ABSTRACT

ANALYSIS OF THE OXIDATION OF ISOTOX® BY IMMOBILIZED BACTERIA

by
Chad Sheng

The purpose of this work is to show the effects of bio-oxidation of a pesticide through a spiral wound bioreactor using immobilized mixed culture bacteria. The pesticide used for this study is Isotox® and its active ingredients are acephate or O,S-dimethyl acetylphosphoramidothioate and vendex or hexakis (2-methyl-2-phenylpropyl)-distannoxane. Qualitative analysis of the product indicated presence of sulfate.

A consistent and unique DO pattern for Isotox® showed at least 4 minima, whereas phenol has only one minimum. The consistency of the DO pattern for Isotox® has been observed for over 30 injections into the bioreactor system. For both an acclimated and an unacclimated system, the basic DO pattern was consistent.

The analysis of bio-oxidized reservoir water showed concentrations in the ranges of parts per billion (ppb). About 90% of the vendex injected was adsorbed by the bacteria as indicated by tin analysis of the product water. A single batch of 16.4784 g dry biomass immobilized on a spiral bioreactor was able to effectively remove 2338 ml of the pesticide containing over 1000 ppm tin. Adsorption is on the order of 0.086 g tin per gram dry bacteria.

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by
Chad Sheng

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This thesis is dedicated to
my Lord, Jesus Christ

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CHAPTER 1

INTRODUCTION

1.1 Wastewater Treatment

Conventional wastewater treatment consists of primary and secondary treatment. At times a tertiary treatment may be employed to polish the effluent. Primary treatment removes the most easily removed waste from wastewater. Primary treatment methods include: screening, skimming the water surface of light density wastes such as oil, sedimentation, and neutralization. Secondary treatment is a biological process which removes the suspended solids in wastewater. An aeration basin and free cell microbes are usually used during this process. The microbes mineralize organics and also lower the BOD in the system. From the secondary treatment process, sludge will form and settle. A secondary clarifier can separate the sludge from the wastewater. Tertiary treatment may be used to further treat the wastewater, by phosphorous and nitrate removal, filtration, centrifugation, photolysis, etc...

Wastewater treatment plants, like other chemical plants, have an optimal design performance at a given concentration of a given pollutant. It is unlikely for a wastewater plant to handle all concentrations of all pollutants and release effluents within legal limits. Wastewater plants are, therefore, designed to handle certain pollutants within a certain concentration level. Influents with pollutant concentrations exceeding these limits will not be properly treated. This results in effluent concentrations which exceed the designed effluent limit, and probably the legal requirement.

Conventionally, pre-treatment processes are settling basins designed to remove most of the coarse materials prior to entry to a treatment plant. The pre-treatment plant can be located on the discharger's plant site. Cases where the effluent from an industry into a wastewater treatment contains effluent concentrations exceeding allowable influent into the treatment plant demand more complicated methods of pre-treatment than settling. Biological treatment can be used to reduce the effluent concentration. Since biological methods of removal are already used in the secondary treatment process, a pre-treatment process involving biological means can only improve the overall efficiency of treatment.

1.2 Advantages of Immobilized Microorganisms

Immobilization is a process in which free cell microorganisms attach onto a support. Although use of free cell bacteria has been the conventional method of bio-oxidation, immobilized bacteria have several advantages over the free cell bacteria system. In immobilized systems, bacteria are not washed out with the effluent stream, but adhere onto the support. There are two obvious benefits from this. First, less replenishment of new bacteria will be needed. Second, it eliminates the need to separate the free cell bacteria from the effluent stream. Immobilized systems also provide ease of operation and maintenance. Since the concentration of bacteria is relatively constant, the immobilized system can be cleaned and re-started rapidly with less downtime. The immobilized system can be treated essentially as a part of a unit in a process. It has been published by Gourdon, Rus, Bhende, and Sofer [10] that immobilized systems are also more resistant to

heavy metal poisoning than free cell systems. This implies a longer lifetime of microbe usage in immobilized systems.

1.3 Advantages of Spiral Polymeric Sheet Support

There are various methods of immobilizing microorganisms. The most popular methods involve immobilization within calcium alginate beads and immobilization onto polymeric sheet or other inert substances such as diatomaceous earth. Calcium alginate beads contain bacteria that are entrapped within the beads. Bacteria can also be immobilized onto polymeric sheet. The surface of the polymeric sheet is ribbed. The polymeric sheet is wound at a constant spacing gap between the adjacent layers. Fluid flows down through the center, from the origin of winding, and flows through the gap, in a spiral fashion out of the support column. Bacteria are immobilized on the surface of the sheet.

Advantages of using polymeric sheet include greater mass transfer rates, increased oxygen availability and longer lifetime of support material. For bacteria entrapped within the bead, the gel decreases the mass transfer rate of the substrate to the bacteria. Using polymeric sheet as support, the bacteria are adsorbed onto the surface of the polymer, where there is direct contact between the substrate and bacteria as opposed to beads where the substrate must diffuse through the bead. By the same reasoning, oxygen availability can also be greater with polymeric sheet than with free cells. A problem with entrapment of bacteria within the bead is the growth of bacteria. As substrates are consumed by the bacteria, bacterial growth will start. This will cause the alginate bead to break apart due to bacterial growth and result in a shorter bead lifetime. This problem

does not occur within the polymeric sheet since the bacteria are on the surface of the membrane. As bacterial growth starts, bacterial build-up will be away from the polymeric surface. The height of bacterial growth on the surface will be limited by the flow of the fluid. As the height exceeds a certain limit, it will be washed off by the fluid. Thus, performance of the polymeric sheet will be predictable.

1.4 Dissolved Oxygen Patterns

Dissolved oxygen (DO) measurements are monitored with respect to a baseline. The baseline is the steady state DO in the fluid, with no substrates in the reservoir water. As substrates are added into the reservoir water, oxygen is used by the bacteria to consume the substrates. As a result, DO concentration in the reservoir water will decrease. Upon completion of biodegradation, the DO level will return to the baseline level. Jung [1] has shown that complete mineralization of phenol is achieved when the baseline is reached and has stabilized.

A typical dissolved oxygen curve from a spiral wound polymeric sheet bioreactor for phenol is a single "dip." The results of phenol bio-oxidation have been quantified by Woods [7] and Borkowski [6]. Woods and Borkowski show a repetitive DO pattern with phenol as a single "dip." Biodegradation of Isotox® exhibits a completely different pattern from phenol. This would support Jung's [1] observation that different compounds produced different DO patterns.

1.5 Isotox®

Isotox® is a brand of pesticide that is easily purchased in stores. It contains 2 active ingredients, acephate and vendex. The IUPAC name for acephate is O,S-dimethyl acetylphosphoramidothioate, and for vendex it is hexakis (2-methyl-2-phenylpropyl) distannoxane. The chemical structures of these compounds are shown in Figure 1 and 2. The composition of Isotox® is 8% (w/w) acephate and 0.5% (w/w) vendex, with the remaining 91.5% (w/w) being inerts.

From Børkowski [6], acephate is a potential leacher into groundwater in New Jersey, but has not yet been detected in New Jersey groundwater. However, Cairns, Siegmund, Doose and Oken [9] from the Food and Drug Administration (FDA) report that there is an increase in the amount of acephate detected on vegetation. In some cases, 3% of the samples surveyed by FDA contained acephate levels exceeding the legal requirement.

The use of organotin has also increased dramatically, because of its multiple applications. Organotin compounds are used as stabilizers in PVC, in the room temperature 'vulcanization' of silicone, and as agricultural biocides, wood preservatives and glass coatings. As a result of its increase in applications, wastewater treatment plants have also noticed an increase in the level of tin in influent wastewater.

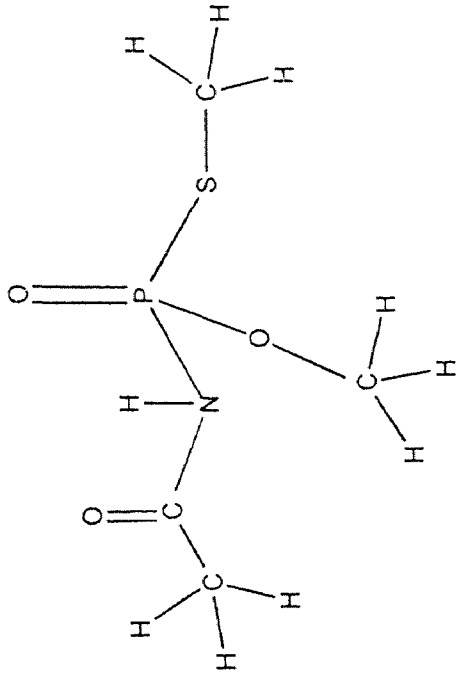

FIGURE 1. Molecular structure of acephate (o,s-dimethyl acetylphosphoramidothioate).

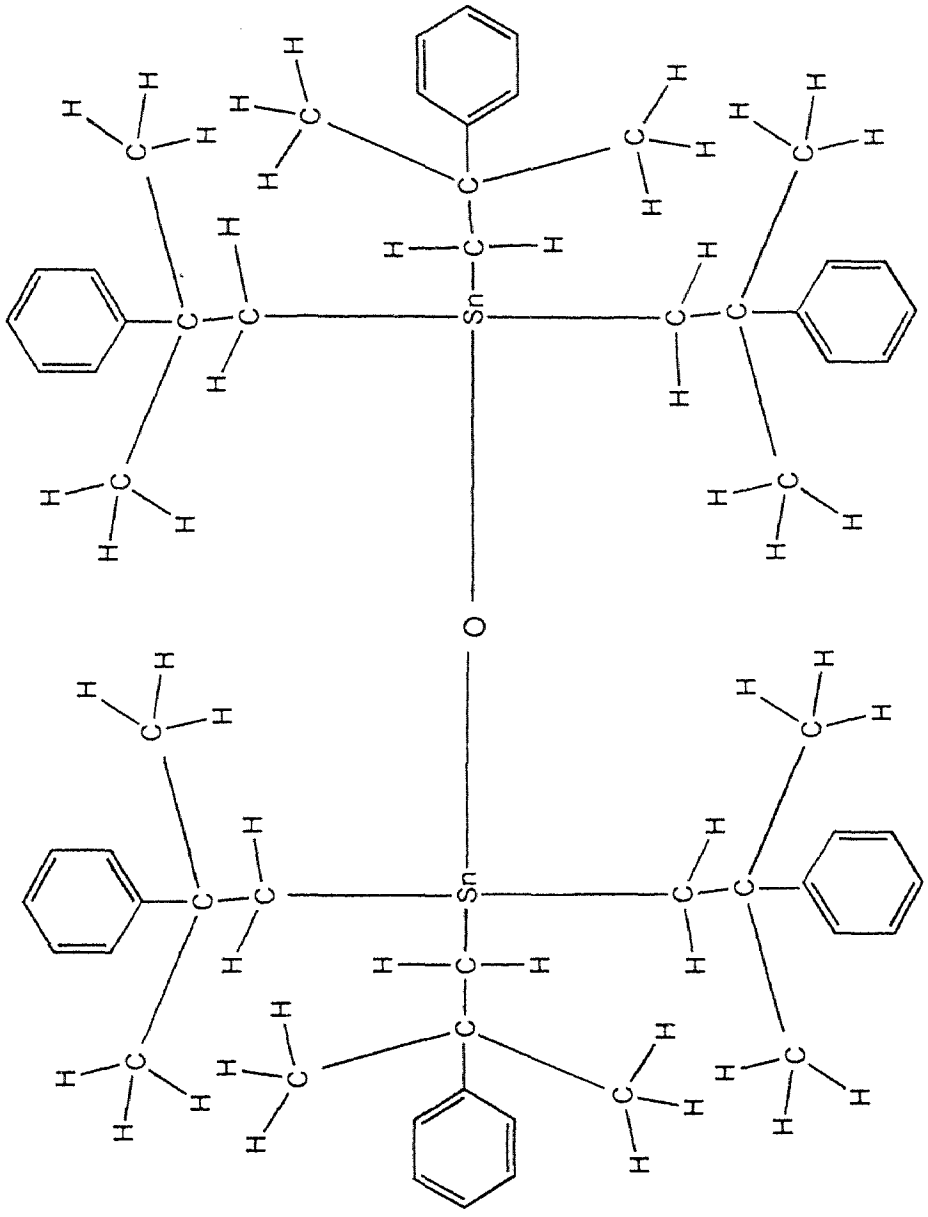

FIGURE 2. Molecular structure of vendex, hexakis (2 methyl-2-phenylpropyl) distannoxane.

CHAPTER 2

LITERATURE SURVEY

Only a small amount of literature on Isotox® has been published. The literature search in this work concentrated on heavy metals, especially tin which is found in Isotox®, and its effects on microorganisms.

Rivera [14] has reported a method of removing zinc in an upflow, packed-bed anaerobic bioreactor (ANFLOW). The mechanism for removal is divided into four distinct processes under anaerobic conditions: adsorption, incorporation into cell, precipitation and entrapment into biofloc, and chelation of soluble metal by organic or inorganic ligands. The bioreactor was packed with Raschig rings with an inlet flow from the bottom. There were 12 sampling ports located at various heights along the bioreactor. Samples were extracted from various ports and analyzed on an atomic absorption spectrophotometer. Rivera's results for overall zinc removal efficiency was over 95% for inlet concentrations greater than 100 mg/L, about 90% for 300 mg/L, and 85% for 1000 mg/L. The reaction time allowed for 100 mg/L was 35 days, for 300 mg/L 26 days, and for 1000 mg/L 13 days. For low concentrations, Rivera reports that most of the adsorption occurred at the inlet. However, for high concentrations the adsorption concentrations increased near the inlet first and then near the outlet. The sorption factor was modeled using a Freundlich isotherm.

