsions, coma, green urine and possible death from respiratory failure. The average fatal dose is 15 grams but 1 gram deaths have been reported [16].

Phenol is primarily used as a disinfectant for toilets, floors, drains, etc. It is also used in the manufacture of colorless or light colored artificial resins. Therapeutic uses are as a topical anesthetic in aqueous solution, topical antiseptic and topical antipruritic.

Four easily obtainable, over the counter pesticides (Acephate, Carbaryl, Chlorpyrifos, and Diazinon) were obtained to determine the affect on the oxygen uptake pattern and therefore bio-oxidation.

Acephate, C₄H₁₀NO₃PS, also known as phosphorothioic acid O,O-diethyl O-(3,5,6-trichloro-2-pyrdinyl) ester is a white solid with solubility in water of 790,000 ppm at 20°C. The solution used in this experiment contains 8.0% Acephate. It is classified by NJDEP as a potential leacher, or a potential groundwater contaminant in New Jersey, but has not yet been detected in New Jersey groundwater. It is used on New Jersey vegetables and soybeans and its trade name is Orthene.

Carbaryl, C₁₂H₁₁NO₂ or 1-naphthyl N-methylcarbamate, is the active ingredient in Sevin (5% Carbaryl-used in this experiment), one of the most widely used insecticides on the market. NJDEP has reported that carbaryl is not a leacher and not a NJ ground water
threat. Other characteristics of carbaryl include its non-persistence due to fast microbial
degradation, and its non toxicity to mammals.

Chlorpyrifos, C9H11Cl3NO3P5, is also known as phosphorothioic acid O, O-diethyl
O-(3,5,6-trichloro-2 pyridinyl) ester. It is the active ingredient in Dursban and used for
mosquito, fly and other household pest control. It has been classified by NJDEP as not a
likely leacher and a small threat to NJ groundwater. In NJ it is used on field corn,
soybeans, vegetables and fruits. It is not soluble in water (0.4 ppm at 23 degrees Celsius).
A 0.5% Chlorpyrifos solution was used in this experiment.

Diazinon, C12H21N2O3, or phosphorothioic acid O,O diethyl O-[6-methyl-2-(1-
methylethyl)-4-pyrimidinyl] ester has a solubility in water of 40 ppm at 20 C. The
concentration of diazinon in the spray used in this experiment is 25%. It is recommended
by the manufacturer that this product be diluted at a rate of 1:384 for most domestic
applications. Diazinon is the active ingredient in Basudin and Sarolex pesticides. It is
normally used for the control or soil insects and household pests, and is used by NJ
farmers on field corn, vegetables, and fruit. DEPE reports that Diazinon is not a threat to
NJ groundwater because it is tightly absorbed by soil and degrades rapidly. It has been
reported that Diazinon degrades rapidly with parathion hydrolase, an enzyme produced by
a mixed culture of *pseudomonas sp.* [15,16].
RESULTS AND DISCUSSION

5.1 Effect of the Recirculation Flow Rate for Bio-Oxidation

Figure 7 shows the effect of recirculation flow rate through the biosupport on reaction rate for each injection made of phenol. The flow rate was controlled for each injection by adjusting the bypass valves on the pump outflow apparatus, and monitoring the change in water pressure through the biosupport. It was noted that generally the water pressure increased with flow rate but no specific relationship between flow and pressure was observed. At maximum pressure of 20 psi, the flow rate was measured at 1750 ml/min during the 1.0 gram phenol injections, and only 900 ml/min during the 0.5 gram injections.

Another variable, besides pressure, influenced the flow rate through the reactor support. It is speculated that the biomass configuration on the biosupport arranged itself to obstruct flow at times. Thus, adjusting the control valves and coordinating the pressure did not have a consistent effect on flow rate and therefore, reproducing specific flow rates was impossible.

It is normally expected that reaction rates initially increase with flow rate because of better mass transfer. Subsequently, rates reach a plateau when substrate and product transfer to the biosurface is no longer limiting. Rates may decrease at higher flows due to the stripping of biomass from the support surface, or as a result of substrate inhibition.

