Capacity of Bark Mulch to Deoxygenate Groundwaters Remediated Anaerobically in Biobarrier Walls

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ABSTRACT

Chlorinated solvents, such as tetrachloroethene (PCE) and trichloroethene (TCE), are regarded as major contaminants in groundwater, and multiple technologies have been developed to remediate them. Among these, bioremediation via anaerobic reductive dechlorination has received the most attention. One method for applying it to dilute contaminant plumes is via installation of a Permeable Reactive Barrier (PRB), a permeable wall, perpendicular to groundwater flow, within which the anaerobic reductive dechlorination process occurs to remediate chloroethenes reaching it. The PRB is constructed of a slowly degradable, particulate organic material such as pine-bark mulch.

The reductive dechlorination process requires a highly reducing environment (anaerobic condition). Consequently, the reactive barrier must be able to remove dissolved oxygen from the plume as the groundwater reaches the wall, and establish a suitable anaerobic condition within the wall to support the desired, reductive processes. In fact, the amount of electron donor needed to remove several milligrams per liter of oxygen is going to be far greater than the amount of electron donor needed to support the reductive dechlorination of ppb levels of chlorinated ethenes.

The overall purpose of this research was to explore the capacity of mulch (pine bark) to serve as electron donor to sustain aerobic microbes in the removal of oxygen from groundwaters flowing to a biobarrier wall intended for anaerobic reductive dechlorination.

Bottle studies were conducted over 110 days with different amounts of mulch, and under different conditions of inoculation, nutrient addition, and liquid exchange (to simulate leaching). The following conclusions were reached: (1) Inoculation with KB1®,

a *Dehalococcoides*-containing mixed culture commonly used for bioaugmentation at sites intended for anaerobic bioremediation, made no difference in initiating O_2 consumption in mulch; (2) the oxygen-consuming capacity of mulch was estimated to be 31.2 mg O_2 per gram (dry wt.); (3) liquid exchange had no effect on cumulative O_2 consumption, suggesting that leaching of electron donor was not significant; and (4) supplementation with nitrogen (as ammonium) and phosphorus (as orthophosphate) had a mixed – and therefore uncertain -- effect on rate of oxygen uptake by pine bark mulch.

BIOGRAPHICAL SKETCH

At five o'clock June 8, 2008, Chinese annual college entrance examination was over as a fierce battle. Standing on the turn point of my life, I started to consider the direction of my future. It was a real hard work for me, as I had a large range of interest. To be a basketball star, to be a famous artist, to be a professional in environmental protection, or not to be, that was a question. Finally, smelling the stink of drainage ditch, and thinking of the swaying plastic bags in tree branches, my family and I agreed to choose environmental engineering as my perspective career in a month-long brainstorm meeting.

Filled with excitement and enthusiasm, I began to concentrate my undergraduate study on environmental engineering at Beijing University of Chemical Technology, which was an academic milieu replete with the different academic thoughts, the positive atmosphere for research, and the active international academic exchanges. Over the four academic years, I got my bachelor degree there. Along with the accumulation of knowledge, I gradually laid a solid foundation for further study and the research project.

Although the undergraduate study endowed me a great ability to conduct the exploration in Environmental Engineering, I was still far away from being a qualified professional, who should be able to deal with the issues our society faces. Therefore, I am determined to study further.

Fortunately, Cornell University embraced me and let me pursue my master's degree here. What is more, I feel so lucky to be supervised under Prof. James M. Gossett as one of his last two graduate students. Not only his professional and accomplished

knowledge in chloroethene bioremediation, but I also learn the correct attitude as a researcher from him, which is priceless.

Now, I'm getting closer and closer to graduation, and my next goal will be getting a job in America, relating to Environmental Engineering. Therefore, I can fully apply my knowledge and engineering techniques within the most advanced and mature conditions.

To my family

Runtian

ACKNOWLEDGEMENT

First, I want to express my deepest gratitude to Professor James M. Gossett. Without his patient guidance and caring, I would never have been able to finish my dissertation so efficiently and successfully. As a Chinese student who never has studied abroad, I often made mistakes in written and oral English, especially during my first semester. However, he never complained about that over these two years, and he provided an excellent atmosphere for doing research. Whenever I had questions, whether about my research or non-academic issues, he was always glad to help me.

What is more important, he is not only my major advisor in academic matters, but also my life mentor. I can see, from his example, the correct attitude towards work, and also, this was the first time I realized what a good teacher should be.

Additionally, I'm grateful to Professor Ruth Richardson. She always came up with valuable recommendations for my research in our group meetings, and I've learned a lot about bio-marker and molecular methods from her.

Also, I want to give my thanks to Yitian Sun, who is my lab partner. We started out on the same topic, and although we later travelled different research paths, we still often discussed together our respective research projects.

Finally, I would like to thank my parents. They always supported and encouraged me with their best wishes, cheering me up, and stood by me through the good times and bad.

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LIST OF ABBREVIATIONS

PCE	**Tetrachloroethene**
TCE	**Trichloroethene**
PRB	**Permeable Reactive Barrier**
VOCs	**Volatile Organic Chemicals**
MCL	**Maximum Contaminant Level**
DCE	**1,2-Dichloroethene**
VC	**Vinyl Chloride**
EPA	**Environmental Protection Agency**
cDCE	***cis*-1, 2- Dichloroethene**
tDCE	***trans*-1, 2- Dichloroethene**
PVC	**Poly Vinyl Chloride**
GAC	**Granular Activated Carbon**
SVE	**Soil Vapor Extraction**
AFB	**Air Force Base**
DNAPL	**Dense Non-Aqueous Phase Liquid**
ETH	**Ethene**
DHC	***Dehalococcoides***
TOC	**Total Organic Carbon**

CHAPTER 1 INTRODUCTION

1.1 Context

Chlorinated solvents, such as tetrachloroethene (PCE) and trichloroethene (TCE), are commonly encountered in preparations used for a wide variety of commercial and industrial purposes, including degreasers, cleaning solutions, paint thinners, pesticides, resins, glues, and a host of other mixing and thinning solutions.

In the past, chlorinated solvents were often directly dumped into landfills, stored in disposal tanks that leaked, or accidently spilled on the ground.[15] Unfortunately, excessive exposure to these untreated pollutions may lead to a series of symptoms, such as nausea, unconsciousness or even cancer. As a consequence, multiple technologies were developed to remediate chlorinated-solvent contamination in subsurface soils and groundwaters, including in situ and ex situ alternatives.

With focus on preventing further migration of dissolved contaminants, one of the cost-effective and sustainable methods for containment, attracting increased attention, is the permeable biological reactive wall, or permeable reactive barrier (PRB). An in situ biological reactive wall consists of a porous barrier, perpendicular to the groundwater flow, in which microorganisms within the wall material are the functional units to remove contaminants (chlorinated solvents in this thesis).

The common biological technology to address chlorinated ethene contaminants is anaerobic reductive dechlorination, a reductive process requiring a highly reducing environment (anaerobic condition). Thus, a PRB must be designed to supply sufficient organic electron donor, not only to reduce the targeted chlorinated ethenes, but also to

reduce competing electron acceptors incident to the PRB (e.g., oxygen and nitrate). One way to do this is to construct the PRB largely from an organic particulate source, such as pine-bark mulch. The reactive barrier must be able to remove dissolved oxygen from the plume as groundwater reaches the wall, and establish a suitable anaerobic condition further inside the wall to support the desired, reductive processes.

In many instances, the amount of electron donor needed to remove several milligrams per liter of oxygen that accompanies the chloroethene contaminant is going to be far greater than the amount of electron donor needed to support the reductive dechlorination of ppb levels of chlorinated ethenes. Thus, the need for sustained removal of oxygen can ultimately be what limits the useful life of a reactive biobarrier wall. Therefore, the sustainability of pine-bark mulch to consume oxygen under different conditions of inoculation, nutrient amendment, and liquid exchange (simulating possible leaching of donor) was investigated in experiments performed in serum bottles over a 110-day period.

1.2 Objectives

The overall purpose of the experiments described here was to explore the capacity of mulch (pine bark) to serve as electron donor to sustain aerobic microbes in the removal of oxygen from groundwaters flowing to a biobarrier wall intended for anaerobic reductive dechlorination.

Specific questions addressed were as follows: (1) what is the ultimate electron-donor capacity (BOD_L) per gram (dry weight) mulch; (2) is inoculation necessary (or even helpful), or is there a sufficient population of indigenous, oxygen-utilizing organisms in mulch; (3) do additions of nitrogen and phosphorus (N, P) affect the rate or

extent of oxygen consumption, or does mulch contain sufficient amounts of these important nutrients; and (4) does movement of water through mulch cause removal (leaching) of significant electron-donor materials?

CHAPTER 2 BACKGROUND

2.1 Chlorinated Ethenes

2.1.1 Types, Uses and Health Concerns

Chlorinated ethenes play important roles in modern society. [1, 5] These chemical compounds are ethenes in which some or all hydrogens are substituted by chlorines; examples include tetrachloroethene (PCE) and trichloroethene (TCE). [1] Since WWII, chlorinated solvents are commonly encountered in preparations used for a wide variety of commercial and industrial purposes, including degreasers, cleaning solutions, paint thinners, pesticides, resins, glues, and a host of other mixing and thinning solutions. These uses originate from the chlorine-containing chemical structures of the chloroethenes, which make them dissolve organic materials like fats and greases efficiently and serve as raw materials or intermediates in producing other chemicals.[1] Among the compounds in this family, PCE and TCE are among the most frequently detected volatile organic chemicals (VOCs) in ground water.[3-5] While monitoring the reductive dehalogenation of PCE and TCE , 1,2-dichloroethene (DCE) and vinyl chloride (VC) are commonly observed simultaneously or downstream, as they are intermediate and final products in a biotransformation process between PCE and ethene.[6]

PCE is a non-flammable, colorless heavy liquid with a mild odor. It is miscible with most organic solvents and exhibits high solvency for most other organic compounds. It is soluble only to about 150 mg/L in water, which means it can often be found as a dense non-aqueous phase liquid in soil and groundwater, as well as in dissolved form in adjacent groundwater. [7, 8] As mentioned above, PCE is widely used as a dry-cleaning solvent and has almost replaced all other solvents in this field because of its non-

flammable and stable properties. Because of its high stability, PCE is often added to TCE for metal degreasing. [8] Exposure to PCE in air at a concentration above 200 ppm may cause depression of the central nervous system. Different from other chlorinated ethenes, PCE does not cause liver and kidney injury, even at an excessive exposure. When the PCE content rises to a high level, people who inhale it may experience nausea and gastrointestinal upset, in addition to the anesthesia and incoordination. [9] The maximum contaminant level (MCL) for PCE in groundwaters is 5 μg/L.[10]

TCE is also a dense liquid, but with a sweet, chloroform-like odor. [10] The major usage of TCE is as a solvent for degreasing in the metal industry. Besides, it also has been used as an extraction solvent in solvent formulations for rubbers and elastomers and as an anesthetic. [10] The exposure to TCE should be controlled, as a result of the typical solvent property of TCE on the skin and eyes. However, the skin-exposure problem is not as serious when comparing to the inhalation problems that TCE causes. In fact, people inhaling TCE may experience a series of symptoms, like visual disturbances, mental confusion and fatigue; sometimes nausea and vomiting are observed at higher levels. [10] What's more, TCE may trigger liver injury or cancer to rats or other laboratory animals. The U.S. Environmental Protection Agency (EPA) recently released its Toxicological Review of Trichloroethylene, which represents the first time that TCE has been classified as a human carcinogen, regardless of the route of exposure. [11] The MCL for TCE in groundwaters is 5 μg/L.[10]

1,2-Dichloroethenes (*cis* and *trans*) often exist as an isomeric mixture during the production of chlorinated hydrocarbons, via side-reactions. [8] Of the two isomers, *cis*-1, 2-DCE (cDCE) and *trans*-1, 2-DCE (tDCE), the *trans* isomer is more reactive than the *cis*

isomer, and its isomerization can be achieved at high temperature and in the presence of bromine or alumina. [8] The industrial product always contains both isomers with a boiling range of 45– 60 ◦ C, while they can be separated by fractional distillation if needed. Most toxicological evaluations for DCE have been on mixed isomers (*cis*- and *trans*-), which indicated a rather low toxicity to animals from inhalation. Anesthesia was the only obvious symptom, in contrast to other chlorinated ethenes. [12] The MCLs for cDCE and tDCE in groundwaters are 70 µg/L and 100 µg/L, respectively.[10]

Lastly, vinyl chloride is a colorless flammable gas with mildly sweet odor and is not stable at high temperatures. [13, 14] However, if oxygen and air are excluded, dry and purified VC is extremely stable and noncorrosive. [8] VC is well known for its widespread polymeric product, Poly Vinyl Chloride (PVC), produced from a series of polymerization reactions based on its vinylic double bond. As all the other chlorinated ethenes, when inhaled at an excessive concentration, VC can cause anesthetic effects. At even higher concentration, cancers and deaths have been reported from massive exposures. [8] The maximum MCL for VC in groundwaters is 2 µg/L.[10]

2.1.2 Environmental Contamination

In the past, used chlorinated solvents were directly dumped into landfills, stored in disposal tanks that often leaked, or accidently spilled on the ground. [15] And with long-term storage of chlorinated ethenes, decomposition can occur if the stored material is infiltrated with water or free acids, with insufficient stabilizers; this can result in a contamination problem because the decomposed product can corrode the storage tanks. [16]

Indiscriminate disposal and leakage became major causes of soil and groundwater contamination, which had not generally been mentioned before 1961. That's when a Symposium on "Ground Water Contamination" was sponsored by the U.S. Public Health Service. [17,18] If discharged onto soil, the movement and fate of chlorinated ethenes are determined by their physical, chemical, and biological properties and by site hydrogeological characteristics.[17] Among multiple layers in soil, neat chlorinated solvents may move downward through sand and particles because their high densities facilitate their movement through the pores between layers, even easier than water. However, when they meet a fine clay layer, they either pool on top of clay, or seek a downward passage around a clay layer. Either fate will have a strong influence on the shape of the contamination plume, which is the key consideration in determining effective modes of remediation. [19]

2.2 Cleanup Technologies for Chlorinated Ethenes

2.2.1 Common Types of Cleanup Technologies

There are multiple technologies available to remediate chlorinated-solvent contamination, including in situ and ex situ alternatives. Many of the more economical remediation technologies are of comparatively recent development. Brief descriptions of some typical treatment methodologies are presented below. [20]

Conventional Pump-and-Treat	Flushing
Ex situ Air Stripping	Cosolvent Flushing
Ex situ Activated Carbon Adsorption	Surfactant Flushing
Ex situ Catalytic Oxidation	
Air Injection	In Situ Thermal Technologies
Vapor Extraction	Hot Fluid Injection–Air, Water, Steam
Bioventing	Electrical Resistive Heating
Air Sparging	Thermal Conductive Heating
Biodegradation	In Situ Chemical Processes
Aerobic Cometabolism	Oxidative Chemical Processes
Anaerobic Reductive Dehalogenation	Reductive Chemical Processes

Table cited from [17] Groundwater Contamination by Chlorinated Solvents

2.2.1.1 Conventional Pump-and-Treat

The initial approach applied to remediation of chlorinated solvents was to remove contaminated soil and subsurface solids from the high-concentration, "source" zone, then pump the contaminated groundwater up to the surface for subsequent treatment (pump-and-treat). Between 1982 and 1992, conventional pump-and-treat systems represented 73% of the cleanup technologies, and they may still represent the most widely used method to remediate contaminated groundwater nowadays. [19] Even at sites where other technologies dominate, there remains a role for pump-and-treat for plume containment. Few sites have only one remediation technology employed.

Generally, conventional pump-and-treat used for chlorinated solvents involves extracting contaminated water from an aquifer and replacing it with clean water. The water source might be adjacent surface or ground water, or the ex situ treated water reinjected. In some cases, extracted water is treated and discharged to existing sewers or spread on soil.

The most common, ex situ treatment for extracted groundwater has been air-stripping, followed by adsorption from the air stream on Granular Activated Carbon

(GAC). [17] However, experience has demonstrated that it is difficult for pump-and-treat systems to achieve cleanup goals, due to the complexity of geological settings and slow rates of contaminant desorption processes from the aquifer solids matrix. [20] Typically, groundwater contaminant concentrations rebound after a pump-and-treat system is turned off. Furthermore, very large extractions are required to contain even small plumes of contaminants.

2.2.1.2 Air-Injection Systems

Air-Injection approaches are technically based on the physical properties (i.e., volatility) of chlorinated solvents and their intermediate degradation products since they can be readily removed by air stripping.

2.2.1.2.1 Vapor Extraction and Bioventing

Soil Vapor Extraction (SVE) was initially applied to remove chlorinated solvents from the vadose zone where air movement through soil was induced by blower or vacuum at a sealed wellhead. [21] Sometimes, SVE is operated in conjunction with groundwater extraction. The purpose of groundwater extraction here is to lower the groundwater surface so that the contaminants will be exposed to passing air through the vadose zone, and then removed by the SVE system.

Bioventing is a similar method to SVE except that the air is introduced at controlled rates (and with addition of moisture and nutrients, where necessary) that do not result in escape of volatiles to the atmosphere, but rather promote aerobic oxidation of the stripped VOCs before they would otherwise reach the atmosphere. [22] Wilson and Ward became the first to propose this process to remediate contaminated hydrocarbons in the vadose zone. [23] This method requires that the volatile contaminants be readily

biodegradable aerobically, and hence is more applicable to hydrocarbons than to chlorinated solvents. However, VC might be remediated this way. Higher-chlorinated ethenes such as cDCE or TCE require addition of cosubstrates such as toluene or methane for aerobic biodegradation, complicating the application of bioventing. PCE is not aerobically biodegradable, and therefore bioventing is inapplicable to its remediation.

2.2.1.2.2 Air Sparging

Air sparging is another in situ method, taking advantage of the volatility of these compounds. [24] In this method, air is initially compressed, then injected into an aquifer formation or specially designed wells. After that, the rising air forms a rising cone pattern, carrying and transferring adsorbed contaminant to the vadose zone, followed by SVE for ex situ treatment. Another scheme uses two parallel wells, one located in groundwater (the lower well), moving through the aquifer upwards to the higher layer in order to remove adsorbed contaminants, where the upper well is set to collect them. [25] In-well air sparging (also referred to as in-well vapor stripping) is generally designed with a lower screen located in the aquifer and an upper screen located in the vadose zone. In an earlier design, a pump was also applied to draw water through the lower screen into the well followed by the transportation in well. [26] The transported groundwater was brought to a zone where air stripping occurred, then the treated water passed through the upper screen and into the vadose zone where it trickled back down to the groundwater. Later designs could achieve both water circulation through the well in an upward-movement and air stripping of contaminants from the upward water at the same time by an air-life pump. [27] Furthermore, the separated contaminated air is commonly cleaned with GAC.

2.2.1.3 Biodegradation

Although the discovery of abiotic and biological transformations of chlorinated solvents in the early 1980s helped researchers understand the origins of the many transformation products of chlorinated products in groundwater, little attention was paid to using the knowledge for bioremediation of contaminated sites since the transformation products (e.g., VC) were even more hazardous than the original contaminants. [17]

2.2.1.3.1 Aerobic Degradation

In 1985, John and Barbara Wilson demonstrated that TCE could be co-metabolically degraded aerobically. [28] In their research, methane was added as the primary substrate to sustain aerobic oxidation of the co-substrate (TCE). The initial step in TCE oxidation was shown to be catalyzed by methane monooxygenase, an enzyme known to initiate methane oxidation, forming TCE epoxide, an unstable chemical that degraded to other smaller chemical compounds that were either further degraded abiotically, or could be utilized as an energy source by microorganisms present in soil. [17] Since it was perceived to be better to degrade TCE aerobically all the way to non-harmful products, rather than simply transform it to other hazardous compounds (such as VC in biological reductive dechlorination), this process received a great deal of interest and potential evaluation as a groundwater remediation technology.

To evaluate the feasibility of using cometabolism to remediate field contamination, field pilot studies were conducted. [29, 30] Among these studies was a full-scale evaluation at Edwards Air Force Base (AFB), California, using dual recirculation wells for plume migration control. This study used a more efficient substrate, toluene, instead of methane to fulfill the metabolic substrate role, even though toluene is a known

toxic compound. [31] As a result, aerobic cometabolism was successfully applied to remove 97% of TCE from locations contaminated with 1,000 to 1,200 μg/L TCE. Contaminated groundwater with higher TCE concentrations would be difficult to treat because of the limited solubility of oxygen, which in turn affects the concentration of toluene that could be used in the process. [17] What's more, the contaminants that can be remediated effectively by the cometabolism are restricted to TCE and lesser-chlorinated intermediate degradation products (DCEs and VC).

2.2.1.3.2 Anaerobic Reductive Dehalogenation

PCE (and the less-chlorinated ethenes) can be transformed to nontoxic ethene (ETH) through reductive dechlorination processes. [17, 33-35, 46-48] Under anaerobic conditions, PCE can be microbiologically transformed by sequential reductive dechlorination processes to less-chlorinated compounds (TCE, DCEs, VC, ETH) with a supply of sufficient electron donors. [17, 39, 49] At many sites, the conversion of VC to ETH is the rate-limiting (or at least the most problematic) step, which limits the whole remedial process. [32] The dechlorination reactions are mediated by several microbial types, chiefly those called *Dehalococcoides* (DHC).

Anaerobic bioremediation had not been considered as a viable alternative to aerobic cometabolism because the reductive dechlorination process was initially reported to stop at VC, a known carcinogen. However, in 1989, Freedman and Gossett reported the complete, reductive dechlorination of PCE to non-toxic ethene, renewing interest in this once-discarded, anaerobic technology. [32] Consequently, natural and enhanced attenuation of chlorinated solvents through anaerobic biological processes became an alternative remediation approach, by which all chlorinated solvents could be transformed

anaerobically with relatively higher concentration limits. (i.e., concentration even higher than 100 mg/L could readily be biodegraded). [17, 33] Since high concentrations of chlorinated solvents no longer presented a problem, reductive dehalogenation drew much attention to be developed as a sustainable treatment technology, giving hope to enhance the dissolution and hence to reduce the persistence of dense non-aqueous phase liquid (DNAPL) sources of plume contamination.

Further research into biological reductive dechlorination by the first isolated organism capable of reducing PCE to cDCE (*Dehalobacter restrictus*) showed that the mediating microorganisms used higher-chlorinated ethenes (PCE, TCE) as electron acceptors, with H_2 as electron donor, in a redox process that served as a source of energy for organism growth. [34] However, these isolates were not capable of complete dechlorination to ethene, but stopped at cDCE. In 1997, an organism called *Dehalococcoides ethenogenes* (now *Dehalococcoides mccartyi)* was isolated, using H_2 to convert PCE and TCE to ethene, coupling each step of the reduction to growth, except for the last step of VC's transformation to ethene, which was carried out cometabolically. [35] Later-isolated strains of closely related organisms were found to couple growth to the last step (VC to ethene). [36, 37] In field treatments, *Dehalococcoides* were not always present at a specific site, and this deficiency could be remedied through bioaugmentation – the use of mixed laboratory cultures containing *Dehalococcoides* species, inoculated into the contaminated site. [38]

In reductive dehalogenation, the chlorinated compounds are used as electron acceptors, which means an electron donor and carbon source are required as a part of the biodegradation process. Acetate is the carbon source directly used by *Dehalococcoides*

and *Dehalobacter*. While molecular hydrogen is the electron donor directly used by *Dehalococcoides* and *Dehalobacter*, it is generally impractical to deliver hydrogen to a remediation process due to its low solubility and mass transfer difficulties. Soluble organic substrates can satisfy the carbon and electron donor requirements because under anaerobic conditions, most organic substrates are fermented to acetate and hydrogen. In "enhanced" anaerobic bioremediation, a variety of potent organic substrates, such as lactate, butyrate, ethanol, methanol, molasses or vegetable oils are applied. In "natural attenuation" of chlorinated solvents in aquifers by reductive dechlorination, organic substances either native (e.g., humics) or co-contaminating (e.g., hydrocarbons such as benzene, ethylbenzene, toluene, or xylenes) can provide the necessary carbon and hydrogen electron donor. However, in many instances these native or contaminating sources prove incapable of long-term sustenance of the reductive process, resulting in incomplete remediation. [17]

2.2.2 Permeable Reactive Barrier

At many PCE- or TCE-contaminated sites, the parent contaminant persists as a separate liquid phase, (referred to as DNAPL), which commonly results in a contaminated plume of groundwater downgradient from the source zone, fed by the slow dissolution of the source. [39,40] It's often difficult to locate and remove the source chlorinated solvents; therefore, an available plume containment methodology is necessary, one that can be sustained over a very long period.

With focus on preventing further migration of dissolved contaminants, one of the cost-effective and sustainable methods for containment, attracting increased attention, is the permeable biological reactive wall. Compared with other traditional treatments (e.g.

pump-and-treat or air sparging), permeable reactive walls have several obvious advantages, including lower energy and maintenance costs, treatment in situ, no requirements for aboveground facilities, and no treated water reinjection.

As contaminated ground water moves through a permeable reactive wall, under either natural or induced hydraulic gradients, the biodegradation occurring in the barrier scavenges the contaminants transferred by plume, and the treated water emerges from the down-gradient side of the permeable wall. [41-44] In some cases, when the source itself has been removed or contained, reactive barriers can serve as a remedial technology, rather than merely as a containment technology. For example, if the plume has migrated beneath developed land it might be impractical to install extraction/injection wells, but it might be practical to intercept the plume at some point along the edge of development. If the regulatory decision-makers agree with a long time-scale for remediation, a reactive barrier would be appropriate.

An in situ biological reactive wall consists of a porous barrier, perpendicular to the groundwater plume, in which microorganisms within the wall material are the functional units to remove contaminants (chlorinated solvents in this thesis). Compared to active, hydrologic barriers, reactive walls commonly minimize the need for mechanical systems, [45] and they are also designed to provide required microbial growth medium (substrates) when installed. In theory, biological reactive walls would require less operation and maintenance compared to other bioremediation technologies.

The major applications of current reactive walls to treat groundwater contamination are not biological (e.g., use of zero-valent iron walls). However, the types of reactive walls, regardless of their treating mechanism, are relatively similar. Among

the configuration options, permeable reactive trenches and funnel-and-gate systems are the most common. [45]

Installation of a permeable reactive trench involves digging a trench below the depth of furthest contamination and back filling with permeable material (Figure 2.1).

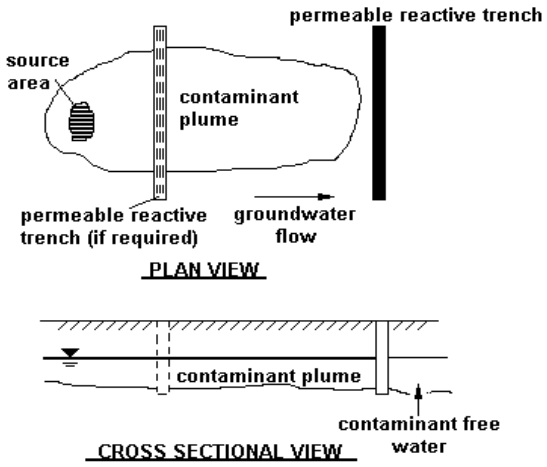

Figure 2.1 Plan and cross-sectional view of a permeable reactive trench. [45]

On the other hand, a funnel-and-gate system is more complex than a permeable reactive wall because it is constructed with impermeable walls on each side of permeable reactive gate, which helps direct the flow of contaminated groundwater through the gate in a stabilized pathway (Figure 2.2). Both systems require a supply of reactive materials.

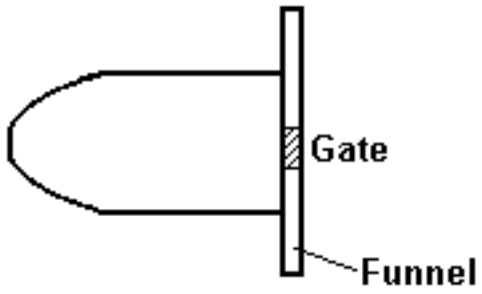

Figure 2.2 Simplistic funnel-and-gate system. [45]

The funnel-and-gate system can be applied to treat more than one contaminant-type, using multiple gates. A specific gate for each contaminant is placed in series (one

gate in front of the next) to remediate the plume. [45] Generally, there is an advantage of the funnel-and-gate system over the permeable reactive trench with respect to reactor media replacement; replacing a relatively "small" gate is easier and less expensive than replacing the entire trench media.

2.3 Analysis of Bark Mulch as Electron Donor

2.3.1 Selection of Organic Mulch

A highly reducing environment (anaerobic condition) is a basic requirement for the reductive dechlorination process, while many groundwaters contain at least small concentrations of dissolved oxygen. Consequently, a reactive barrier designed to operate via reductive dechlorination must be able to remove dissolved oxygen from the plume as the groundwater reaches the wall, and establish a suitable anaerobic condition within the wall to support the desired, reductive processes. In many instances, the amount of electron donor needed to remove several milligrams per liter of oxygen is going to be far greater than the amount of electron donor needed to support the reductive dechlorination of ppb levels of chlorinated ethenes. Thus, the need for sustained removal of oxygen can ultimately be what limits the useful life of a reactive biobarrier wall. At what point is the electron donor supplied with the barrier material effectively exhausted?

As the functional part of a permeable reactive barrier (PRB), choosing an available and cost-effective reactive material as electron donor is very important. Among the various reactive fill materials (e.g. iron, chitin, compost, or mulch) for permeable reactive walls, organic mulch is perhaps the alternative with the lowest cost [50]. Organic mulch consists of insoluble carbon biopolymers that are enzymatically hydrolyzed during decomposition to release aqueous total organic carbon (TOC), including more readily

fermentable molecules. [51] The released TOC can be utilized by aerobic microorganisms to reduce O_2, as well as electron donor to transform electrophilic contaminants via reductive pathways. [52]

Over the last decade, organic mulch PRBs, or biowalls, have received increased interest as a relatively inexpensive slow-release electron donor technology for addressing contaminated groundwater, especially for electrophilic compounds, such as chlorinated-solvent contaminants. [53] In fact, several mulch biowall projects are currently under way at several U.S. Department of Defense facilities. However, at the present time, there are limited guidelines available for the design or installation of mulch PRBs since only a few have been published in the technical literature. [51]

In general, the benefits of using mulch as electron donor in barrier walls are:

(1) Mulch is relatively inexpensive and readily available from local sources, where it is often regarded as a waste product. [54] (2) Mulch is rich in carbon, nitrogen and other chemical nutrients as well as utilizable bioenergy. [55] (3) Its relatively low rate of degradation is actually beneficial, in comparison to some other alternatives such as vegetable oil; there is expected to be only a slow decline in performance over a long period of running time, and almost no operation and maintenance required. With more rapidly available donors, such as vegetable oil, the donor is made available at a rate far in excess of what is needed to remove oxygen and reduce chloroethenes; this causes a great deal of methane production, which itself is a concern in groundwaters, since the massive bubble formation impedes hydraulic conductivity, and accumulation of methane in basements presents an explosion hazard.

2.3.2 Mulch Structure and Decomposition

Organic mulch is composed of lignocellulosic materials. These materials are lignin, a three-dimensional biopolymer with a high degree of aromaticity, and complex carbohydrates (i.e., polysaccharides). Among the multiple components of mulch, cellulose and xylans are the most prevalent, consisting of glucans and glycans, respectively. Note that glycans are also known as hemicelluloses. In wood-based mulches, the amount of each of these three components on a dry weight basis ranges from 15 to 40 percent for lignin, 35 to 55 percent for cellulose, and 5 to 25 percent for hemicellulose. [56]

The overall structure of a plant is strengthened primarily by its cell walls, in which cellulose and hemicelluloses are the dominant components. In addition, cell walls also contain lignin, which begins to deposit once the plant begins its maturation process [57]. As far as recalcitrant chemical structure is concerned, the high aromatic content of lignin offers the most formidable challenge. Lignin is degraded only by nonspecific extracellular oxidative enzymes found in soil [58], which are secreted most commonly by fungi (e.g., lignin peroxidase). Because of these constraints, lignin decomposition always requires aerobic conditions. [51]

Cellulose can form distinct crystalline structures that are somewhat recalcitrant to decomposition because these regions allow little or no penetration of water. The decomposition of cellulose can occur under both aerobic and anaerobic conditions [59]. Enzymes that decompose cellulose are broadly grouped under the name "cellulases." Using a combination of different cellulases, microbes can completely decompose cellulose. [58, 59].

Research has indicated [51] that the decomposition of mulch tends to occur in the following preferential order: hemicellulose > amorphous cellulose > crystalline cellulose >

lignin. The decomposition order in organic mulch materials is influenced not only by the arrangement of the carbon biopolymers in wood, but also by the actual chemical structure of the biopolymer.

CHAPTER 3 MATERIALS AND METHODS

3.1 Experimental Strategy & Program of Study

The overall purpose of the experiments described here was to explore the capacity of mulch (pine bark) to serve as electron donor to sustain aerobic microbes in the removal of oxygen from groundwaters flowing to a biobarrier wall intended for anaerobic reductive dechlorination. Specific questions addressed were as follows: (1) what is the ultimate electron-donor capacity (BOD_L) per gram (dry weight) mulch; (2) is inoculation necessary (or even helpful), or is there a sufficient population of indigenous, oxygen-utilizing organisms in mulch; (3) do additions of nitrogen and phosphorus (N, P) affect the rate or extent of oxygen consumption, or does mulch contain sufficient amounts of these important nutrients; and (4) does movement of water through mulch cause removal (leaching) of significant electron-donor materials?

A first experiment was conducted to explore the possible benefits of nutrient (N, P) supplementation and inoculation. Glass bottles (160-ml) with approximately 10 g of dry mulch were prepared with dechlorinated tap water, either with or without nutrient (N, P) supplementation. With each of these nutrient categories, two inoculation conditions were studied: bottles were either uninoculated or inoculated with KB-1® (SiREM, Guelph, Ontario, Canada), a *Dehalococcoides*-containing mixed culture widely used to biodegrade chlorinated ethenes. [60] Triplicates were prepared of each condition. To simulate potential loss of substrate through leaching, these bottles were also subjected to regular water exchange (a volume of water was removed and replaced with equivalent volume of either dechlorinated tap water or dechlorinated tap water supplemented with N, P, as appropriate).

After running this experiment for a few weeks, the performances appeared quite similar: the cumulative oxygen consumptions and rates were nearly identical under all conditions of inoculation or nutrient supplementation. It also became apparent that 10 g of mulch would require too extensive a period to assess accurately the depletion of electron donor. Furthermore, it was decided that some bottles should be run without regular exchange of water, for comparison to bottles in the first experiment.

Consequently, a second experiment was conducted, in which different amounts of mulch were put into bottles (approximately 1 g, 2 g, 5 g, or 10 g, dry weight). Based on the fact that KB1 inoculations didn't show any effect in Experiment 1, there were no inocula used in this second experiment. Half the bottles received N, P supplementation. Water exchange was not employed in any bottles, allowing some assessment of that factor by comparing results across the two experiments. Triplicates were employed for all conditions in Experiment 2.

With both experiments, whenever oxygen levels neared depletion, caps were opened to allow introduction of ambient air, bottles were recapped, and monitoring resumed.

For the entire experimental processes, gas chromatography was utilized to monitor oxygen levels in bottles, allowing calculation of the cumulative oxygen consumption in each.

3.2 Experimental Setup

3.2.1 Materials

3.2.1.1 Mulch

Pine-bark mulch was obtained from a local store, (Ithaca Agway). This mulch had a rich brown color and was made from 100% southern pine bark. It was stored in thermostatic chamber with a temperature of 22 ˚C. Chip size averaged about 12 mm. Prior to using the mulch, its ambient moisture-content was measured so that mulch contributions to total water in bottles could be calculated. Six mulch samples were oven-dried (Fisher Scientific) at 104 ˚C overnight. From the weight differences before and after drying, a mean moisture-content value (± standard deviation) was calculated, which was 35.98% (w/w) ± 1.49%.

3.2.1.2 Water

Tap water was used in experiments because it better simulated natural groundwater than would distilled water. However, before use, the tap water was dechlorinated by allowing it to sit for a few days in an open container. A standard test kit (HACH) was used to verify that the resultant, total chlorine was low enough for subsequent use.

3.2.1.3 Nutrient Solution

Some bottles received a nutrient solution. This was prepared as a 100-fold concentrate, for convenience, containing 100 mg/L P and 500 mg/L N. It was prepared from K_2HPO_4 (A. C. S.) and NH_4Cl (A. C. S.) (Fisher Chemicals) Whenever used, this stock solution was diluted with dechlorinated tap water to achieve 1 mg/L P and 5 mg/L N as delivered to experimental bottles.

3.2.1.4 KB-1®

A KB-1-containing culture (SiREM Guelph, Ontario, Canada) was prepared for use in some bottles of Experiment 1. As used, it was a 1000X dilution (either in dechlorinated tap water or nutrient solution, as appropriate) of original, KB-1.

3.2.1.5 Marble Chips

Marble chips (Fisher Scientific) were added to all the experimental bottles. Commonly, organic acids could be generated as intermediate products during biodegradation process, which may significantly alter the pH environment in experimental bottles, inhibiting growth of microbes. Thus the usage of marble chips ($CaCO_3$) was to keep pH value consistent at an acceptable level (6.2 – 6.9).