Fent and Müller [13] have reported that organotin compounds have been detected in wastewater treatment plants in Zurich, Switzerland. The origin of the organotin

compounds was not determined, but they provided several possibilities, including PVC processing industries, PVC piping, leaching from PVC related products, and use of organotins as homogeneous catalysts for silicones and polyurethane foam. The reported concentrations in pre-treatment water were on the order of 120 - 1000 ng/L. The organotins were primarily removed with the sludge in the primary clarifier. The main mechanism for organotin removal was by adsorption onto the sludge.

Donard, Quevauviller, and Bruchet [15] in Bordeaux, France also noticed an increase in the amount of tin in wastewater. Like Fent and Müller, they also suggested that an increase in inorganic and organic tin are due to an increase in the application of tin in industries. They sampled at 7 locations within the waste treatment plant: influent, primary clarifier, activated sludge, sludge digestion and thickening, thermal processing, in between the influent and primary clarifier and in between activated sludge and sludge thickening. From their analysis, they found that inorganic tin was the predominant species. The concentration levels were all below one μg/L, but significant in the ng/L range. Donard *et al.* found that tin was removed progressively throughout the whole process. This observation differs from what Fent and Müller reported. Fent and Müller reported that tin was predominantly removed by adsorption onto solids. Donard *et al.* also found that some of the tin formed volatile compounds. They reported that SnH_4 and $(CH_3)SnH_3$ were detected under anaerobic conditions. The boiling points for these tin compounds are -52 and -1.4°C. Donard *et al.* calculated that they had at least 150 ng/L of tin in gaseous form in the samples they collected.

Argaman, Hucks, and Shelby [12] discussed the effects of using organotin as an agent in marine antifouling paint in wastewater treatment. They performed their analysis on a municipal wastewater treatment plant in Tennessee. Tributyl tin oxide (TBTO) was detected in the wastewater. TBTO has the following chemical structure,

$$(CH_3CH_2CH_2CH_2)_3\text{-Sn-O-Sn-}(CH_3CH_2CH_2CH_2)_3.$$

It was determined that tin with a coordination number of four, where 3 bonds are attached to organic moieties and the remaining bond is anionic, is the most toxic, and persistent in the environment. Argaman concluded that unacclimated sludge shows an inhibition at concentration as low as 25 μg/L. However, after the sludge has been acclimated, their tolerance of TBTO increased to over 8000 μg/L It was observed that after treatment, toxicity decreased considerably and systems fed with 100 μg/L showed no toxicity in their effluent. . A bioassay performed on fathead minnows showed a TBTO 96 hour LC_{50} of 45-200 μg/L.

Gourdon, Rus, Bhende, and Sofer [11] analyzed and reported the effects of cadmium uptake in mixed culture, free cell microorganisms. Cadmium, a biotoxic heavy metal, was found to inhibit both respiration and extracellular protein production. However, Gourdon *et al.* reported there may be some resistance to cadmium at higher concentrations. Biosorption of cadmium at concentration levels below 30 mg/L were shown to have a Freundlich pattern. The Freundlich equation assumes an initial linear relationship between the biosorbed concentration and the concentration in the water.

Over 95% of injected cadmium was adsorbed by the microorganisms during the first 5-10 minutes.

Gourdon, Rus, Bhende, and Sofer [10] did a comparative study on cadmium uptake between free and immobilized cells. Methods of preparing the microorganisms were identical. For immobilization, microorganisms were immobilized within pellets using sodium alginate, and calcium chloride. The rate of metal adsorption was faster than with free cell microbes, previously reported to be 5-10 minutes. Immobilized bacteria required about 15 hours to reach the same equilibrium point. Gourdon *et al.* hypothesized that this was probably caused by diffusional resistance of the metal through the pellet. At low pH, a higher concentration of cadmium was found in solution, rather than on the bacteria. One explanation for this observation is that there is competition between cadmium and free protons from the acid on the available binding sites.

CHAPTER 3

OBJECTIVES

The primary objective of this study has been to determine the oxidative biodegradability of Isotox®, a pesticide, using a spiral wound polymeric immobilized bioreactor. A list of specific objectives discussed within this thesis are to:

1. Investigate whether a definitive DO pattern is associated with the bio-oxidation of Isotox®,
2. Observe the time of duration for each reaction,
3. Modify the aeration system to increase the efficiency of air delivered to the bioreactor,
4. Determine in a qualitative/semi-quantitative manner the minimum volume for acclimation to take place,
5. Present a quantitative method to correlate amount of oxygen required to oxidize Isotox® through calibration by total oxidation of phenol,
6. Identify any concentration dependence of tin adsorption onto bacteria, and
7. Determine the efficiency of tin adsorption from wastewater using this bioreactor.

CHAPTER 4

MATERIALS AND EXPERIMENTAL METHODS

4.1 Microorganisms and Immobilization

The activated sludge used in this study consists of mixed culture microorganisms that have been obtained from the Parsippany Troy Hills Water Pollution Control Plant in New Jersey. The activated sludge was sieved through a screen size opening of 297 μm and washed with 0.25% saline solution. It was then acclimated with 50 ml of oily industrial waste and 5 ml ethanol at a constant air bubbling rate.

The microorganisms are immobilized onto a polymeric sheet in a series of 4 injections. The reservoir (see Figure 3) contained about 40 L of water. The sludge is injected into the reservoir and allowed to recirculate past the polymeric sheet. The microorganism attaches onto the polymeric sheet naturally. The spirally wound sheet serves as an immobilized cell bioreactor.

The flow rate of the water recirculated through the reactor ranged from 200 to 2000 ml/min at 6 psi. Microorganism injections were made at least 1 day apart from each other to allow for even distribution and immobilization onto the biosupport. The amount of dry biomass injected for the first injection was 4.0796 g, the second was 4.8880 g, the third was 3.5796 g and the fourth was 3.9312 g, for a total of 16.4784 g dry biomass injected. All the bacteria injected were attached onto the biosupport.

4.2 Determining the Amount of Dry Biomass

The amount of dry biomass per injection was determined by mixing the activated sludge in an Osterizer Galaxie blender. The sludge was blended for 15 seconds to ensure a uniform solution. A 10 ml volumetric pipette was used to collect 10 ml of the uniform solution. The sample was then heated in a Stabil-Therm Gravity Oven, manufactured by Blue M Electric Company, at 108°C for over 24 hours. The dry biomass was determined by weighing the dried sample.

4.3 Polymeric Sheet and Bioreactor

The sheet has a porosity of 75 - 80%, with a pore size of 1.2 - 1.5 μ, and a chemical composition of 55% silica and 45% PVC. [2]. The hollow center core has a diameter of about 1 inch. The dimensions of the polymeric sheet are 2.5 feet x 20 feet, spirally wrapped into a cartridge with a 1.2 mm spacing gap between the adjacent layers. The effective possible surface area is 100 ft^2.

The cartridge used has one inlet through the top. Fluid is pumped through the top and flows into the hollow core of the column. The fluid then traverses along with the spiral, until it exits at the end of the spiral. This method allows for the effective possible surface area of close to 100 ft^2 since both sides of the spiral layer are contacted by the fluid.

The cartridge becomes a bioreactor when microorganisms are attached onto the sheet. The polymeric sheet becomes saturated with immobilized microorganisms and acts as a bioreactor.

4.4 Experimental Setup

The overall bio-oxidation system consists of a reservoir, a centrifugal pump, aeration system, bioreactor, and a dissolved oxygen (DO) analysis system. Figure 3 illustrates the overall configuration. The reservoir water is pumped through the centrifugal pump and into the top of the bioreactor. There are two bypass valves between the centrifugal pump and the bioreactor. These bypass valves serve two purposes; first to control the amount of flow into the bioreactor and secondly, to create a constant stirring in the reservoir to ensure a uniform concentration of substrate into the bioreactor and increase aeration. The flow rate of the reservoir water is determined by a pressure gauge and can be controlled by the two bypass valves.

Periodic measurements of the flow rate, and volume of the reservoir water were made. The pressure gauge reading throughout the study was kept at 4 to 7 psi.

Aeration of the reservoir water is provided by inserting an air line directly into the piping before the bioreactor and after the two bypass valves. This is to ensure maximum dissolved oxygen within the reservoir water. To increase the air contact with reservoir water, an aquarium 1" air stone was inserted onto the airline within the pipe. With the addition of the air stone, the air flow rate needed was between 150 - 223 ml air/min.

Flow rate through the reactor was determined by measuring the outlet volumetric flow rate from the bioreactor. To achieve this, a small plastic catch basin with an outlet on the side was used. The basin served 3 purposes; first to separate reservoir water from the bio-oxidized products, secondly to recirculate the product into the reservoir water,

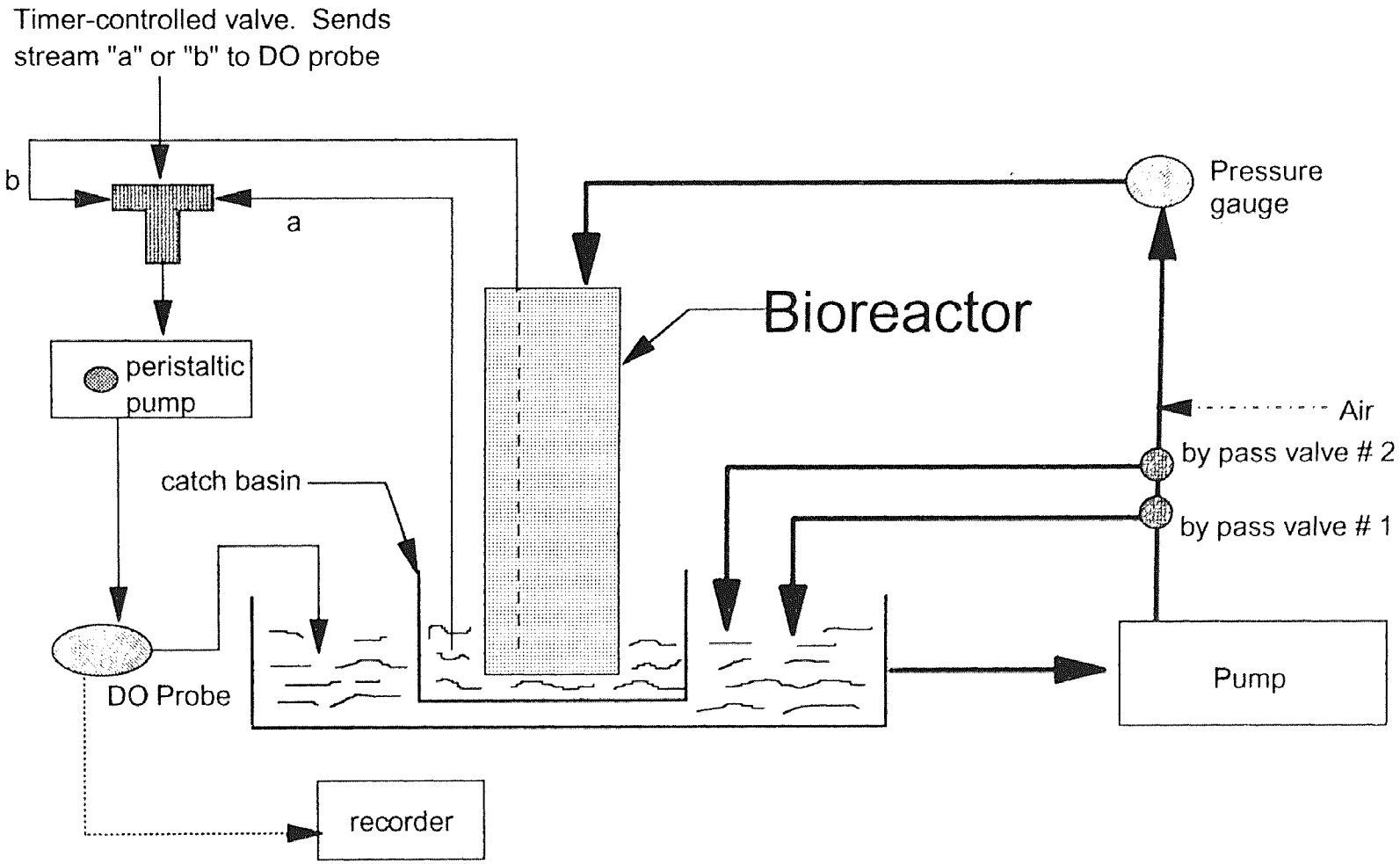

FIGURE 3. Bioreactor System Experimental Setup. Water is continuously recycled from the reservoir, through the bioreactor cartridge and back via a catch basin. The sensor alternately detects oxygen at the entrance and the exit of the bioreactor. A large decrease in the oxygen concentration indicates a high oxidation rate.

and thirdly as a means of measuring the flow rate through the cartridge, situated within the basin. Temperature was measured by a thermometer in the basin. The temperature range of the system varied between 30 - 40 °C.

Substrate is injected into the system in solution form. If the substrate is a solid, it is dissolved using the reservoir water as the solvent. If the substrate is a liquid, a small amount of reservoir water and the liquid substrate are mixed in a beaker first to observe for effects and to dilute the mixture for better mixing. The mixture is then uniformly poured into the reservoir.