For each phenol amount injected, the reaction rates increase with flow. However, maximum reaction rate due to optimum flow is not clear from the data. The 2.5 gram
phenol injections indicate a reaction rate decrease above 800 ml/min, and for 1.5 gram injections reaction rates decrease above 800 ml/min also. This corresponds to the expectations outlined in the previous paragraph.

However, the 1.0 gram phenol injections appear to increase after 1400 ml/min, and the 0.5 gram and 5.0 gram injection data do not indicate an achieved maximum reaction rate. The flow rate for which biomass is stripped from the support is about 5,000 ml/min, and is therefore not observed in this figure. It can be concluded that reaction is independent of recirculation rate at these flow levels.

There are additional factors which influence to a small degree the isolated effect of flow rate on reaction rate. The reaction rate may be influenced by the amount of enzymes in the reservoir at the time of injection. It was observed that the color of the reservoir water was yellowish at times of some injections. This indicates the existence of enzymes in the reactor solution which would in effect degrade phenol while in the reservoir. Also, biomass which may have been stripped from the reactor support surface may enter the reactor solution and continue to degrade the substrate similar to a free microorganism reactor. Finally, biomass growth and residue buildup inside of the spiral configuration may block spacing passageways and, therefore, limit the flow of substrate containing solution.

5.2 Effect of Substrate (Phenol) Concentration for Bio-Oxidation

Figure 8 shows the relationship between the amount of phenol injected into the reactor solution and average biodegradation rate. The average biodegradation rate was calculated by averaging the reaction rates for each amount of phenol injected. As expected from the
literature, reaction rates increase from zero with substrate concentration, level off to a maximum and may decrease with additional substrate concentration due to substrate inhibition. The average biodegradation rate maximizes after 1.5 gram of phenol injected into the 42 liter reactor solution, thus indicating that the optimum operating range with respect to substrate concentration begins at 1.5 gram of phenol in 42 liters reactor solution (36 ppm).

Initially, rate increases with concentration and subsequently decreases when substrate inhibition possibly occurs. The entire operating range from 0 - 5.0 grams phenol in 42 liters (0 ppm - 119 ppm) is satisfactory. The optimum operating range may continue beyond the 5.0 gram injection amount.

5.3 Quantitation Method 1: Utilizing The Area Under The Dissolved Oxygen Curve

For each injection of phenol, a curve representing the DO output was drawn from the DO probe readout (see sample calculations for details). Quantitation Method 1 utilizes this area between the DO curve and baseline to represent the amount of oxygen consumption by the bacteria, and further to represent the amount of phenol injected. Figure 9 shows the DO curve areas for the 0.5 gram phenol injections. Theses curves are generated using the data obtained by the DO probe readout (Figures 5 and 6). The DO probe measures the concentration of oxygen in the reactor solution, and the deviation from baseline indicates an oxygen depletion. This drop in oxygen concentration is converted to grams of oxygen depleted which is assumed to be proportional to the rate of oxygen consumption by the microorganisms. Therefore, the area under the DO curve is assumed to be proportional to the amount of oxygen consumed by the microbes and can be
correlated with amount of phenol using the stoichiometric relationship of phenol oxidation. For example, 1 gram of phenol theoretically requires 2.408 grams of oxygen for complete oxidation.

Bacteria

\[ \text{C}_6\text{H}_6\text{O} + \frac{71}{2}\text{O}_2 \rightarrow 6\text{CO}_2 + 3\text{H}_2\text{O} \]

The average areas of each group of phenol injections (Figures 15-18) were plotted with their corresponding amount of theoretical oxygen consumed to obtain Figure 2. The best fit line was superimposed over the points, and the slope of the line was found to be 0.478 grams of oxygen consumed / oxidation area (min*g). This number, K1, represents the conversion factor between area under the DO curve and the amount of oxygen consumed by the bioreactor. For example, a DO curve with area of 2 min*g would correspond to 0.956 grams of oxygen consumed and a predicted phenol amount of 0.379 grams. Tables 1 to 6 show the results of Quantitation Method 1, 2 and 3 with averages and standard deviations.