3.2.2 Setup of Bottles

3.2.2.1 Bottle Preparation

As mentioned above, two separate experiments were conducted. For Experiment 1, four different conditions were investigated (each in triplicate), using 160-ml serum bottles (Wheaton, borosilicate glass) sealed with Teflon-lined, grey-butyl rubber serum stoppers and aluminum crimps (Fisher Scientific): Mulch+Water; Mulch+Water+KB1; Mulch+Water+N,P; and Mulch+Water+N,P+KB1. Each bottle had approximately 10 g (dry weight) mulch and 2.5 g marble chips. The overall steps and quantities are shown below.

1. Added sufficient, ambient-moisture mulch to yield approximately 10 g dry weight (weights of ambient-moisture mulch corresponding to 10 g dry weight were determined from the average moisture-content value calculated previously); calculated water weight in mulch (w_m, g) and added 2.5g marble chips into a 160-ml bottle (it is

inadvisable to use oven-dried mulch in biodegradation experiments, because oven-drying can adversely affect degradability by decreasing pore size [61]).

2. Weighed the bottle + mulch + marble chips + w_m (w_1, g).

3. Filled the bottle with the appropriate solution for each experimental condition (dechlorinated tap water with/without KB1; or with/without KB1 + N, P); weighed it again (w_2, g).

4. Removed 50 ml solution with pipette, producing a gaseous volume in the bottle, V_g = 50 ml.

5. Sealed the bottle with serum stopper and aluminum crimp.

6. Volume of water in bottle

$$V_w, \text{ml} = (w_2 - w_1 + w_m)/0.9977 - 50 \qquad [3.1]$$

where 0.9977 is the density of water at 22˚C.

7. Put them upside-down and inclined (fixed by pre-installed clamps) on orbital shakers (Innova 2000 Platform Shaker, New Brunswick Scientific) at 120 rpm in a dark isothermal chamber (22 ˚C). This inverted and inclined orientation produced liquid-surface disturbance during orbital shaking, which provided better gas-solution mass transfer. Quantities are presented in Table 3.1.

Table 3.1 V_w (ml) and V_g (ml) for each bottle in Experiment 1.

	water+mulch			water+mulch+N,P			water+mulch+KB1			water+mulch +N,P+KB1		
Number	1	2	3	1	2	3	1	2	3	1	2	3
Weight before adding water (g)	120	120	119	122	120	121	120	121	120	120	120	119
Weight after adding water (g)	256	256	254	257	256	257	256	256	257	254	255	255
Water added (g)	136	136	136	135	135	136	136	136	135	137	134	135
Vg (ml)	50	50	50	50	50	50	50	50	50	50	50	50
Water in mulch (g)	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
Vw (ml)	90.9	90.9	89.9	89.9	90.9	90.9	90.9	89.9	91.9	88.9	89.9	90.9

For Experiment 2, 24 160-ml serum bottles were separated into two groups, with or without N, P supplementation. Furthermore, four different conditions were conducted in each group (each condition in triplicate): 1 g mulch; 2 g mulch; 5 g mulch; and 10 g mulch (all as equivalent dry weight) and 2.5 g marble chips. Other than the different quantities of mulch used, the setup procedure was the same as that described above for Experiment 1. The overall quantities are shown below (Table 3.2 and 3.3).

Table 3.2 V_w (ml) and V_g (ml) for Experiment 2 (without N,P)

	water+mulch (1g dry)			water+mulch (2g dry)			water+mulch (5g dry)			water+mulch (10g dry)		
Number	1	2	3	1	2	3	1	2	3	1	2	3
Weight before adding water (g)	106	104	105	108	107	106	111	112	112	117	118	117
Weight after adding water (g)	263	261	262	261	261	260	257	258	258	252	253	252
Water added (g)	157	157	157	157	153	154	154	146	146	146	135	135
Vg (ml)	50	50	50	50	50	50	50	50	50	50	50	50
Water in mulch (g)	0.46	0.46	0.46	0.92	0.92	0.92	2.3	2.3	2.3	4.6	4.6	4.6
Vw (ml)	108	108	108	104	105	105	99	99	99	90	90	90

Table 3.3 V_w (ml) and V_g (ml) for Experiment 2 (with N,P).

	water+mulch (1g dry)+N,P			water+mulch (2g dry)+N,P			water+mulch (5g dry)+N,P			water+mulch (10g dry)+N,P		
Bottle number	1	2	3	1	2	3	1	2	3	1	2	3
Weight before adding water (g)	106	104	105	108	107	106	111	112	112	117	118	117
Weight after adding water (g)	263	262	262	261	260	260	258	257	257	256	254	253
Water added (g)	157	157	158	157	153	153	154	147	145	145	139	136
Vg (ml)	50	50	50	50	50	50	50	50	50	50	50	50
Water in mulch (g)	0.46	0.46	0.46	0.92	0.92	0.92	2.3	2.3	2.3	4.6	4.6	4.6
Vw (ml)	108	109	108	104	104	105	100	98	98	94	91	91

3.2.2.2 Bottle Operation and Oxygen Analysis

For Experiment 1, total oxygen in bottles was determined from headspace analysis of a sample acquired with a locking, gas-tight syringe (Pressure-Lok, 500-µl RN

0.029-inch x 0.012-inch x 2-inch side-port needle, Gas Syringe A-2). Samples were analyzed for oxygen (1 test/day to 1 test/4 days) via gas chromatography (HP 5890 Series II), equipped with a 3-ft x 1/8-inch stainless-steel column packed with 60/80 Molecular Sieve 5A (Supelco, Inc.), as described previously. [62] The overall steps and quantities were as follows:

1. Unloaded bottle from the orbital shaker, let it settle down for 10 min.

2. Flushed the analytical syringe with pure N_2 (Airgas) twice (a nitrogen tank was located next to the GC for convenience), then immediately sampled either 0.1-ml or 0.25-ml headspace from an experimental bottle, depending on the expected gaseous O_2 concentration (C_g, mg/L) -- if Cg < 32 mg/L, then a 0.25-ml headspace sample was used.

3. Injected headspace sample to GC, recording the peak height of oxygen, and calculated C_g by application of a standard curve (shown below in the section, "Oxygen Standard")

4. Calculated total O_2 ($Mt.O_2$, mg) in the bottle via Equation 3.2.

$$Mt.O_2 = C_g V_g + C_w V_w = C_g (V_g + V_w/H_c) \qquad [3.2]$$

in which, C_w is aqueous dissolved oxygen concentrations (mg/L), related to Cg by the pseudo-dimensionless Henry's constant (H_c), where $C_g = H_c C_w$ At 22 ˚C, $H_c = 31.676$ [mg/L gas per mg/L water], based on an oxygen solubility in pure water (1 atm of air) of 8.743 mg/L. Note that V_g and V_w for use in Equation 3.2 were previously calculated (see Tables 3.1 and 3.2).

5. Whenever the oxygen content of a bottle became too low ($Mt.O_2 < 1$ mg), it was reopened and refreshed with atmospheric air. Also, in Experiment 1, each reopened bottle had 15 ml of supernatant extracted and replaced with equivalent volume of fresh

solution, by pipette. After refreshment, the bottle was moved back to the dark isothermal chamber, shaken 15 min on the orbital shaker, then sampled and analyzed again.

6. Tested the extracted liquid with pH meter (Denver Instrument) to monitor the pH value.

For Experiment 2, the same operations were employed as in Experiment 1 except there was no liquid removal/refreshment for any bottle.

3.3 Analytical Methods

3.3.1 Oxygen Measurement

Oxygen standardization was achieved by delivering known volumes of air to serum bottles (without liquid), which had been purged for 30 min with pure N_2 (Airgas). The ideal gas law was applied (with local measurement of temperature and barometric pressure) to calculate moles of O_2 added to these standards, and thus to calculate resulting volumetric gaseous concentrations (Cg, mg/L or mmol/L). [62] A locking, gastight syringe was used to sample the standards of known Cg concentration while the related GC reading (Peak Height) could be read on a computer. With 5 groups of triplicated standards, linear regression was performed between Cg (x) and Peak Height (y). To gain more accurate results, two standard calibrations were performed to deal with different ranges of O_2 concentrations — one for high oxygen levels (0.1 ml headspace per injection), the other one for low oxygen levels (0.25 ml headspace per injection).

Table 3.4 Cg and GC reading for high oxygen level.

O_2 percentage	Triplicated Samples	V O_2 delivered (mL)	n O_2 delivered (mmol)	Cg (mmol/L), x	GC Reading for Peak Height, y
0(pure N2)	1	0	0	0	8.6
	2				8.4
	3				8.3
5%	1	8	0.3316	2.073	268.5
	2				269.3
	3				270.1
10%	1	16	0.6632	4.145	501.5
	2				499.6
	3				503.9
15%	1	24	0.9948	6.218	711.4
	2				699.1
	3				705.5
20.95% (Pure air)	1	33.52	1.3894	8.684	1175
	2				1178
	3				1184

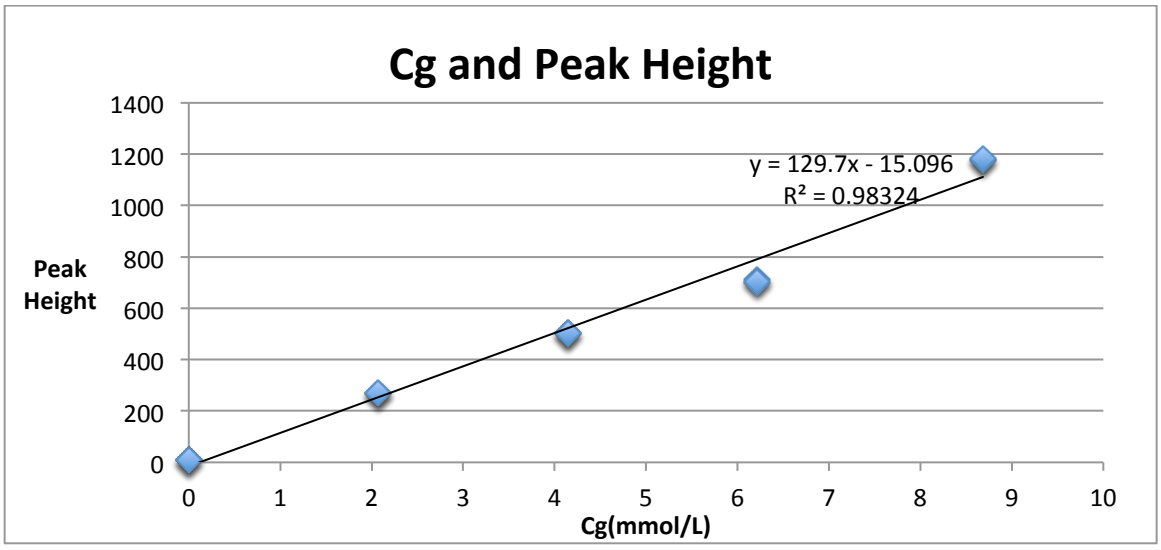

Figure 3.1 Liner regression between Cg and peak height for high oxygen level.

Table 3.5 Cg and peak height for high oxygen level.

O_2 percentage	Triplicated Samples	V O_2 delivered (mL)	n O_2 delivered (mmol)	Cg (mmol/L), x	GC Reading for Peak Height, y
0(pure N2)	1	0	0	0	12.1
	2				11.4
	3				11.4
0.0625%	1	0.1	0.004145	0.026	21.4
	2				22.1
	3				21.7
0.3125%	1	0.5	0.02073	0.13	53.8
	2				52.6
	3				53.4
1.25%	1	2	0.0829	0.518	173
	2				176.7
	3				174.7
2.50%	1	4	0.1658	1.036	322.2
	2				326.4
	3				322.6

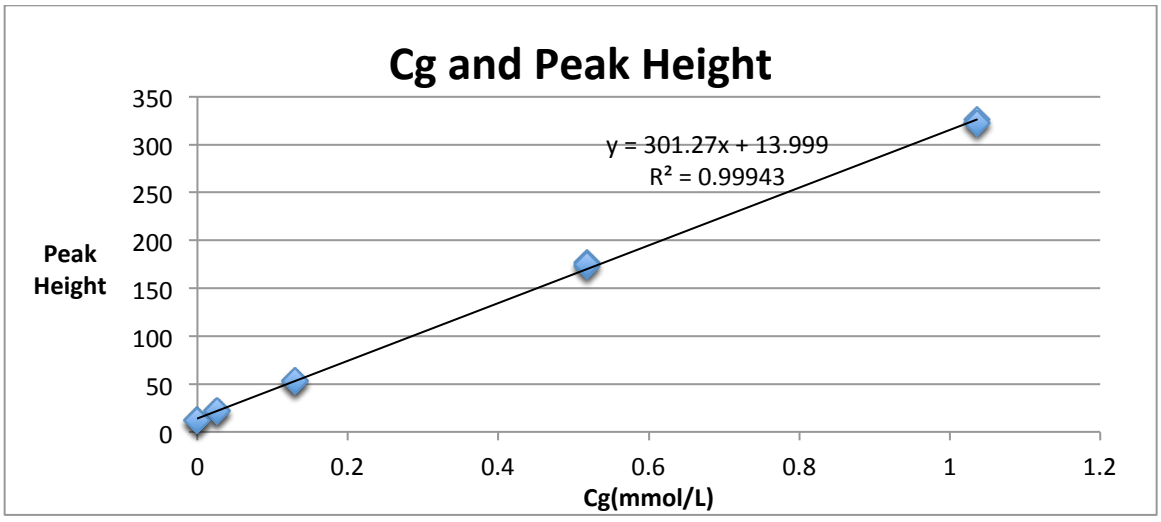

Figure 3.2 Liner regression between Cg and peak height for low oxygen level.

3.3.2 Statistical Analysis

Time-course plots for all bottles (cumulative O_2 consumed vs. time) were created. A simple, first-order model was employed to describe oxygen degradation kinetics – essentially the standard model used to describe BOD kinetics:

$$\frac{dL}{dt} = kL \qquad [3.3]$$

where L = amount of organic substrate remaining (in mg BOD_L units); and k = first-order decay coefficient (day^{-1}). The integrated form for this is

$$y = L_0\left(1 - \exp\left[-kt\right]\right) \qquad [3.4]$$

where y = the cumulative oxygen consumed (mg) in time, t (days); and L_0 = degradable substrate (mg BOD_L) present at $t = 0$.

For statistical analysis, a software product called XLSTAT (Addinsoft SARL) was used to determine best-fit values of L_0 and k for data from individual bottles, fitted to Eq[3.4]..

XLSTAT was further applied to do significance-testing (t-tests) between different sample groups to address questions posed as research objectives.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Cumulative O_2 Consumption Data

Ahead of data analysis, the depletion-curve data (Mt.O_2 vs. time) measured by GC for each bottle (Appendix 1) was processed and transformed into curves of cumulative O_2 consumption versus time, for both Experiment 1 and Experiment 2 (Figures 4.1 through 4.3). Note that two of the triplicate bottles in Type "M+W" from Experiment 1 were broken ("M+W Cum 2" and "M+W Cum 3") soon after beginning, and therefore neither of these was available for further analysis.

Curiously, two bottles (both coincidentally the No. 2 replicates of 10-g mulch bottles in Experiment 2 – with and without N, P, respectively) display sawtoothed, "bumpy" patterns of cumulative O_2 consumption (Figures 4.2 and 4.3). Such a pattern resulted from an extremely slowed degradation rate as oxygen concentration became low – ca. 1 mg/L -- in these bottles, almost akin to a threshold level of oxygen below which it could not be utilized (see Figures A1.8 and A1.12 in Appendix 1). Similar behavior was evident in some other bottles at other g-mulch levels (e.g., see Figures A1.5 and A1.9 for 1-g mulch bottles). It's a baffling observation, because it is not something seen in all bottles. A possible explanation lies in the heterogeneity of the mulch particles – i.e., large particles might have a slow intraparticle O_2-transfer, manifesting itself as a very large half-velocity constant with respect to bulk-phase, dissolved oxygen. If some bottles received larger particles of mulch than others, it might explain the idiosyncratic behavior.

pH was measured in Experiment 1 whenever bottles were opened for reaeration and liquid exchange, while one-time pH measurements were made for bottles in Experiment 2 at completion of the experimental run. In all cases, pH was observed to

center around 6.3, with an interval between 6.2 and 6.4, suggesting that pH wasn't a factor adversely affecting biodegradation. Because no bottles were run without marble chips, we cannot be sure such buffering was required, and its value should be explored in future experiments.

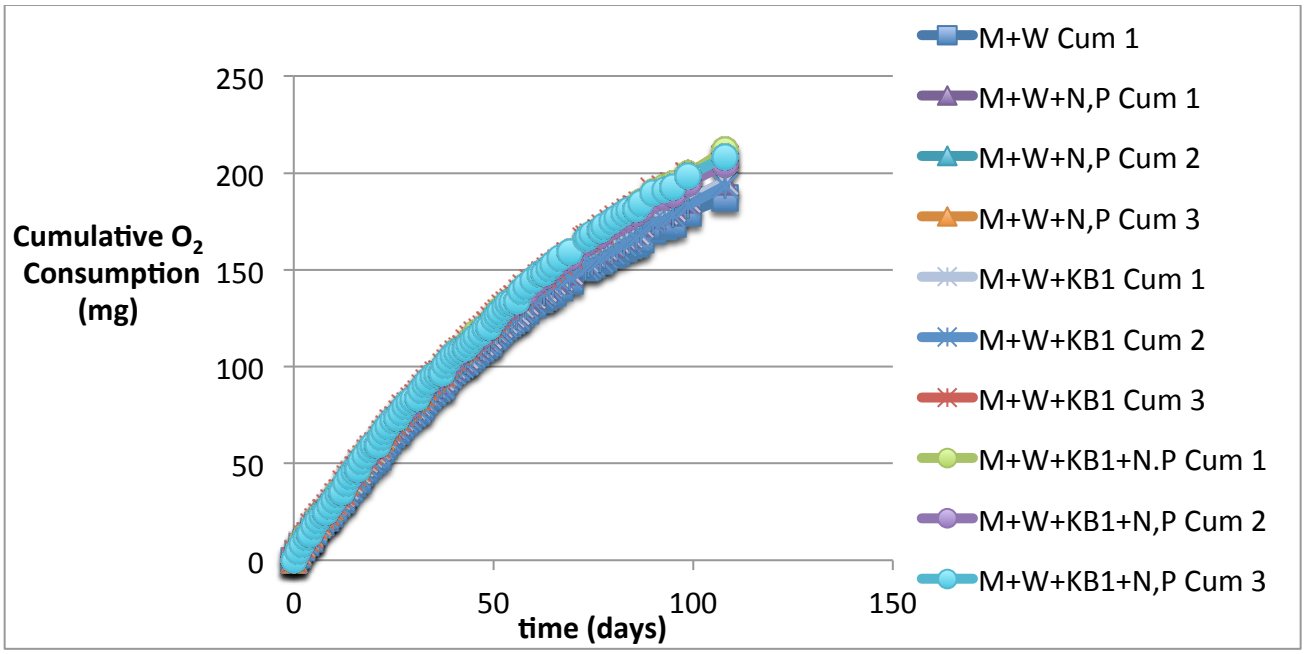

Figure 4.1 Cumulative O_2 consumption results for Experiment 1.

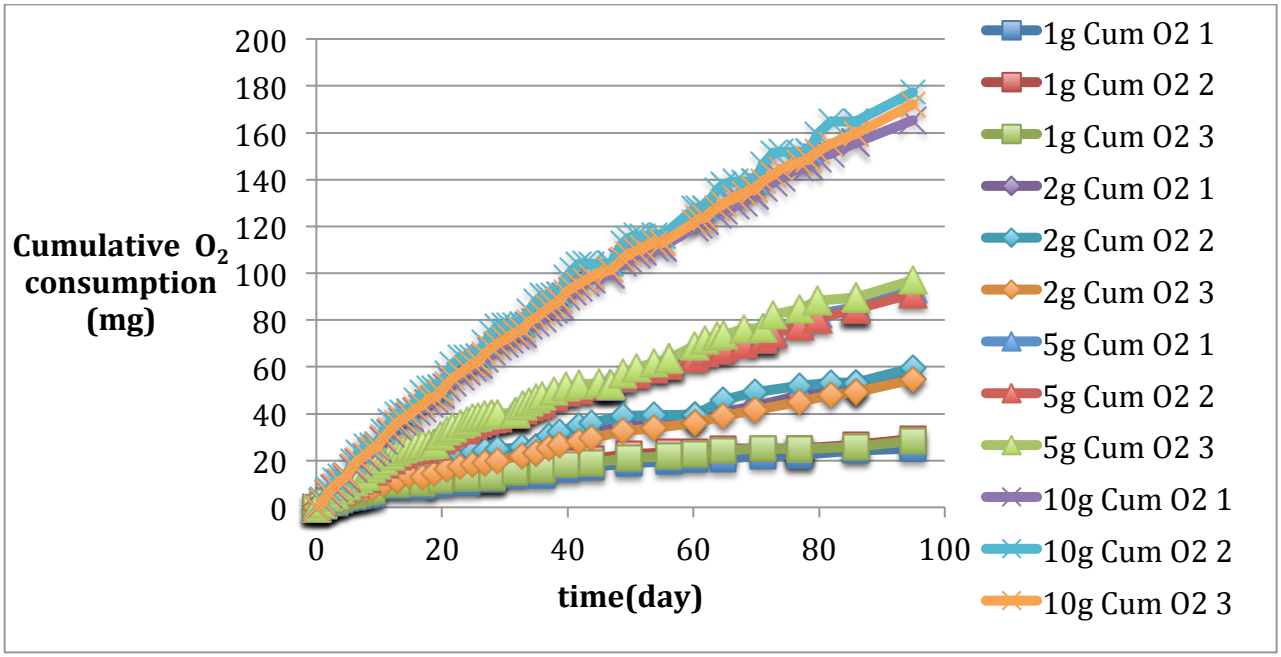

Figure 4.2 Cumulative O_2 consumption results for part 1 of Experiment 2 (without N, P).

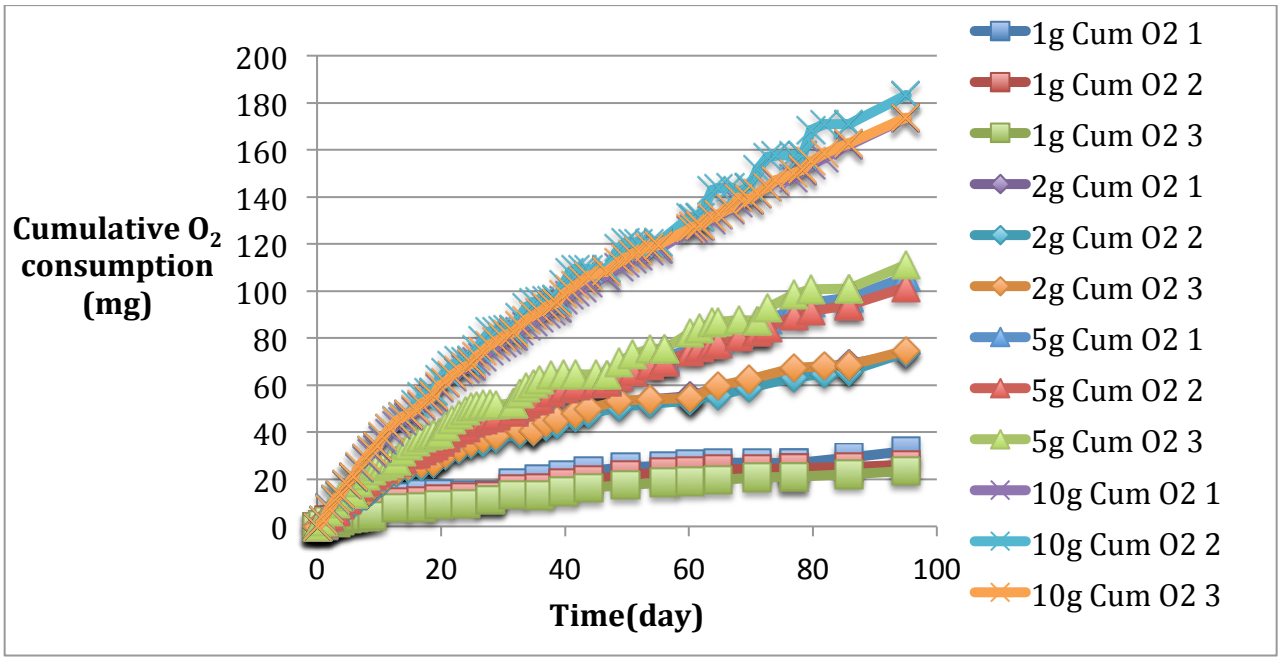

Figure 4.3 Cumulative O_2 consumption results for part 2 in Experiment 2(with N, P).

4.2 Effect of KB1 Inoculation

In Experiment 1, four different conditions were investigated (each in triplicate): Mulch+Water; Mulch+Water+KB1; Mulch+Water+N,P; and Mulch+Water+N,P+KB1. Comparison of bottles that received KB1 inoculum with their corresponding bottles that did not, could be used to determine whether or not KB1 inoculation affected mulch biodegradation. If inoculation mattered, it would be expected to manifest itself at the earliest time-points – i.e., inoculation might have sped the initiation of degradation and oxygen consumption.

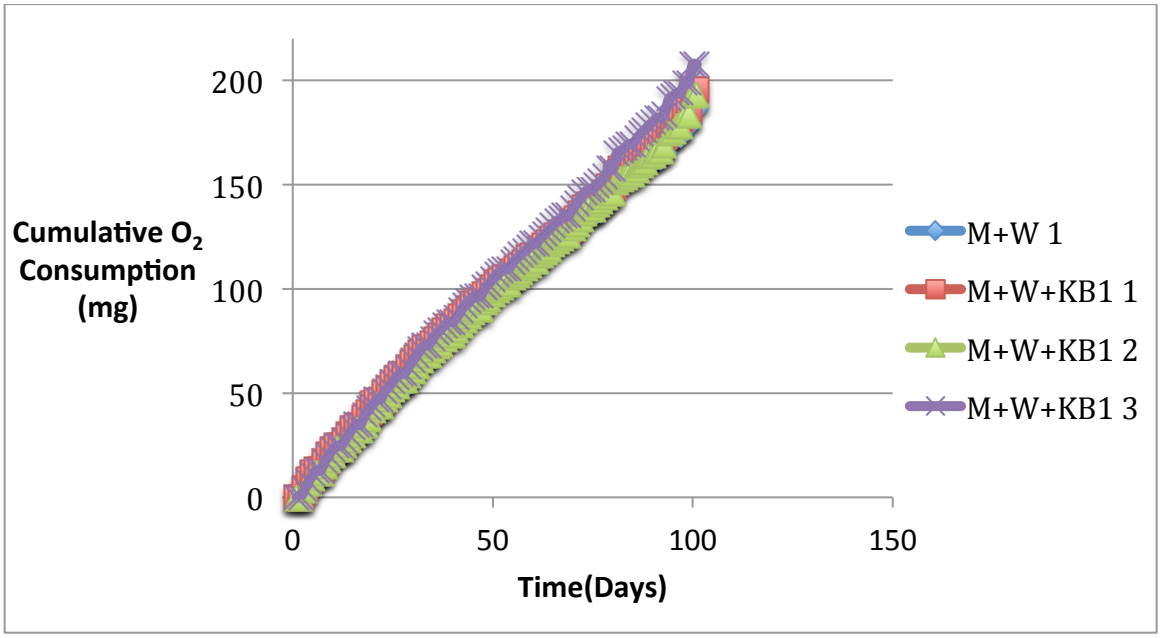

Figure 4.4 Cumulative O_2 consumption in Experiment-1 bottles, with or without KB1 inoculations (without N, P).

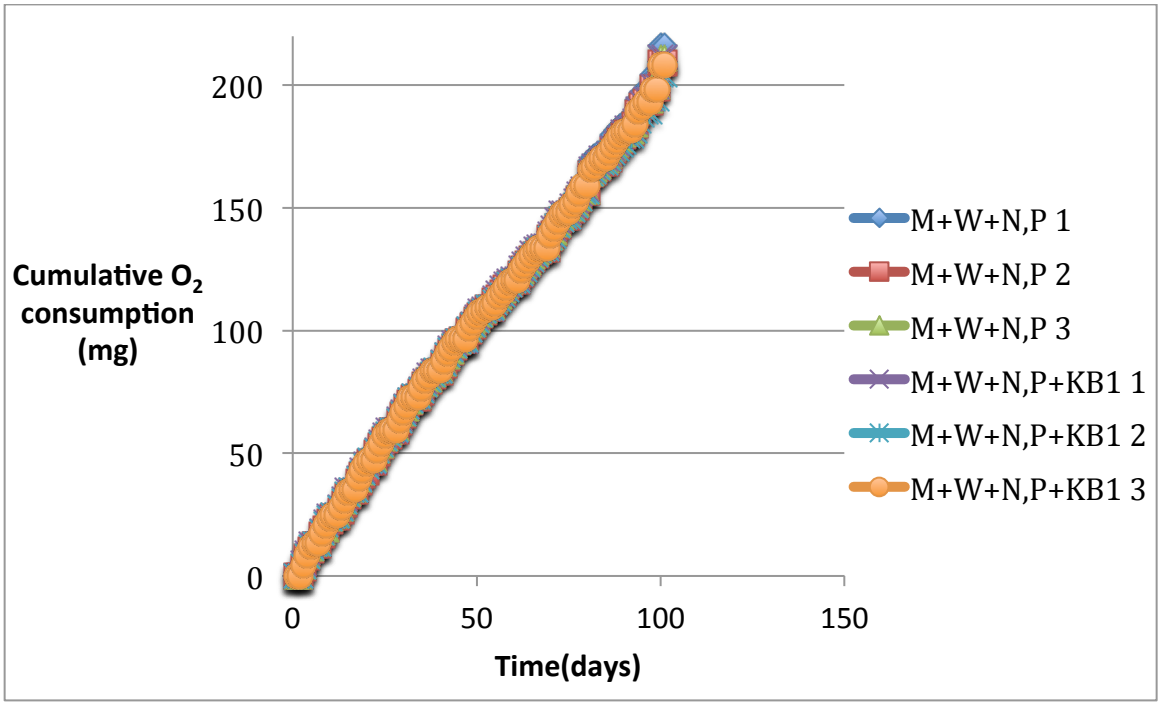

Figure 4.5 Cumulative O_2 consumption in Experiment-1 bottles, with or without KB1 inoculations (with N, P).

It is apparent from Figures 4.4 and 4.5 that KB1 inoculation made no difference in initiating O_2 consumption in mulch. Apparently there is a large, robust population of indigenous microbes in mulch to consume oxygen without need for inoculation. Note that KB1 is an ostensibly anaerobic culture, but would be expected to contain facultatives capable of O_2 utilization.

Given that KB1 inoculation had no discernible effect on O_2 consumption (either with respect to rate or extent), we subsequently ignored KB1's presence in bottles and grouped all bottles together by nutrient condition (i.e., W+M or W+M+N, P), regardless of inoculation condition. This, then, provided 4 replicates of the W+M condition and 6 replicates of the W+M+N, P condition for subsequent statistical comparisons.

4.3 Inherent L_0/g for Bark Mulch

4.3.1 Analysis of L_0/g Data

Non-linear regression was applied to each bottle, fitting the measured cumulative O_2 consumptions with Equation [3.4]; model-fits for all bottles are available in Appendix 2. Values of k and L_0 for each bottle were thus estimated, and L_0/g was simply calculated by dividing the L_0 by the known mass (dry wt.) of mulch in the bottle (Table 4.1).

Table 4.1 Estimates of *k* and L_0/g for each bottle.

Bottle	Actual dry weightof added mulch (g)	*k* (d^{-1})	L_0 (mg)	L_0/g (mg O_2/g dry wt)
W+M 1	9.35	0.0110	270.1	28.9
W+M+N, P 1	9.35	0.0093	339.5	36.3
W+M+N, P 2	9.35	0.0099	316.6	33.9
W+M+N, P 3	9.35	0.0102	313.8	33.6
W+M+KB1 1	9.35	0.0116	271.2	29.0
W+M+KB1 2	9.35	0.0107	280.4	30.0
W+M+KB1 3	9.35	0.0103	311.9	33.4
W+M+KB1+N, P 1	9.35	0.0102	313.7	33.6
W+M+KB1+N, P 2	9.35	0.0109	296.1	31.7
W+M+KB1+N, P 3	9.35	0.0105	307.2	32.9
1g 1 (without N, P)	0.93	0.0225	27.6	29.5
1g 2 (without N, P)	0.93	0.0254	30.2	32.4
1g 3 (without N, P)	0.93	0.0237	30.1	32.2
2g 1(without N, P)	1.87	0.0138	71.6	38.3
2g 2(without N, P)	1.87	0.0143	76.0	40.6
2g 2(without N, P)	1.87	0.0090	90.9	48.6
5g 1 (without N, P)	4.67	0.0133	121.8	26.1
5g 2 (without N, P)	4.67	0.0131	121.2	25.9
5g 3 (without N, P)	4.67	0.0120	136.9	29.3
10g 1 (without N, P)	9.35	0.0104	260.5	27.9
10g 2 (without N, P)	9.35	0.0117	257.1	27.5
10g 3 (without N, P)	9.35	0.0104	267.6	28.6
1g 1 (with N, P)	0.93	0.0280	31.9	34.1
1g 2 (with N, P)	0.93	0.0269	28.5	30.5
1g 3 (with N, P)	0.93	0.0214	26.4	28.3
2g 1(with N, P)	1.87	0.0239	77.2	41.3
2g 2(with N, P)	1.87	0.0232	75.5	40.4
2g 3(with N, P)	1.87	0.0236	78.0	41.7
5g 1 (with N, P)	4.67	0.0180	118.5	25.4
5g 2 (with N, P)	4.67	0.0187	113.2	24.2
5g 3 (with N, P)	4.67	0.0201	119.1	25.5
10g 1 (with N, P)	9.35	0.0146	220.7	23.6
10g 2 (with N, P)	9.35	0.0146	233.9	25.0
10g 3 (with N, P)	9.35	0.0157	213.7	22.9

Subsequently, means and 95% confidence intervals of L_0/g for each bottle type were computed (Figure 4.6). All were based on triplicates, except for "W+M" and "W+M+N, P" in Experiment 1, which had four and six replicates, respectively, as described in Section 4.2.

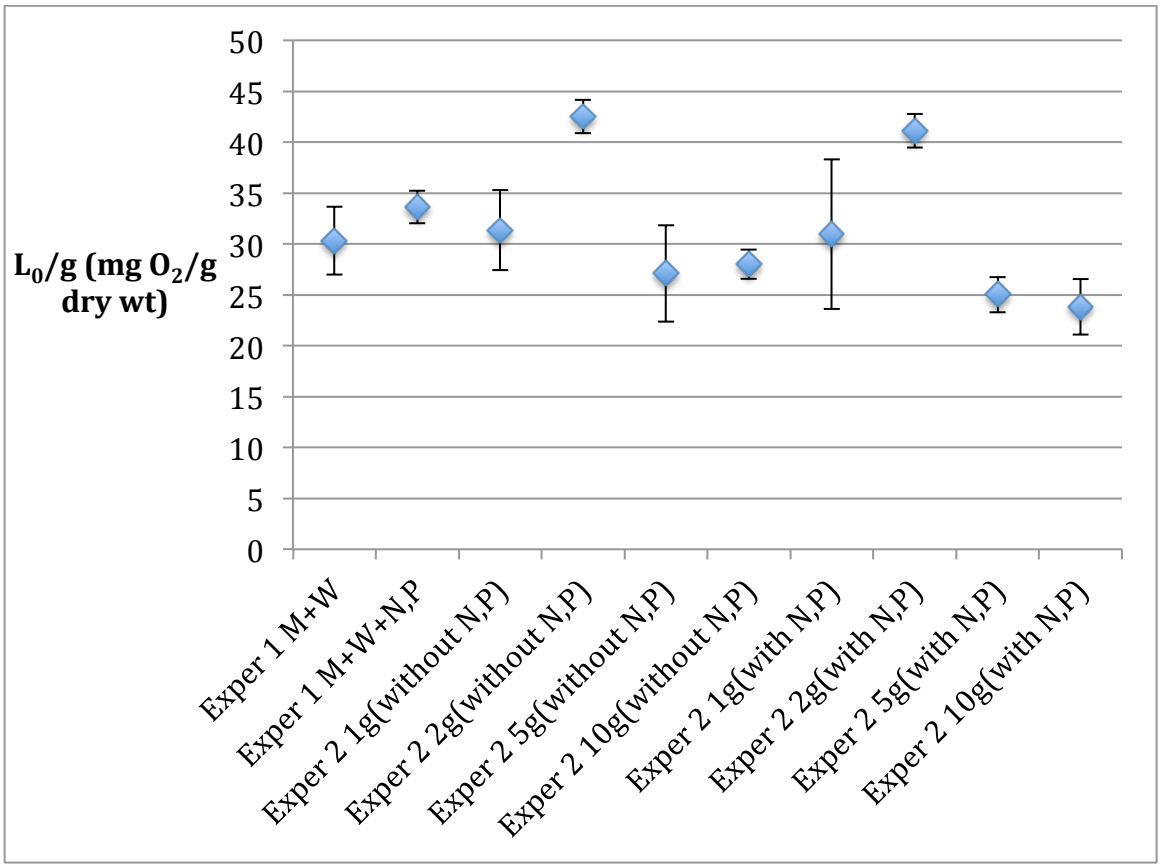

Figure 4.6 L_0/g for different types. Error bars are 95% CIs.

It's apparent from Figure 4.6 that the 2-gram bottles of Experiment 2 were outliers – their 95% CIs do not overlap any of the others. Therefore, these two types were discarded from further data analysis, resulting in Figure 4.7. We have no conclusive explanation for the anomalous results from 2-g mulch bottles. Perhaps an error was made in delivering mulch to them in bottle preparation.