All experimental preparations were made under a ventilating hood. The majority of the experimental set up was situated within a ventilated hood, with the exception of the dissolved oxygen analysis system. The DO analysis system was located outside of the hood, next to the bioreactor.

4.5 Dissolved Oxygen Analysis System

The DO analysis system consists of two sampling inlets (see Figure 4), an alternating time delayed flow switch, a timer, peristaltic pump, flow damper, DO probe, and a recorder. One of the sampling inlets is located within the hollow core of the bioreactor. This sampling inlet is denoted as the reactor sampling inlet. The DO reading from the core is assumed to be the dissolved oxygen in the liquid prior to bio-oxidation, per pass. The second inlet samples the product, which has undergone bio-oxidation. This sampling inlet will be denoted as the cartridge outlet. An alternating time delayed flow switch is used to control the sampling time as well as to allow the use of only one DO

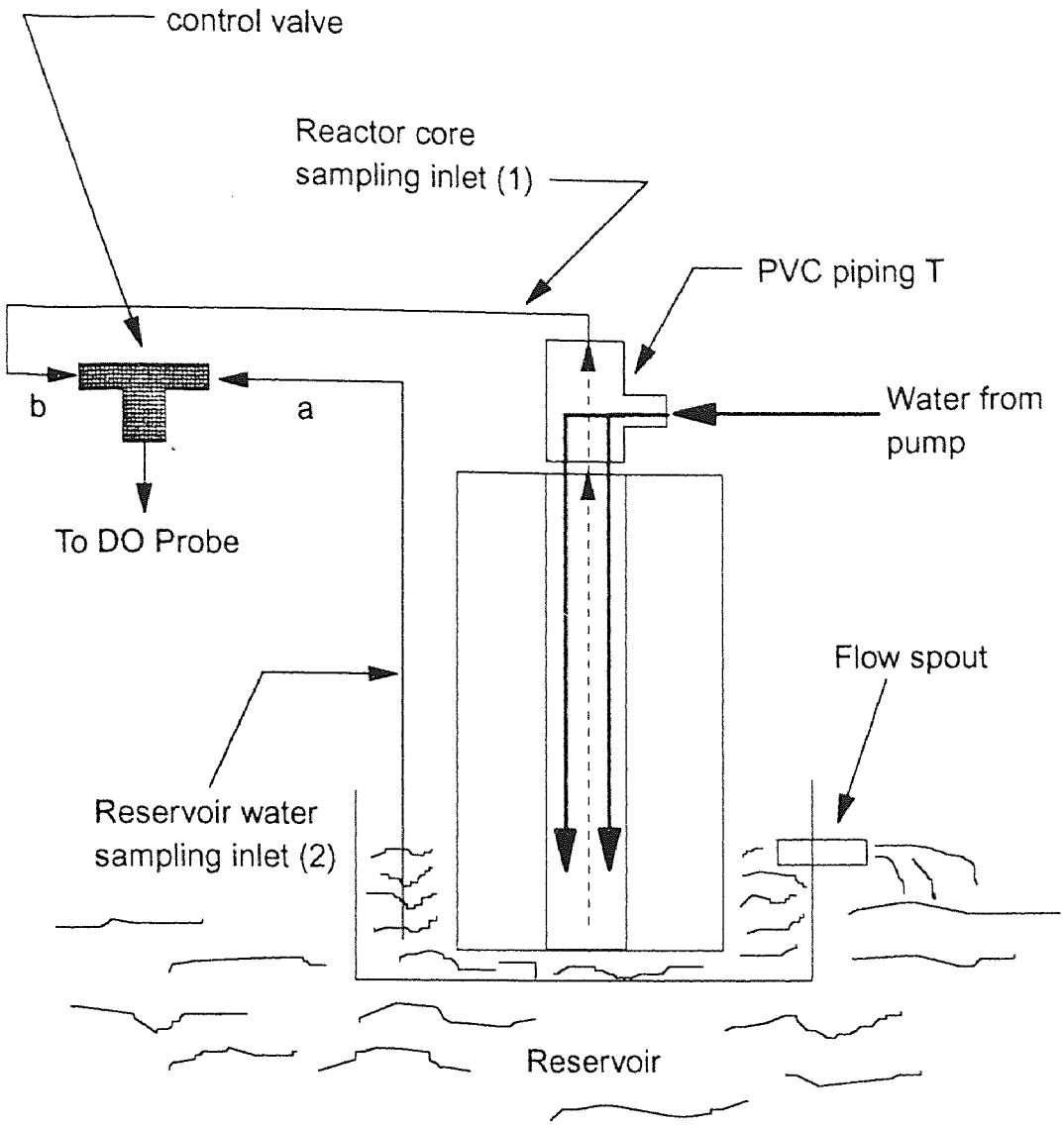

FIGURE 4. Detail of Oxygen Sampling. One sampling inlet is at the core, within the bioreactor. The second sampling inlet point is located outside the bioreactor within the catch basin. Flow rate from the spout is measured by stopwatch and beaker.

probe. The alternating time delay for each inlet is 10 minutes. Samples from the two inlets are withdrawn from the system by the peristaltic pump. A flow damper is used since the flow exiting from a peristaltic pump is in pulses. The flow damper reduces the pulse and provides a more constant flow rate into the DO probe. The DO probe and the recorder continuously analyze and record the DO concentration within the system. The water, after being analyzed by the DO probe, is returned to the reservoir.

4.6 Dissolved Oxygen Measurements

Due to the two inlet sampling points in the DO analysis system, the recorder will record, what appears to be, an oscillation between two boundaries. (Please refer to Figures 7, 8 and 9). The upper boundary is the DO concentration of the fluid at the core of the bioreactor, before any bio-oxidation has taken place. The lower boundary is the DO concentration after bio-oxidation has taken place. At steady state, the upper and lower boundary level are constant. A baseline is determined by the midpoints of these upper and lower boundary levels, prior to any injection and after the completion of a reaction. It can be assumed that this baseline represents the saturated dissolved oxygen level. The zero point of the recorder is determined by shorting the recorder.

A typical injection of a substrate, like phenol, will result in a DO pattern. (Please refer to Figure 7). The decrease in the DO concentration is caused by the consumption of oxygen by the immobilized microorganisms. The curve represents the oxidation of the substrate. Upon completion of the oxidation process, the DO curve returns to the baseline level.

The pattern of the substrate oxidation reaction and the baseline are determined, relatively, by their position from the zero. This pattern is then calibrated by using phenol, which has been studied and shown by both Lakhwala [2] and Jung [1] to go to complete mineralization for such a bioreactor system. The oxygen consumed by the substrate is then determined by stoichiometric chemistry.

4.7 Measurement of Consumed Oxygen by Weighing of DO Curve

The DO curves and baselines are measured and plotted using the same grid units for all curves and baselines. This is to ensure consistency of data between different injection volumes. For each injection plotted on a graph paper, rather than maximizing the DO pattern on each graph, the same units for each grid are used for all the injections. This will allow a comparison between all the different injections to be established.

In order to determine the correct grid units to be used for all the injections, standard axis values have to be determined. DO was measured directly off the recorder paper in millimeters. The maximum DO value, comparing all DO curves and baselines, was used. It should be mentioned that the injection that requires the maximum reaction time is not necessarily the injection that has the maximum DO dip measurement. This will establish the boundaries and sets as the basis for all DO curves.

The individual curves and baselines were plotted on a standardized axis and the curves were cut out and weighed. Phenol injections and its corresponding DO pattern and reaction times were also plotted on a graph using the standardized axis. This is a method to calibrate the DO associated with the pesticide injections with a known DO, namely that

of phenol. An Ainsworth Type 21 N balance with a 0.0001 g precision was used to determine the individual paper weight. The accuracy of this method is 2-5%. [5].

4.8 Qualitative Analysis of Sulfate Ion and Sulfur

Analysis of bio-oxidized product for sulfate ions, SO_4^{-2}, was performed using wet chemistry. A 1 ml sample of the product was collected and acidified by adding 1 ml of 6 M HCl. A few drops of barium chloride, $BaCl_2$, were added to the sample. If sulfate ions are present, a white precipitate will form. [20]

Sulfur analysis was also performed on the bio-oxidized product. Sodium extract is prepared by placing Na metal into a fusion tube along with a small sample of the bio-oxidized product. The contents are then heated over a Bunsen burner until red hot. The fusion tube, along with the Na extract are then placed into a porcelain dish containing distilled water and covered with a porcelain cover. It may be necessary to break the fusion tube to allow Na extract to react with DI water. After completion of reaction between Na extract and DI water, the porcelain dish is heated to concentrate the solution. Lead acetate and acetic acid are added to the concentrated solution. Black coloration or precipitation will indicate the presence of PbS, or the presence of sulfur in the sample. [17]

4.9 Acid Washing

Adsorption of heavy metals by bacteria has been long known. Gourdon, Rus, Bhende, and Sofer [10 and 11] have shown that cadmium, lead, chromium and other heavy metals

adsorb readily to immobilized bacteria. The removal of adsorbed heavy metals from the cartridge can be achieved using chemical methods.

This method required decreasing the pH of the fluid to acidic conditions. A Corning Ion Analyzer 250 was used to measure the pH of the reservoir water. 6 M HCl was added into the reservoir water to attain the desired pH level, usually pH 3.0. The acidic reservoir water was allowed to circulate through the system for about 1 - 2 hours to ensure thorough contact with microorganisms. Samples of the washed reservoir water were collected for tin analysis. The reservoir water was replaced with normal tap water, which may be considered essentially free of heavy metals. The tin adsorbance and acid washing for cumulative additions of the pesticide is shown in Figure 5.

4.10 Preparation for Tin Analysis

20 Ml samples of the reservoir product were collected and frozen until ready for analysis. Prior to analysis, the samples were defrosted at room temperature overnight. The samples were then acidified with 2 ml of concentrated HCl (12 N HCl) and analyzed using an atomic absorption spectrophotometer (AA), as discussed later.

The tin standards were prepared according to the method outlined by Standard Methods [21] 1.000 G tin metal was dissolved in 100 ml 12 N HCl. The tin-HCl solution was then diluted in a 1000 ml volumetric flask with distilled water. The standard solution contained 1000 ppm Sn. If dilution was necessary, the dilution was done using 1:9 concentrated acid to distilled water

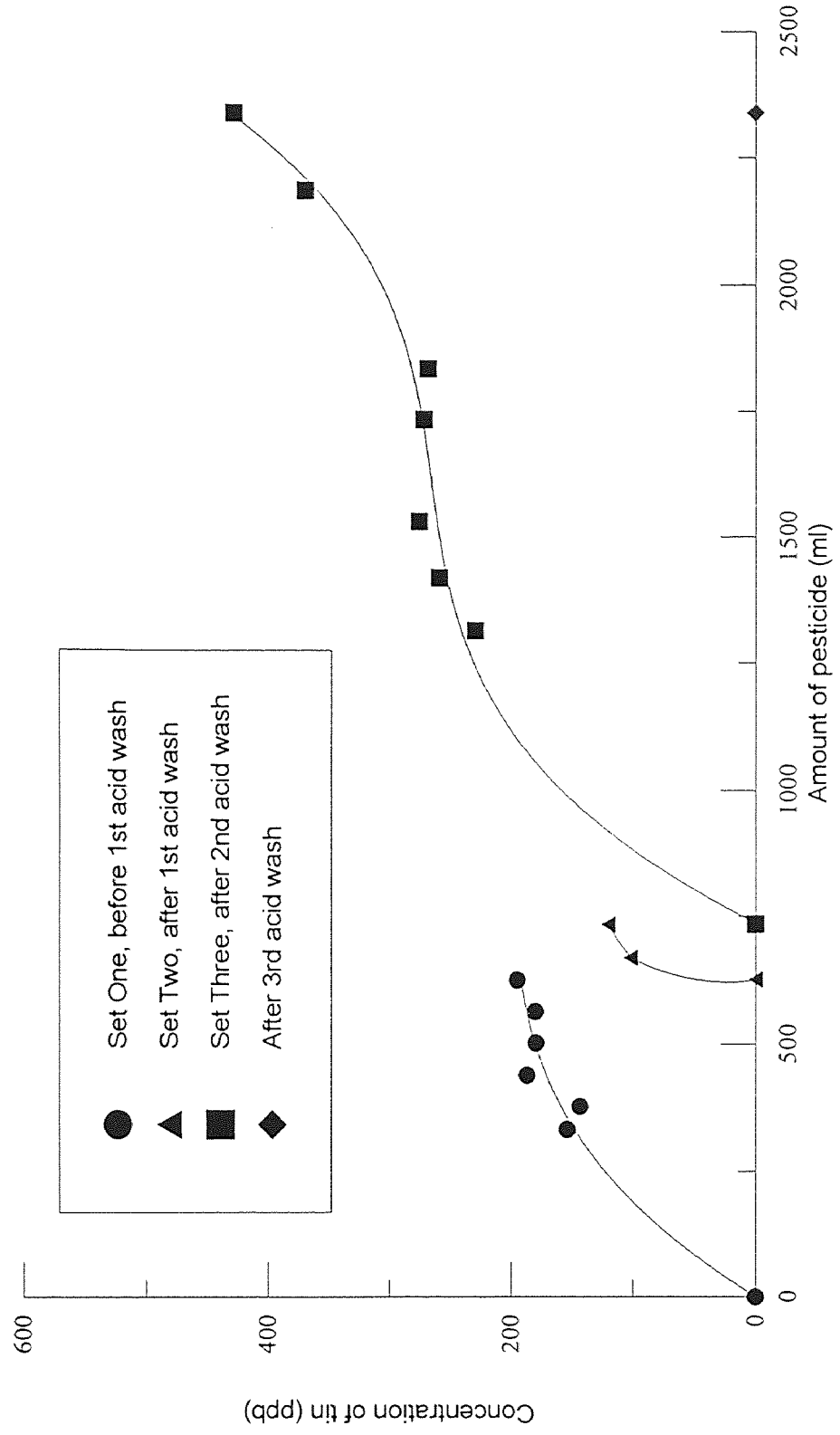

Figure 6. Post Reaction Tin Concentration in Reservoir. Corrected total tin in reservoir water is shown for cumulative injections of pesticide. After every acid wash, the reservoir was essentially be tin-free. Tap water used in this study contained measurable traces of tin at about 70 ppb.