5.4 Quantitation Method 2: Area Under DO Curve with Baseline CPP Correction

A second method of utilizing the DO output to represent the amount of phenol degraded was investigated utilizing baseline Conversion Per Pass (CPP). It was noticed during experimentation that the DO probe readout for similar injections varied when the baseline (DO probe readout before injection) thickness varied. The points used in obtaining the DO curve from Method 1 were divided by the thickness of the baseline DO output. Figure 10 shows the resulting DO curves with the baseline offset. As in Method 1, the altered data were plotted for each injection and the areas and standard deviations were
Slope, $K_1 = 0.478$ grams Oxygen Consumed / Oxidation Area (min*g)

Figure 2 Quantitation Method 1: DO Readout Area vs. Theoretical Oxygen Consumed. The slope of the best fit line is conversion constant $K_1$, which relates the area measured from DO readout to theoretical oxygen consumed.
calculated for each group of phenol injection (Figures 19-22 and Tables 1-6). The average areas were then plotted with corresponding amount of theoretical oxygen consumed and the best fit line was superimposed (Figure 3). The slope of this line, K2, represents the conversion factor for Quantitation Method 2. K2 was determined to be 0.450 grams oxygen consumed / oxidation area with baseline CPP offset. It can be easily determined from Figure 3 that this method does not produce an accurate correlation between DO area and oxygen consumed.

5.5 Quantitation Method 3: Area Under DO Curve with Initial Slope Correction

It was noticed during experimentation that some injections of phenol produced an immediate decrease in DO readings while other injections decreased at a lower DO rate, most likely due to the vitality of the microorganisms at the time of injection. The area curves from Method 1 were plotted together with the 1.0 gram phenol injection curve to determine the relative difference in initial slope. Figure 11 compares multiple area curves for both 0.5 and 1.0 grams of phenol. The two 0.5 gram areas with lower slopes (flow rates: 560 ml/min and 760 ml/min) were multiplied by an arbitrary number of 1.3. It was similarly found that two 2.5 gram injections (flow rates: 380 ml/min and 1090 ml/min) had a lower initial slope, and their areas were also multiplied by 1.3. All 5.0 gram phenol injection areas (with the exception of flow rate 1200 ml/min) were likewise multiplied by 1.3 because of their lower initial slope as compared to 1.0 gram phenol slopes. Higher initial slopes as compared to the 1.0 gram curves are multiplied by a correction of 0.8, but no slopes in this category were found. DO areas with slopes which do not deviate beyond the 1.0 gram range are not corrected (multiplied by 1.0).
Figure 3 Quantitation Method 2: DO Readout Area with Baseline Conversion Per Pass Offset vs. Theoretical Oxygen Consumed. The slope of the best fit line is conversion constant K2, which relates the adjusted area from DO readout to oxygen consumed.
The new average areas were then calculated and plotted with their corresponding oxygen amount to obtain Figure 4. The error distance (deviation from the best fit line) due to this method is reduced. However, because of the arbitrary and complicated nature of this method, it is not recommended for \((\text{area})/(\text{grams of oxygen consumed})\) correlation. The slope of the line, \(K3\), represents the conversion factor of Quantitation Method 3. \(K3\) was found to be 0.407 grams of oxygen consumed / DO area with initial slope offset (min\(*\)g).

### 5.6 Pesticide Injections

Figure 12 shows the resulting DO curves for three successive injections of the pesticide Acephate. It can be determined that the introduction of Acephate produces bio-oxidative activity among the microorganisms in the bioreactor. However, it cannot be concluded that total mineralization of Acephate is completed because analytical methods for substrate disappearance were not within the scope of this work. Because several distinct peaks appear on the original DO output (and can be seen in the Figure 13 DO curve), the microorganisms seem to respond by changing DO consumption in stages, indicating the possible appearance of various by-products of Acephate degradation.

Figure 13 shows the resulting DO curve of one injection of the pesticide Diazinon. Again, the curve indicates bio-oxidative activity of the microorganisms, and the various peaks may indicate the oxidative response of Diazinon by-products. However, no conclusive determination can be made concerning the complete mineralization of Diazinon. Further, it was noted during the Diazinon injection that a milky white substance formed in the reservoir and persisted well beyond the DO readout return to baseline. This
Figure 4 Quantitation Method 3: DO Readout Area with Initial Slope Offset vs. Theoretical Oxygen Consumed. The slope of the best fit line is conversion constant K3, which relates the adjusted DO readout with oxygen consumed.
may indicate a developed by-product of Diazinon biodegradation that cannot be oxidized by these microorganisms. Because of this occurrence, the reactor water was replaced and no further injections of Diazinon were made.