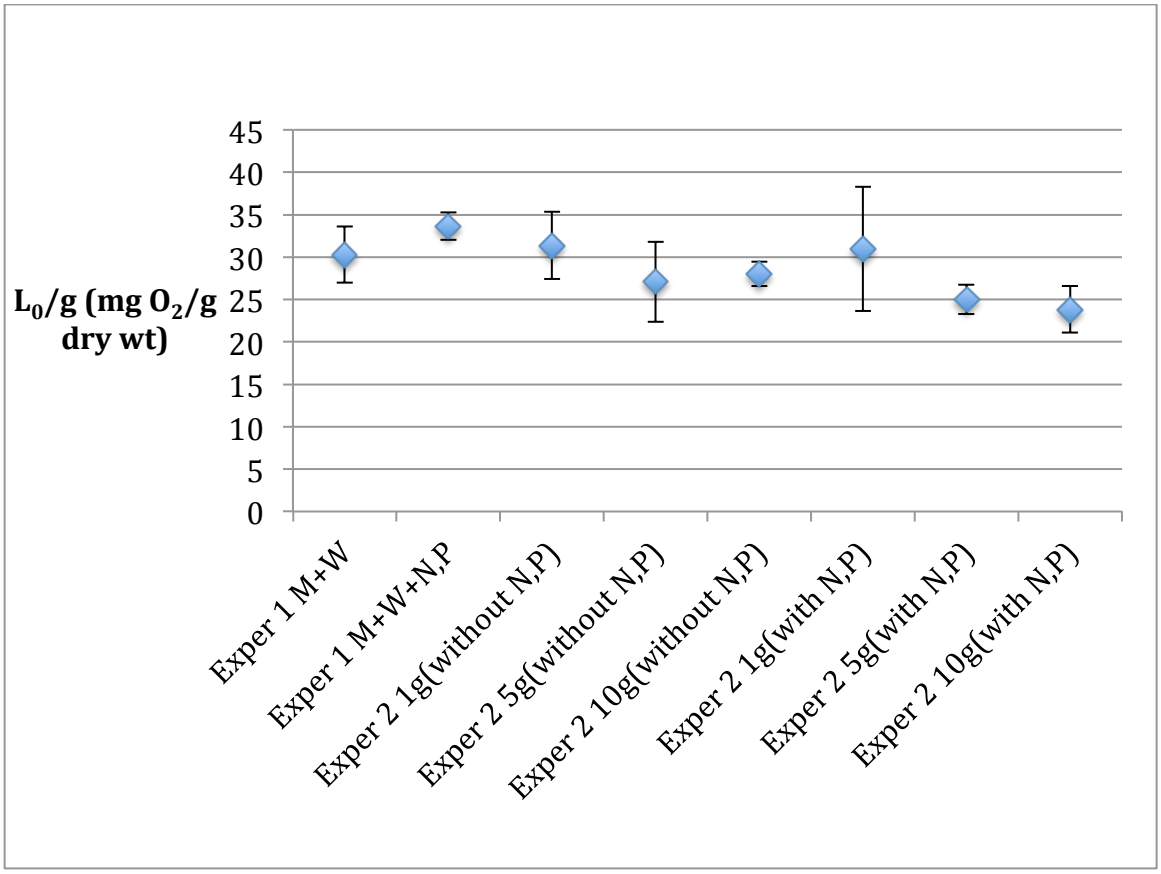

Figure 4.7 L_0/g for different types after removing outliers.

When the first-order degradation model was fit to data using simultaneously both L_0/g and *k* as fitting parameters, a relationship (albeit complex) was seen between them (Figure 4.8). There is clearly correlation between L_0/g and *k*, but its magnitude and direction appear to depend on the gram mulch per bottle. The correlation is clearly negative for 10-g data; it's clearly positive for 1-g data; and it's transitional for 5-g data. This suggests at least two different difficulties:

(1) Instability of parameter estimation when data have not been obtained at cumulative oxygen consumption values that approach L_0. This makes it difficult to independently, robustly estimate the two parameters, and you'd expect a negative correlation (the same data are almost equally well-fit if the estimate of L_0 is increased

slightly while the estimate of *k* is decreased slightly). This would be more of a problem as the g mulch per bottle increases, because the bottles would have achieved a lower fraction of possible cumulative O_2 consumption by the end of experiment. It's hard to get a good estimate of L_0 if none of my data approach it.

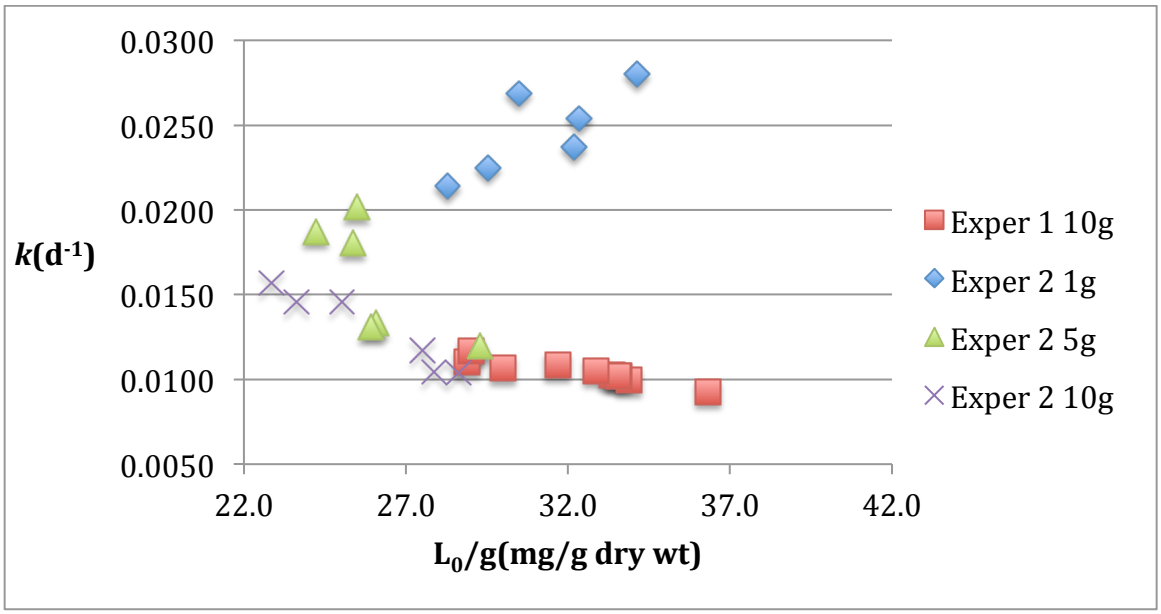

Figure 4.8 Relationship between estimated *k* and L_0/g based on two-parameter, non-linear regression.

(2) The positive correlation between *k* and L_0/g in bottles containing 1gram mulch suggests, perhaps, some inadequacy in the simple, first-order model. There is a negative correlation between apparent L_0/g and g mulch/bottle in Experiment 2, which suggests a flaw in the simple, first-order model (Figure 4.9). Regardless, the first-order model is judged to reasonably fit the data (see Figures of Appendix 2).

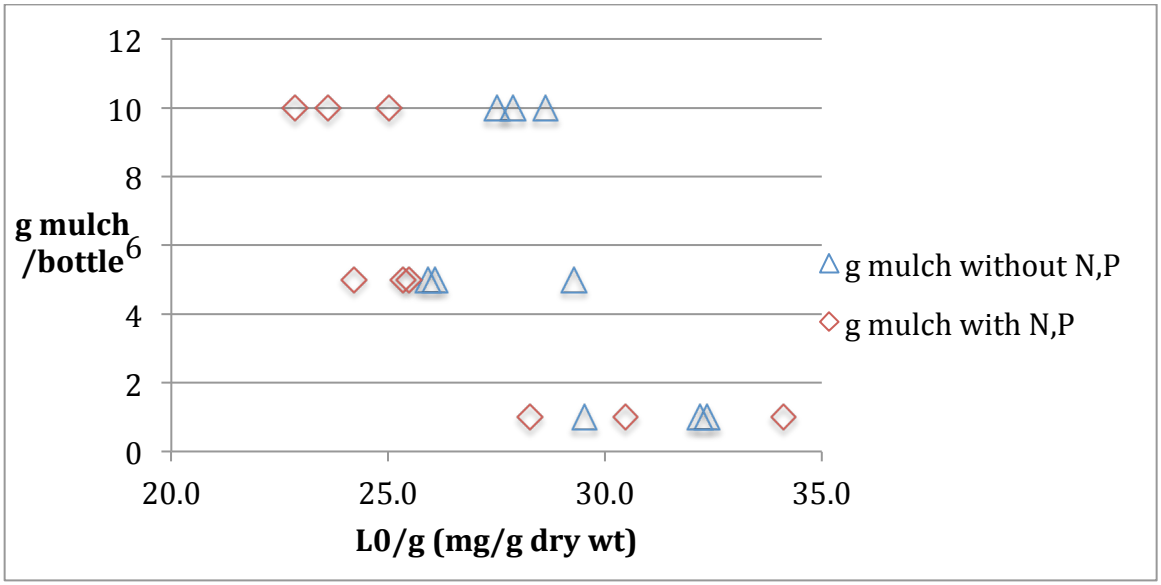

Figure 4.9 Correlation between apparent L_0/g and g mulch/bottle in Experiment 2.

4.3.2 Best Estimate of L_0/g

Since value of L_0/g represents the degradable capacity of mulch, it is regarded as an inherent value -- it theoretically should not be affected by the number of grams of mulch in a bottle (which should merely affect how long it takes to reach capacity), and it should not be affected by nutrient supplementation (which should affect rate, but not ultimate extent, though nutrient deficiency might be expected to result in complex kinetics – e.g., initially higher *k*, then later, lower *k*).

Given the dependencies evident in Figure 4.8, we judged that the best estimate of inherent L_0/g would be obtained by considering bottles that had proceeded nearest to completion (or capacity) and which did not experience exchange/replenishment of water that could have removed some electron donor. These would be the 1-gram-type bottles of Experiment 2.

However, the 1-gram types of Experiment 2 included two conditions – one that contained N and P and the other one didn't (both in triplicate). As mentioned above, we

did not expect nutrient supplementation to affect inherent L_0/g, and therefore would expect that the triplicates of both nutrient conditions could be pooled for purposes of estimating mulch capacity. A two-tailed t-test (alpha = 0.05) was conducted (Appendix 4, Section A4.1.1), which showed no statistical difference between L_0/g values from these two nutrient conditions. Consequently, all six bottles of 1-gram type in Experiment 2 were used from Table 4.1 to obtain a best estimate of inherent L_0/g for mulch (with 95% confidence intervals), and the result was 31.2 ± 2.2 mg/g.

4.4 Effect of Water Exchange on Mulch Degradation

Bottles in Experiment 1 were operated with periodic removal and replenishment of liquid, somewhat simulating the exchange of water that would occur through a mulch barrier wall. Water flow through mulch could potentially remove some electron donor from the wall via leaching. Bottles in Experiment 2 were not subjected to such exchange.

To verify the effect of water exchange on mulch degradation, a two-tailed t-test (alpha = 0.05) was conducted. The relevant data for comparison were 10-gram bottles (without N and P) from Experiment 1 to 10-gram bottles (without N and P) in Experiment 2. The 10-gram bottles from these two experiments that had N, P could not validly be compared because the total N and P amounts were so much different between the two experiments. In other words, bottles in Experiment 1 with N and P not only had water exchange but also much higher N and P than corresponding bottles with N and P from Experiment 2 because nutrient solution was employed for this type of bottles in Experiment 1. Thus, they differed in more than just the factor of water exchange. Results from the t-test (Appendix 4, Section A4.1.2) showed no effect of water exchange on Lo/g at the 95% level of confidence. The likely explanation is that hydrolysis of

particulate substrate in mulch is rate-limiting, and soluble substrate is degraded as soon as it is produced, with very little loss of electron donor through leaching.

4.5 Effect of N and P on Mulch Degradation Rates

The most likely effect of N and P would be on mulch degradation rates (*k*), not L_0/g values. To explore the possible effect of N, P on *k*-values, the data from all bottles (except the 2-gram types that were discarded as outliers) were fit again to the first-order model [Equation 3.4], but this time the inherent L_0/g value (31.2 mg/g) from previous analysis was input, and only the *k*-value was used as fitting parameter in non-linear regression (Table 4.2). This approach – using a fixed L_0/g – was intended to remove the artifactual errors in k-values caused by the inverse correlation seen previously in Figure 4.8 at high-gram-mulch values, where data ended far from exhaustion of mulch capacity. Curves for individual bottles fit this way are shown in Appendix 3.

Statistical t-tests (two-tailed, alpha = 0.05) were conducted between different types to explore the possible significance of N and P on mulch degradation rates, including "M+W vs. M+W+N, P in Experiment 1", "1g mulch+W vs. 1g mulch+W+N, P" in Experiment 2", "5g mulch+W vs. 5g mulch+W+N, P in Experiment 2" and "10g mulch+W vs. 10g mulch+W+N, P in Experiment 2". (Appendix 4, Section A4.2)

In the end, only "M+W vs. M+W+N, P in Experiment 1" and "5g mulch+W vs. 5g mulch+W+N, P" showed significant differences on degradation rates (*k*) while the other two conditions did not. Therefore the effect of N and P is uncertain from a statistical perspective. However, from a practical perspective, it appears that N, P supplementation is unimportant. This is evident from comparing Figures 4.2 and 4.3.

Table 4.2 Estimated *k* with fixed, inherent L_0/g by non-linear regression.

Bottle type	Inherent L_0/g (mg/g dry wt)	Actual dry weight of added mulch (g)	L_0 (mg) for each bottle	Estimated k (d^{-1})
Exper 1 M+W	31.2	9.35	291.7	0.0099
Exper 1 M+W+N,P 1	31.2	9.35	291.7	0.0115
Exper 1 M+W+N,P 2	31.2	9.35	291.7	0.0112
Exper 1 M+W+N,P 3	31.2	9.35	291.7	0.0113
Exper 1 M+W+KB1 1	31.2	9.35	291.7	0.0105
Exper 1 M+W+KB1 2	31.2	9.35	291.7	0.0101
Exper 1 M+W+KB1 3	31.2	9.35	291.7	0.0113
Exper 1 M+W+KB1+N,P 1	31.2	9.35	291.7	0.0114
Exper 1 M+W+KB1+N,P 2	31.2	9.35	291.7	0.0112
Exper 1 M+W+KB1+N,P 3	31.2	9.35	291.7	0.0114
Exper 2(without N,P) 1g 1	31.2	0.93	29.0	0.0204
Exper 2(without N,P) 1g 2	31.2	0.93	29.0	0.0274
Exper 2(without N,P) 1g 3	31.2	0.93	29.0	0.0253
Exper 2(without N,P) 5g 1	31.2	4.67	145.7	0.0104
Exper 2(without N,P) 5g 2	31.2	4.67	145.7	0.0101
Exper 2(without N,P) 5g 3	31.2	4.67	145.7	0.0110
Exper 2(without N,P) 10g 1	31.2	9.35	291.7	0.0090
Exper 2(without N,P) 10g 2	31.2	9.35	291.7	0.0099
Exper 2(without N,P) 10g 3	31.2	9.35	291.7	0.0100
Exper 2(with N,P) 1g 1	31.2	0.93	29.0	0.0339
Exper 2(with N,P) 1g 2	31.2	0.93	29.0	0.0257
Exper 2(with N,P) 1g 3	31.2	0.93	29.0	0.0180
Exper 2(with N,P) 5g 1	31.2	4.67	145.7	0.0131
Exper 2(with N,P) 5g 2	31.2	4.67	145.7	0.0127
Exper 2(with N,P) 5g 3	31.2	4.67	145.7	0.0146
Exper 2(with N,P) 10g 1	31.2	9.35	291.7	0.0098
Exper 2(with N,P) 10g 2	31.2	9.35	291.7	0.0106
Exper 2(with N,P) 10g 3	31.2	9.35	291.7	0.0100

CHAPTER 5 CONCLUSIONS

Permeable reactive barriers (PRBs) are an attractive method to intercept and treat groundwater contaminated with chlorinated ethenes. Within the PRB, the remedial mechanism would be reductive, anaerobic dechlorination, a process that requires both a source of electron donor and highly anaerobic conditions. Since many groundwaters contain dissolved oxygen, the ability of the PRB to sustain consumption of oxygen and to create a suitable anaerobic environment is a critical requirement.

Because the groundwater concentration of dissolved oxygen (typically in the mg/L range) far exceeds the concentrations of chlorinated ethenes (typically in the 10 to 100 μg/L range), the amount of a PRB's electron donor used for oxygen depletion is expected to far exceed the donor needed for reductive dechlorination. In other words, the deoxygenation occurring in a PRB wall would be the function that determines the sustainability of a PRB (how long a PRB could be used without replenishing electron donor).

Bark mulch is among the donor-matrix materials considered in the construction of PRBs. The sustainability of pine-bark mulch to consume oxygen under different conditions of inoculation, nutrient amendment, and liquid exchange (simulating possible leaching of donor) was investigated in experiments performed in serum bottles over a 110-day period.

The following conclusions were reached:

1) Inoculation with a mixed culture, KB1, made no difference in initiating O_2 consumption in mulch. Although KB1 is an ostensibly anaerobic culture, it's also expected to contain facultatives capable of O_2 utilization. However, from our

experimental results, inoculation with this culture didn't result in a different performance compared with the bottles that received no KB1 inoculations.

2) A first-order model – essentially that typically used for BOD kinetics –

$$y = L_0 * [1 - \exp(-kt)]$$

where: y = mg O_2 consumed in time, t; L_0 = BOD_L initially present in mulch (mg O_2) ; and k = first-order rate constant (d^{-1}),

was judged to fit reasonably the cumulative oxygen-consumption data using simultaneously both L_0 and k as fitting parameters.

3) An inherent electron-donor capacity per gram mulch (L_0/g) was estimated (with 95% confidence interval), and the result was 31.2 ± 2.2 mg O_2/g.

4) Exchange of liquid (somewhat crudely simulating the water exchange that would accompany flow through a PRB) resulted in no statistically significant difference in L_0/g, suggesting that leaching of electron donor from a pine-bark mulch PRB is probably not appreciable. This is likely because the potential rate of particulate-donor hydrolysis is much slower than the potential rate of uptake of the hydrolysis products, and thus the concentration of soluble donor is kept very, very low.

5) Supplementation with nitrogen (as ammonium) and phosphorus (as orthophosphate) had a mixed – and therefore uncertain -- effect on rate of oxygen uptake by microbes utilizing pine bark mulch. While some systems showed statistically significant effects, the magnitude was not judged to be practically significant.

CHAPTER 6 ENGINEERING SIGNIFICANCE

Investigating the ability of a pine-bark mulch PRB to sustainably consume oxygen flowing to it is the primary purpose of this research. Having measured a value for the inherent L_0/g of this material, we can provide estimates of a PRB's useful life as a function of the velocity and dissolved oxygen concentration in the groundwater reaching the PRB.

Consider a PRB of 1-m thickness, and let's look at the groundwater reaching a 1-m^2 section (perpendicular to groundwater flow). In essence, we are considering the performance of a 1 m^3 volume of PRB (Figure 6.1).

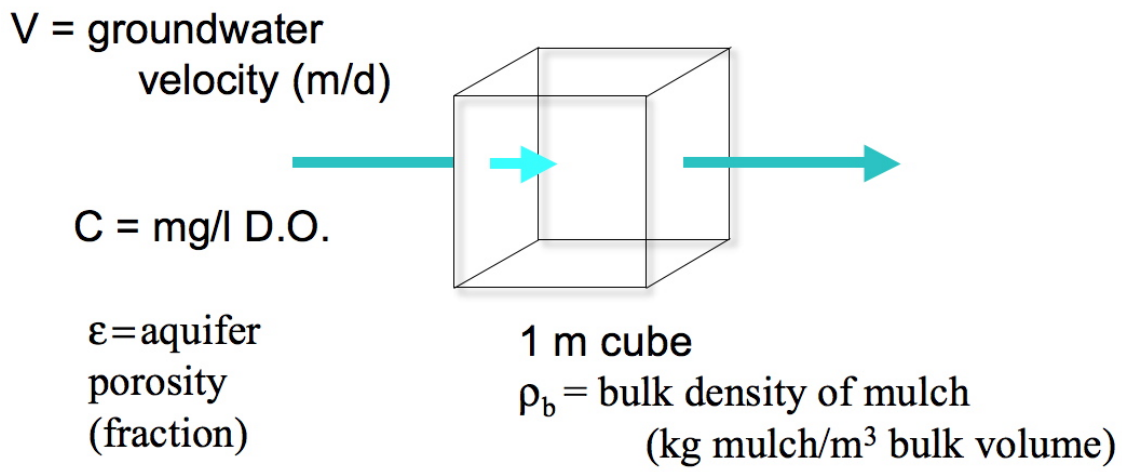

Figure 6.1 Schematic representation of a section of a PRB.

Whatever happens to the 1-m^2 surface facing groundwater flow is what happens throughout the length and depth of the PRB (assuming one uses an appropriate average velocity). What is the mass/time of O_2 that enters that 1 m^2 surface of the PRB?

The flow, Q (m^3/d), entering the 1 m^2 surface would be V (m^3 water d^{-1} m^{-2} cross-sectional pore area) * ε (m^2 cross-sectional pore area m^{-2} nominal area). Therefore, the mg/d of O_2 reaching the 1-m^2 surface of the PRB is:

$$\text{mg/d } O_2 \text{ per m}^2 \text{ PRB} = 1000 \cdot C \cdot V \cdot \varepsilon \qquad [6.1]$$

where the factor 1000 is a conversion of C from mg/L to mg/m^3

The 1 m^3 cube of PRB contains r_b kg dry mulch, with a capacity of 31.2 mg O_2 per gram, or 31,200 mg/kg. Therefore, the capacity of the cube (days) would be:

$$\text{capacity(days/meter thickness)} = \frac{31{,}200 \cdot \rho_b}{1000 \cdot C \cdot V \cdot \varepsilon} \qquad [6.2]$$

or

$$\text{capacity(years/meter thickness)} = \frac{31.2 \cdot \rho_b}{365.25 \cdot C \cdot V \cdot \varepsilon} \qquad [6.3]$$

Table 6.1 shows the effects of groundwater velocity and dissolved-oxygen concentration on the estimated capacity of a bark-mulch PRB, based upon Eq [6.3]. For purposes of calculation, the bulk density of mulch (r_b) was assumed to be 300 kg/m^3 [63], while aquifer porosity (ε) was assumed to be 0.3 [64] Groundwater velocity (V) was assumed to range between 0.2 to 0.7 m/d [65], and dissolved oxygen (C) between 1 to 5 mg/L. [66]

Table 6.1 Multiple PRB capacities with two series variables.

Capacity (year/m) C(mg/L) V(m/d)	1	2	3	4	5
0.2	581	290	194	145	116
0.3	387	194	129	97	77
0.4	290	145	97	73	58
0.5	232	116	77	58	46
0.6	194	97	65	48	39
0.7	166	83	55	41	33

APPENDIX 1. DEPLETION-CURVE DATA.

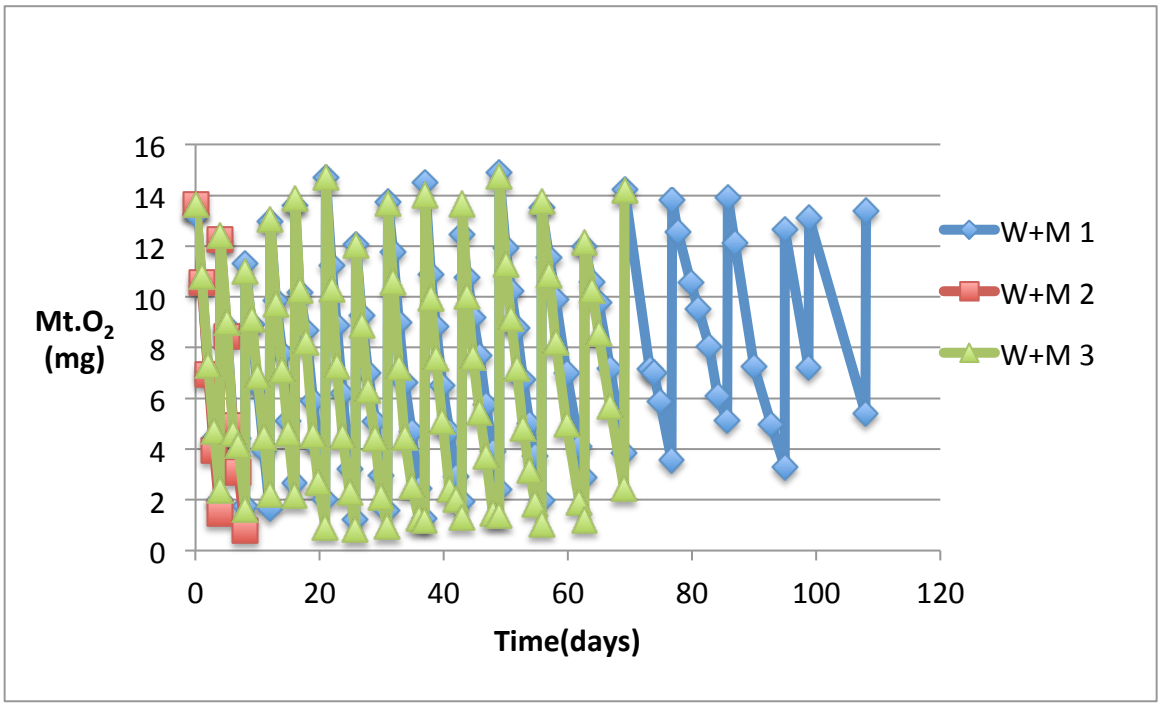

Figure A1.1 Real-time $Mt.O_2$ monitoring for "W+M" in Experiment 1.

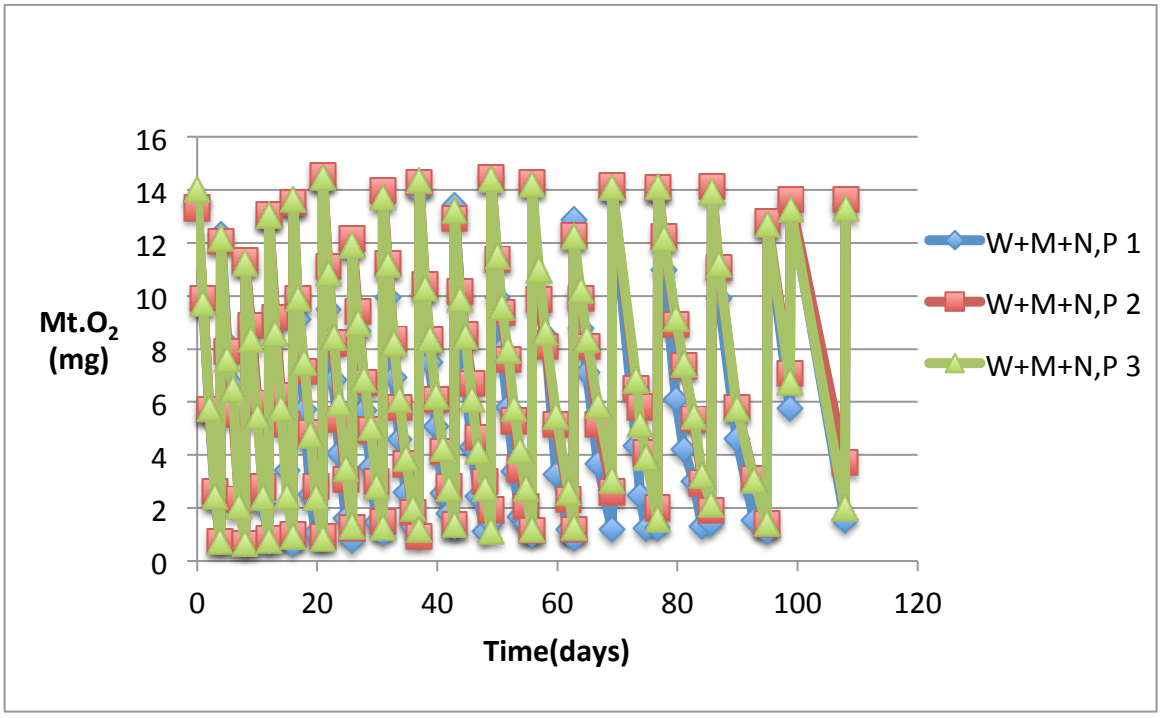

Figure A1.2 Real-time $Mt.O_2$ monitoring for "W+M+N, P" in Experiment 1.

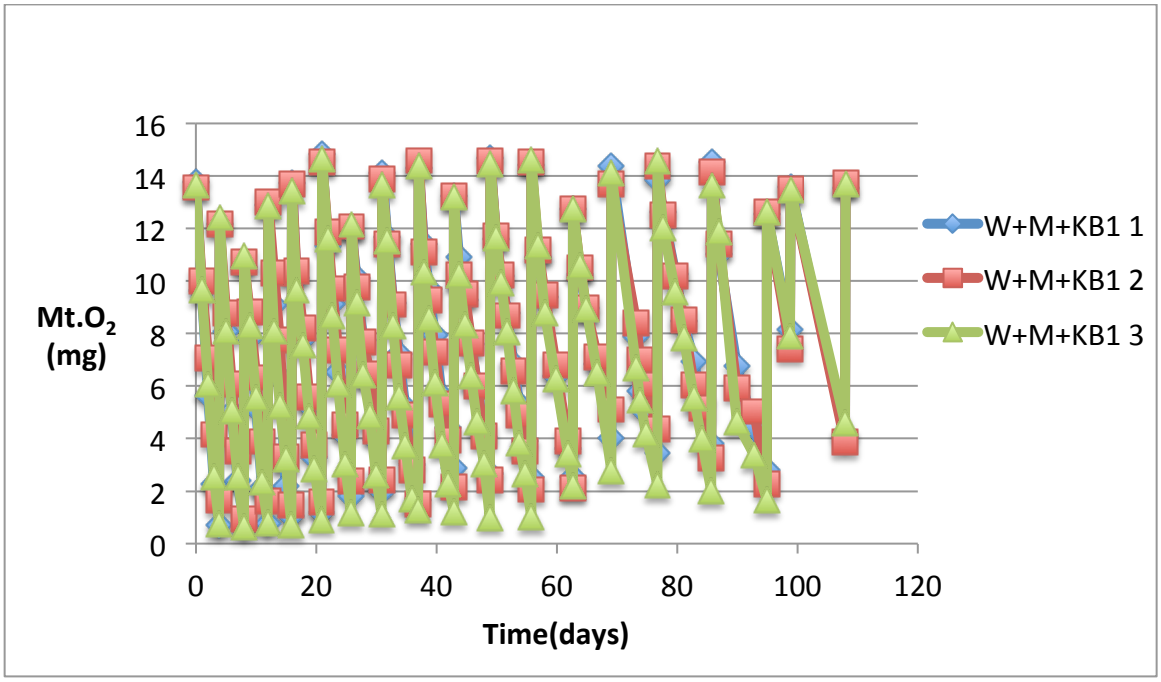

Figure A1.3 Real-time $Mt.O_2$ monitoring for "W+M+KB1" in Experiment 1.

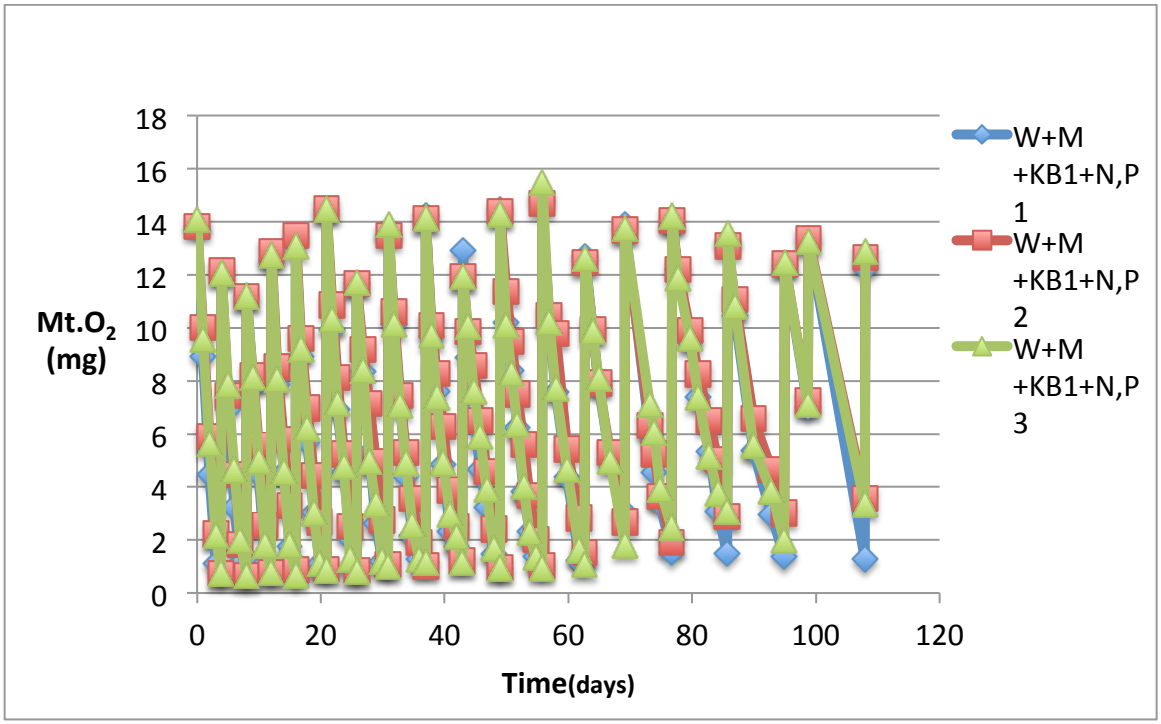

Figure A1.4 Real-time $Mt.O_2$ monitoring for "W+M+KB1+N, P" in Experiment 1.

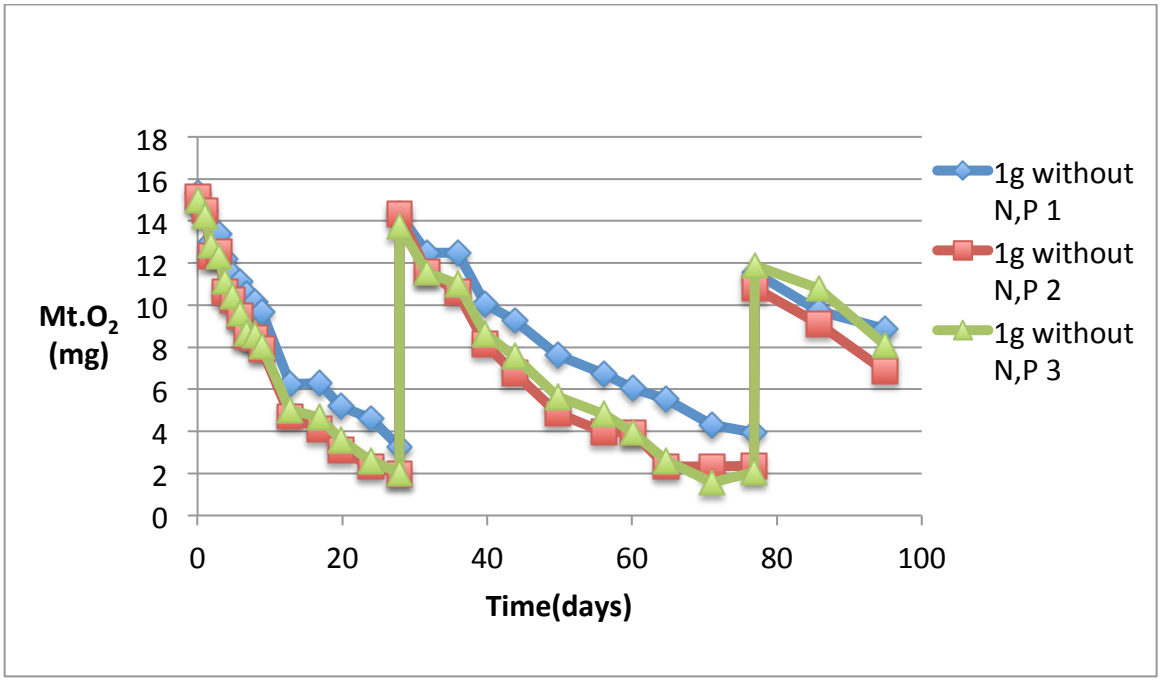

Figure A1.5 Real-time $Mt.O_2$ monitoring for "1g without N, P" in Experiment 2.