4.11 Microorganism Analysis of Tin Adsorbance

Tin adsorbance on microorganisms was analyzed by two methods. The first method consisted of collecting the small amounts of microorganisms that had been washed off of the bioreactor during the acid washing process. Porcelain crucibles were baked in a furnace at 550°C (ashen temperature) overnight. The crucibles were placed in a desiccator to cool for 1 - 2 hours and then weighed. The collected microorganisms were then placed into the crucibles and heated on a hot plate until carbonized. The samples were then placed into the furnace, at ashen conditions, overnight. The crucibles were removed from the furnace and placed into a desiccator for 1-2 hours to cool, and then weighed. The ash was acidified and washed into a 50 ml Nalgene volumetric flask and analyzed by atomic absorption method.

The second method of collecting tin adsorption consisted of cutting 1 square inch of the polymeric sheet. Careful attention was placed on the removal of the sample since some biomass may be removed from the polymeric sheet during handling. The square sheet was placed into a 125 ml Erlenmeyer flask containing 25 ml DI water. The solution was acidified by adding 25 ml of 12 N HCl. The flask was placed in a Lab Line Orbit Environ Shaker for 10 hours at about 150 rpm. The sample was then analyzed by AA.

4.12 Atomic Absorption

Two atomic absorption spectrophotometers were used during this study: a Perkin-Elmer Model 2380 Flame Atomic Absorption (FLAA) and a Smith-Hieftje 12 Thermo Jarrell Ash Graphite Furnace Atomic Absorption (GFAA). The sensitivity of the FLAA is in the

ppm range, while the GFAA has a sensitivity in the ppb range. The accessibility of using the GFAA was limited, and at times FLAA was used instead. The specific settings for the respective AA used were as prescribed by the respective user's manual.

4.13 Substrate Characteristics

The substrate used in this experiment was an over-the-counter pesticide, Ortho Isotox® Insect Killer Formula IV by The SOLARIS Group. Its active ingredients include 8% (wt) acephate (CAS30560191), and 0.5% (wt) vendex (CAS13356086), with the balance of 91.5% (wt) are listed as inert. The exact composition of the inerts was not available, and is considered proprietary by the manufacturer. However, based upon the Material Safety Data Sheet (MSDS) supplied by the manufacturer, two of the inerts are isopropyl alcohol (CAS67630) and pyrrolidinone (CAS872504).

There are several potential health effects that can result from exposure to Isotox®, depending upon the degree of exposure. It can cause eye irritation and permanent damage, i.e. blindness. Some symptoms of exposure to the eyes include pain, tears, swelling, redness and blurred vision. This pesticide is also a skin irritant and can be absorbed through the skin. If it is absorbed through the skin, it can cause damage to internal organs. Some symptoms of skin exposure include feeling of heat, discoloration, swelling and blistering of skin. Ingestion of Isotox® will cause damage to internal organs, depending upon the amount absorbed through the digestion tract. This substance is practically non-toxic to internal organ, if inhaled.

Acephate has a melting point of 64-68°C and is very soluble in water. It has an LD_{50} of 700 mg/kg taken orally in rats. It is an inhibitor of the cholinesterase enzyme found in nervous tissues, plasma, and red blood cells. Target organs in male mice are liver, lungs and kidneys. In female mice, acephate causes liver tumors. No signs of carcinogenicity occurred in male rats or mice, tested over a 2-year study. Acephate also caused pre- and postnatal growth retardation, as well as decreased the live born litter size in rats. [23]

Vendex is a selective organotin miticide. It has a melting point of 138 - 139°C, and is a white crystalline powder. It is insoluble in water and converts to the hydroxide in presence of water. Vendex is non-toxic to bees, but is toxic to fish. It has an LD_{50}, taken orally in rats, of 2630 mg/kg.

Isotox® is toxic to birds and other wildlife. It is also toxic to bees and aquatic organisms. Therefore, it should be kept out of all bodies of water, including sewage and drainage systems.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Effects of Air Flow Rate on Recirculation Bioreactor

The method of aeration in this study differed from the setup used by both Borkowski [6] and Woods [7]. Both Borkowski and Woods aerated their systems within the reservoir, prior to the centrifugal pump. Woods reported that an aeration rate of approximately 4.5 L/min of air was used for their study.

Two different aeration set-ups were used during this study. Both configurations introduced air after the pump, and prior to entrance into bioreactor. These methods allow a greater mixing of air into water prior to the bioreactor. Since oxygen is a co-substrate in an aerobic biodegradation process, an efficient method of oxygen delivery to the microbes is essential.

The first aeration technique used a hard plastic tube placed within the pump outlet pipe, via a PVC piping T. (Please refer to Figure 6). The second set-up is a modified version of the first by addition of an aquarium stone (Figure 6).

The aeration rate used for the first set-up was about 570 ml/min. Phenol was injected into the bioreactor, and the reaction time observed was about the same as those performed by Woods and Borkowski. The pressure drop associated with the addition of aeration tube was negligible.

Next, an aquarium air stone was placed upon the aeration tube, within the pipe. The aeration stone decreased the bubble size and thereby increase the availability of air

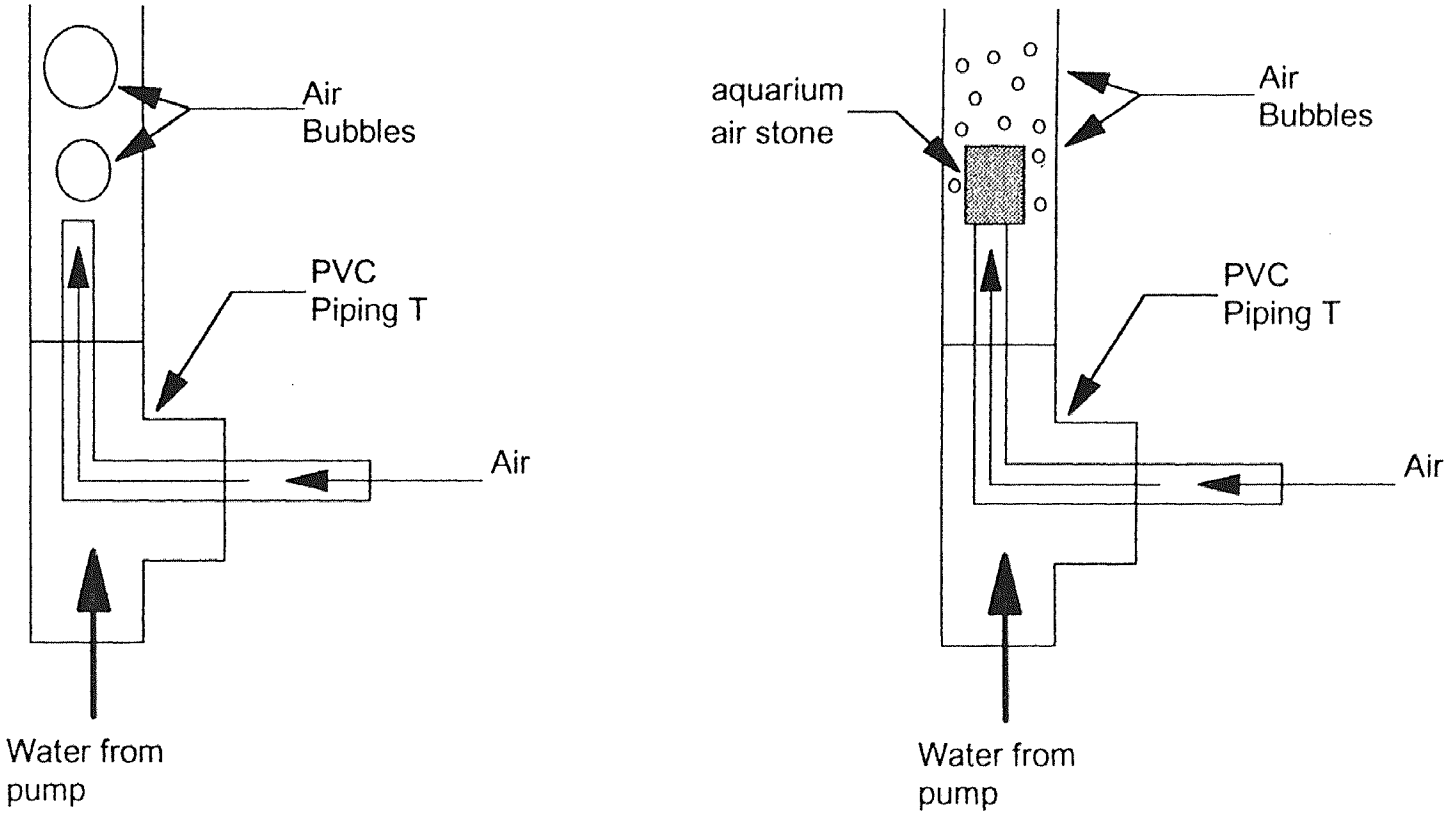

FIGURE 6. Two Methods of Aeration Used. Both systems are essentially the same with the exception of aquarium aeration stone. The stone produces finer bubbles to help increase the mass transfer of oxygen into the water.

into water. The aeration rate, using this set-up was decreased to about 220 ml/min. Results with phenol showed the reaction time to be about the same as with the first set-up. The pressure drop associated with the aeration stone was about 2 psi. However, a decrease of over 50% air requirement was achieved compared to previous set-up and over 95.1% was achieved compared to Woods and Borkowski's setup.

5.2 DO Pattern for Isotox®

A typical DO pattern for phenol is a single dip (Figure 7). In Isotox®, a reproducible DO pattern was observed with all injections. The DO pattern associated with this pesticide differs from phenol in that it has multiple dips before it finally returns to the baseline. A typical Isotox® DO curve is shown in Figure 8. From Figure 8, at least 4 distinct dips or minima occur within one injection. A DO curve for an organic mixture containing hexane, 2-butanone, ethanol, 2-propanol, and ethyl acetate produced only one and maybe 2 dips (Figure 9). The exact cause of why Isotox® consistently produced at least 4 dips was not determined within this course of study. It is very difficult to determine this effect since the complete composition of the pesticide is not known.

A possible theory is the complex composition within Isotox® containing compounds with different chemical groups. In oxidation of phenol, most of the bonds are carbon-carbon and carbon-hydrogen bonds. The organic mixture injected also contained compounds with similar bond structure, i.e. carbon-hydrogen, or carbon-oxygen. However, in Isotox®, vendex contains not only carbon-hydrogen bonds, but tin-oxygen, and tin-carbon bonds. Acephate contains sulfur, phosphorous and nitrogen

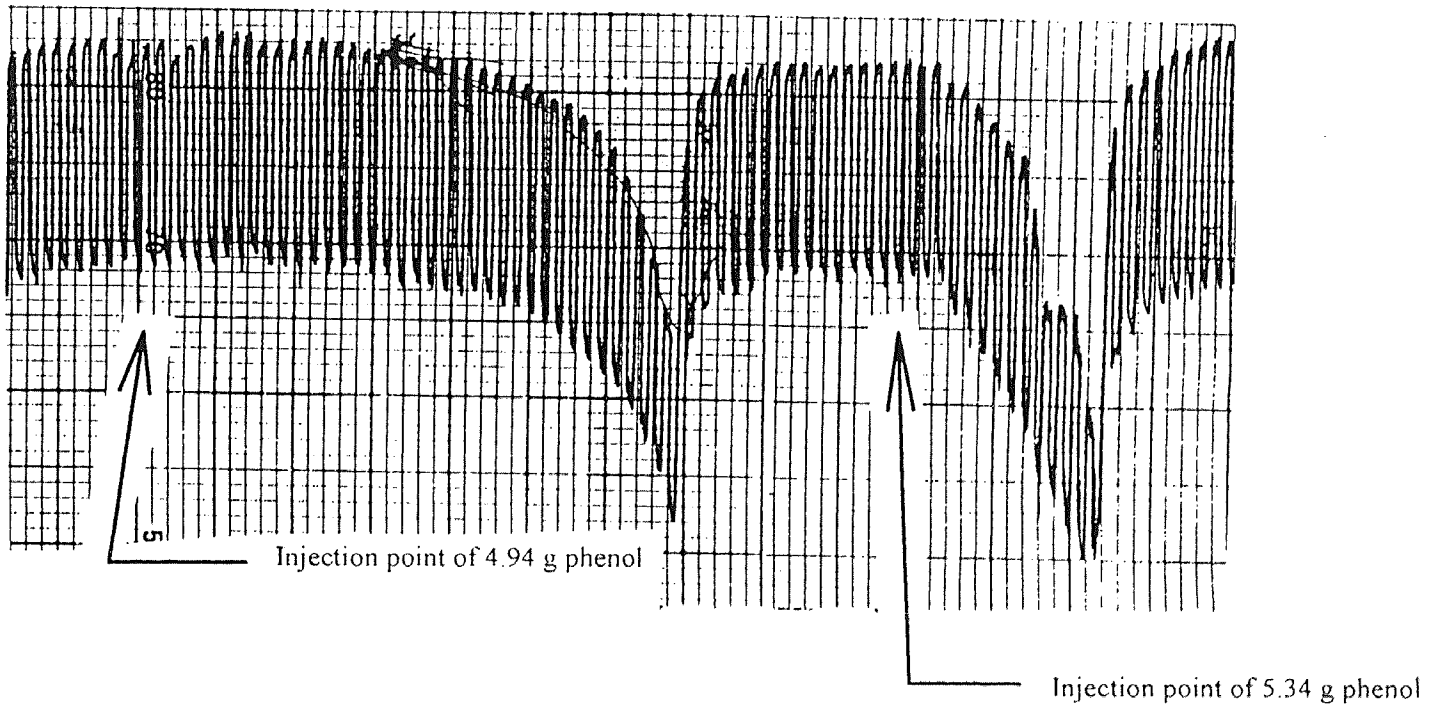

FIGURE7. Typical Bio-oxidation Pattern for Phenol. Direct readout of DO versus time is shown for two phenol injections. The system is unacclimated prior to first injection. The unacclimated system is injected with 4.94 g phenol (left arrow). The freshly acclimated system is next injected with 5.34 g phenol (right arrow). Both injections show a single "dip" in DO pattern. For the unacclimated system, bio-oxidation reach a maximum 700 minutes after injection. The acclimated system reaches a maximum bio-oxidation 240 minutes after injection.