Figure 14 shows the resulting DO curves for three injections of the pesticide Chlorpyrifos. The curves indicate that the presence of Chlorpyrifos stimulates bio-oxidative activity. As before, it can not be conclusively determined that complete mineralization of Chlorpyrifos was achieved. However, disappearance of odor and return of DO baseline indicate possible mineralization.

Two injections of the pesticide Carbaryl were performed on the bioreactor. 5 grams and 20 grams of Sevin 5.0% solid mixture Carbaryl (0.25 grams Carbaryl and 1.0 gram Carbaryl, respectively) were dissolved in a 1 liter beaker of water and injected into the bioreactor. Both resulting DO curves remained at baseline with no deviation, and the reservoir water remained murky until replaced. The presence of Carbaryl did not stimulate any measurable bio-oxidative activity of the microorganisms. This is ironic because Carbaryl has been reported to be very biodegradable [15].

5.7 Qualitative Determinations from DO Probe Readout

Figure 6 shows the DO probe readout for three sample injections of phenol: a 5 gram injection and two 0.5 gram injections. The 5 gram phenol injection DO measurements curve down after injection, then appear to level off and finally return sharply to baseline. After the 0.5 gram phenol injection, DO measurements are quickly reduced at injection and then gradually return to baseline. By noting the characteristics of these phenol injections, no conclusive correlation can be made to qualitatively identify the substrate by
analyzing the DO readout. However, it can safely be concluded that a simple organic compound is being degraded because of the simplicity of the peak. The DO oxidative pattern produced by Acephate (Figure 12) indicates that a more complex compound, or a multitude of compounds is being oxidized, because of the various DO peaks throughout the reaction.

5.8 Quantitation Method Summary

The following tables compare the results of each quantitation method. For each method, the DO areas were obtained from the DO curves for each reaction, and each area was multiplied by the appropriate K conversion factor to obtain the measured oxygen (K1=0.478, K2=0.450, K3=0.407). This amount of oxygen was then divided by 2.408 g O₂/g phenol to give the predicted or measured amount of phenol. The percent errors of phenol degradation prediction are compared in Table 6. The percentage of standard deviation related to average predicted phenol are shown for each injection amount, and the average of these percentages is used to indicate the predictability error % for the three quantitation methods.
### Table 1 Quantitation Method Comparison for 0.5 Gram Phenol Injections
(Theoretical Oxygen Consumption = 1.204 grams)

<table>
<thead>
<tr>
<th>Flow (ml/min)</th>
<th>METHOD 1</th>
<th>METHOD 2</th>
<th>METHOD 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DO Area min* g</td>
<td>Measured Oxy,. g</td>
<td>Measured Phenol, g</td>
</tr>
<tr>
<td>360</td>
<td>3.71</td>
<td>1.77</td>
<td>0.735</td>
</tr>
<tr>
<td>480</td>
<td>2.78</td>
<td>1.33</td>
<td>0.552</td>
</tr>
<tr>
<td>560</td>
<td>2.89</td>
<td>1.38</td>
<td>0.573</td>
</tr>
<tr>
<td>760</td>
<td>2.84</td>
<td>1.36</td>
<td>0.565</td>
</tr>
<tr>
<td>900</td>
<td>2.37</td>
<td>1.13</td>
<td>0.469</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>2.92</td>
<td>1.40</td>
<td>0.581</td>
</tr>
<tr>
<td><strong>Std. Dev.</strong></td>
<td>0.436</td>
<td>0.208</td>
<td>0.0865</td>
</tr>
</tbody>
</table>