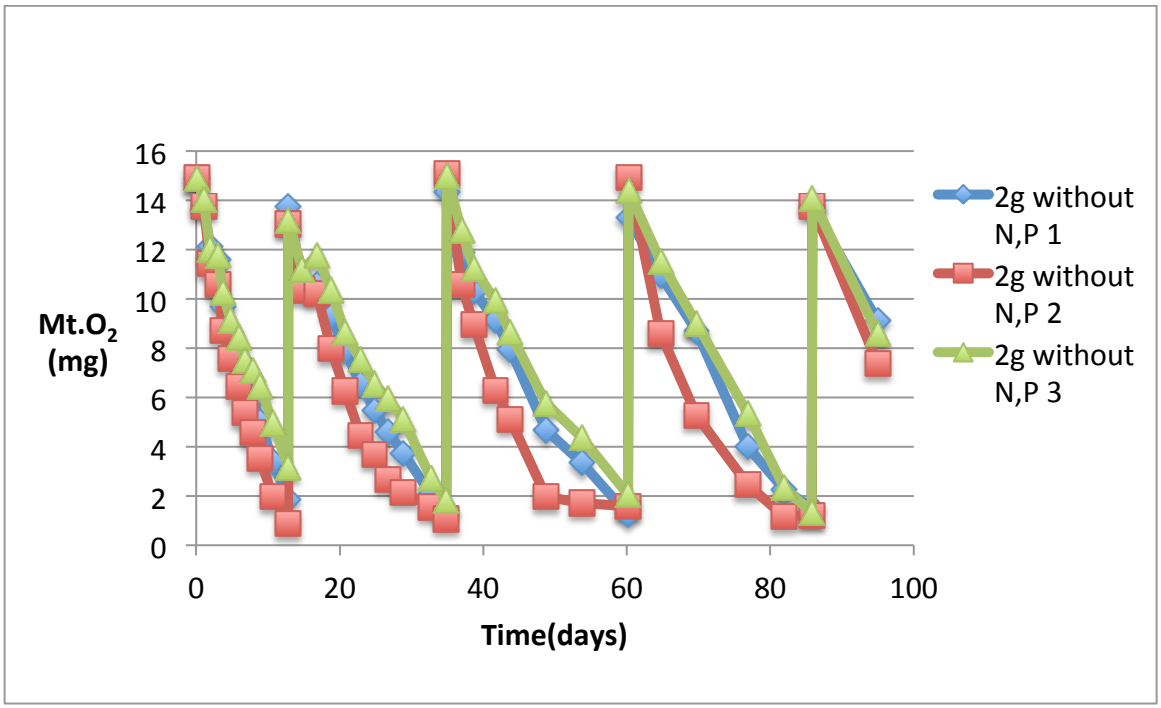

Figure A1.6 Real-time $Mt.O_2$ monitoring for "2g without N, P" in Experiment 2.

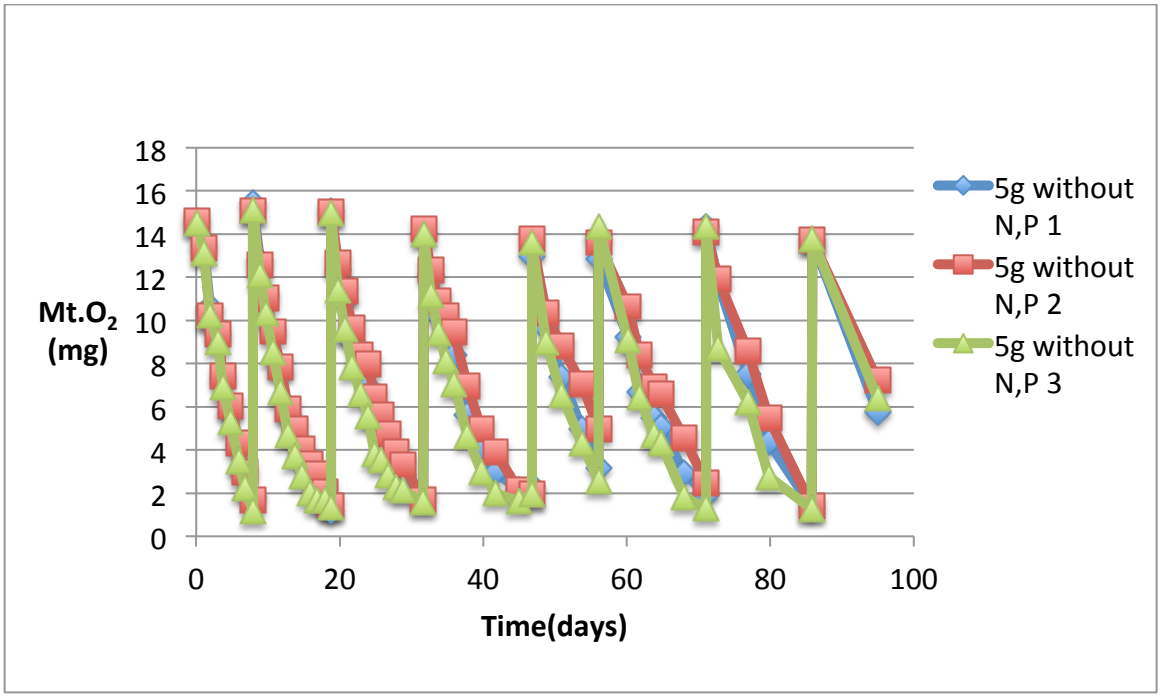

Figure A1.7 Real-time $Mt.O_2$ monitoring for "5g without N, P" in Experiment 2.

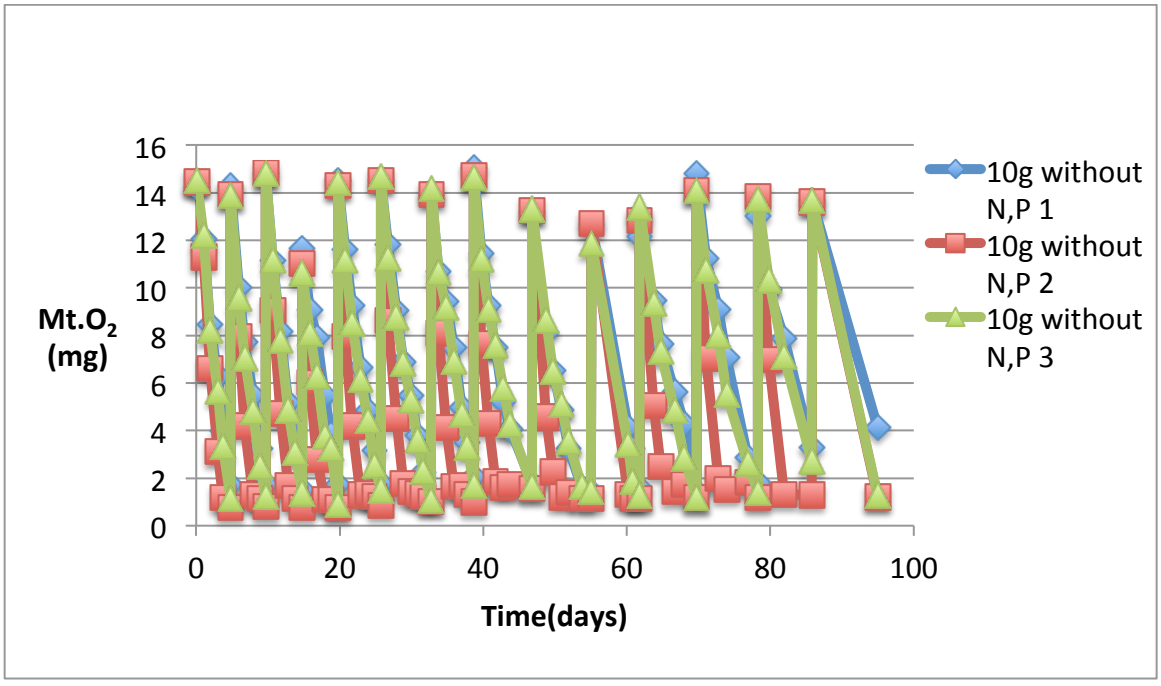

Figure A1.8 Real-time $Mt.O_2$ monitoring for "10g without N, P" in Experiment 2.

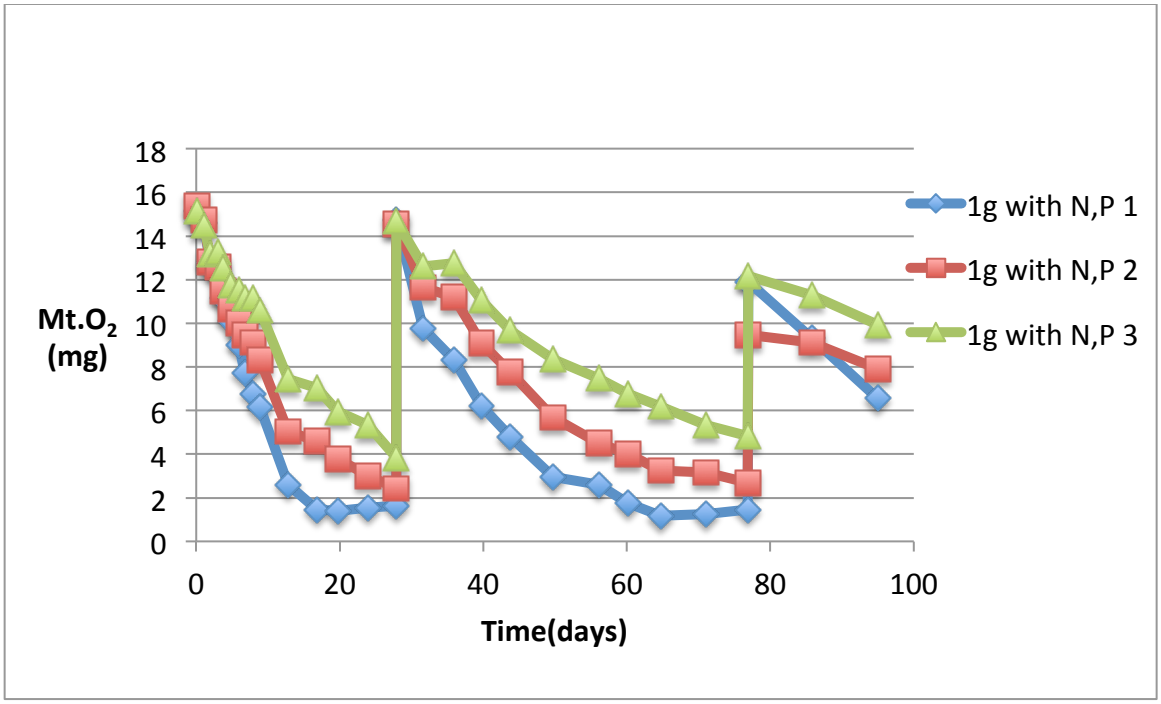

Figure A1.9 Real-time $Mt.O_2$ monitoring for "1g with N, P" in Experiment 2.

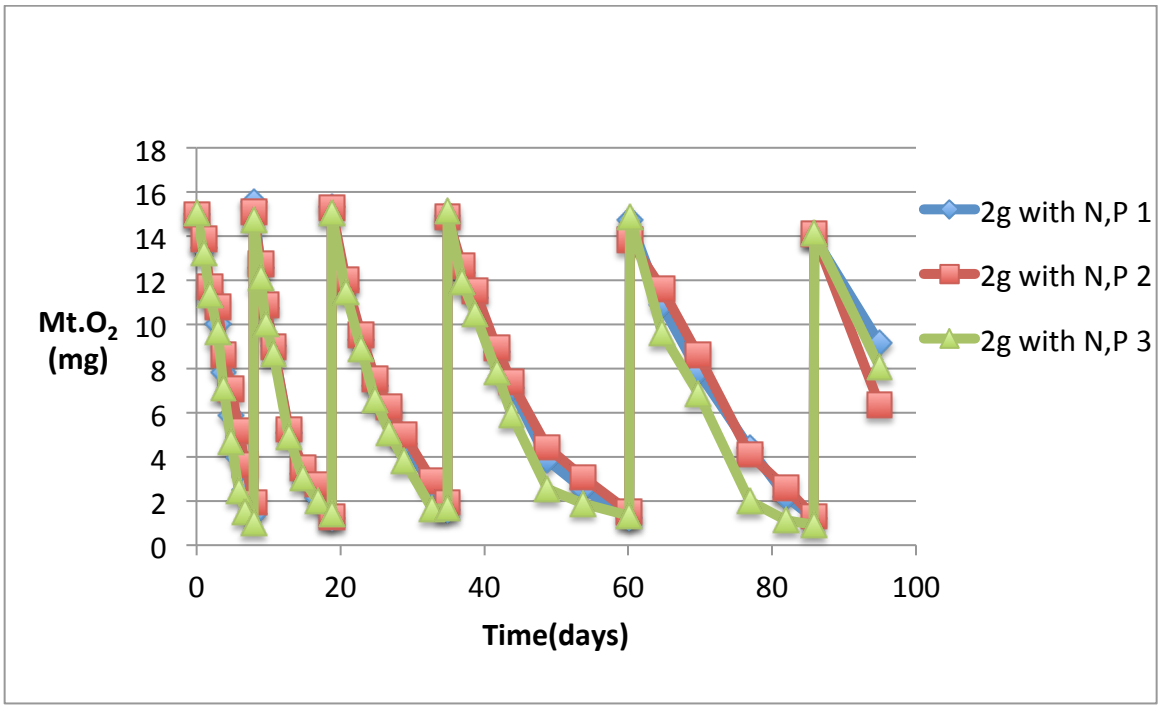

Figure A1.10 Real-time $Mt.O_2$ monitoring for "2g with N, P" in Experiment 2.

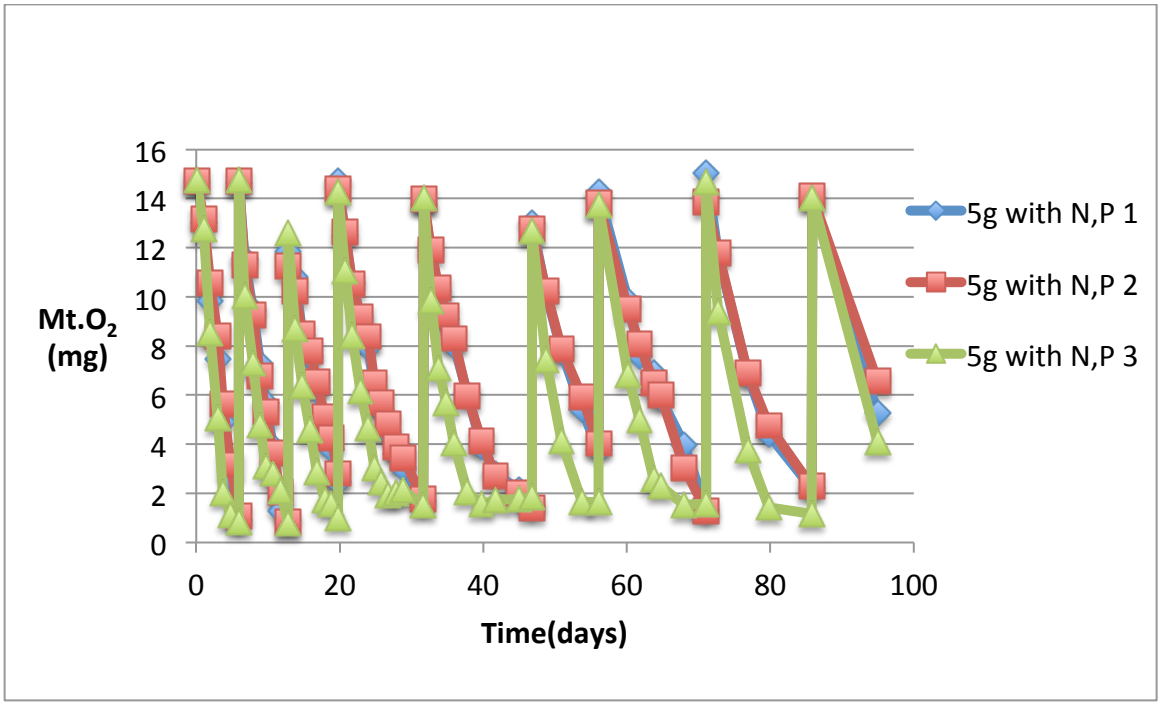

Figure A1.11 Real-time $Mt.O_2$ monitoring for "5g with N, P" in Experiment 2.

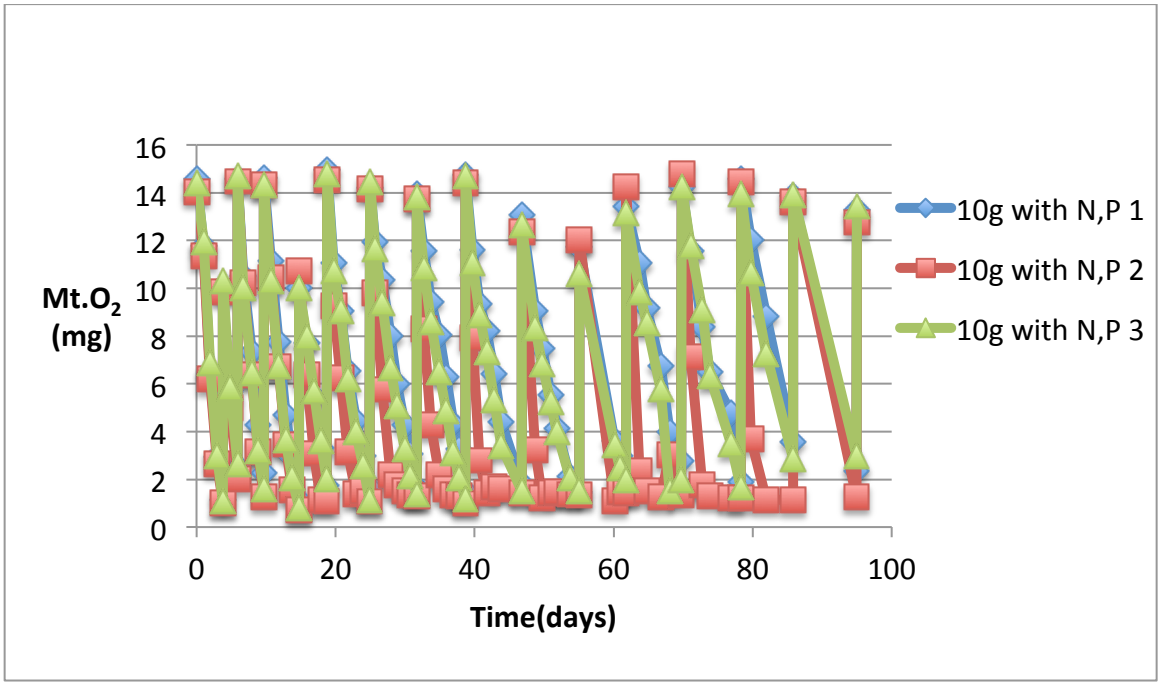

Figure A1.12 Real-time $Mt.O_2$ monitoring for "10g with N, P" in Experiment 2.

APPENDIX 2. FIRST-ORDER MODEL-FITTING USING TWO FITTING PARAMETERS (k AND L_0).

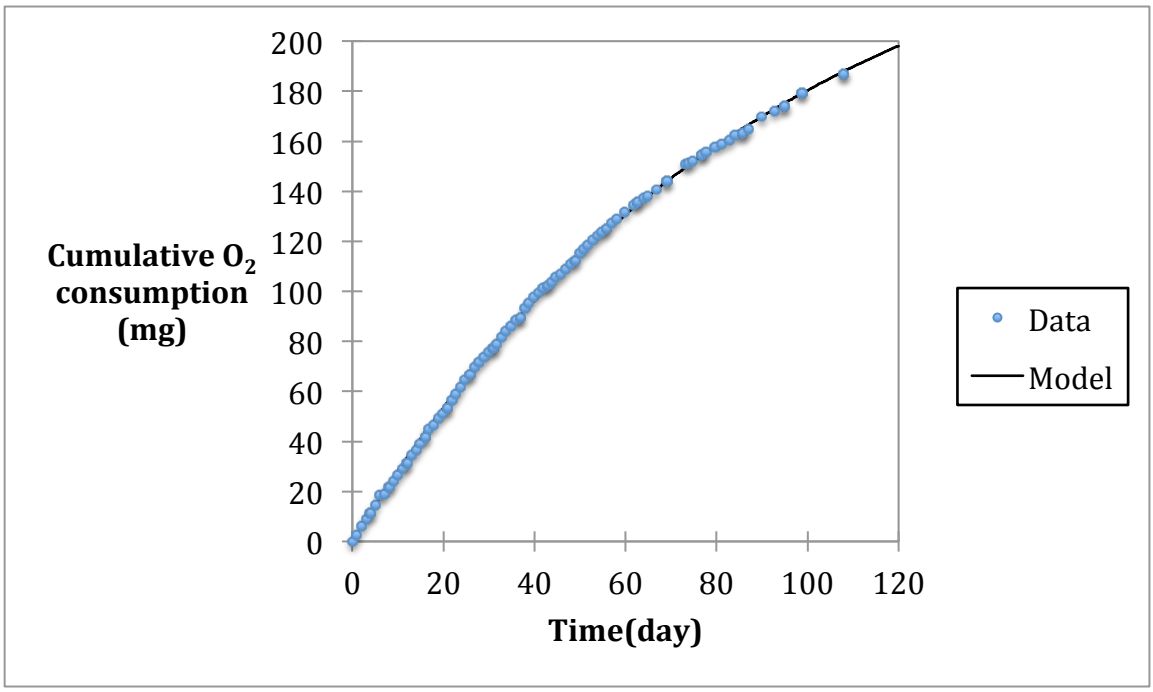

Figure A2.1 Cumulative O_2 consumption for "M+W 1" in Experiment 1.

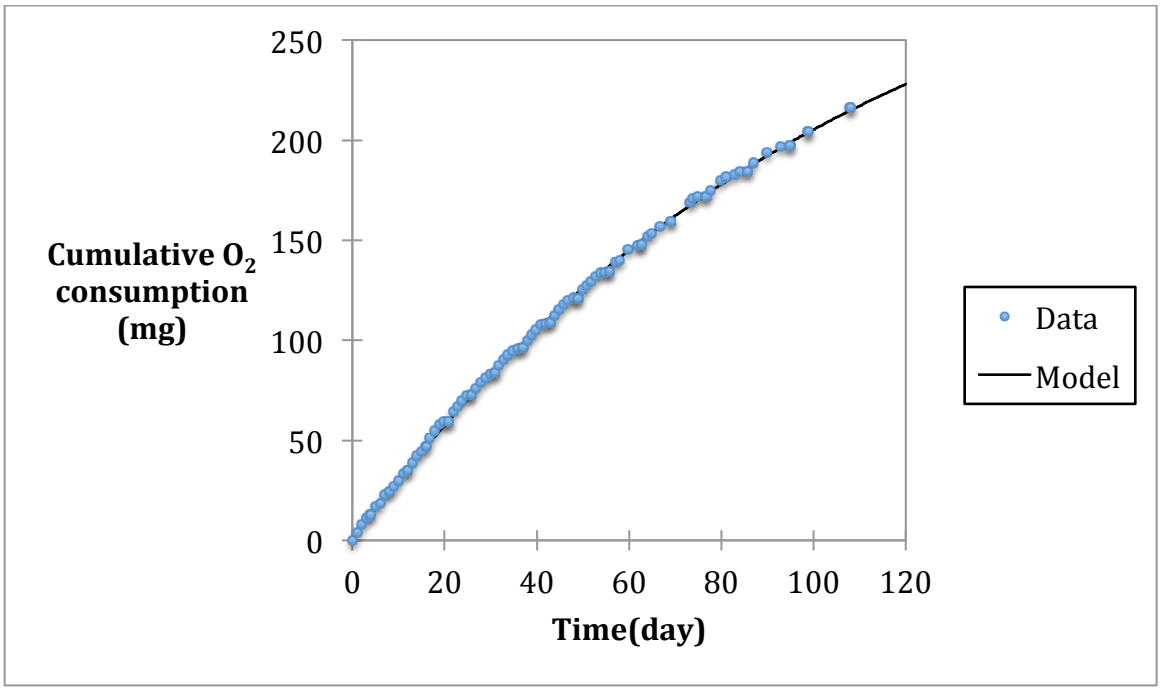

Figure A2.2 Cumulative O_2 consumption for "M+W+N, P 1" in Experiment 1.

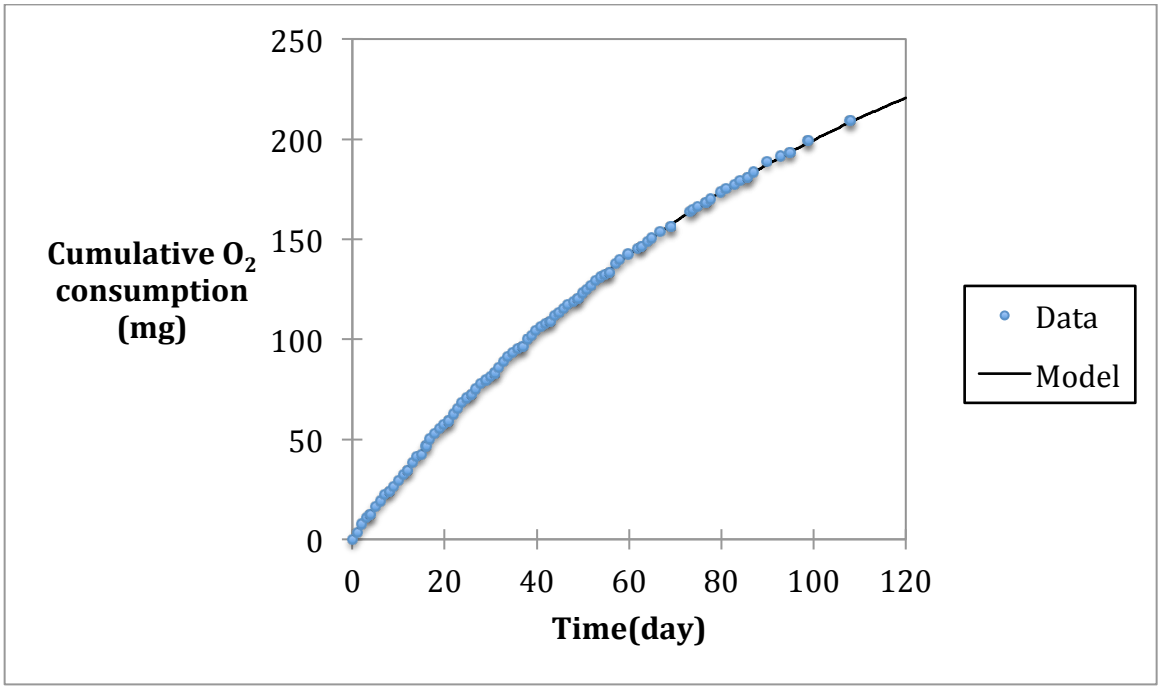

Figure A2.3 Cumulative O_2 consumption for "M+W+N, P 2" in Experiment 1.

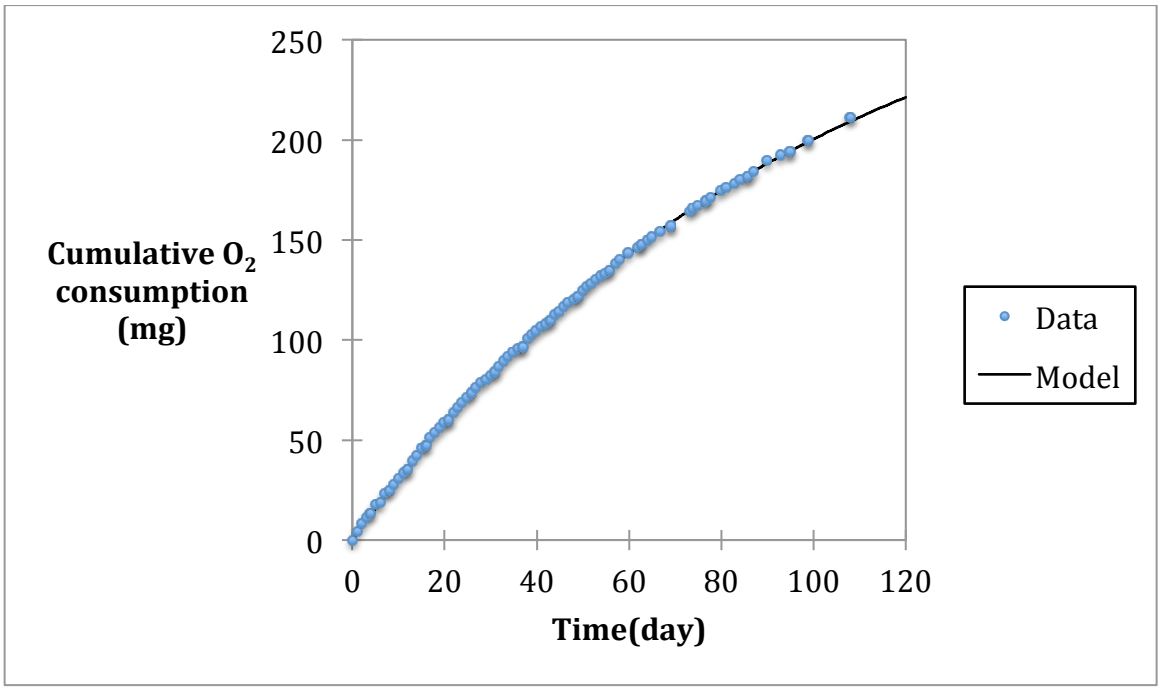

Figure A2.4 Cumulative O_2 consumption for "M+W+N, P 3" in Experiment 1.

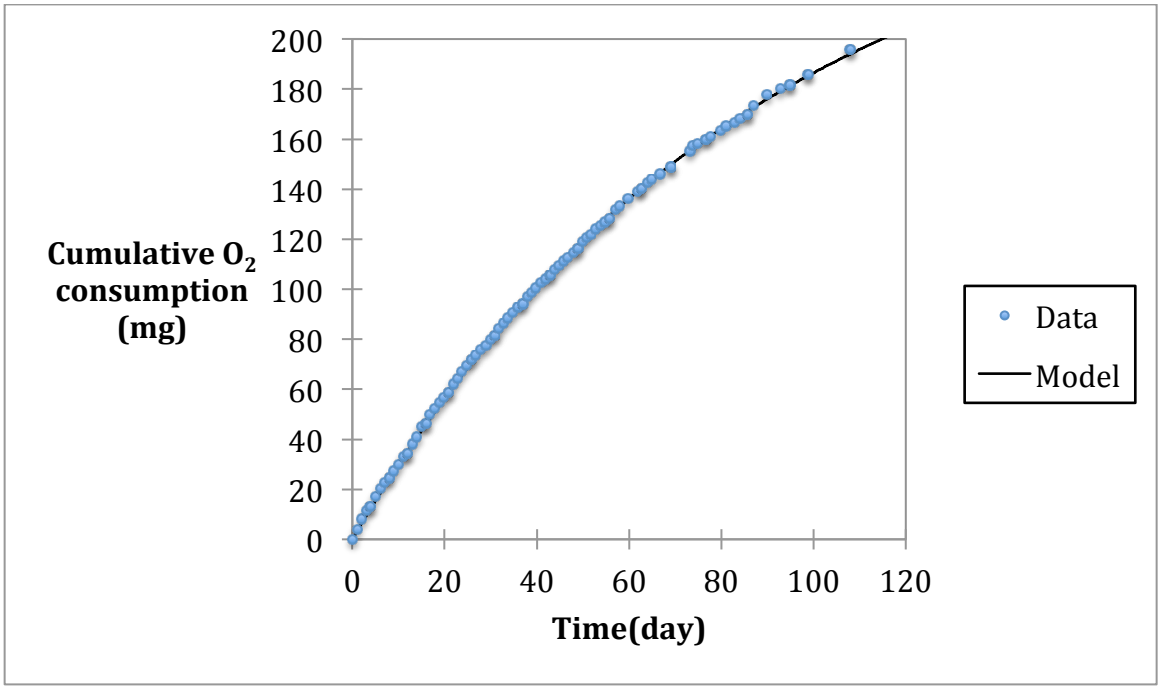

Figure A2.5 Cumulative O_2 consumption for "M+W+KB1 1" in Experiment 1.

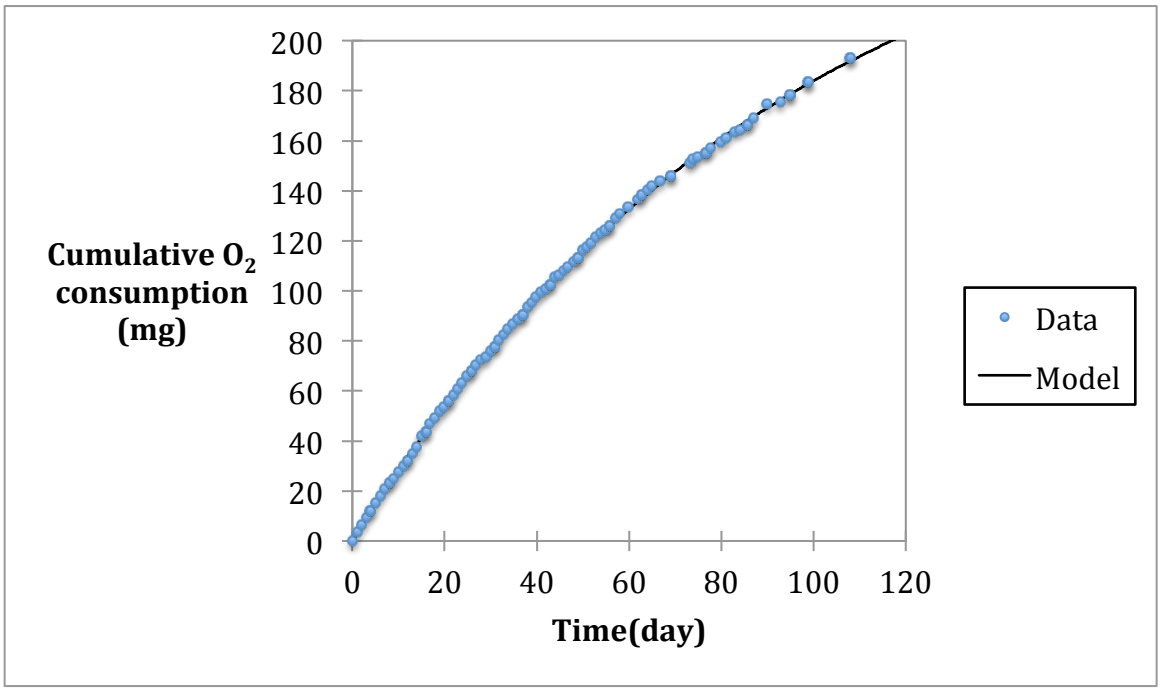

Figure A2.6 Cumulative O_2 consumption for "M+W+KB1 2" in Experiment 1.

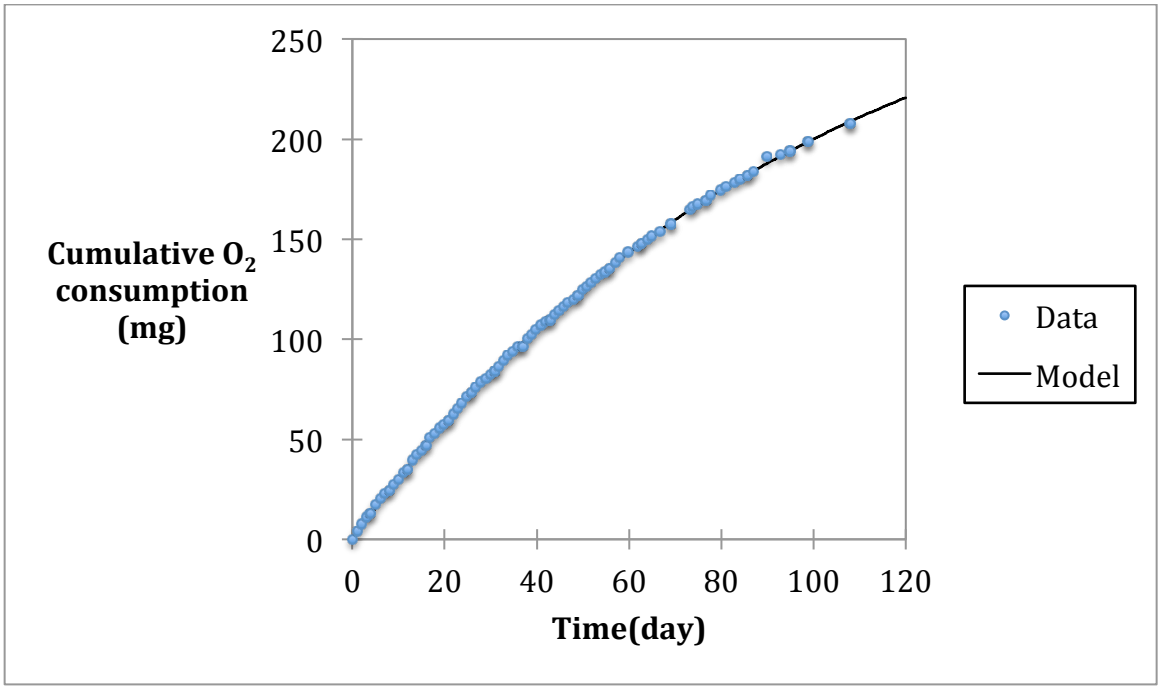

Figure A2.7 Cumulative O_2 consumption for "M+W+KB1 3" in Experiment 1.