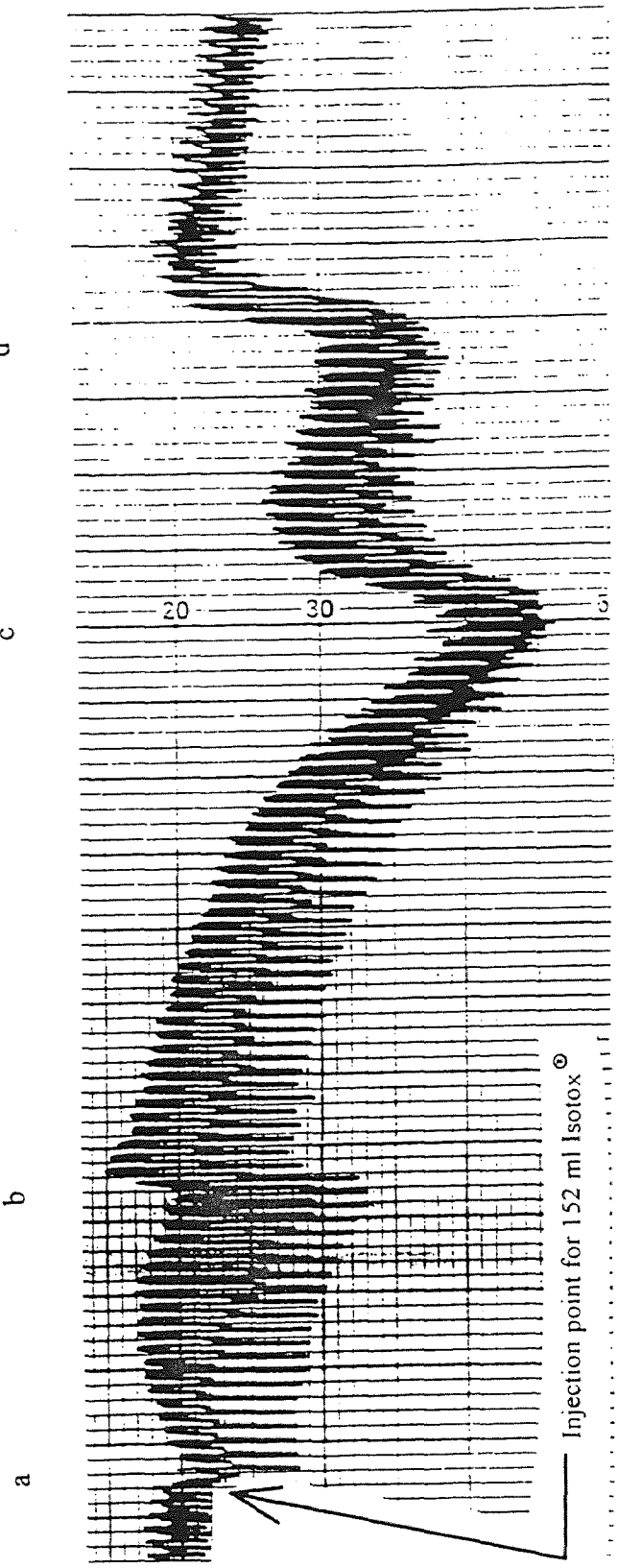

Figure 8. Typical Organotin Pesticide Injection Pattern. Four distinct "dips" are shown at points a-d. The injection was 152 ml pesticide added at the arrow on left. The reaction time needed for complete oxidation was 1750 minutes.

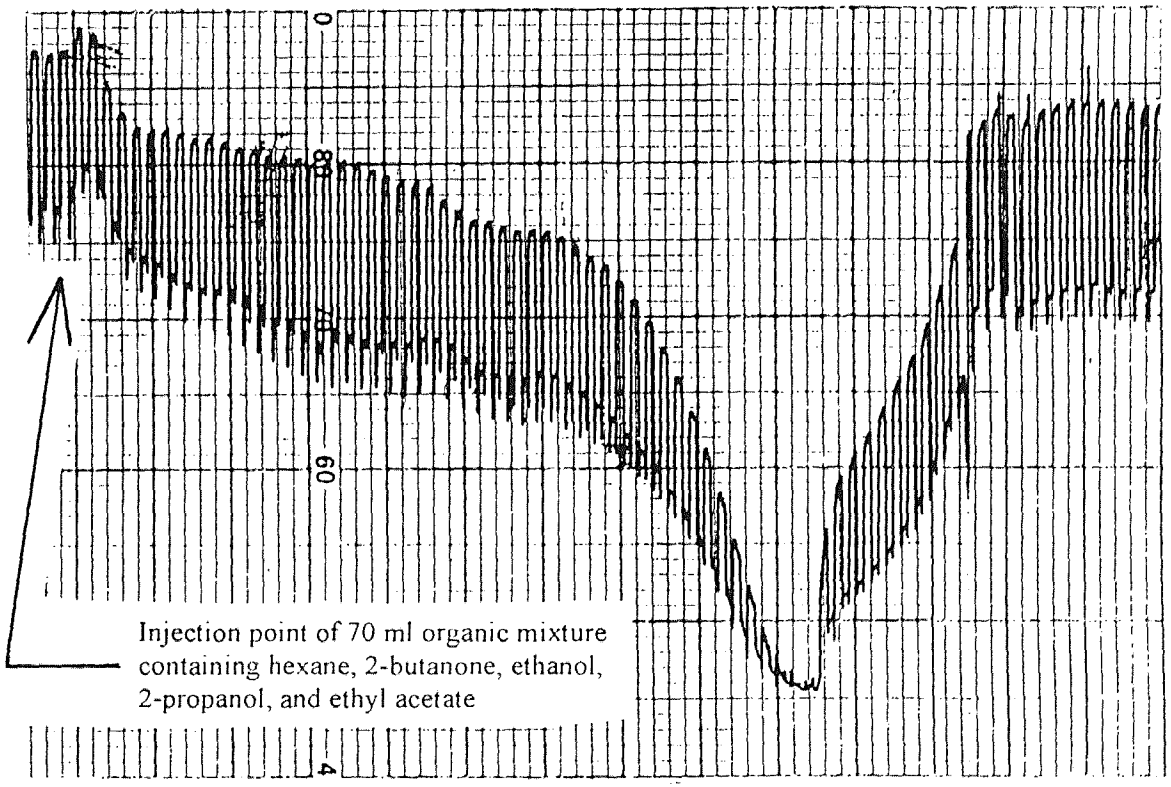

FIGURE 9. Organic Mixture Pattern. An organic waste mixture from NJIT freshman Chemistry laboratories containing hexane, 2-butanone, ethanol, 2-propanol and ethyl acetate. The injection volume was 70 ml. There is one distinct "dip" in its DO pattern. A possible second "dip" is located between the injection point and the first distinct "dip." Sharp drop indicates intesified rate of oxidation.

bonds in addition to carbon-hydrogen and carbon-oxygen bonds. With such complex and different types of bonds, the oxidation rate associated with each type of bond would be different. If one type of bond is significantly more difficult to bio-oxidize than, say the carbon-hydrogen bond, it may be possible that after complete oxidation of that moiety has been completed, the "difficult" bond may not have begun to oxidize yet. This delay allows the reactor water time to re-aerate and increase its DO concentration, contributing to the pattern formation.

5.3 Reaction Rates of Isotox®

Isotox® injections into the bioreactor system were recorded and the reaction times needed for completion were compared. The injections were categorized into 3 groups: below 50 ml, between 50 to 100 ml, and over 100 ml injected. The reaction times for degradation of the pesticide were determined by the DO curve. The reaction is considered complete when the DO returns to the baseline.

For unacclimated systems, the reaction time needed is longer than for an acclimated system. An acclimated system, in this study, is defined as a system that has had a previous injection of the same substrate without introducing a different substrate in-between the two injections. From Figure 10, the results show longer reaction times for unacclimated systems. However, for injections below 50 ml, this was shown to be the opposite case. The sole "acclimated" data point below 50 ml was an injection of 40 ml. It was injected at the completion of a 20 ml injection. In all cases for acclimation at higher injection volumes, acclimation took place after the completion of first injection.

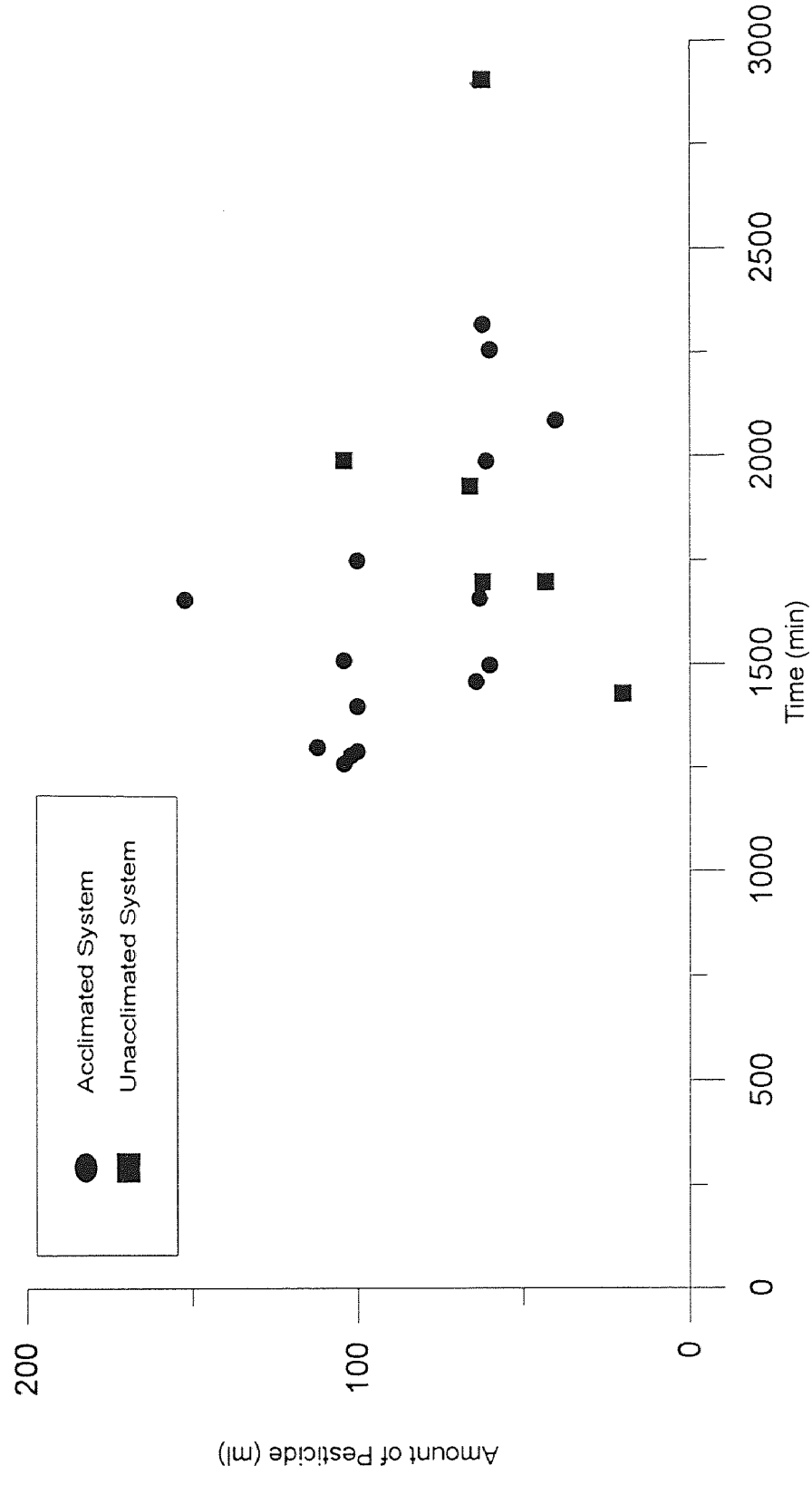

Figure 10. Pesticide Oxidation Reaction Time. The oxidation reaction time needed for individual pesticide injection volumes for both an acclimated and unacclimated bioreactor. The reaction time needed for completion of reaction varied between 1250 to 2250 minutes, despite the volume of injection. Pesticide volume represents additions of concentrated pesticide directly from the commercial, concentrated form.