### Table 2 Quantitation Method Comparison for 1.0 Gram Phenol Injections
(Theoretical Oxygen Consumption = 2.408 grams)

<table>
<thead>
<tr>
<th>Flow (ml/min)</th>
<th>METHOD 1</th>
<th>METHOD 2</th>
<th>METHOD 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DO Area min* g</td>
<td>Measured Oxy,. g</td>
<td>Measured Phenol, g</td>
</tr>
<tr>
<td>460</td>
<td>10.6</td>
<td>5.07</td>
<td>2.11</td>
</tr>
<tr>
<td>540</td>
<td>8.21</td>
<td>3.92</td>
<td>1.63</td>
</tr>
<tr>
<td>1220</td>
<td>7.05</td>
<td>3.37</td>
<td>1.40</td>
</tr>
<tr>
<td>1430</td>
<td>7.08</td>
<td>3.38</td>
<td>1.40</td>
</tr>
<tr>
<td>1500</td>
<td>6.13</td>
<td>2.93</td>
<td>1.22</td>
</tr>
<tr>
<td>1860</td>
<td>7.19</td>
<td>3.44</td>
<td>1.43</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>7.70</td>
<td>3.68</td>
<td>1.53</td>
</tr>
<tr>
<td><strong>Std. Dev.</strong></td>
<td>1.43</td>
<td>0.68</td>
<td>0.282</td>
</tr>
</tbody>
</table>
### Table 3: Quantitation Method Comparison for 1.5 Gram Phenol Injections
(Theoretical Oxygen Consumption = 3.612 grams)

<table>
<thead>
<tr>
<th>Flow (ml/min)</th>
<th>METHOD 1</th>
<th>METHOD 2</th>
<th>METHOD 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DO Area min(^\text{g})</td>
<td>Measured Oxy. (^\text{g})</td>
<td>Measured Phenol. (^\text{g})</td>
</tr>
<tr>
<td>650</td>
<td>8.09</td>
<td>3.87</td>
<td>1.61</td>
</tr>
<tr>
<td>820</td>
<td>4.38</td>
<td>2.09</td>
<td>0.868</td>
</tr>
<tr>
<td>1050</td>
<td>6.74</td>
<td>3.22</td>
<td>1.34</td>
</tr>
<tr>
<td>1120</td>
<td>8.18</td>
<td>3.91</td>
<td>1.62</td>
</tr>
<tr>
<td>1200</td>
<td>6.77</td>
<td>3.24</td>
<td>1.35</td>
</tr>
<tr>
<td>Average</td>
<td>6.83</td>
<td>3.26</td>
<td>1.35</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>1.37</td>
<td>0.655</td>
<td>0.272</td>
</tr>
</tbody>
</table>

### Table 4: Quantitation Method Comparison for 2.5 Grams Phenol Injections
(Theoretical Oxygen Consumption = 6.02 grams)

<table>
<thead>
<tr>
<th>Flow (ml/min)</th>
<th>METHOD 1</th>
<th>METHOD 2</th>
<th>METHOD 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DO Area min(^\text{g})</td>
<td>Measured Oxy. (^\text{g})</td>
<td>Measured Phenol. (^\text{g})</td>
</tr>
<tr>
<td>380</td>
<td>7.67</td>
<td>3.67</td>
<td>1.52</td>
</tr>
<tr>
<td>400</td>
<td>15.0</td>
<td>7.17</td>
<td>2.98</td>
</tr>
<tr>
<td>900</td>
<td>25.1</td>
<td>12.0</td>
<td>4.98</td>
</tr>
<tr>
<td>1090</td>
<td>12.8</td>
<td>6.12</td>
<td>2.54</td>
</tr>
<tr>
<td>Average</td>
<td>15.1</td>
<td>7.22</td>
<td>3.00</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>6.35</td>
<td>3.04</td>
<td>1.26</td>
</tr>
</tbody>
</table>
Table 5 Quantitation Method Comparison for 5.0 Gram Phenol Injections  
(Theoretical Oxygen Consumption = 12.04 grams)

<table>
<thead>
<tr>
<th>Flow (ml/min)</th>
<th>METHOD 1</th>
<th>METHOD 2</th>
<th>METHOD 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DO Area</td>
<td>Measured</td>
<td>DO Area</td>
</tr>
<tr>
<td></td>
<td>min*g g</td>
<td>Oxy., g</td>
<td>min*g/cm</td>
</tr>
<tr>
<td>527</td>
<td>20.9</td>
<td>9.99</td>
<td>4.15</td>
</tr>
<tr>
<td>660</td>
<td>20.7</td>
<td>9.89</td>
<td>4.11</td>
</tr>
<tr>
<td>660</td>
<td>26.0</td>
<td>12.4</td>
<td>5.15</td>
</tr>
<tr>
<td>700</td>
<td>25.1</td>
<td>12.0</td>
<td>4.98</td>
</tr>
<tr>
<td>800</td>
<td>24.4</td>
<td>11.7</td>
<td>4.86</td>
</tr>
<tr>
<td>1200</td>
<td>19.9</td>
<td>9.51</td>
<td>3.95</td>
</tr>
</tbody>
</table>