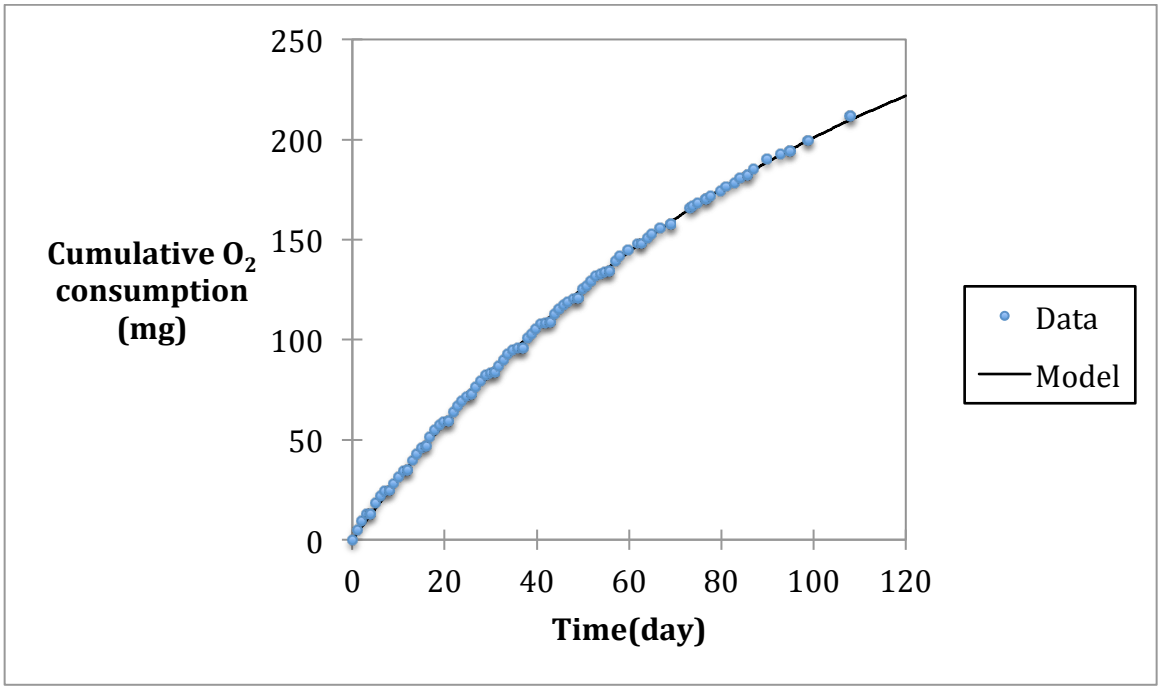

Figure A2.8 Cumulative O_2 consumption for "M+W+KB1+N, P 1" in Experiment 1.

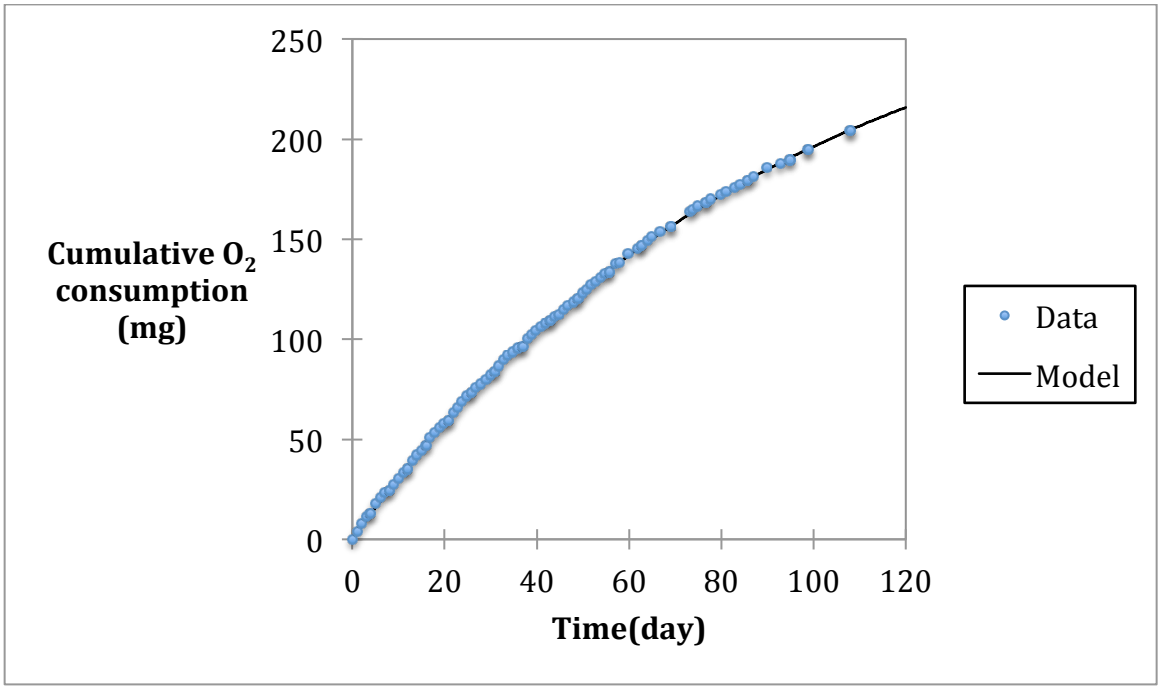

Figure A2.9 Cumulative O_2 consumption for "M+W+KB1+N, P 2" in Experiment 1.

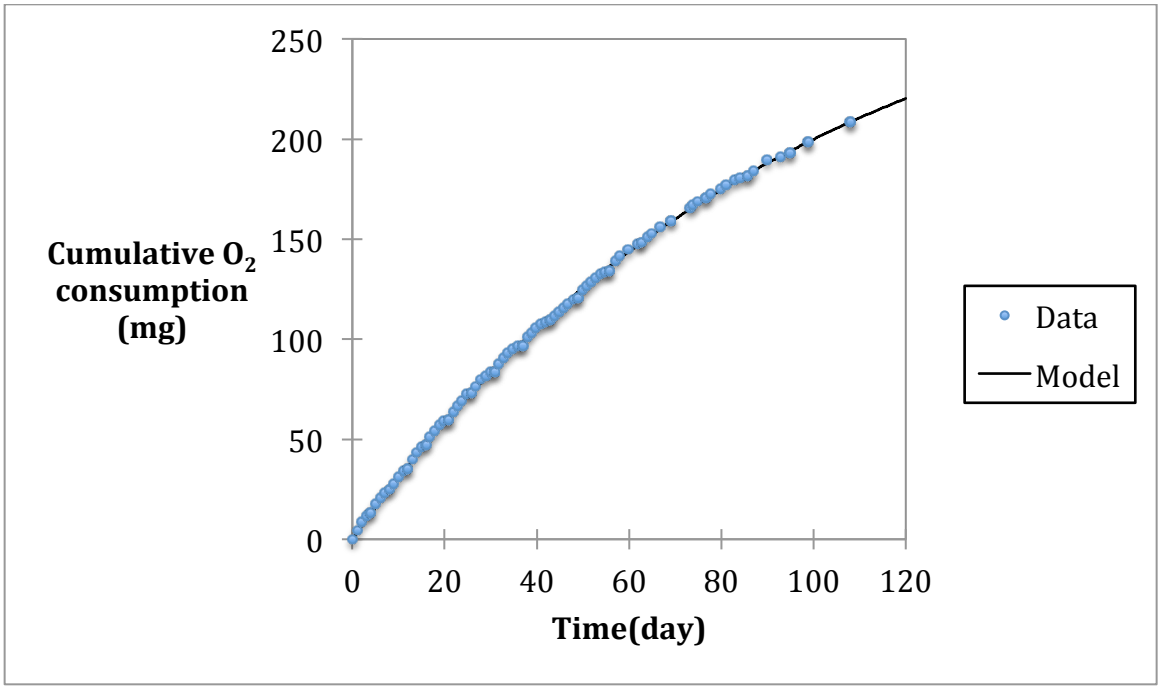

Figure A2.10 Cumulative O_2 consumption for "M+W+KB1+N, P 3" in Experiment 1.

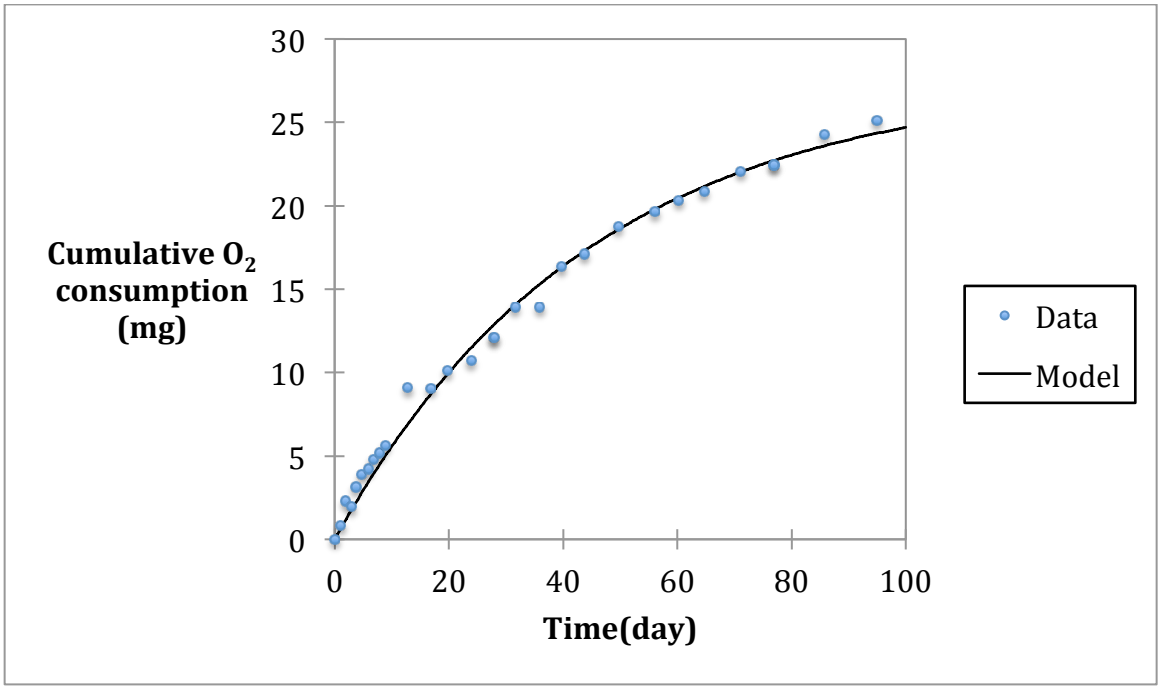

Figure A2.11 Cumulative O_2 consumption for "1g without N, P 1" in Experiment 2.

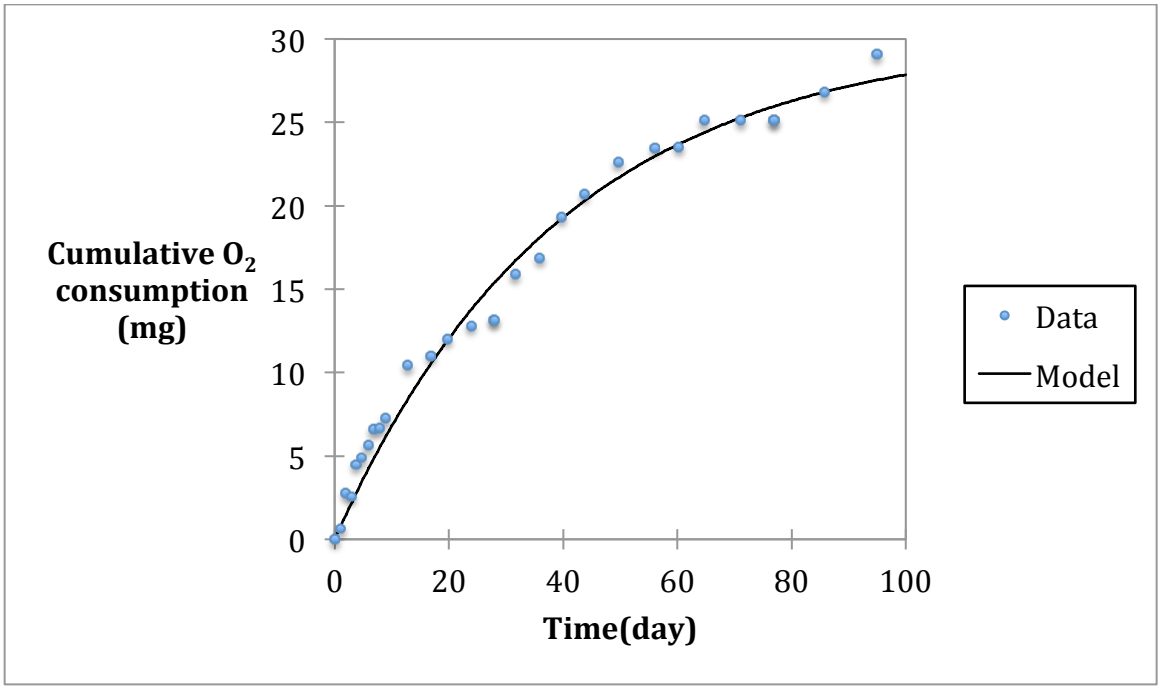

Figure A2.12 Cumulative O_2 consumption for "1g without N, P 2" in Experiment 2.

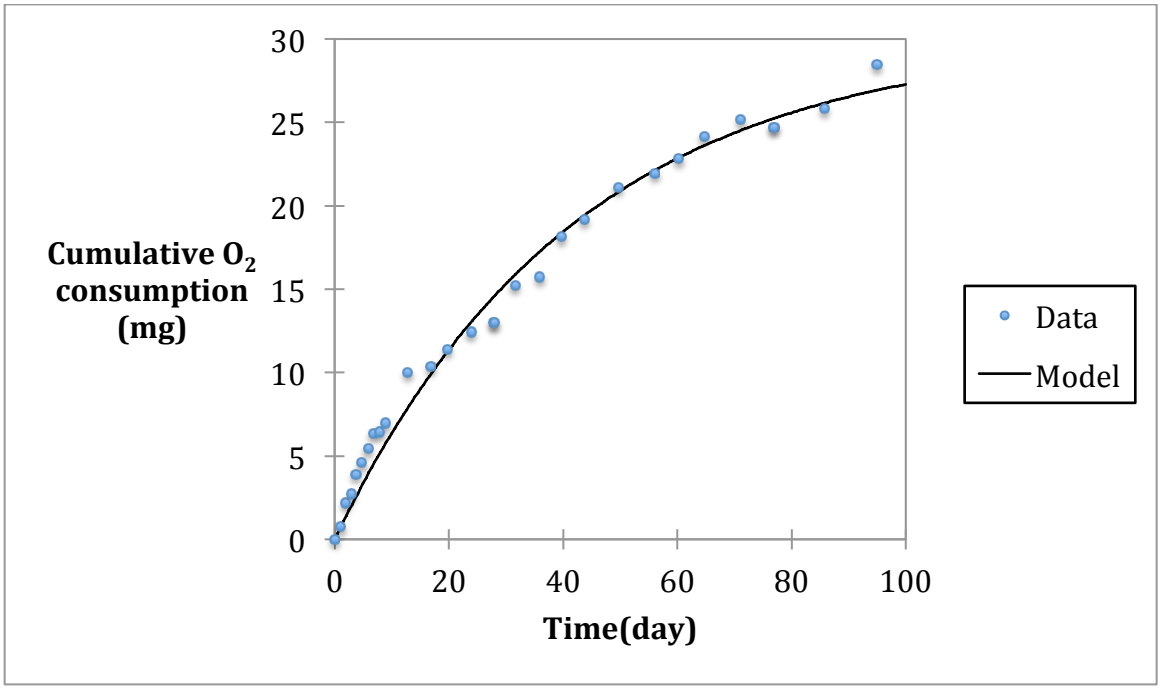

Figure A2.13 Cumulative O_2 consumption for "1g without N, P 3" in Experiment 2.

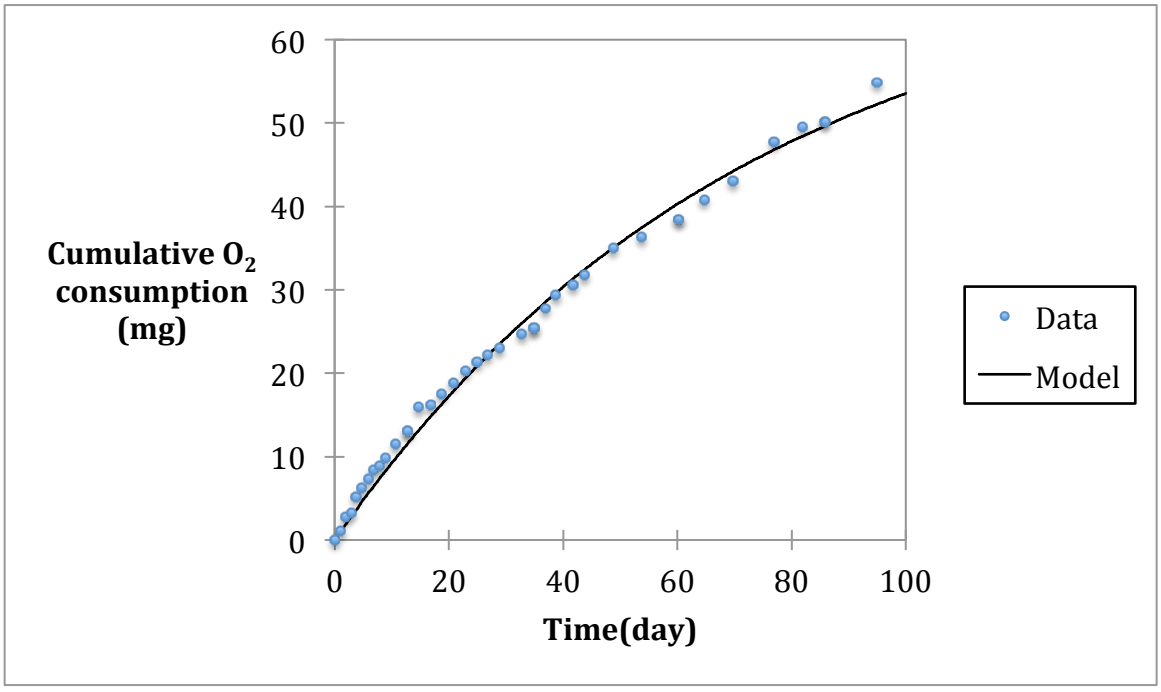

Figure A2.14 Cumulative O_2 consumption for "2g without N, P 1" in Experiment 2.

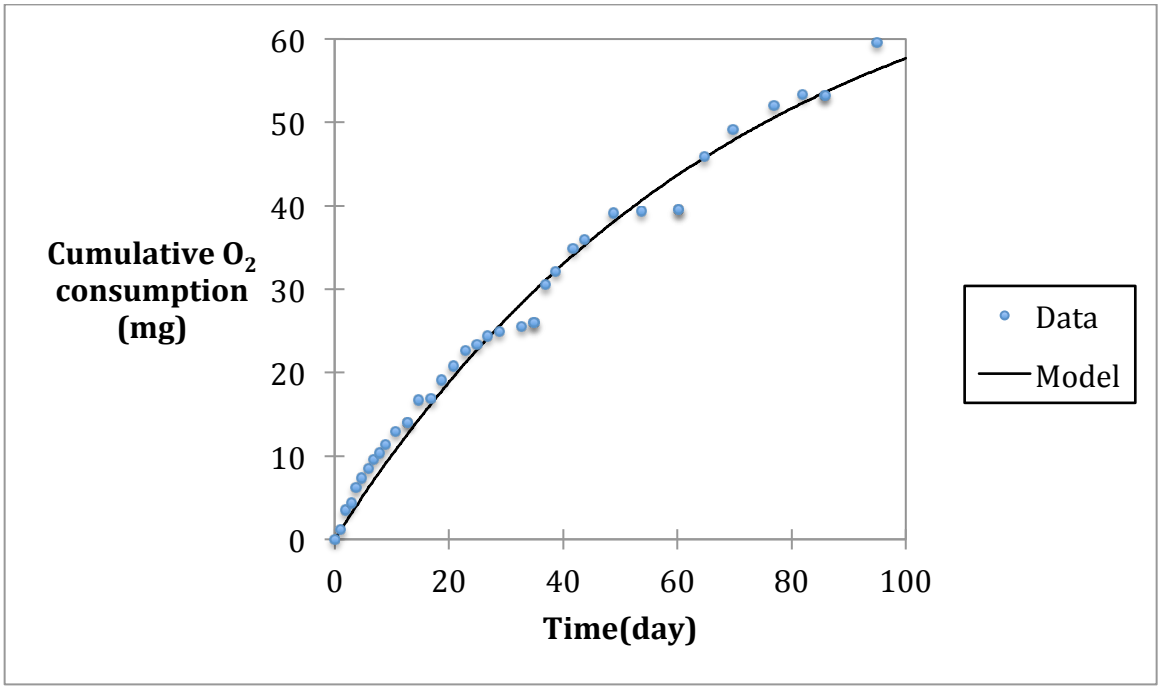

Figure A2.15 Cumulative O_2 consumption for "2g without N, P 2" in Experiment 2.

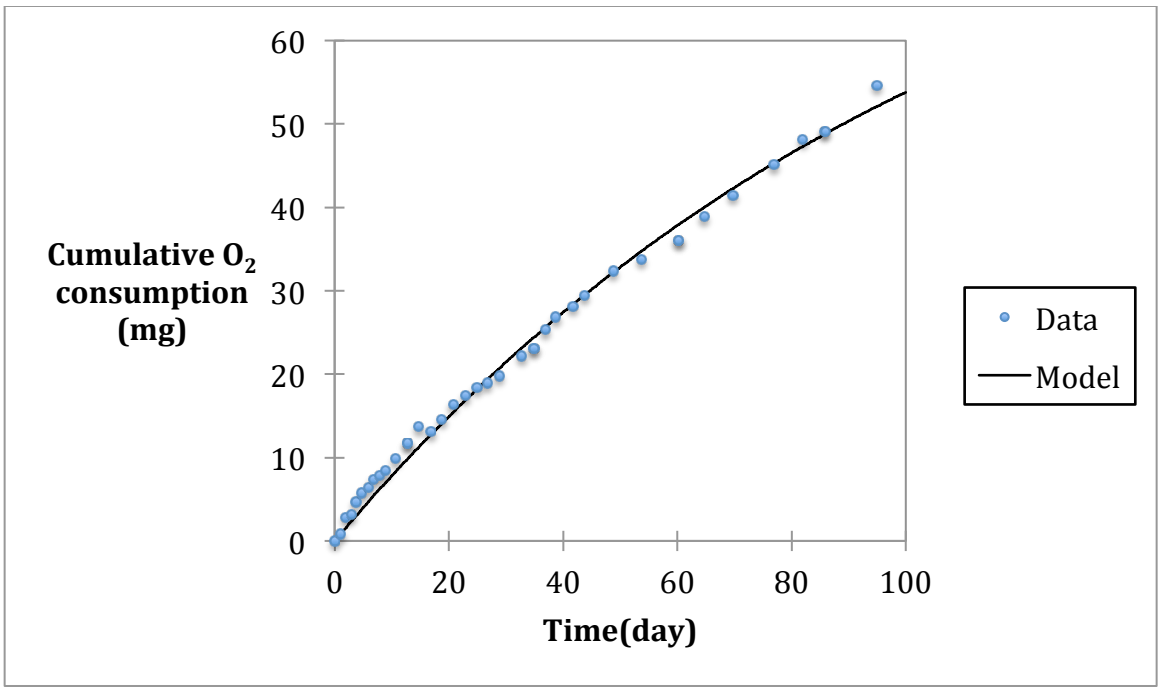

Figure A2.16 Cumulative O_2 consumption for "2g without N, P 3" in Experiment 2.

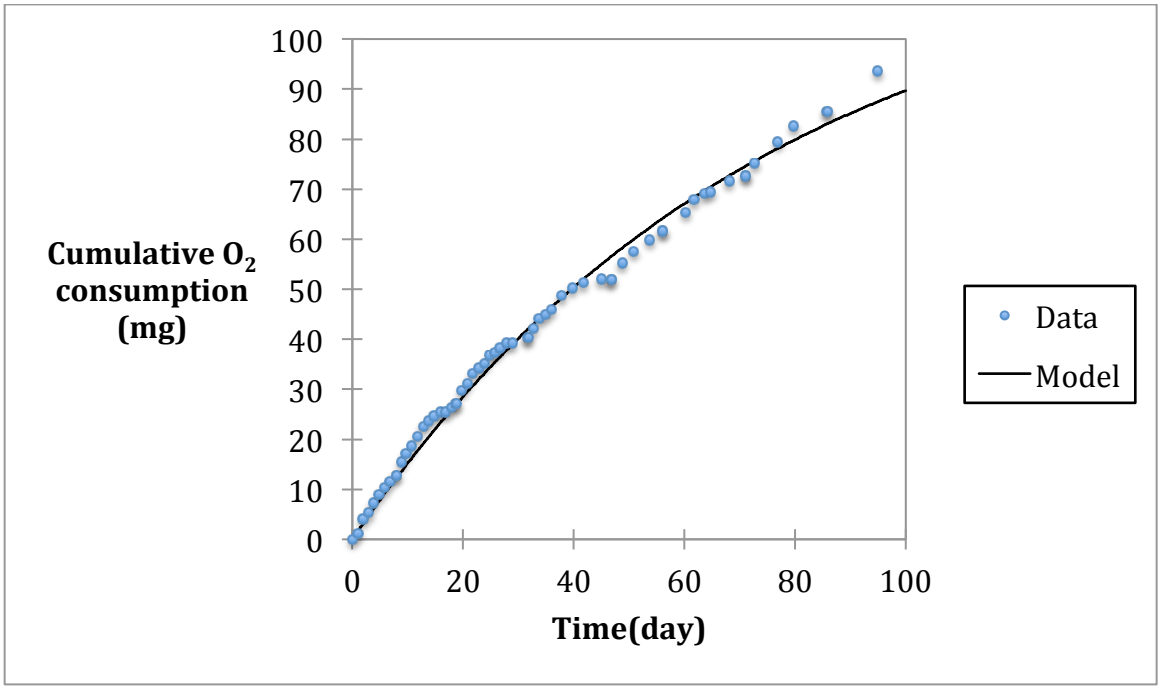

Figure A2.17 Cumulative O_2 consumption for "5g without N, P 1" in Experiment 2.

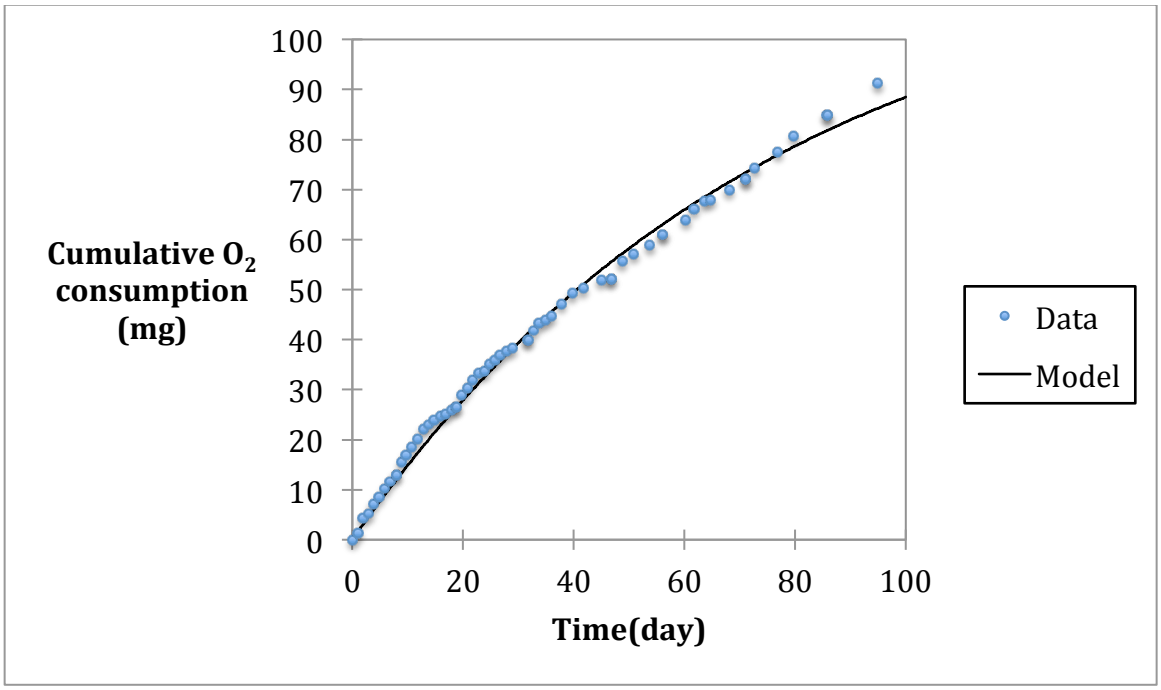

Figure A2.18 Cumulative O_2 consumption for "5g without N, P 2" in Experiment 2.

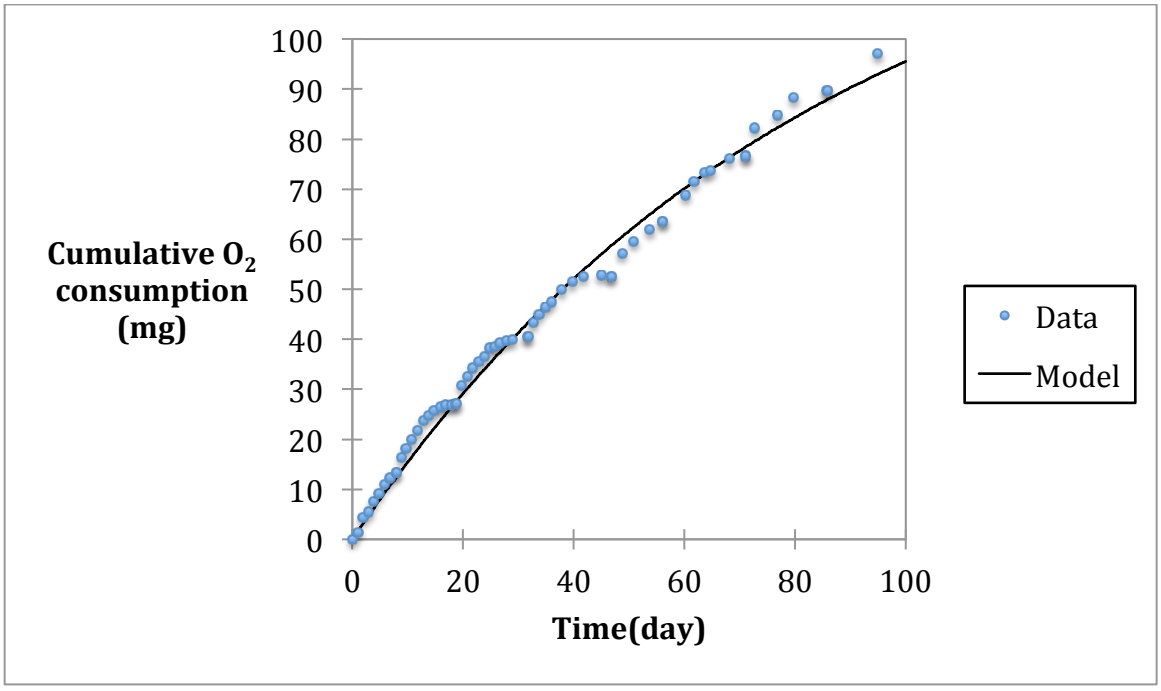

Figure A2.19 Cumulative O_2 consumption for "5g without N, P 3" in Experiment 2.

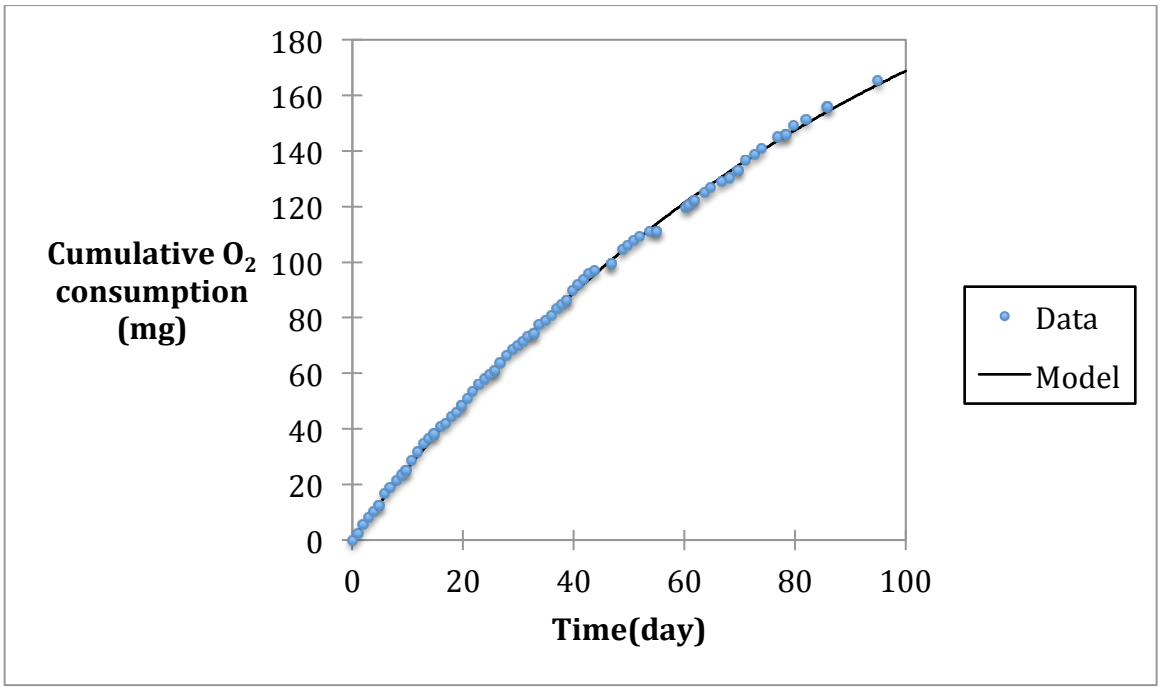

Figure A2.20 Cumulative O_2 consumption for "10g without N, P 1" in Experiment 2.

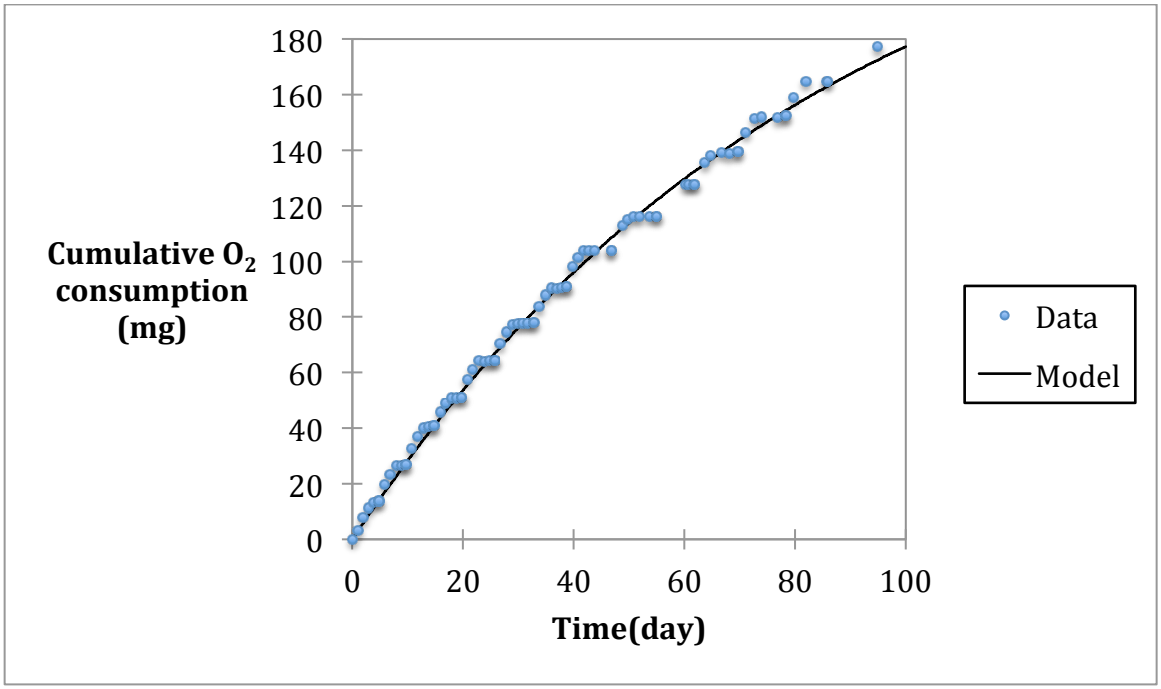

Figure A2.21 Cumulative O_2 consumption for "10g without N, P 2" in Experiment 2.

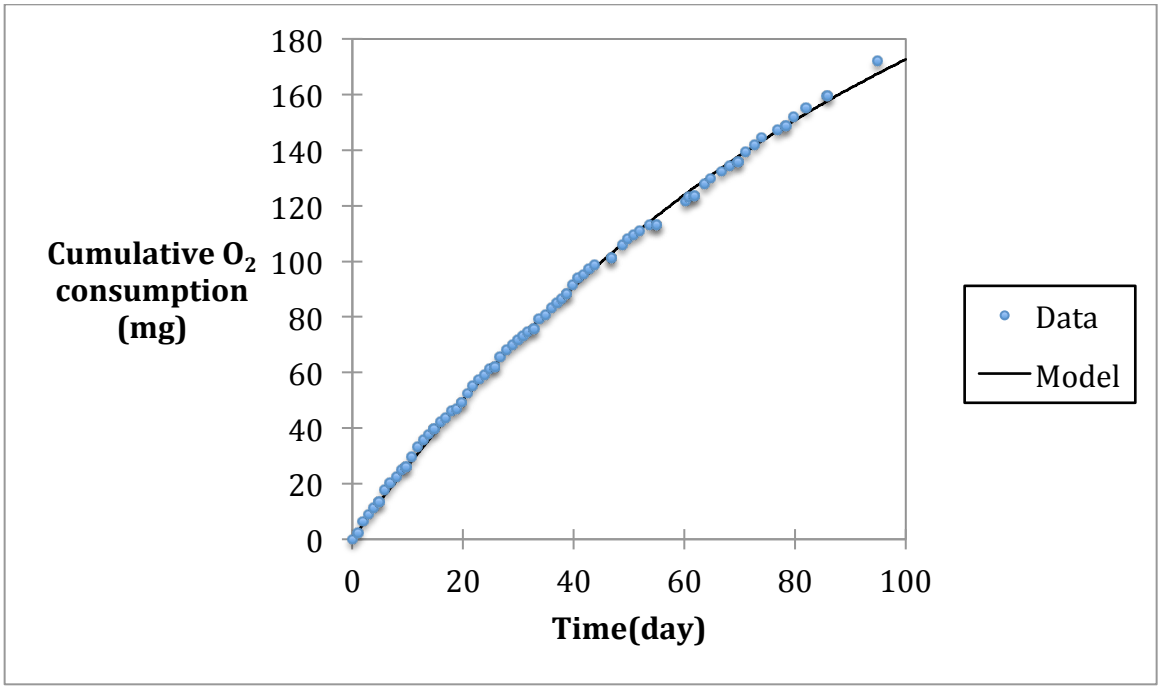

Figure A2.22 Cumulative O_2 consumption for "10g without N, P 3" in Experiment 2.