At low concentrations, this was not obvious. This would suggest that there is probably a minimum volume of Isotox® needed for acclimation to take place. The minimum cumulative volume required for acclimation is about 60 ml. It was observed that after a cumulative volume of 60 ml was injected, the following injection would react similar to that of an acclimated system. An acclimated system would show an immediate decrease in the DO concentration upon injection, as recorded by the DO probe.

Another result, shown in Figure 10, is that the reaction time needed for all the injections falls within the same range. Regardless of the volume of injection, the reaction time range, for all volumes of substrate injected, is between 1250 - 2250 minutes. Two hypotheses are proposed to explain this finding. One hypothesis is that the reaction time and concentration of the substrate are independent or nearly independent of each other. A second hypothesis, is that mass transfer of substrate to the bacteria is lower at lower concentrations. From Figure 10, the majority of reaction times for injection volumes over 100 ml were generally faster than for 60 ml. Based upon the information available, the second hypothesis is more likely.

5.4 Statistical Analysis of Total Oxygen Consumed by the Bioreactor

A statistical analysis of the baselines for each injection was performed. A graph of the baseline was plotted, using the standard axis described in Section 4.7. A cut-out of this baseline was weighed. [For details on baseline graphing and weighing, see Methods]. The respective reaction time and the weight of the baselines were plotted using error bars set

at 1.0 standard deviation. All the data fell within a best-fit line determined by linear regression method (Figure 11).

There are several advantages in performing a statistical analysis on the baseline over DO curve. The baseline is more consistent than the DO curve. There are many factors that influence the DO curve, such as the different volumes of injection, the condition of the bacteria (if it is acclimated or not), and the concentration of the substrate. Analysis of the baseline is simpler, and represent the overall viability of the system. In correlating various DO injections, the baseline was used as the basis of reference; since the baseline essentially represents the saturated dissolved oxygen in water.

The two sets of data, one each for uncorrected and corrected runs, were normalized and amount of oxygen consumed per unit injection volume calculated. The least squares method of linear fitting was performed on both sets of data. For corrected runs it was (0.0067 ± 0.0028, $N = 21$) and for uncorrected runs it was(0.0082 ± 0.0041, $N = 21$). Based upon mean standard deviation calculations, the corrected data set was about 8.7% better than raw data.

5.5 Method of Correlating Oxygen Consumed by Isotox®

The dissolved oxygen consumed by the bioreactor to biodegrade Isotox® was plotted and the resultant curve weighed. This weight was correlated to number of moles of oxygen consumed through a known amount of phenol. Since complete mineralization of phenol by the same bacteria has been shown by Jung [1], the weight of the DO curve from

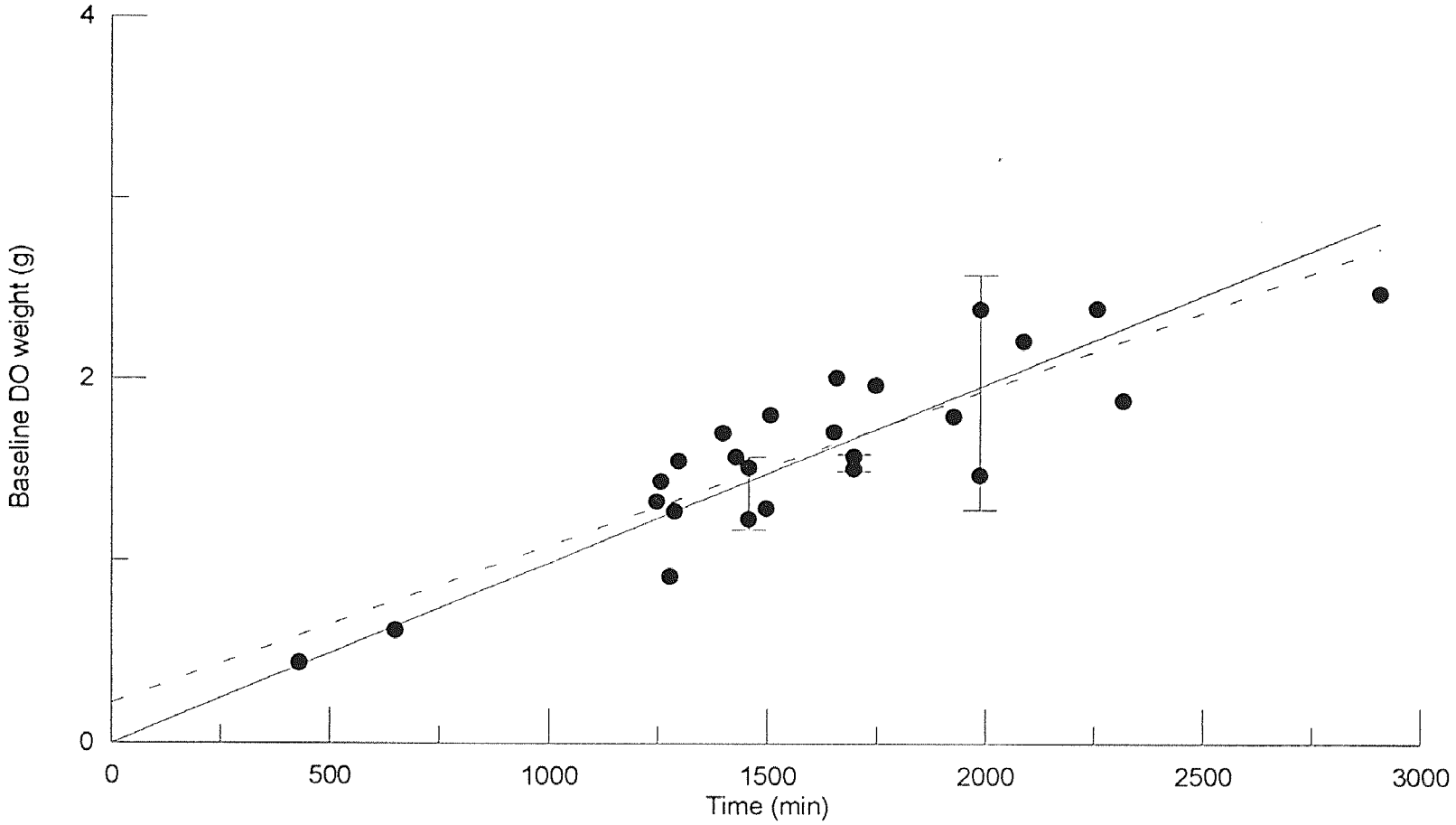

Figure 11. Analysis of Integration Technique. A statistical analysis of the oxidation reaction in a spirally wound polymeric bioreactor, using a 1.0 standard deviation error bar. The oxidation reaction is based upon the DO baseline associated with the respective pesticide injection.

phenol represents the oxygen required to mineralize phenol. Phenol undergoes the following oxidation reaction,

$$C_6H_5OH + 7\ O_2 \rightarrow 6\ CO_2 + 3\ H_2O.$$

The DO pattern resulting from a known mass of phenol injected represents a molar ratio of 1:7. The DO curve weight resulting from phenol can be determined by a balance. A relationship between the weight of the DO curve and moles of oxygen consumed is then established.

In establishing a correlation between the pesticide and oxygen consumed, 2 sets of phenol DO curves are needed: an acclimated system and an unacclimated system. This is due to the fact that for an unacclimated system, the reaction time needed is longer than an acclimated system. For this study, 4.94 g of phenol was injected into an unacclimated system and 5.34 g of phenol was injected into an acclimated system. The paper weight for 4.94 g was 0.0417g and for 5.34 g it was 0.0410 g. The method for determining the amount of oxygen is shown in Appendix A.

5.6 Dissolved Oxygen Requirement for Isotox®

The amount of oxygen needed to consume Isotox® was determined for both unacclimated and acclimated systems at different volumes of Isotox® injection. The results are presented in Figure 12. Two sets of linear best fits through the origin are presented, and it should be noted that for the unacclimated system the best fit was at a steeper slope. A

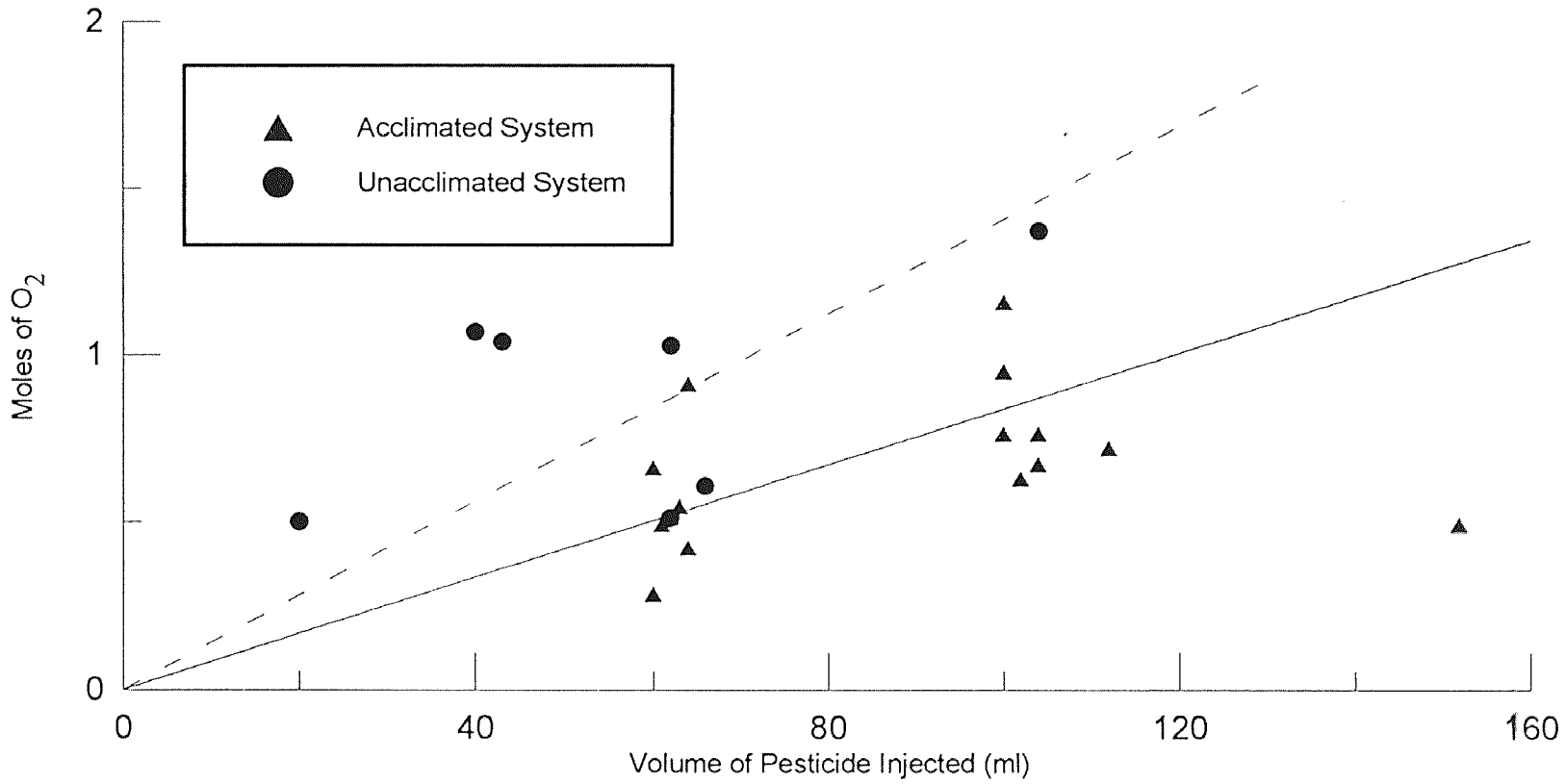

Figure 12. Oxygen Requirements for Acclimated and Unacclimated Systems. The total amount of oxygen consumed, per injection, by bioreactor to mineralize pesticide, for both an acclimated and an unacclimated system is presented. A linear regression fit, forced through the origin, has been determined for the bioreactor. The linear regression fit, forced through the origin, for the unacclimated system is shown in dashed lines. The unacclimated system requires more oxygen, indicating a probable utilization for the synthesis of enzymes.

steeper slope shows a greater oxygen requirement, which is consistent with the phenol data from above. One theory is that for an acclimated system, the bacteria already have the necessary enzymes to oxidize the substrate. For an unacclimated system, the bacteria will be required to make those enzymes. These enzymes, or process of making these enzymes would require oxygen. As a result for unacclimated systems, a slight increase in oxygen consumption is observed.

The exact ingredients within the pesticide were not revealed by the manufacturer, due to company confidentiality. Therefore, the exact oxidation reaction for Isotox® cannot be determined. However, based upon the dissolved oxygen curve, the oxygen consumed ranged from approximately 0.25 to 1.2 moles for injection volumes ranging from 20 ml to 152 ml (Figure 13). The stoichiometric amounts required are from 0.25 to 1.5 moles of O_2, as will be shown below.