Average: 22.8 | 10.9 | 4.53 |
Std. Dev: 2.43 | 1.16 | 0.481 |

Table 6 Summary of Measured Phenol for Each Quantitation Method

<table>
<thead>
<tr>
<th>Amount of Phenol Degraded (g)</th>
<th>Quantitation Method 1</th>
<th>Quantitation Method 2</th>
<th>Quantitation Method 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured Amount of Phenol (g)</td>
<td>Standard Deviation % of Average</td>
<td>Measured Amount of Phenol (g)</td>
</tr>
<tr>
<td>0.5</td>
<td>0.581</td>
<td>14.9</td>
<td>0.465</td>
</tr>
<tr>
<td>1.0</td>
<td>1.53</td>
<td>18.4</td>
<td>1.05</td>
</tr>
<tr>
<td>1.5</td>
<td>1.35</td>
<td>20.1</td>
<td>0.681</td>
</tr>
<tr>
<td>2.5</td>
<td>3.00</td>
<td>42.0</td>
<td>3.80</td>
</tr>
<tr>
<td>5.0</td>
<td>4.53</td>
<td>10.6</td>
<td>3.91</td>
</tr>
</tbody>
</table>

Average Standard Deviation % Error: 21.2 | 25.4 | 21.1

5.9 Using Quantitation Method 1 on Pesticide Injections

Quantitation Method 1 was used to analyze the DO curves generated for the three pesticides: Acephate, Diazinon, and Chlorpyrifos. Tables 7-9 show the results of this analysis. The amount of O2 utilized by the microorganisms was predicted by multiplying the DO area (min*g) by K1 (0.478 g O2/min*g). Then the predicted amount of pesticide was found by the stoichiometrical relationship to oxidation (see appendix for sample
calculations). It was found that the method satisfactorily predicted the amount of Acephate injected into the reactor solution (5.0 % - 37.2 % error). Quantitation Method 1 also satisfactorily predicted the amount of Chlorpyrifos injected into the reactor solution (8.5 % - 20.5 % error). However, the error of prediction for Diazinon was approximately 72 %. It had been observed during the Diazinon run that a white color appeared in the reservoir solution indicating that total mineralization did not occur. The large error between the amount injected and amount predicted by Quantitation Method 1 supports this observation.

Table 7 Application of Quantitation Method 1 to Acephate DO Curves

<table>
<thead>
<tr>
<th>Amount Injected of Active Ingredient, g</th>
<th>DO Curve Area min*g</th>
<th>Measured O2, g (X K1)</th>
<th>Predicted Amount Degraded</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.28</td>
<td>2.648</td>
<td>1.266</td>
<td>0.804</td>
<td>37.2</td>
</tr>
<tr>
<td>2.56</td>
<td>8.853</td>
<td>4.232</td>
<td>2.689</td>
<td>5.0</td>
</tr>
<tr>
<td>3.84</td>
<td>11.287</td>
<td>5.395</td>
<td>3.428</td>
<td>10.7</td>
</tr>
</tbody>
</table>

Table 8 Application of Quantitation Method 1 to Diazinon DO Curve

<table>
<thead>
<tr>
<th>Amount Injected of Active Ingredient, g</th>
<th>DO Curve Area min*g</th>
<th>Measured O2, g (X K1)</th>
<th>Predicted Amount Degraded</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.835</td>
<td>6.581</td>
<td>3.146</td>
<td>1.335</td>
<td>72.4</td>
</tr>
</tbody>
</table>

Table 9 Application of Quantitation Method 1 to Chlorpyrifos DO Curves

<table>
<thead>
<tr>
<th>Amount Injected of Active Ingredient, g</th>
<th>DO Curve Area min*g</th>
<th>Measured O2, g (X K1)</th>
<th>Predicted Amount Degraded</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0967</td>
<td>0.300</td>
<td>0.143</td>
<td>0.105</td>
<td>8.5</td>
</tr>
<tr>
<td>1.547</td>
<td>2.378</td>
<td>1.370</td>
<td>1.01</td>
<td>11.4</td>
</tr>
<tr>
<td>1.818</td>
<td>6.216</td>
<td>2.971</td>
<td>2.191</td>
<td>20.5</td>
</tr>
</tbody>
</table>
CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

This study has demonstrated the ability of the spirally wound immobilized bioreactor to oxidize a model compound, phenol, and has demonstrated the ability of utilizing dissolved oxygen data over the course of degradation as a useful source of quantitative information regarding the substrate.