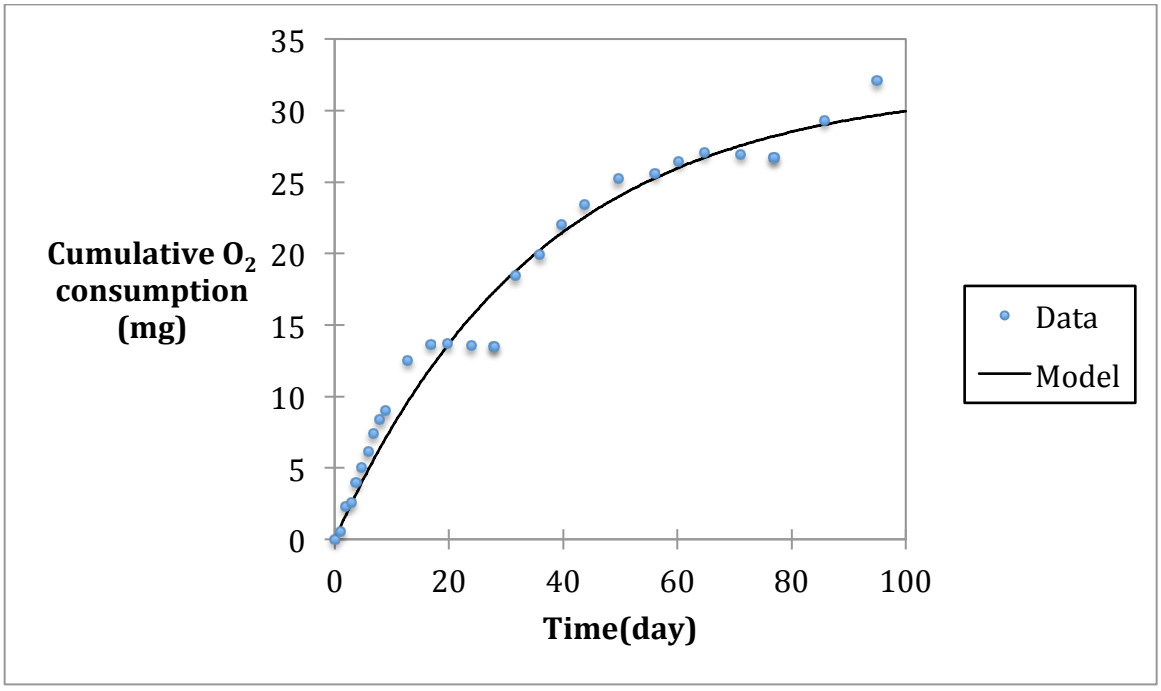

Figure A2.23 Cumulative O_2 consumption for "1g with N, P 1" in Experiment 2.

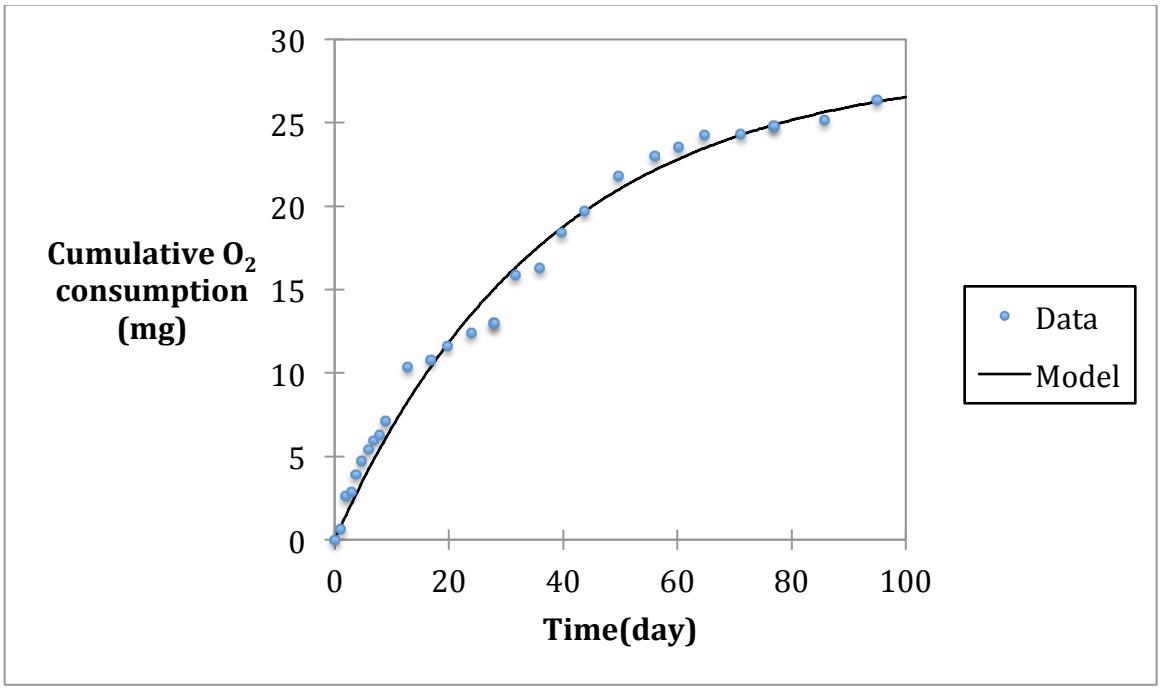

Figure A2.24 Cumulative O_2 consumption for "1g with N, P 2" in Experiment 2.

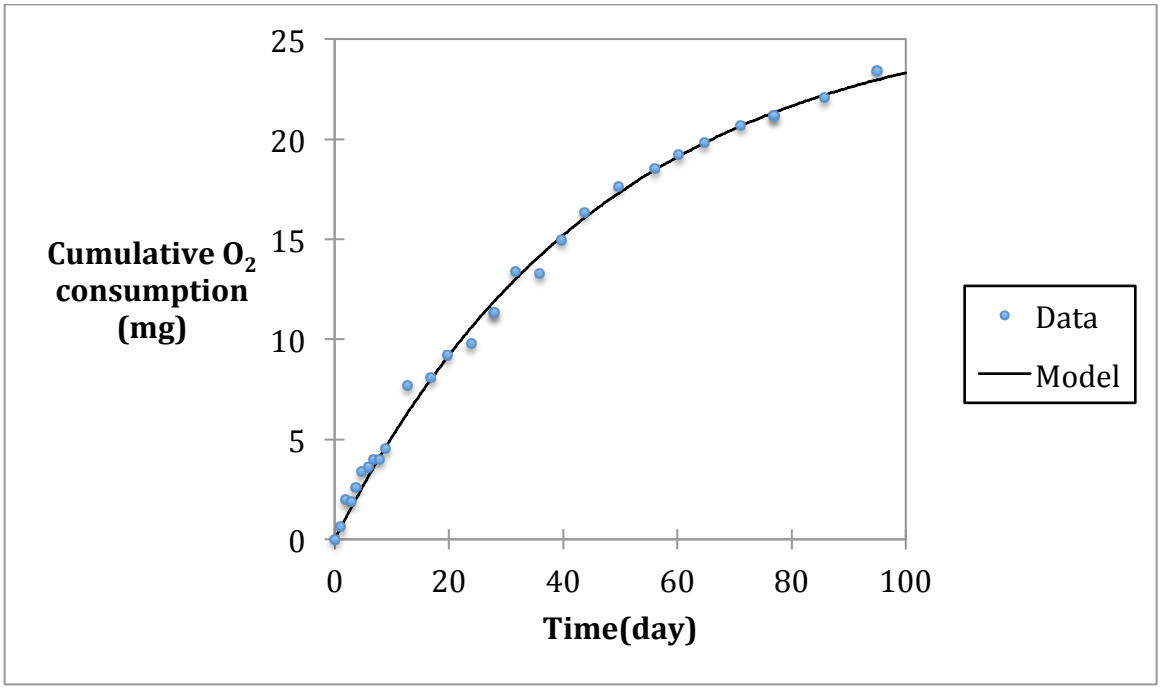

Figure A2.25 Cumulative O_2 consumption for "1g with N, P 3" in Experiment 2.

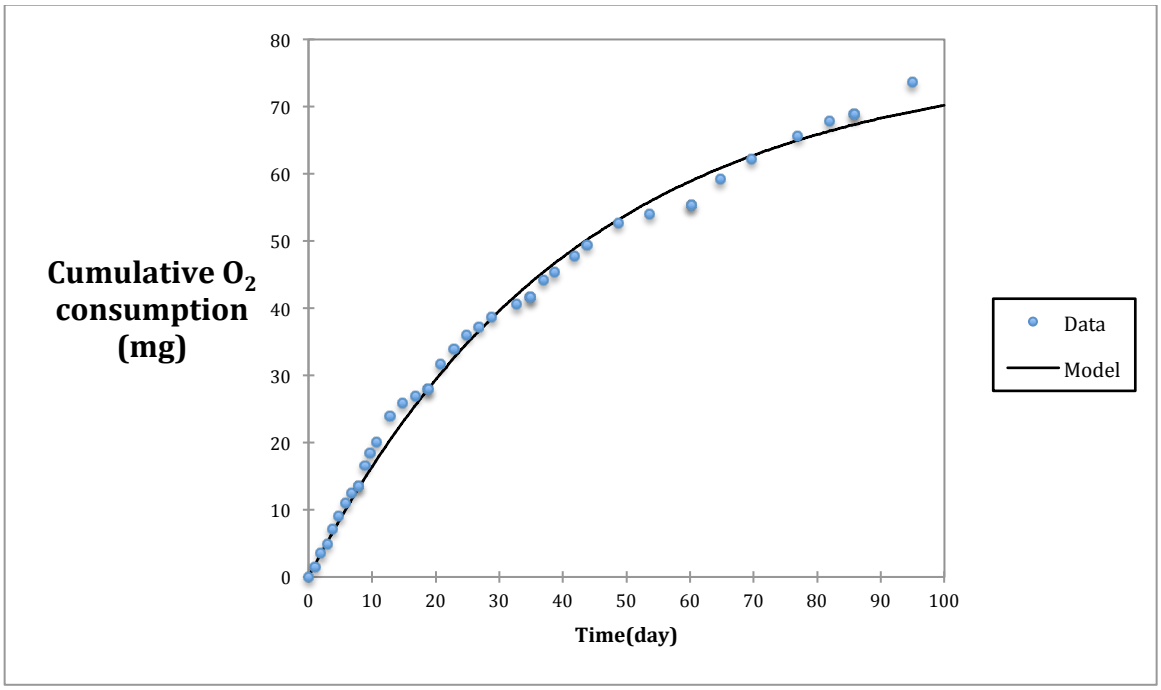

Figure A2.26 Cumulative O_2 consumption for "2g with N, P 1" in Experiment 2.

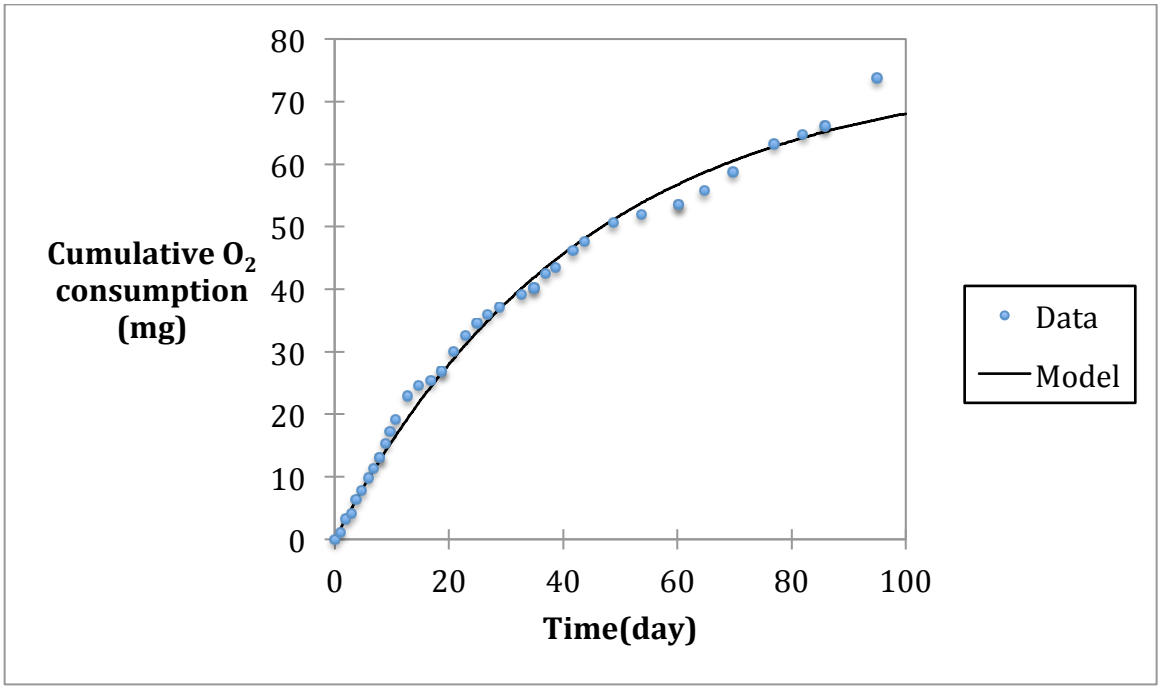

Figure A2.27 Cumulative O_2 consumption for "2g with N, P 2" in Experiment 2.

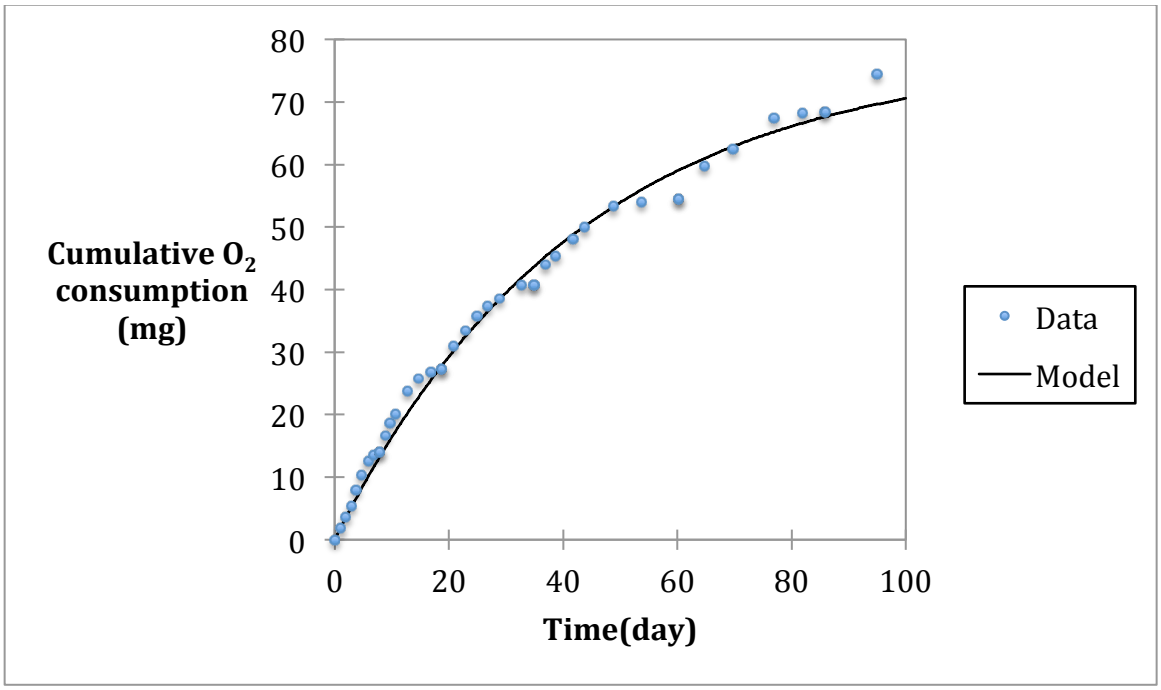

Figure A2.28 Cumulative O_2 consumption for "2g with N, P 3" in Experiment 2.

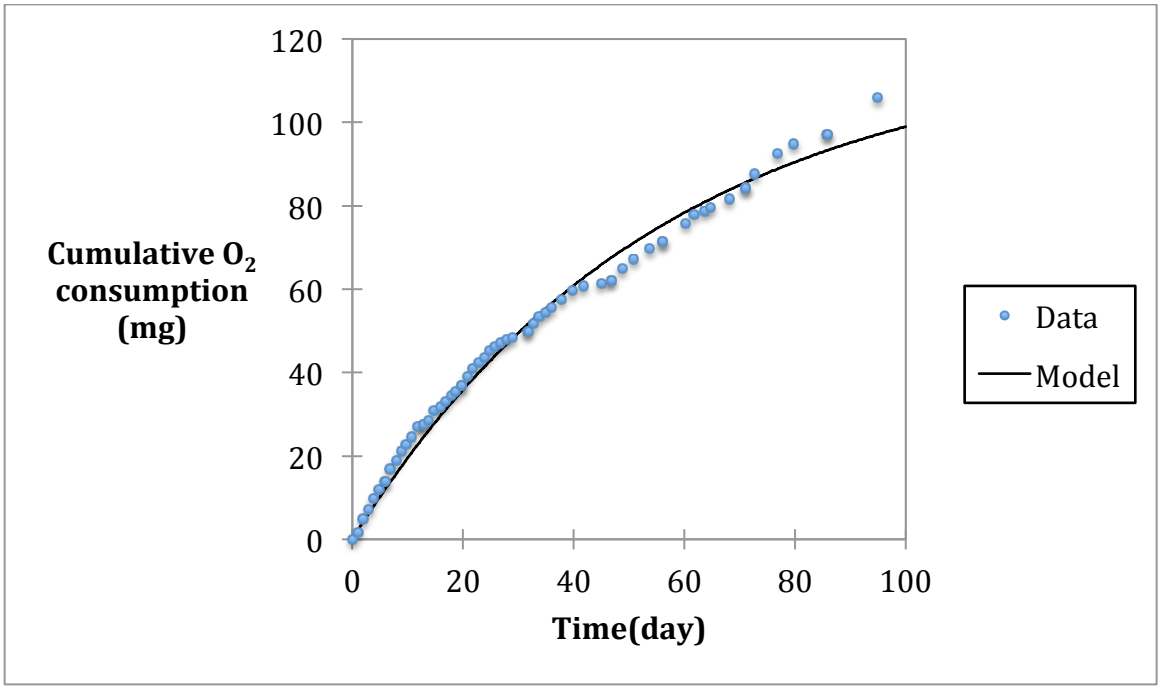

Figure A2.29 Cumulative O_2 consumption for "5g with N, P 1" in Experiment 2.

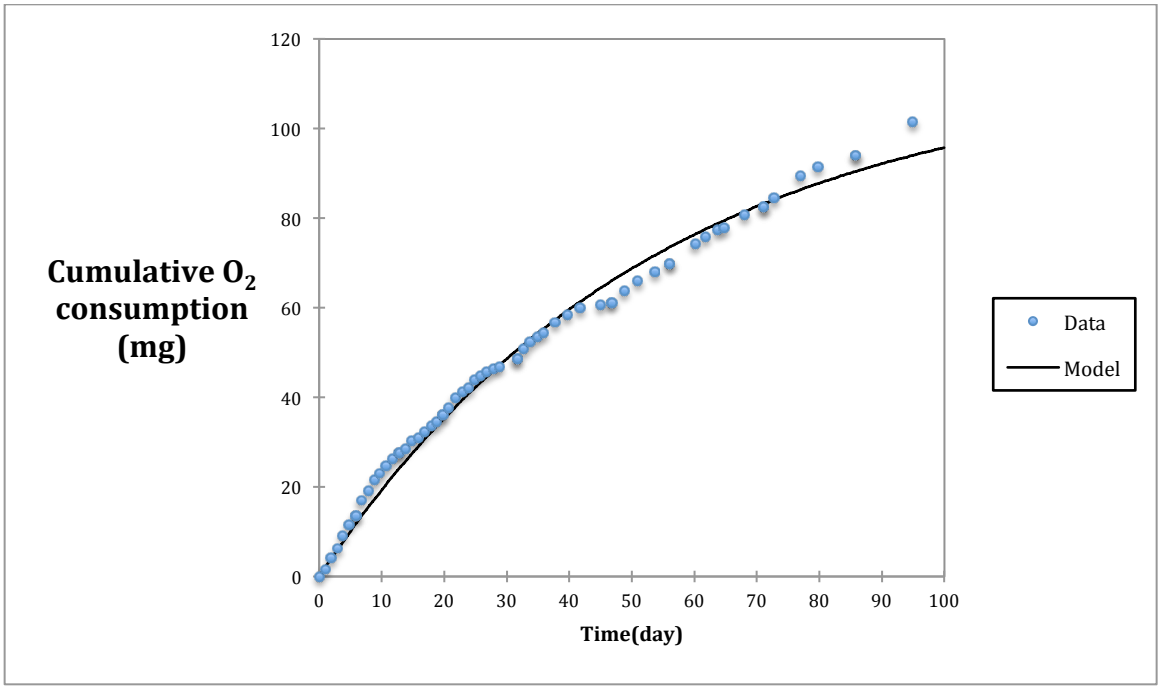

Figure A2.30 Cumulative O_2 consumption for "5g with N, P 2" in Experiment 2.

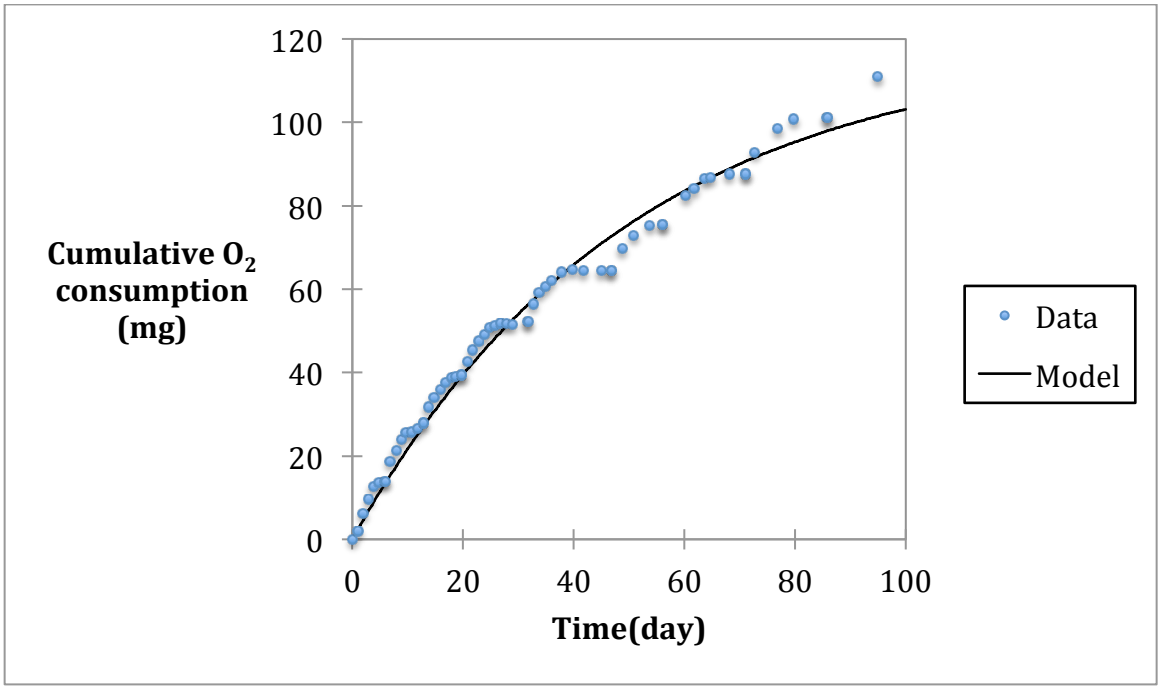

Figure A2.31 Cumulative O_2 consumption for "5g with N, P 3" in Experiment 2.

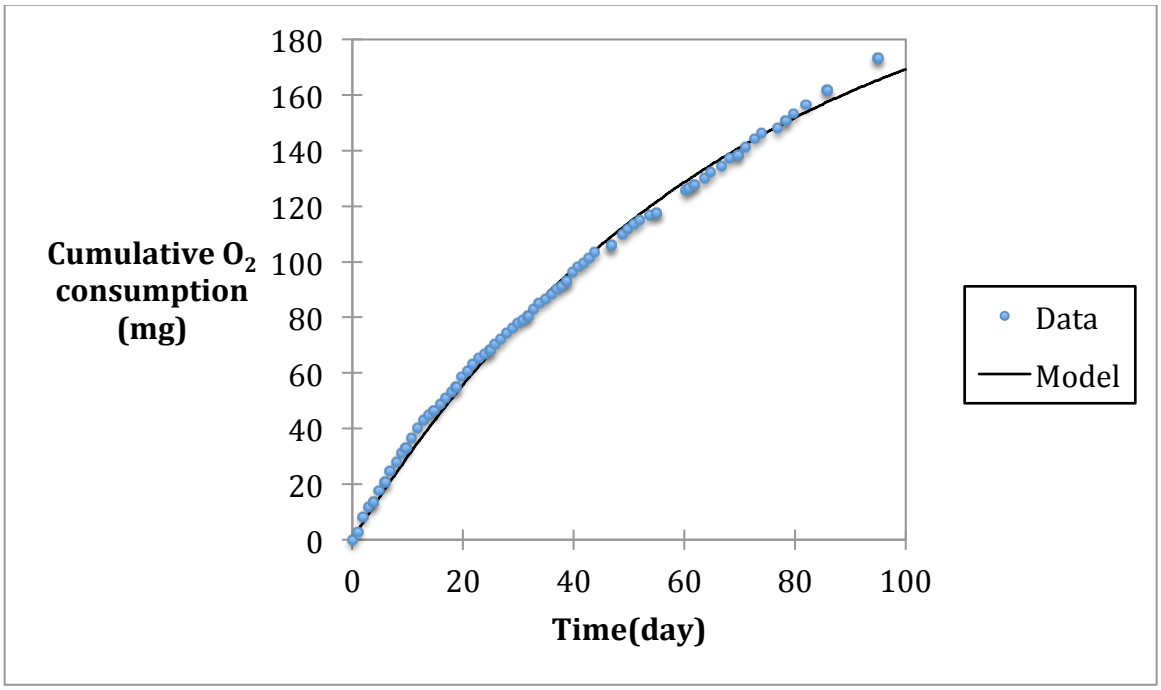

Figure A2.32 Cumulative O_2 consumption for "10g with N, P 1" in Experiment 2.

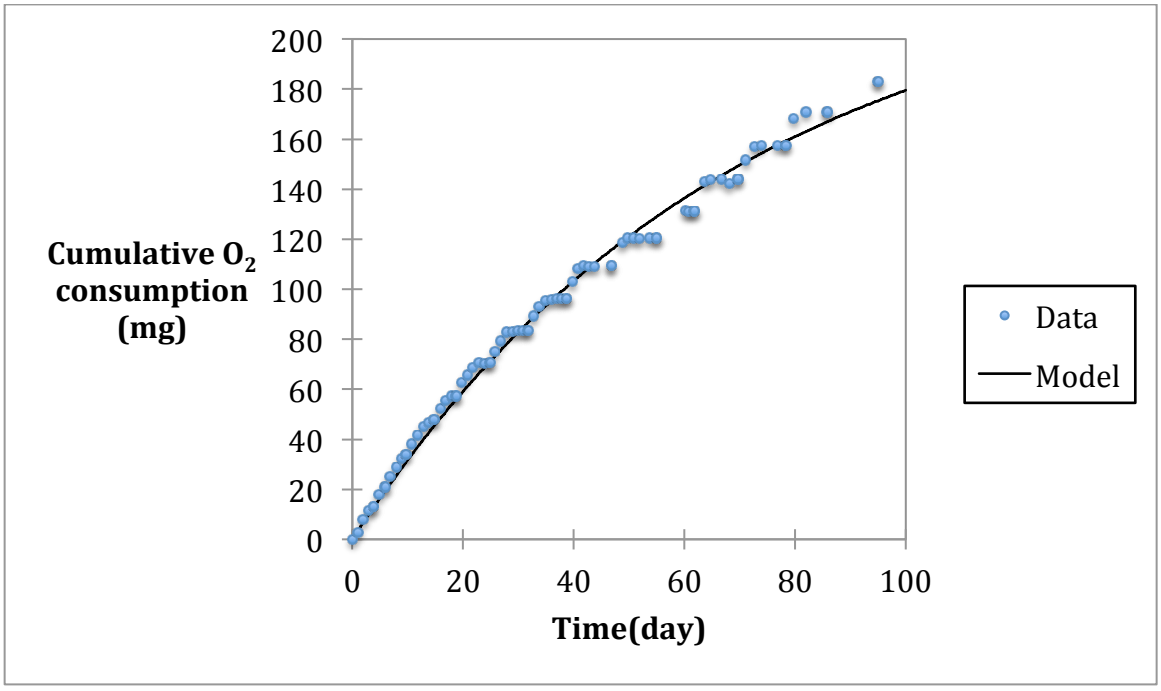

Figure A2.33 Cumulative O_2 consumption for "10g with N, P 2" in Experiment 2.

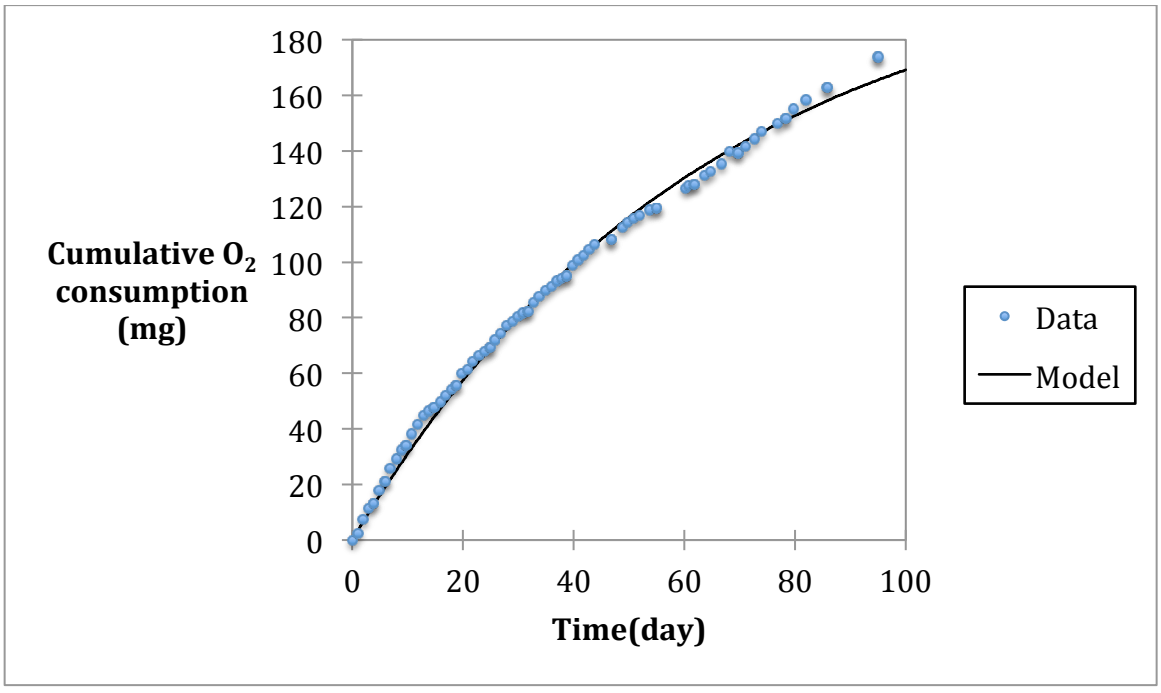

Figure A2.34 Cumulative O_2 consumption for "10g with N, P 3" in Experiment 2.

APPENDIX 3. FIRST-ORDER MODELING USING ONE FITTING PARAMETER (*k*).

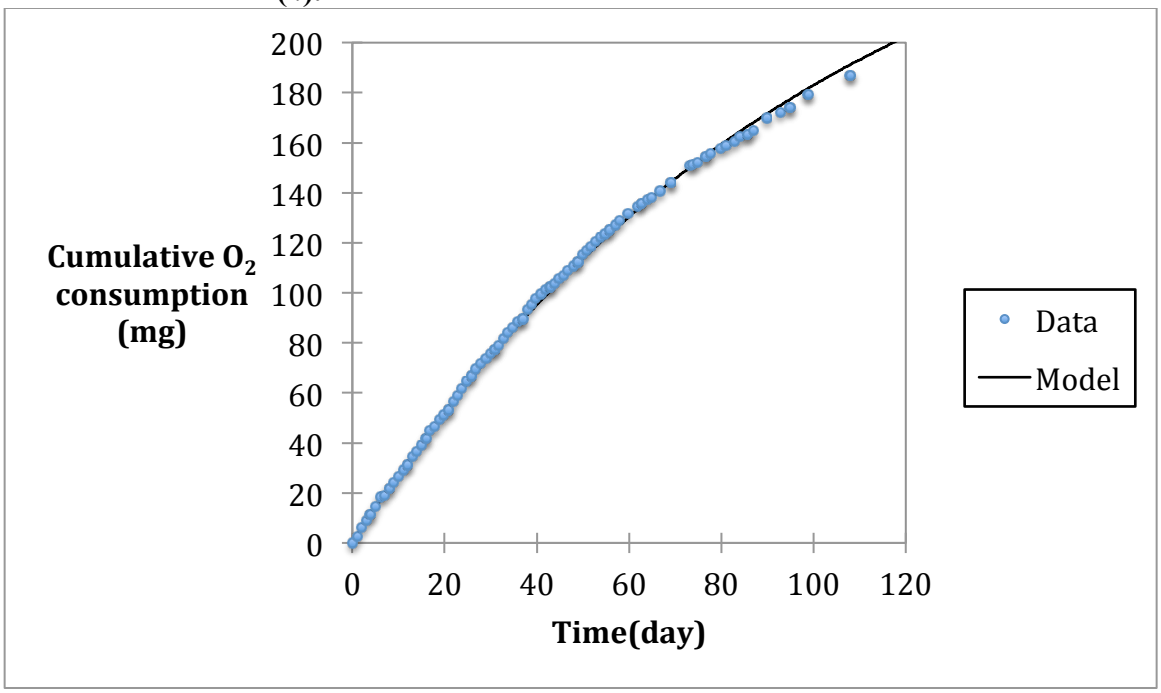

Figure A3.1 Cumulative O_2 consumption for "M+W 1" in Experiment 1.

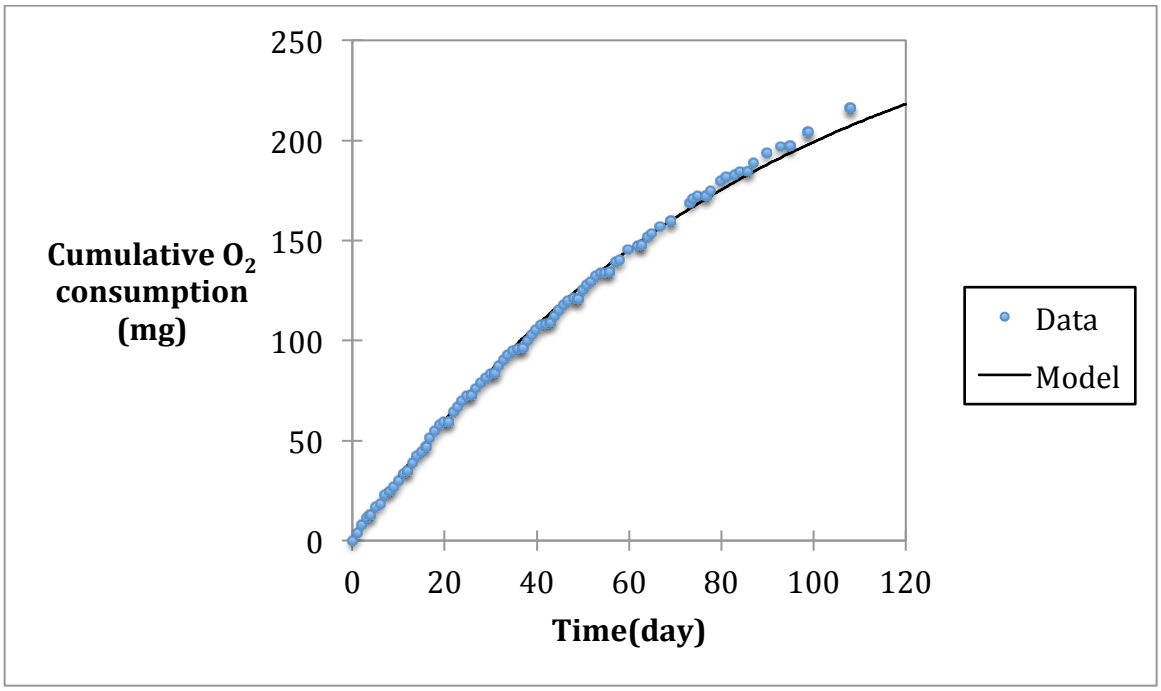

Figure A3.2 Cumulative O_2 consumption for "M+W+N, P 1" in Experiment 1.