One of the active ingredients in Isotox® is vendex, an organotin biocide. The oxidation reaction for vendex, excluding the fate of tin, is,

$$C_{60}H_{78}OSn_2 + 79\ O_2 \rightarrow 60\ CO_2 + 39\ H_2O.$$

The theoretical oxygen requirement for complete oxidation of vendex is 0.120 g-O_2 per g-Isotox® added. The theoretical oxygen requirement for vendex is about 4 moles percent of the total oxygen consumed.

The combined total theoretical oxygen requirement for complete oxidation of the active ingredients in Isotox® is about 0.25 moles O_2 (Figure 13). The theoretical oxygen

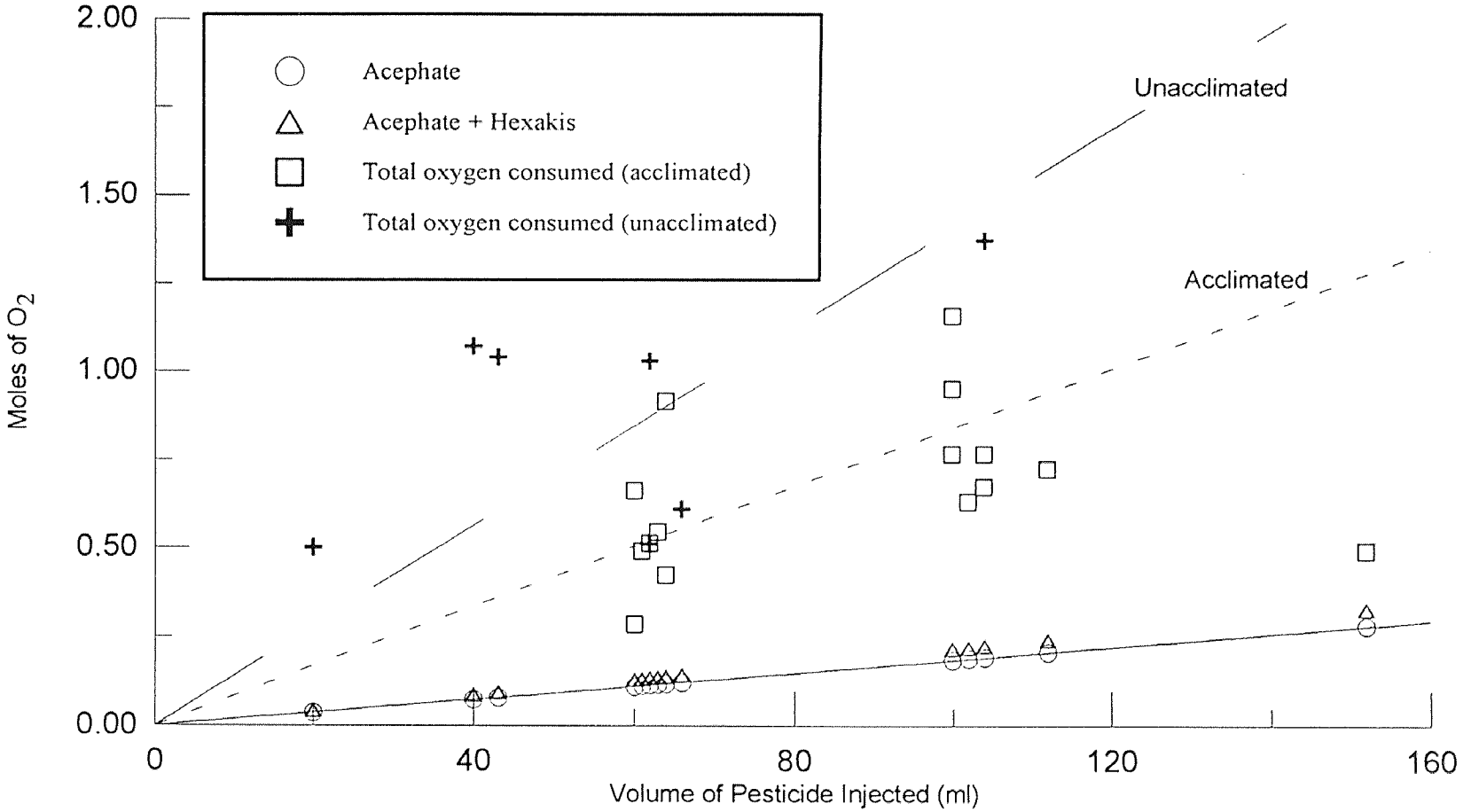

Figure 13. Oxygen Consumed for Pesticide Oxidation. A comparison between the total oxygen consumed and the theoretical oxygen required for oxidation of the active ingredients in the pesticide is presented. The theoretical oxygen requirements were determined by stoichiometry and mass balance.

requirements for the active ingredients were calculated based on stoichiometry and mass balance, taking into consideration only the fate of carbon, oxygen and hydrogen within the active ingredients. The exact fate of phosphorous, nitrogen, and sulfur complexes were not determined, and therefore were not taken into consideration in determining the oxygen required

5.7 Tin Analysis on the Bioreactor System

Tin was adsorbed onto the microorganisms within the first 5-10 minutes. This was in accordance with the effects observed by Gourdon, Rus, Bhende and Sofer [10] for cadmium. Tin samples were collected after 5 minutes of injection, at midpoint of the reaction and at the completion of oxidation, as determined by the DO readings. Tin was then analyzed on an atomic absorption spectrophotometer. The concentrations between the different sampling times showed very small changes in tin concentration.

The amount of tin added into the bioreactor system per injection was determined by mass balance. (Please refer to Appendix A for Sample Calculation). The results from the mass balance are shown in Figure 14. The amount of tin remaining in the reservoir water was analyzed and calculated taking into consideration the reservoir volume. The result from this analysis is shown in Figure 15. The efficiency of tin adsorption onto the bioreactor was determined and plotted in Figure 16. From Figure 16, tin adsorption or tin removal from the wastewater was about 90%.

The accumulation effects of tin adsorbed onto the bacteria were also analyzed. Figure 5 shows the tin concentration in the reservoir water as a function of cumulative

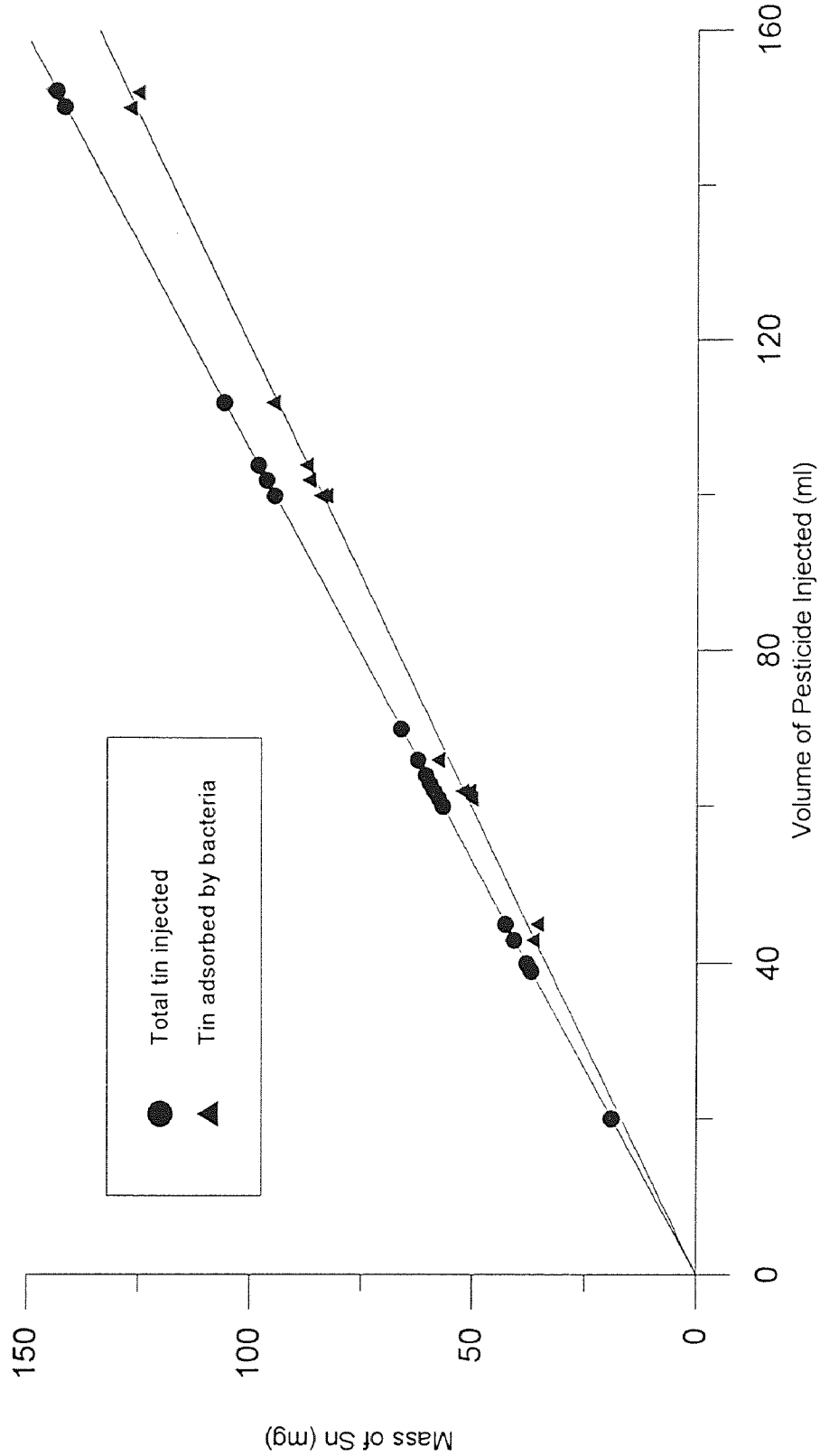

Figure 14. Tin Adsorbtion by Bacteria. The amount of tin injected into the bioreactor system, per injection of pesticide. The amount of tin adsorbed by the bacteria are compared. The mass of tin was determined by mass balance based upon experimental data. 90% Adsorption is observed.

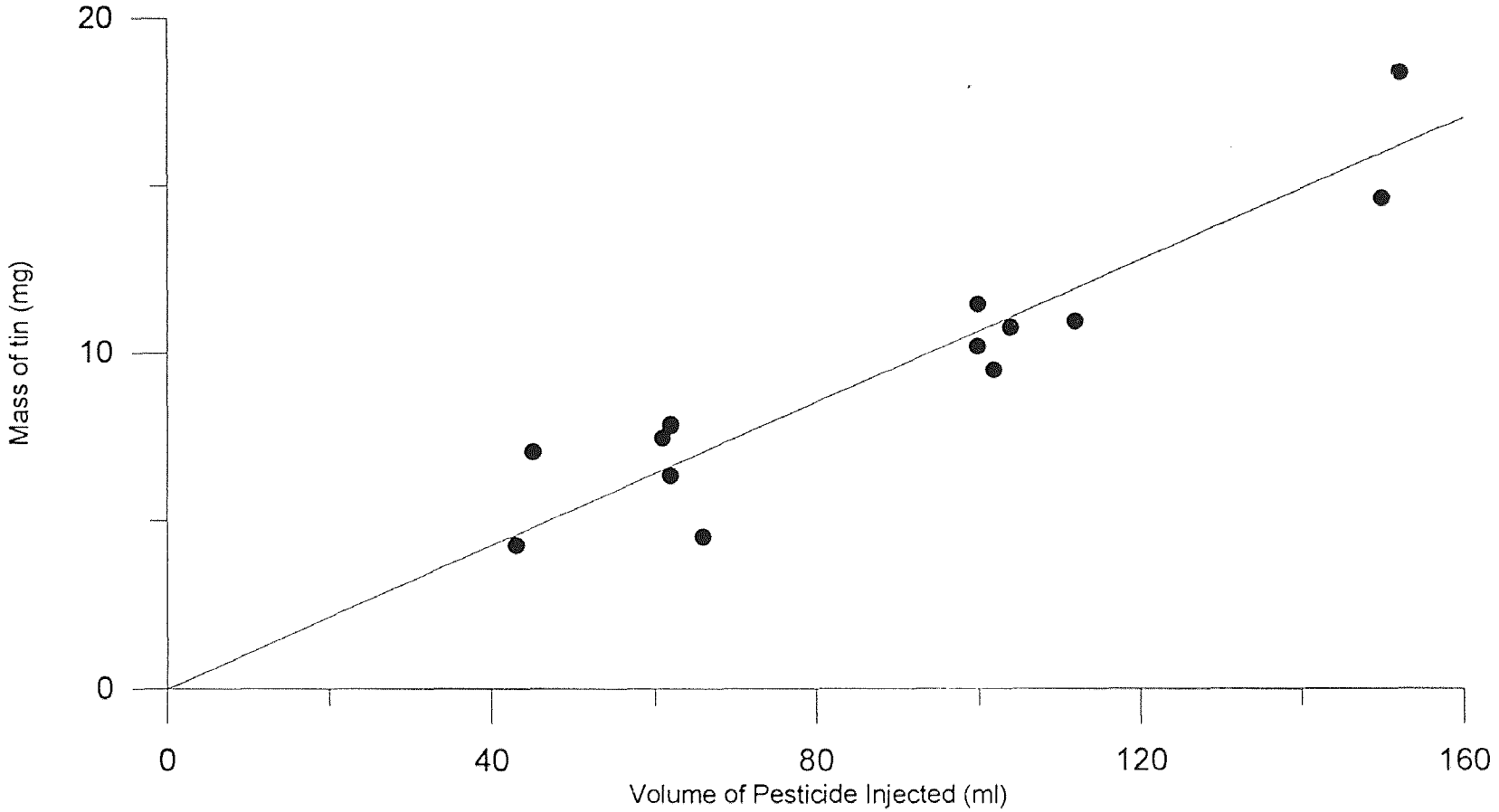

Figure 15. Residual Tin in Reservoir. The amount of tin present in the reservoir water after biodegradation of pesticide is shown. The mass of tin was determined by atomic absorption spectrophotometer and mass balance. This amount represents the less than 10% unadsorbed tin. Typical values of residual tin are in the ppb range.