The biodegradation reaction rate was determined to be independent of recirculation flow rate through the biosupport.

The optimum amount of phenol injected into the 42 liter reactor solution was determined to range from 1.5 grams to 5.0 grams (36 ppm - 119 ppm) and produced an average degradation rate of 0.006 g/min or 0.36 g/hr.

It was determined that the area produced under the dissolved oxygen curve can be used to determine a satisfactory approximation of the amount of oxygen consumed and the amount of phenol injected into the reactor. The mathematical correlation between area under the DO curve and amount of oxygen consumed was determined to be 0.478 grams of oxygen / DO curve area (min*g), using the recommended (because of simplicity) Quantitation Method 1, which utilizes no correction values applied to the DO readout. The error of prediction for this method was found to be 21.2%. Quantitation Method 3 was found to be most accurate, but requires more computation time.

This experimentation also demonstrated that bio-oxidative activity of the microorganisms is produced by the introduction of pesticides Acephate, Chlorpyrifos, and
Diazinon into the bioreactor. Diazinon was shown to produce an undegradable milky white substance in the reservoir solution, and it can be assumed that complete mineralization did not occur. The pesticide Carbaryl stimulated no bio-oxidative activity when introduced into the reservoir solution.

The three pesticides which produced an increased use of oxygen were analyzed using Quantitation Method 1 to measure the amount of substrate injected into the reservoir. It was determined that Acephate and Chlorpyrifos produced relatively accurate measurements of the substrate. Diazinon did not produce accurate results. This supports the observation that complete mineralization of Diazinon did not occur.

This study has also determined that qualitative determinations from the dissolved oxygen patterns cannot be made. However, certain types of compounds are expected to produce patterns based on their complexity.

Further studies are recommended to broaden the observed optimum operating parameters. A pump which can produce higher and easily controlled flow through the reactor should be used to determine the effects of higher recirculation flow rates on bio-oxidation. Also, analytical equipment should be used to verify degradation of the pesticides. The degradation of the pesticides should be further analyzed to determine if by-products are produced and if the dissolved oxygen pattern corresponds to the development of these by-products.
APPENDIX

Sample Calculations

1. The following is a description of the calculations involving the analysis of the dissolved oxygen readout. Figure 5 shows the DO probe readout from two sample phenol injections. This sample calculation will concern itself with the first injection (5.0 grams - 660 ml/min).

The first step in analyzing the DO pattern is determining the baseline before and after injection, or where the reaction begins and ends. The DO pattern deviates from baseline and returns when the reaction is complete. At the beginning and ending baseline, the center of the conversion per pass (CPP) is determined. The CPP is the thickness produced by the oscillating DO measurements of input and output of the biosupport. A line is then drawn connecting the baseline CPP centers, which represents the course of DO measurements if no injection had been made.

The reaction length (time) is divided into a sufficient amount of equal distances. At each division, the length of the reaction (representing time) is recorded. For each division, the center of the CPP is determined, and its distance from the baseline is measured (CPP Midpoint Distance). The CPP Midpoint Distances at the beginning and end of the reaction will be zero. The DO curve utilized in this experimentation is obtained by plotting the measured CPP Midpoint Distances vs. Reaction Time. This DO curve represents the DO pattern for analytical purposes. The areas under each DO curve were
obtained by weighing the curves and converting them from grams to min\*g using the weight of a known area.

Table 10 contains the data obtained from the 5.0 gram injection at 660 ml/min as shown in Figure 5.