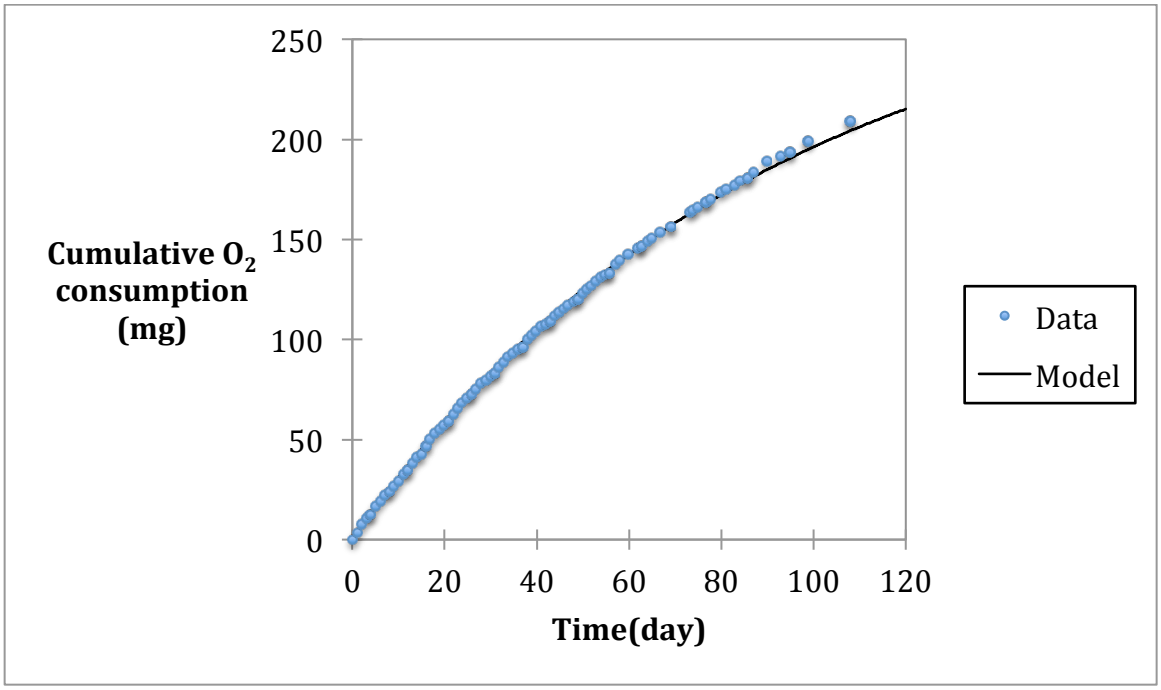

Figure A3.3 Cumulative O_2 consumption for "M+W+N, P 2" in Experiment 1.

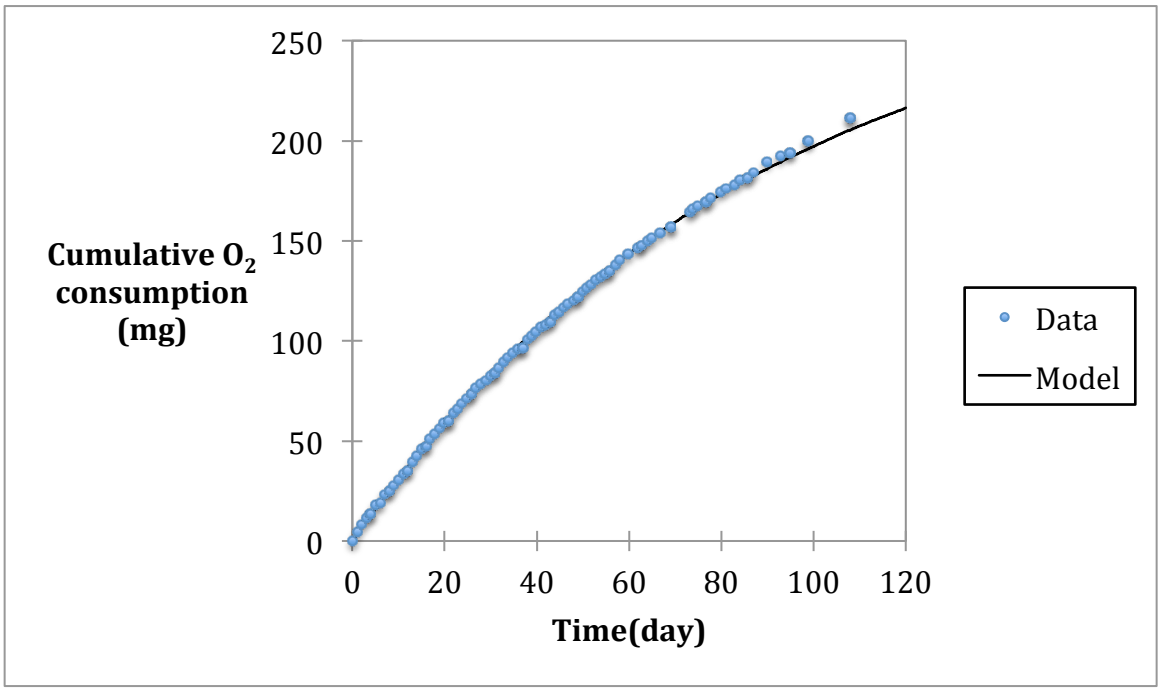

Figure A3.4 Cumulative O_2 consumption for "M+W+N, P 3" in Experiment 1.

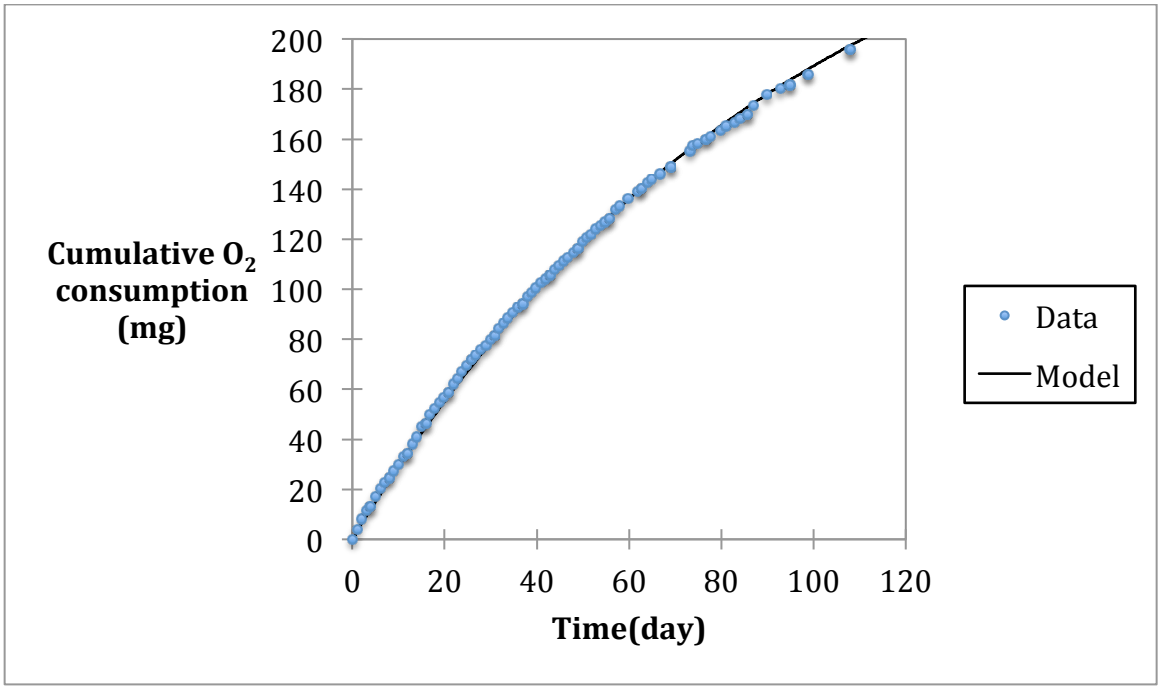

Figure A3.5 Cumulative O_2 consumption for "M+W+KB1 1" in Experiment 1.

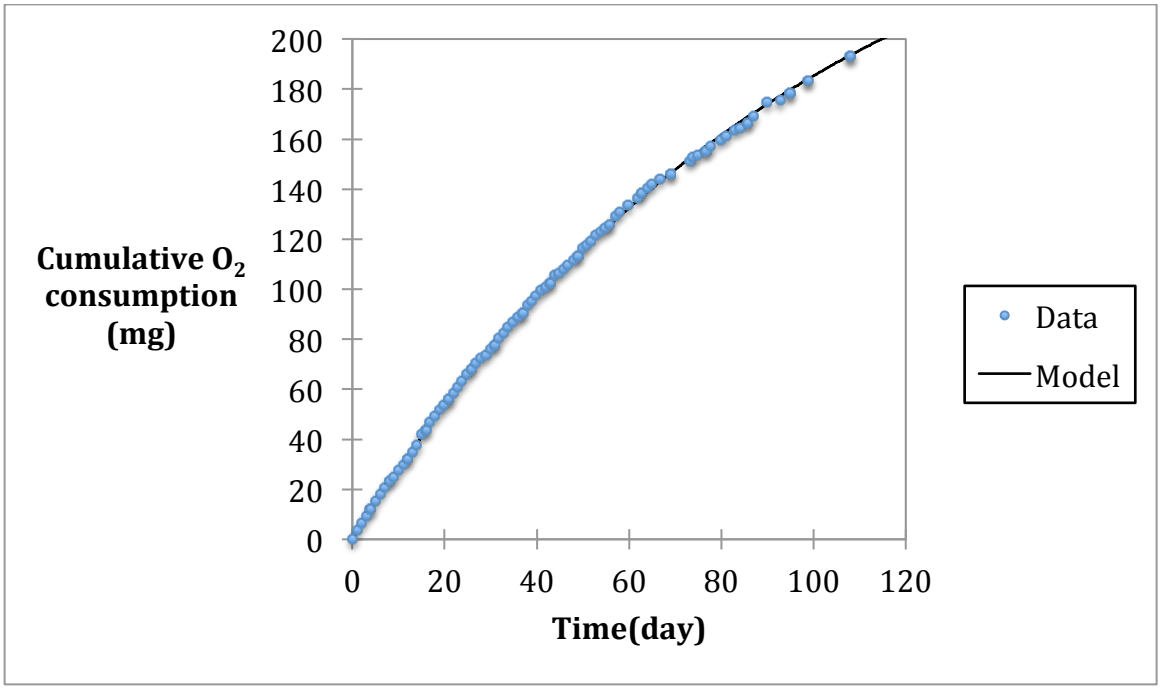

Figure A3.6 Cumulative O_2 consumption for "M+W+KB1 2" in Experiment 1.

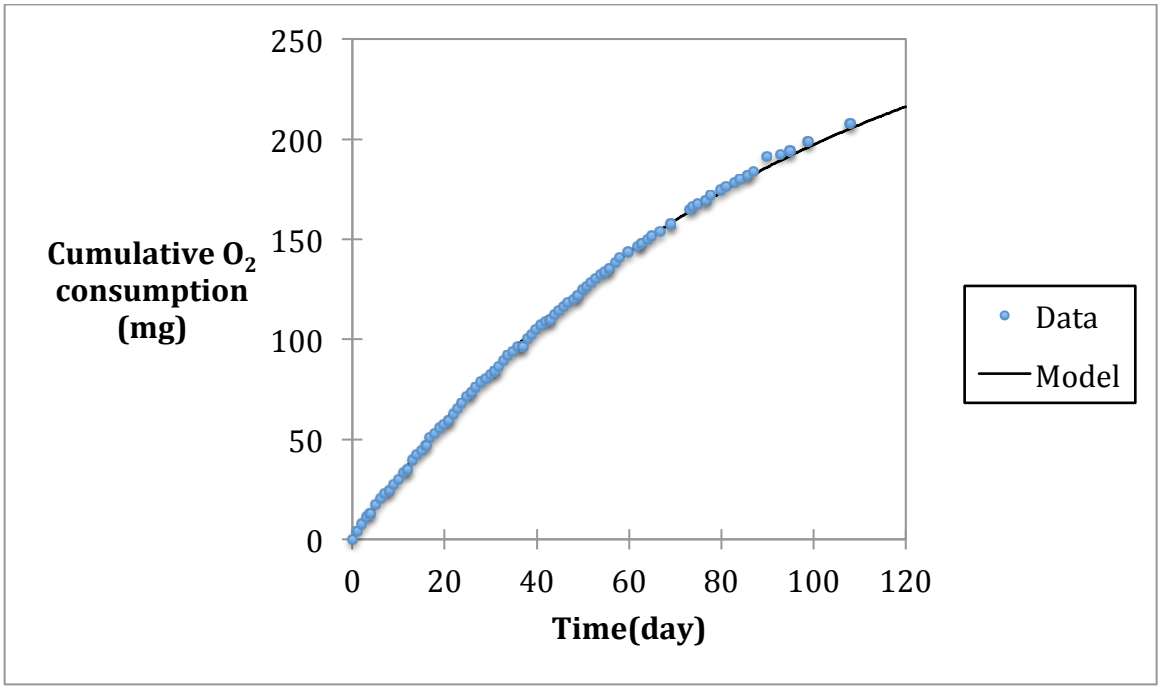

Figure A3.7 Cumulative O_2 consumption for "M+W+KB1 3" in Experiment 1.

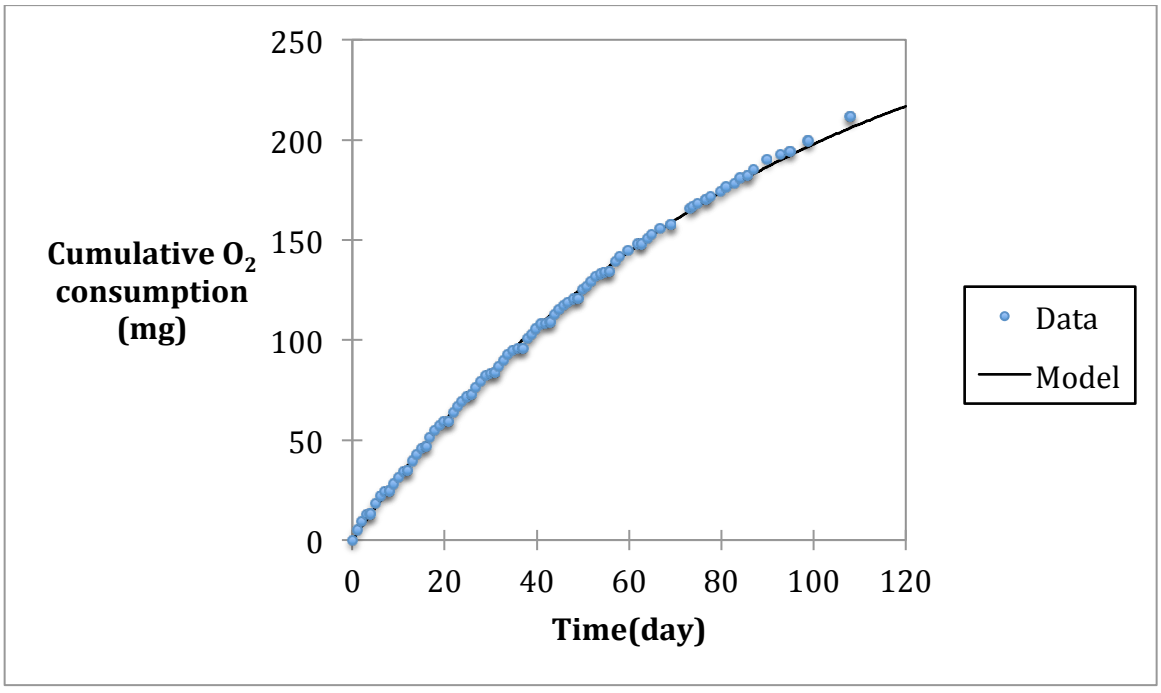

Figure A3.8 Cumulative O_2 consumption for "M+W+KB1+N, P 1" in Experiment 1.

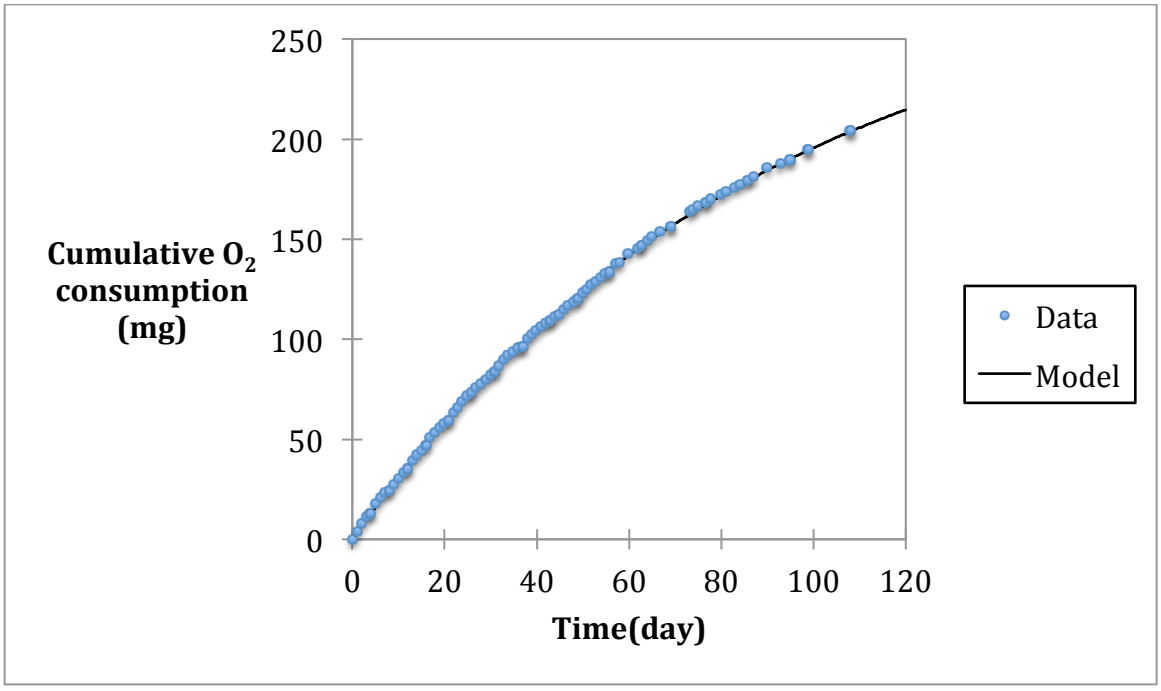

Figure A3.9 Cumulative O_2 consumption for "M+W+KB1+N, P 2" in Experiment 1.

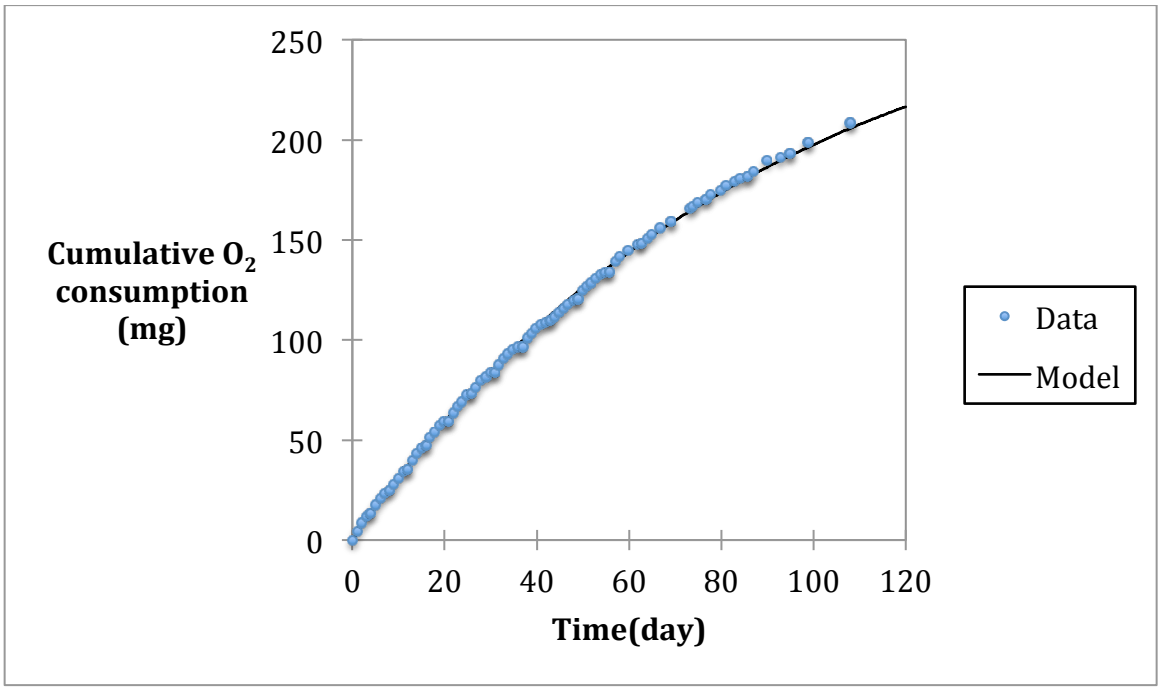

Figure A3.10 Cumulative O_2 consumption for "M+W+KB1+N, P 3" in Experiment 1.

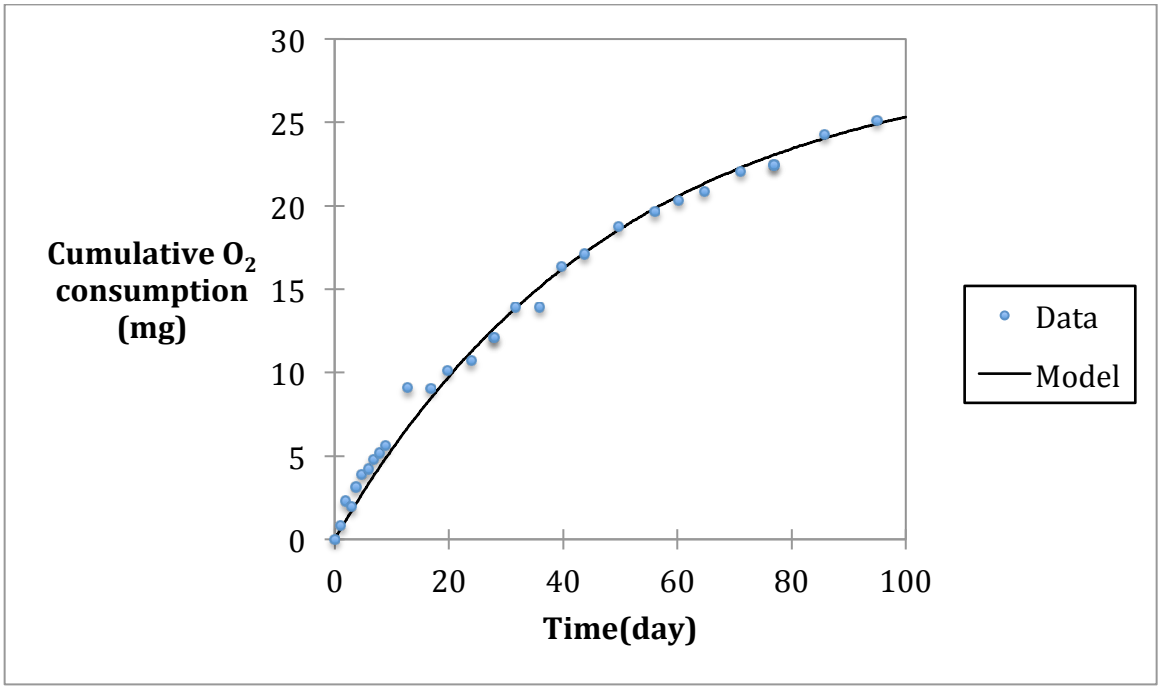

Figure A3.11 Cumulative O_2 consumption for "1g mulch without N, P 1" in Experiment 2.

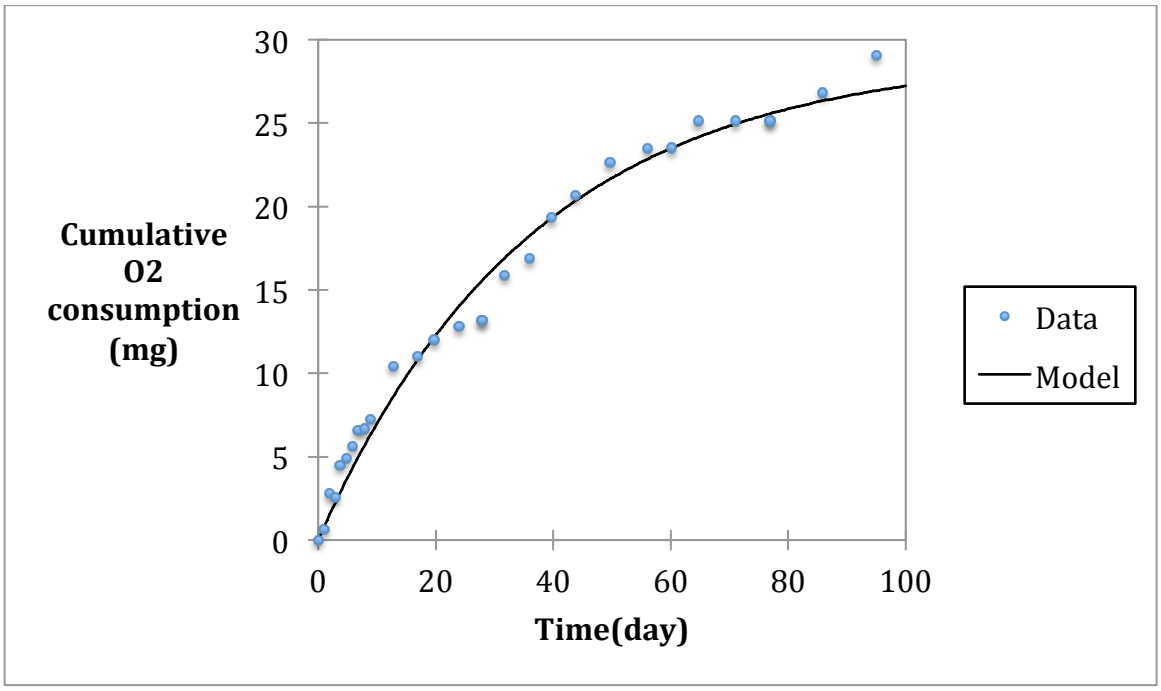

Figure A3.12 Cumulative O_2 consumption for "1g mulch without N, P 2" in Experiment 2.

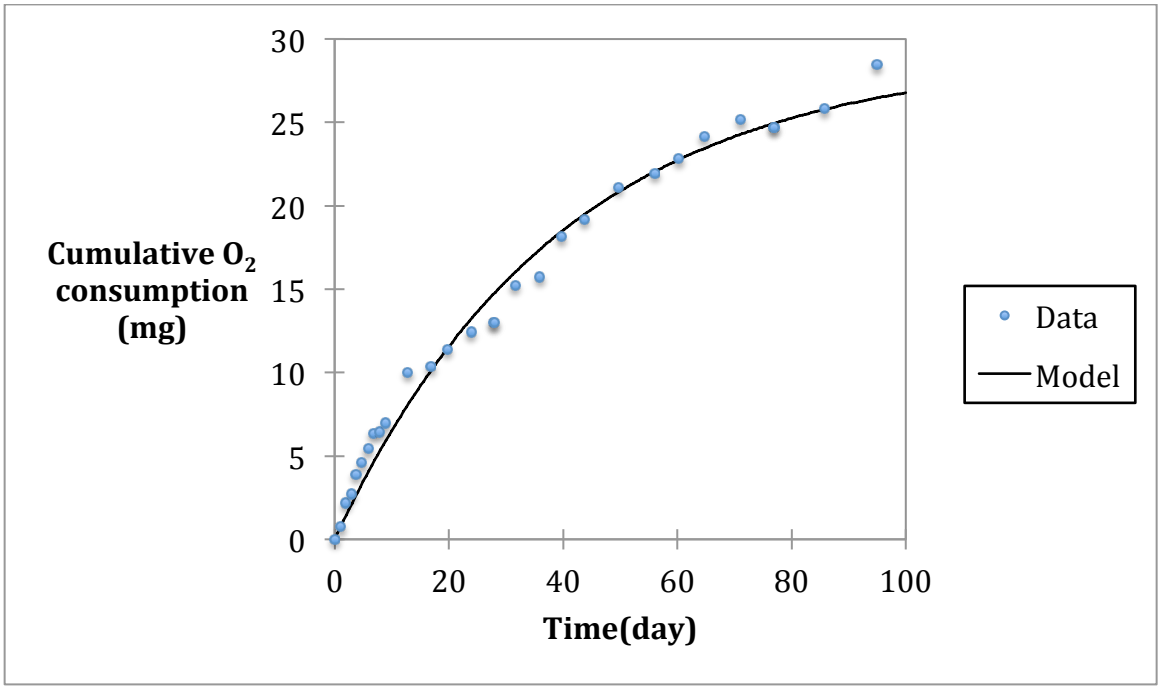

Figure A3.13 Cumulative O_2 consumption for "1g mulch without N, P 3" in Experiment 2.

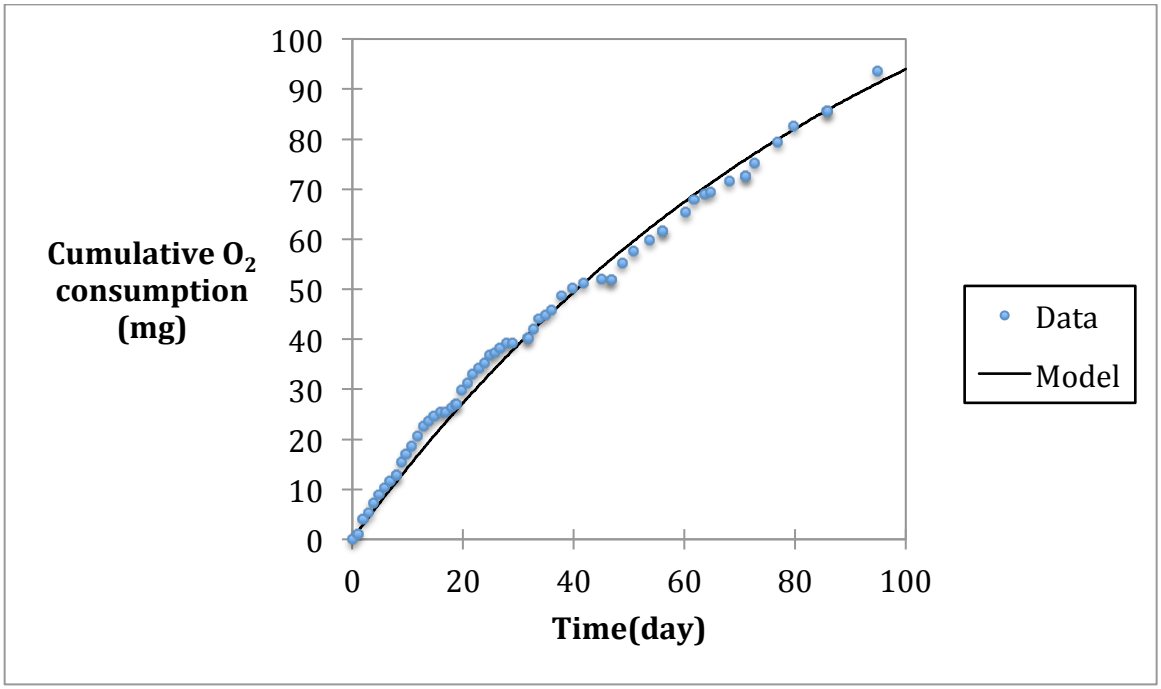

Figure A3.14 Cumulative O_2 consumption for "5g mulch without N, P 1" in Experiment 2.

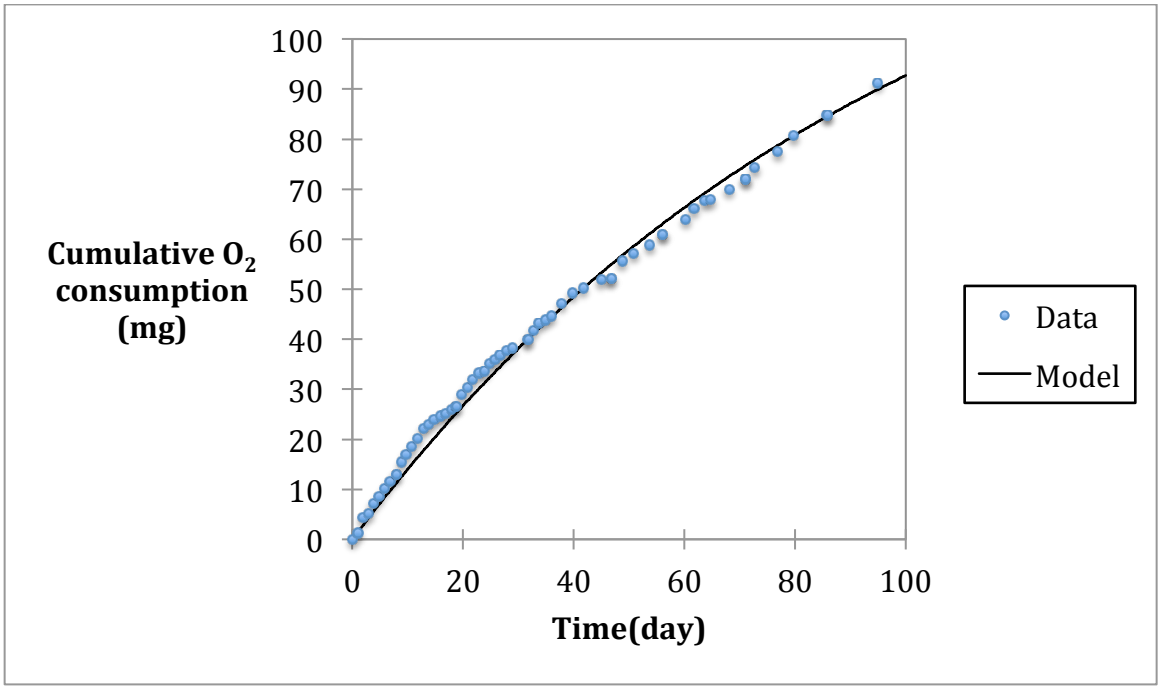

Figure A3.15 Cumulative O_2 consumption for "5g mulch without N, P 2" in Experiment 2.

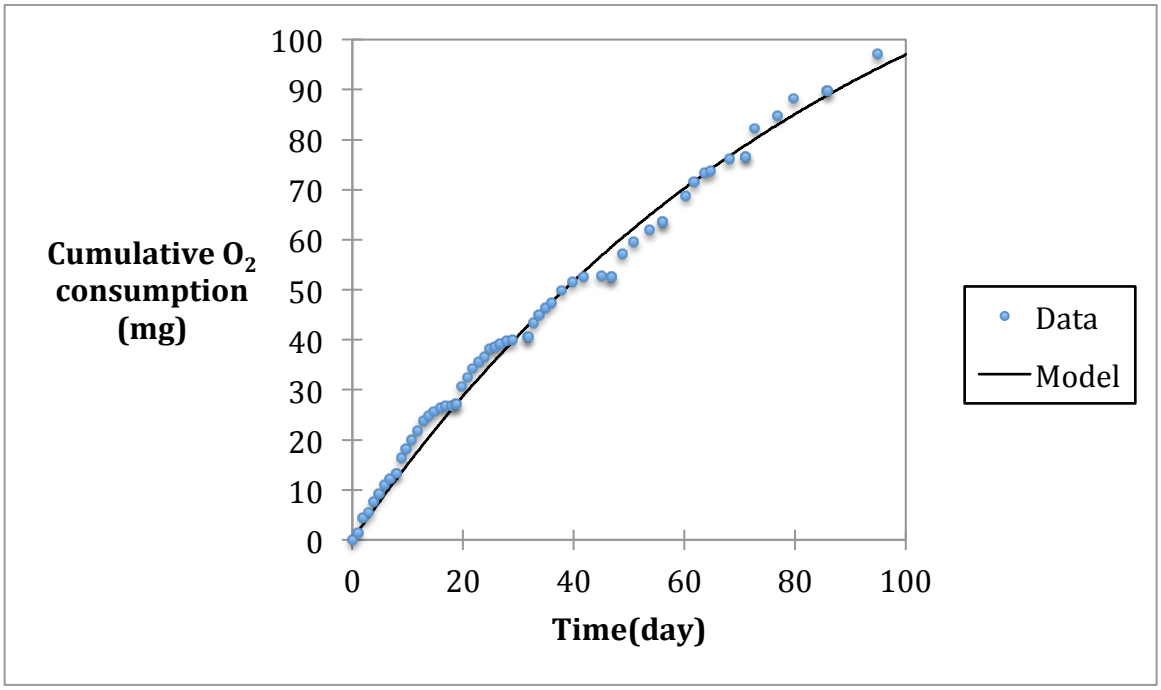

Figure A3.16 Cumulative O_2 consumption for "5g mulch without N, P 3" in Experiment 2.