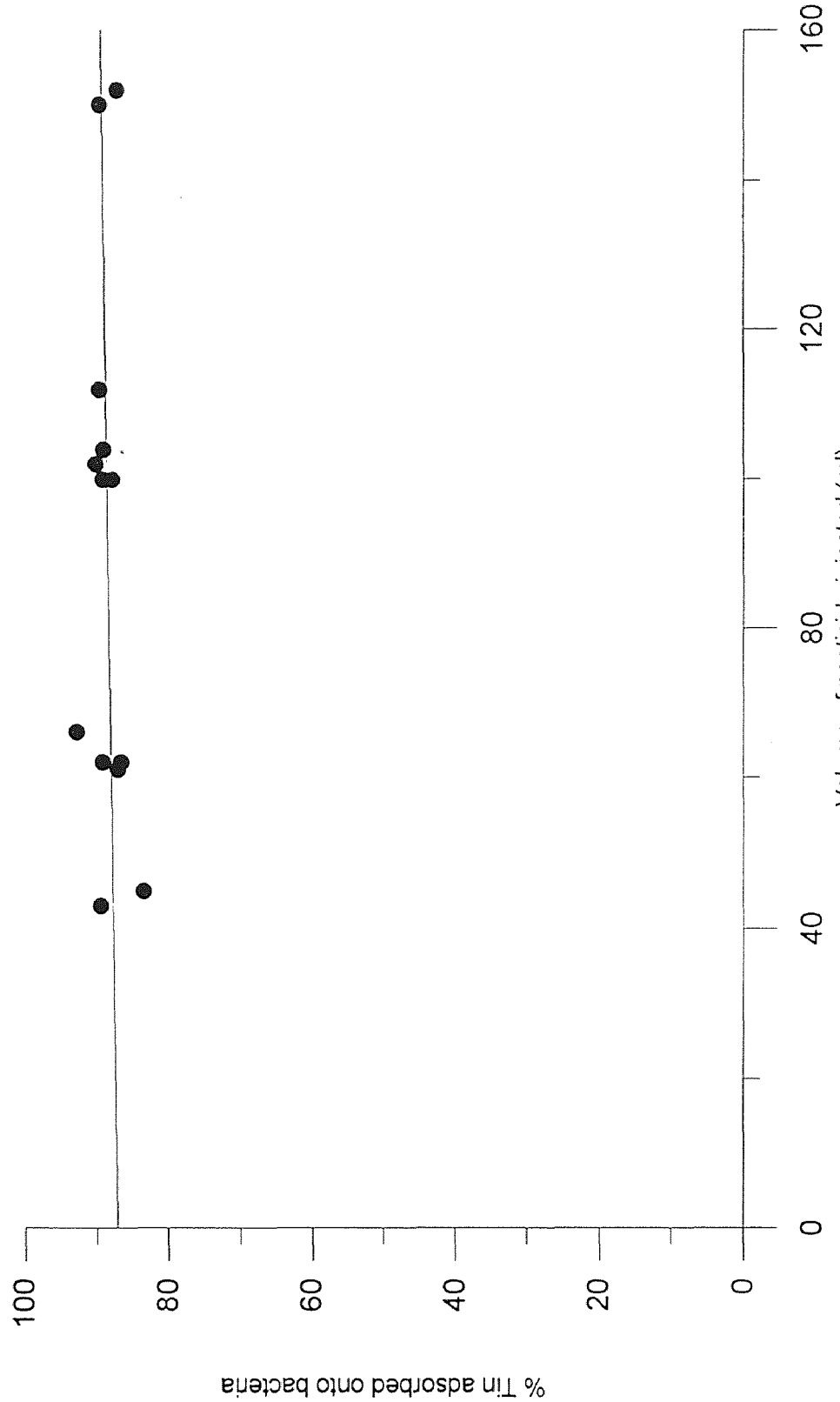

Figure 16. Percent Tin Removal. The effectiveness of tin removal from reservoir water by adsorption onto bacteria. The bacteria were immobilized onto a spirally wound polymeric support.

additions of Isotox®. A positive trend in tin concentration in the reservoir water is evident. However, based upon Figure 16, the amount of tin adsorbed is relatively constant at about 90%. A hypothesis for this behavior is that there may be a chemical equilibrium between the concentration adsorbed and the concentration of the surrounding medium, which in this case is the reservoir water. From Gourdon *et al.* [10], cadmium adsorption followed a Freundlich isotherm.

During tin analysis, three experimental intervals were used. Each interval comprised the cumulative additions of Isotox® before acid washing. At the completion of each acid wash, samples were collected to ensure that the reservoir was tin-free. Tap water samples were also collected to determine the background level of tin. Data collected from the tin-free reservoir and tap water both contained approximately 70 ppb. The corrected tin data for each injection was then determined by subtracting background tin concentration. A graphical representation of the corrected tin concentration in the reservoir for cumulative pesticide injection is shown in Figure 5. From Figure 5, a significant plateau is seen between 1500 to 1750 ml pesticide injected. The reservoir tin concentration did not change during this range. This would suggest that there is an optimum tin removal range. Therefore an optimum range of operation for removal of tin would be to keep the tin concentration in the reservoir between 250 to 300 ppb.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

This study has demonstrated that the effectiveness of organotin removal from a wastewater stream is about 90% removal using a spiral bioreactor. The maximum amount of tin that will adsorb onto bacteria is at least 1218 mg, after additions in excess of 1.6 liters of pure organotin pesticide. The actual maximum amount of tin is likely much higher. Heavy metal inhibition of substrate uptake was not observed; the time required for reaction to reach completion was not affected by tin poisoning. The relative reaction time needed ranged from 1250 - 2250 minutes, regardless of volume of pesticide injected.

A distinct DO pattern has been identified for Isotox®. The DO pattern consists of at least 4 dips during its oxidation reaction.

With the modified aeration set-up, air requirement has decreased from 4.5 L/min to 220 ml/min. A few experimental trials were performed using 150 ml/min air flow rate. However, it was difficult to control low air flow rate due to the pressure from the centrifugal pump causing a back flow of water into the air delivery tube. It is therefore possible that air flow requirement is even less than was used in these experiments.

The main recommendation for future study is to pursue the analysis and identification of the 4 dips caused during bio-oxidation. It may be required to use a more sensitive and versatile analytical instrument as back-up, such as GC-MS, and ICP. During the completion of mineralizing Isotox®, there appear to be suspended solids in the reservoir water. These suspended solids were not further degraded, and appeared to be

yellowish, indicating the possibility of sulfur presence. Incorporating a method of separating the suspended solid from the solution would further refine the water treatment quality. One such set-up would be a microfiltration process in series with the bioreactor. A study on different possible set-ups using different available portable separation techniques in various combinations with the bioreactor to determine optimal design configurations would be useful in refining the water quality.

Further studies of the bioreactor and biodegradability of other pesticides should also be investigated. This will allow a basis for comparative studies of pesticide biodegradability.

APPENDIX

Sample Calculations

1. Determination of molecular weight % of individual atoms in vendex:

Molecular formula of vendex:

$C_{60}H_{78}OSn_2$

Atomic weight:

C = 12.011

H = 1.0079

O = 15.9994

Sn = 118.69

Calculation of molecular weight of vendex:

MW_{Hex} = 60 * (C) + 78 * (H) +1 * (O) + 2 * (Sn)

MW_{Hex} = 60 * (12.011) + 78 * (1.0079) +1 * (15.9994) + 2 * (118.69)

MW_{Hex} = 1052.6556 g/g-mole

$wt\ \% = n * aw / MW_{Hex} * 100\%$

where

n = number of atom of interest in the compound

aw = atomic weight of atom of interest

MW_{Hex} = molecular weight of compound

For carbon, C:

$wt\%_C = 60 * 12.011 / 1052.6556 * 100\%$

$= 68.461\%$

Table A-1. Elemental Information of Vendex $C_{60}H_{78}OSn_2$.

Atom	Number of atom/compound, n	Atomic Weight	Weight Percent, %
Carbon	60	12.011	68.46
Hydrogen	78	1.0079	7.47
Oxygen	1	15.9994	1.52
Tin	2	118.69	22.55

2. Determining the amount of oxygen required for oxidation of Isotox®:

$$n_{Ox,isx} = (N_{std})\ (DO_{std} / DO_{inj})\ (wt_{inj} / wt_{std})$$

where

$n_{Ox,isx}$ = moles O_2 required to oxidize Isotox®, g-moles

N_{std} = moles of oxygen required to oxidize phenol, g-moles

DO_{std} = weight of baseline paper cut-out for phenol, g-paper

DO_{inj} = weight of the baseline paper cut-out for injection, g-paper

wt_{inj} = weight of the DO curve paper cut-out for injection, g-paper

wt_{std} = weight of the DO curve paper cut-out for phenol, g-paper

Table A-2: Dissolved Oxygen Parameters Based on Phenol Injections.

	Acclimated System	Unacclimated System
Amount phenol injected (g)	5.34	4.94
N_{std} calculated (g-moles)	0.34004	0.31494
DO_{std} (g-paper)	0.4225	0.9319
wt_{std} (g-paper)	0.0410	0.0417

For the injection done on 12/15/94: (an unacclimated system)

$n_{Ox,isx}$ = (0.31494 g-mole) (0.9319g-paper / 1.5707g-paper)

(0.1989 g-paper/ 0.0417 g-paper)

= 0.8913 g-mole O_2

3. Determining the amount of oxygen required for oxidation of acephate:

Oxidation reaction, excluding the fate of N, P and S.

$$C_4H_{10}O_3NPS + 5\ O_2 \rightarrow 4\ CO_2 + 5\ H_2O$$

$n_{Ox,acep} = V_{isx}\ \rho_{isx}\ (Wt\%_{Acep}) / MW_{acep} * SR$

where

$n_{Ox,acep}$ = number of moles required to oxidize acephate, g-moles

V_{isx} = volume of Isotox® injected, ml

MW_{acep} = molecular weight of acephate, 183.16 g / g-mole

$Wt\%_{Acep}$ = weight percent of acephate in Isotox®, 8.0%

ρ_{isx} = density of Isotox®, 0.837 g/ml

SR = stoichiometric ratio, 5

For 11/17/94 injection of 45 ml Isotox®:

$n_{Ox,acep}$ = (45 ml) (0.837 g/ml) (0.08) / (183.16) * (5)

= 0.08226 g-moles of O_2

4. Determining the mass of tin per injection:

$$M_{Sn} = (Wt\%_{Hex})\,(V_{isx})\,(\rho_{isx})\,(Wt\%_{Sn})$$

where

M_{Sn} = Mass of tin, mg

$(Wt\%_{Hex})$ = Weight percent of vendex in Isotox®, 0.5%

$Wt\%_{Sn}$ = Weight percent of tin in vendex, 22.55%

For 11/17/94 injection of 45 ml of Isotox®:

M_{Sn} = (0.005) (45 ml) (0.837 g/ml) (0.2255) (1000 mg/g)

= 42.49 mg Sn

5. Determining tin concentration in reservoir after mixing:

Assumptions:

1) Assume all of the tin from the injection are in solution (no adsorbance onto bacteria).
2) Concentration distribution of tin is uniform.

$Con_{Sn} = M_{Sn} / V_{res}$

where

Con_{Sn} = Concentration of tin, mg/L

M_{Sn} = Mass of tin, mg

V_{res} = Volume of reservoir water, L

For 11/17/94 injection of 45 ml of Isotox®:

Con_{Sn} = (42.49 mg) / (39.87 L)

= 1.066 mg/L

6. Determining the mass of Sn detected by AA in reservoir water:

$M_{Sn,AA} = Con_{Sn,AA} * V_{res}$

where

$M_{Sn,AA}$ = mass of tin in reservoir water determined by AA, mg

$Con_{Sn,AA}$ = concentration of tin determined by AA, ppb

For 11/17/94 injection

M_{Sn} = (163.8375 ppb) (42.99L)

= 7.0434 mg

7. Determining amount of tin adsorbed onto bacteria:

$$\Delta M_{Sn} = M_{Sn,inj} - M_{Sn,AA}$$

where

ΔM_{Sn} = tin mass adsorbed by bacteria, mg

$M_{Sn,inj}$ = mass of tin injected into the system, mg

For 11/17/94 injection:

ΔM_{Sn} = 42.4858 mg - 7.0434 mg

= 35.4424 mg

8. Determining the percent of tin adsorbed onto bacteria per injection:

$$\%(ads) = \Delta M_{Sn} / M_{Sn,inj} * 100\%$$

For 11/17/94 injection:

%(ads) = 35.4424 mg / 42.4858 * 100%

= 83.42 %

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