**Table 10** Example Phenol Data for DO Curve Calculations: 5 Grams Phenol - 660 ml/min

<table>
<thead>
<tr>
<th>Point #</th>
<th>Reaction Length, mm</th>
<th>CPP Midpoint Distance, cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.55</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>0.80</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>0.95</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>1.30</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

2. The following is a description of the calculations involved to determine the amount of oxygen needed to fully oxidize Acephate. A similar method was used on the other pesticides. The chemical formula of Acephate is C₄H₁₀NO₃P₅. It was assumed that the constituents are oxidized as follows:

\[
\begin{align*}
C_4 + 4O_2 & \rightarrow 4CO_2 & 4 \text{ moles } O_2 \text{ used} \\
H_{10} + 5/2 \; O_2 & \rightarrow 5H_20 & 5/2 \text{ moles } O_2 \text{ used} \\
NO_3 & \rightarrow NO_2 + 1/2 \; O_2 & 1/2 \text{ moles } O_2 \text{ liberated} \\
P + 3/2 \; O_2 & \rightarrow PO_3 & 3/2 \text{ moles } O_2 \text{ used} \\
S + 3/2 \; O_2 & \rightarrow SO_3 & 3/2 \text{ moles } O_2 \text{ used}
\end{align*}
\]

A total of 9 moles of O₂ is required to oxidize 1 mole of Acephate. This corresponds to 288 grams of oxygen required to oxidize 183 grams of Acephate.
Figure 5 Dissolved Oxygen Probe Readout Sample. (A) is a 5 gram phenol injection and (B) is a 2.5 gram phenol injection.
Figure 6  Dissolved Oxygen Probe Readout Sample.  (A) is a 5 gram phenol injection and (B) and (C) are 0.5 gram phenol injections.  Phenol produces two distinct DO curves.
Figure 7 Recirculation Flow Rate Through The Biosupport vs. Reaction Rate for All Phenol Injections. These scattered data indicate that reaction rate is independent of recirculation rate.
Figure 8: Average Reaction Rates for each Injection Amount in 42 Liters of Reactor Solution.

Initial degradation rates appear to be mass transfer limited and optimum rates (after 1.5 g - 36 ppm) indicate possible substrate inhibition.
0.5 Gram Phenol Dissolved Oxygen Areas. The oxygen depletion is measured from the DO readout. The areas between the baseline and each curve are used in Quantitation Method 1 by relating them proportionally to oxygen consumed by the microorganisms.
Figure 10 0.5 Gram Phenol Dissolved Oxygen Areas for Quantitation Method 2. The oxygen depletion measured from the DO readout was divided by the baseline Conversion Per Pass (CPP) for each reaction. The resulting areas under each curve were then related proportionally to oxygen consumed by the microorganisms.
Figure 11  Quantitation Method 3: Slope Comparison to 1 Gram Phenol DO Curves.
The 0.5 gram phenol DO areas with lower initial slopes were multiplied
by a correction factor of 1.3.
Figure 12 Acephate Pesticide Injections - The rapid depletion of oxygen suggests that biocatalytic activity is stimulated by the successive (16 g, 32 g, 48 g) introduction of Acephate into the reactor solution. Each oxygen depletion peak indicates bio-oxidative response.
Figure 13  Diazinon Injections. Oxygen depletion indicates that bio-oxidative activity is stimulated by the introduction of Diazinon into the reactor solution. The formation of a persistent white color in the reactor solution may indicate that mineralization of Diazinon did not totally occur.
Figure 14 Chlorpyrifos Injections. Oxygen depletion indicates bio-oxidative activity.
Figure 15: 1.0 Gram Phenol Dissolved Oxygen Areas for Quantitation Method 1.
Figure 16 1.5 Gram Phenol Dissolved Oxygen Areas for Quantitation Method 1.
Figure 17 2.5 Gram Phenol Dissolved Oxygen Areas for Quantitation Method 1.
Figure 18 5 Gram Phenol Dissolved Oxygen Areas for Quantitation Method 1.
Figure 19: One Gram Phenol Dissolved Oxygen Areas for Quantitation Method 2.
Figure 20 1.5 Gram Phenol Dissolved Oxygen Areas for Quantitation Method 2.
Figure 21 2.5 Gram Phenol Dissolved Oxygen Areas for Quantitation Method 2.
Figure 22 5 Gram Phenol Dissolved Oxygen Areas for Quantitation Method 2.
BIBLIOGRAPHY