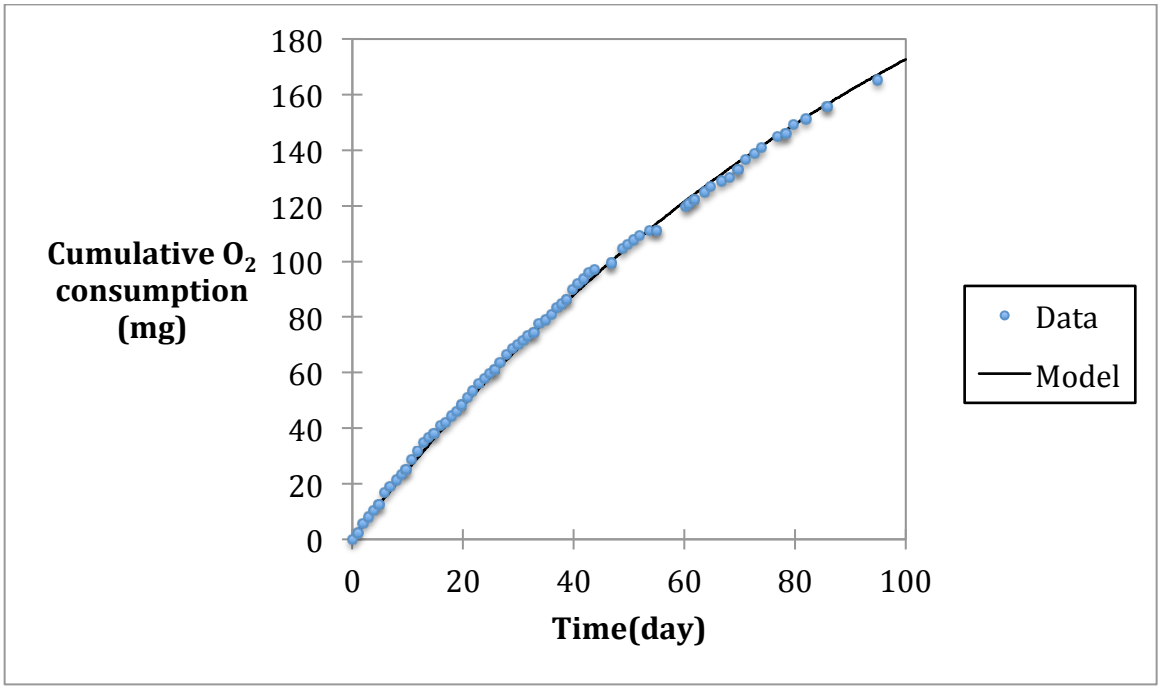

Figure A3.17 Cumulative O_2 consumption for "10g mulch without N, P 1" in Experiment 2.

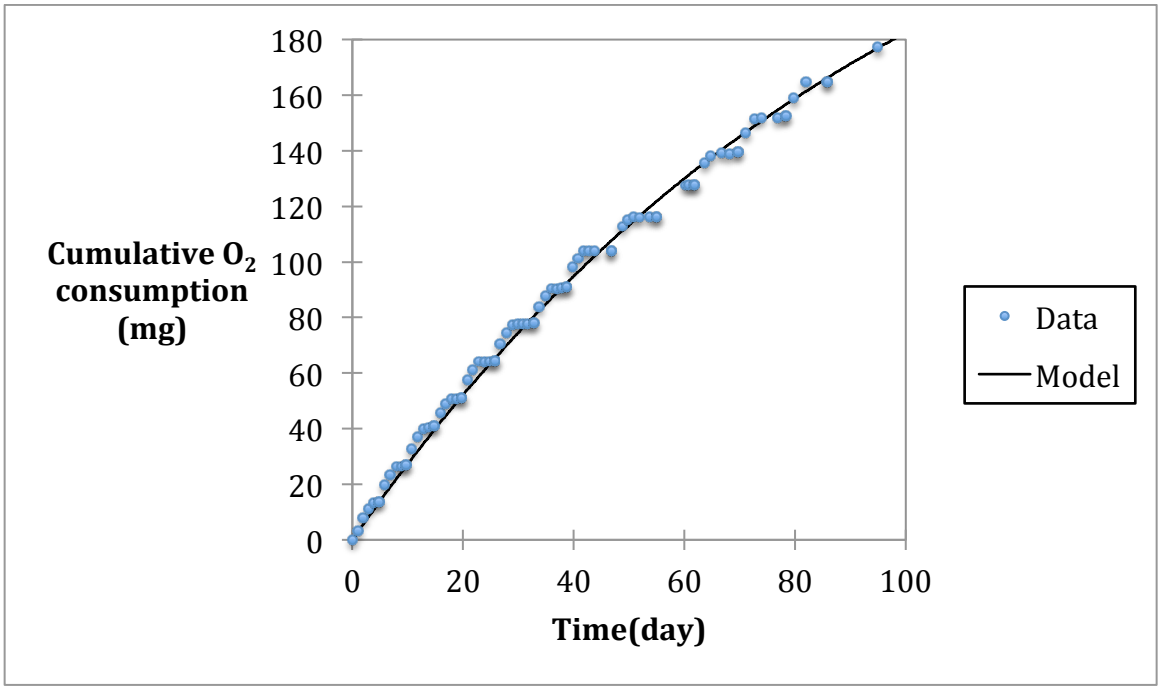

Figure A3.18 Cumulative O_2 consumption for "10g mulch without N, P 2" in Experiment 2.

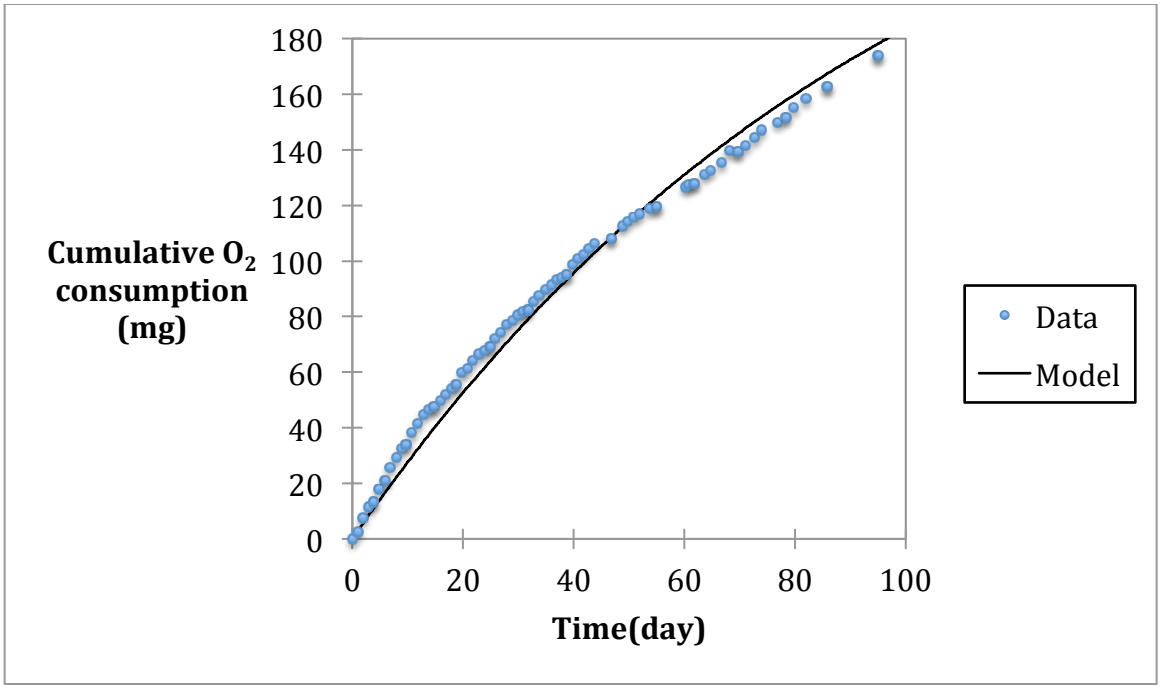

Figure A3.19 Cumulative O_2 consumption for "10g mulch without N, P 3" in Experiment2.

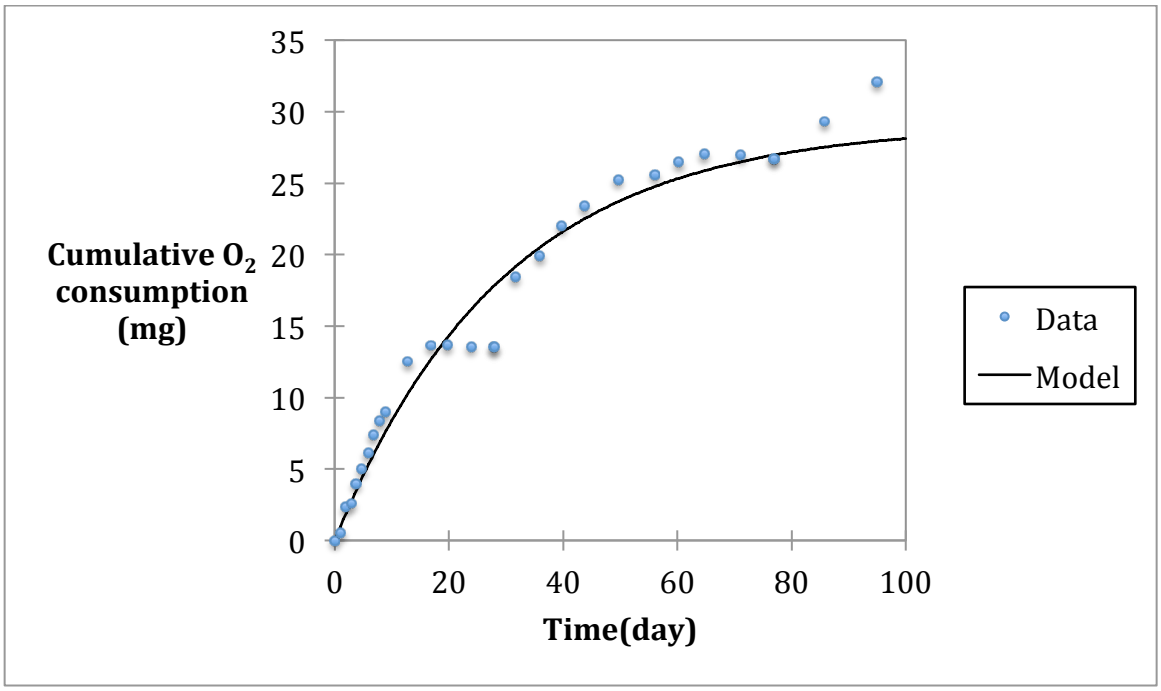

Figure A3.20 Cumulative O_2 consumption for "1g mulch with N, P 1" in Experiment 2.

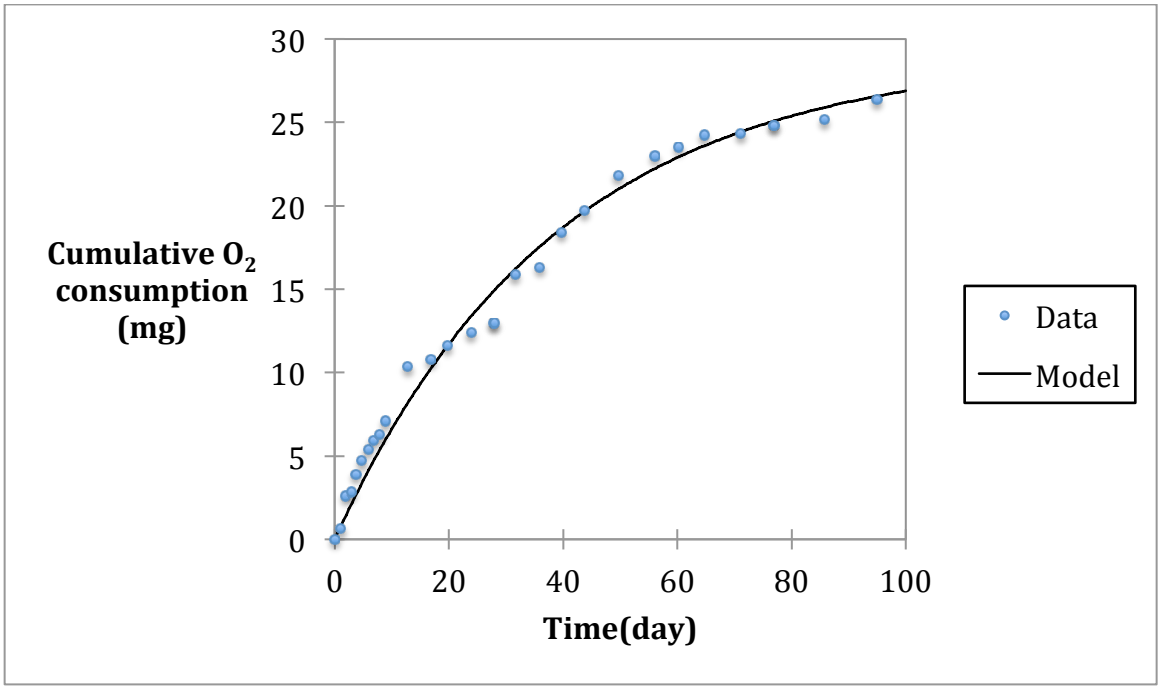

Figure A3.21 Cumulative O_2 consumption for "1g mulch with N, P 2" in Experiment 2.

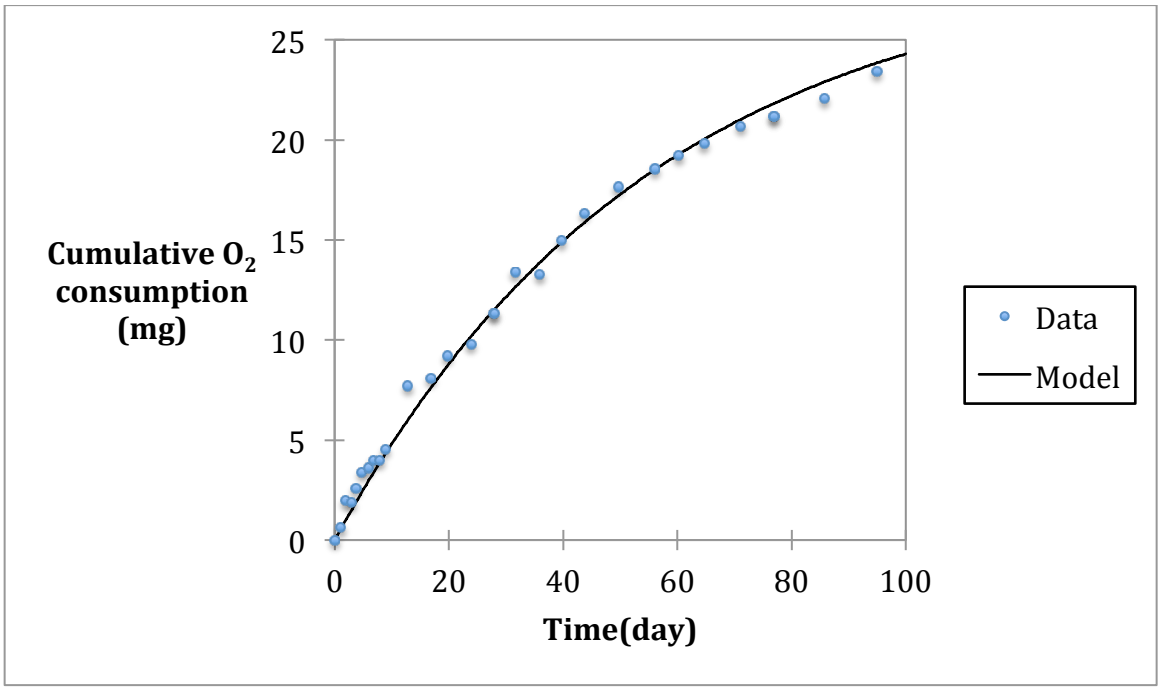

Figure A3.22 Cumulative O_2 consumption for "1g mulch with N, P 3" in Experiment 2.

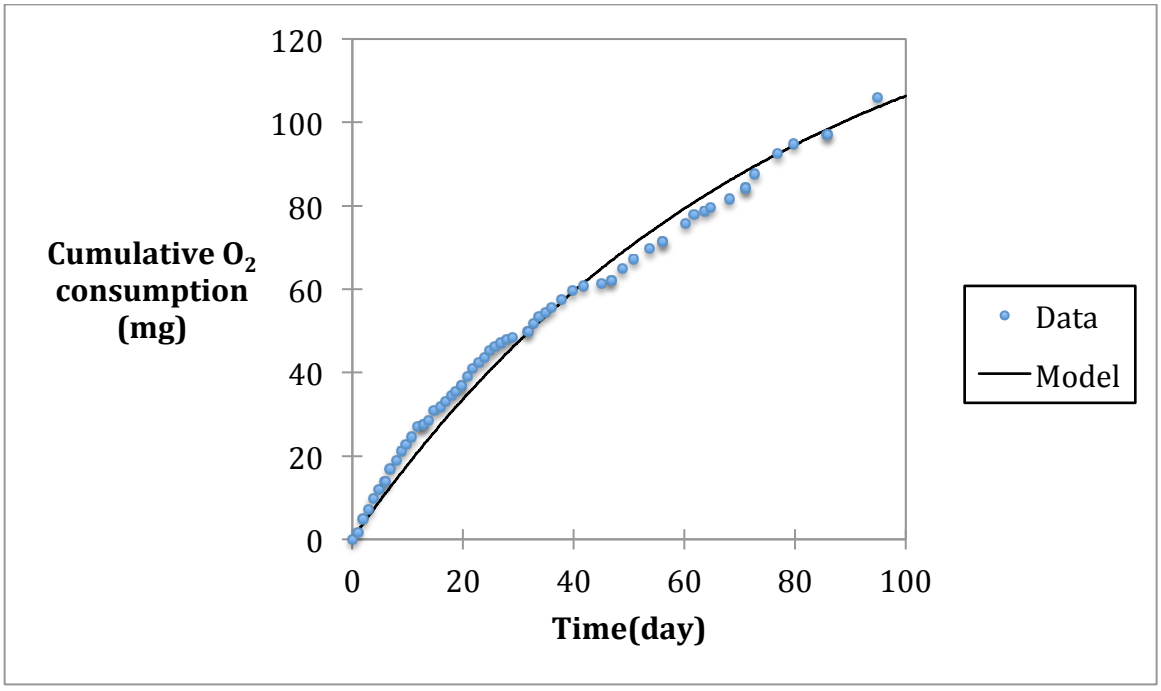

Figure A3.23 Cumulative O_2 consumption for "5g mulch with N, P 1" in Experiment 2.

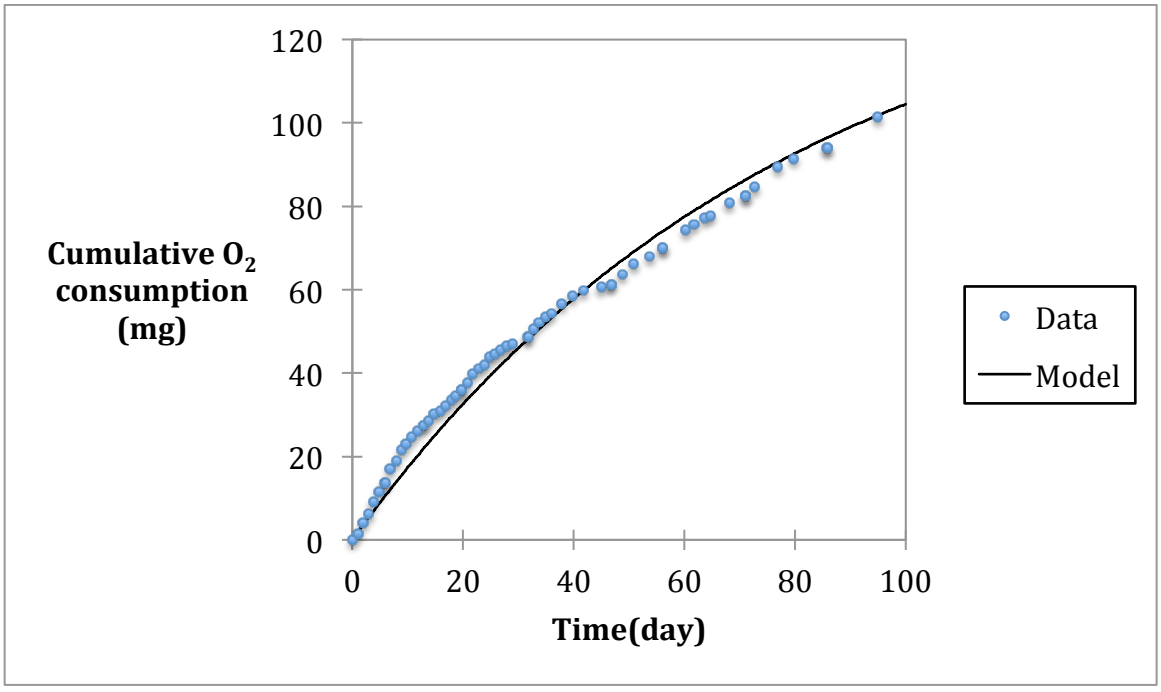

Figure A3.24 Cumulative O_2 consumption for "5g mulch with N, P 2" in Experiment 2.

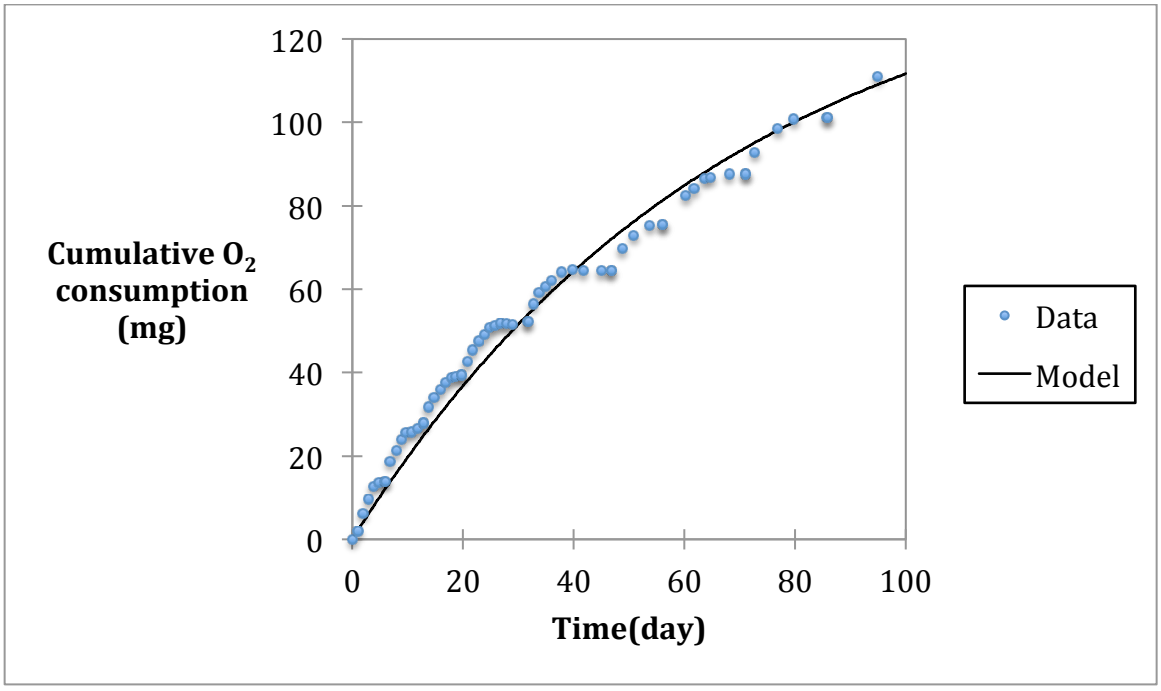

Figure A3.25 Cumulative O_2 consumption for "5g mulch with N, P 3" in Experiment 2.

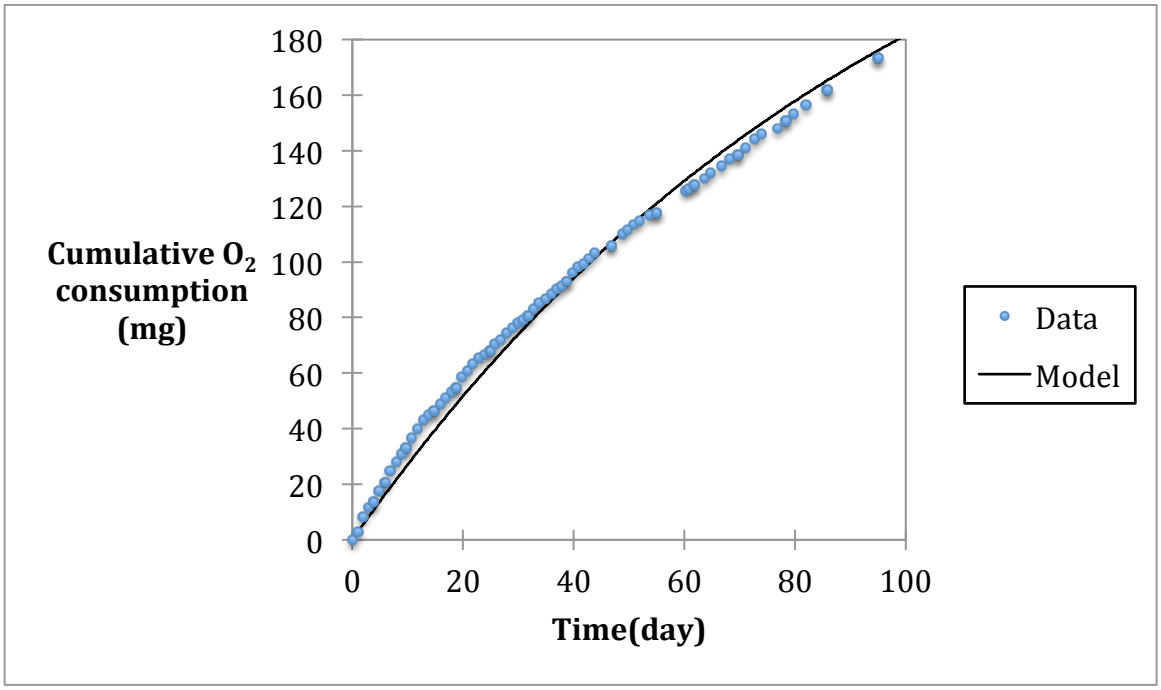

Figure A3.26 Cumulative O_2 consumption for "10g mulch with N, P 1" in Experiment 2.

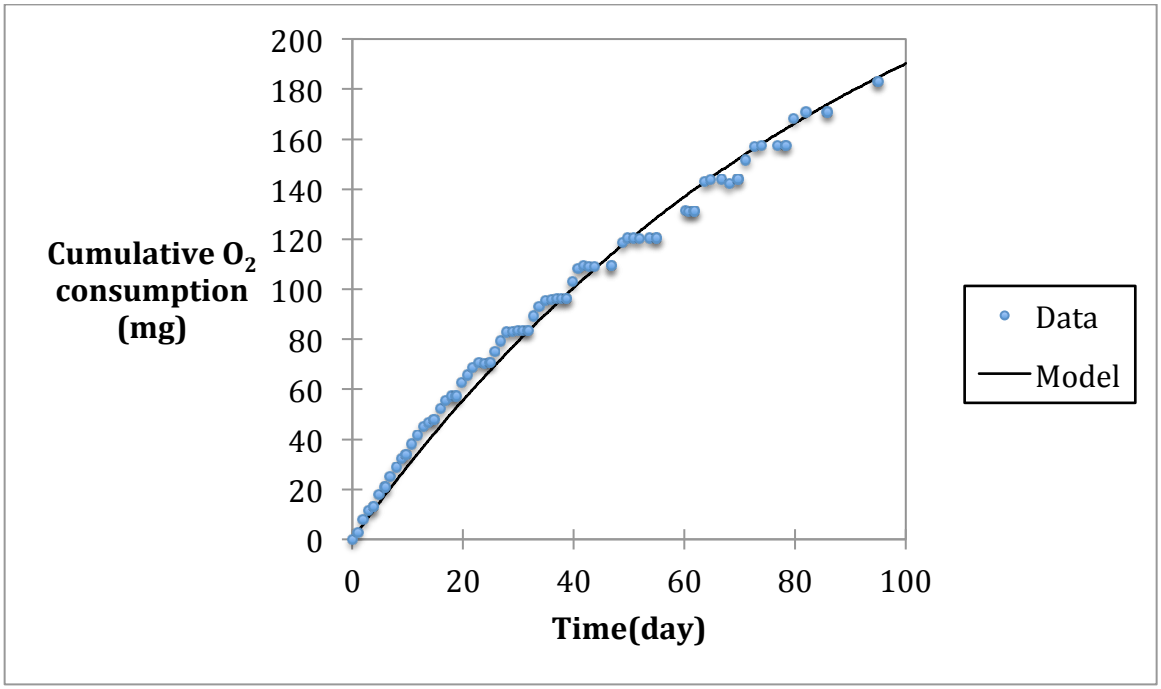

Figure A3.27 Cumulative O_2 consumption for "10g mulch with N, P 2" in Experiment 2.

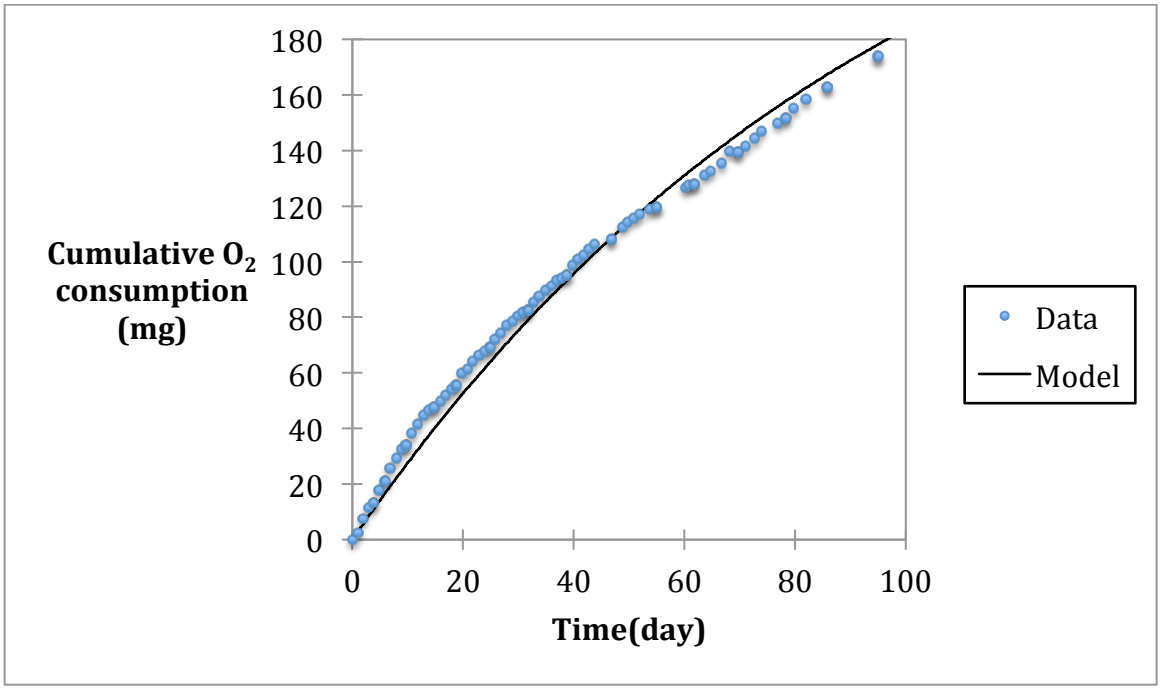

Figure A3.28 Cumulative O_2 consumption for "10g mulch with N, P 3" in Experiment 2.

APPENDIX 4. DETAILED RESULTS OF SIGNIFICANCE TESTING (T-TESTS).

A4.1 Significance Testing of L_0/g.

A4.1.1 T-test on L_0/g between 1g Types (with and without N, P) in Experiment 2.

1g type in Experiment 2 (without N,P)		1g type in Experiment 2 (with N,P)	
Triplicate	Lo/g (mg/g dry wt)	Triplicate	Lo/g (mg/g dry wt)
1g 1	27.6	1g 1	31.9
1g 2	30.2	1g 2	28.5
1g 3	30.1	1g 3	26.4

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Standard Deviation
Lo(mg)/gram	3	0	3	27.604	30.249	29.317	1.485
Lo(mg)/gram(2)	3	0	3	26.429	31.897	28.937	2.762

t-test for two independent samples / Two-tailed test:

95% confidence interval on the difference between the means:
] -4.647 , 5.407 [

Difference	0.380
t (Observed value)	0.210
|t| (Critical value)	2.776
DF	4
p-value (Two-tailed)	0.844
alpha	0.05

Test interpretation:
H0: The difference between the means is equal to 0.
Ha: The difference between the means is different from 0.
As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis
The risk to reject the null hypothesis H0 while it is true is 84.40%.

They are not significantly different.

A4.1.2 T-test on L_0/g between "W+M" Type in Experiment 1 and "10g without N, P" in Experiment 2.

W+M in Experiment 1		10g type in Experiment 2(without N,P)	
Trplicate	Lo/g (mg/g dry wt)	Trplicate	Lo/g (mg/g dry wt)
W+M 1	27	EXP 2 10g without N,P 1	26.1
W+M+KB1 1	27.1	EXP 2 10g without N,P 2	25.7
W+M+KB1 2	28.0	EXP 2 10g without N,P 3	26.8
W+M+KB1 3	31.2		

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Standard Deviation
Lo(mg)/gram	4	0	4	27.000	31.189	28.336	1.957
Lo(mg)/gram(2)	3	0	3	25.709	26.757	26.173	0.535

t-test for two independent samples / Two-tailed test:

95% confidence interval on the difference between the means:

] -0.886 , 5.214 [

Difference	2.164
t (Observed value)	1.824
|t| (Critical value)	2.571
DF	5
p-value (Two-tailed)	0.128
alpha	0.05

Test interpretation:
H0: The difference between the means is equal to 0.
Ha: The difference between the means is different from 0.
As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 12.78%.

They are not significantly different.

A4.2 Significance Testing of *k*.

A4.2.1 T-test between "M+W" Type and "M+W+N, P" Type in Experiment 1 on *k*.

M+W in Experiment 1		M+W+N, P in Experiment 1	
Triplicate	k(d^{-1})	Triplicate	k(d^{-1})
Exper 1 M+W	0.0099	Exper 1 M+W+N,P 1	0.0115
Exper 1 M+W+KB1 1	0.0105	Exper 1 M+W+N,P 2	0.0112
Exper 1 M+W+KB1 2	0.0101	Exper 1 M+W+N,P 3	0.0113
Exper 1 M+W+KB1 3	0.0113	Exper 1 M+W+KB1+N,P 1	0.0114
		Exper 1 M+W+KB1+N,P 2	0.0112
		Exper 1 M+W+KB1+N,P 3	0.0114

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Standard Deviation
k(d-1)	4	0	4	0.010	0.011	0.010	0.001
k(d-1)(2)	6	0	6	0.011	0.012	0.011	0.000

t-test for two independent samples / Two-tailed test:

95% confidence interval on the difference between the means:
] -0.001 , -0.000 [

Difference	-0.001
t (Observed value)	-3.499
|t| (Critical value)	2.306
DF	8
p-value (Two-tailed)	0.008
alpha	0.05

Test interpretation:
H0: The difference between the means is equal to 0.
Ha: The difference between the means is different from 0.
As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.
The risk to reject the null hypothesis H0 while it is true is lower than 0.81%.

They are significantly different.

A4.2.2 T-test between 1g Types (with and without N, P) in Experiment 2 on *k*.

1g type in Experiment 2 (without N,P)		1g type in Experiment 2 (with N,P)	
Triplicate	$k(d^{-1})$	Triplicate	$k(d^{-1})$
1g 1	0.0204	1g 1	0.0339
1g 2	0.0274	1g 2	0.0257
1g 3	0.0253	1g 3	0.018

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Standard Deviation
k(d-1)	3	0	3	0.020	0.027	0.024	0.004
k(d-1)(2)	3	0	3	0.018	0.034	0.026	0.008

t-test for two independent samples / Two-tailed test:

95% confidence interval on the difference between the means:
] -0.015 , 0.012 [

Difference	-0.002
t (Observed value)	-0.298
|t| (Critical value)	2.776
DF	4
p-value (Two-tailed)	0.781
alpha	0.05

Test interpretation:
H0: The difference between the means is equal to 0.
Ha: The difference between the means is different from 0.
As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis
The risk to reject the null hypothesis H0 while it is true is 78.07%.

They are not significantly different.

A4.2.3 T-test between 5g Types (with and without N, P) in Experiment 2 on *k*.

5g type in Experiment 2 (without N,P)		5g type in Experiment 2 (with N,P)	
Triplicate	$k(d^{-1})$	Triplicate	$k(d^{-1})$
Exper 2(without N,P) 5g 1	0.0104	Exper 2(with N,P) 5g 1	0.0131
Exper 2(without N,P) 5g 2	0.0101	Exper 2(with N,P) 5g 2	0.0127
Exper 2(without N,P) 5g 3	0.011	Exper 2(with N,P) 5g 3	0.0146

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Standard Deviation
k(d-1)	3	0	3	0.010	0.011	0.011	0.000
k(d-1)(2)	3	0	3	0.013	0.015	0.013	0.001

t-test for two independent samples / Two-tailed test:

95% confidence interval on the difference between the means:
] -0.005 , -0.001 [

Difference	-0.003
t (Observed value)	-4.665
|t| (Critical value)	2.776
DF	4
p-value (Two-tailed)	0.010
alpha	0.05

Test interpretation:
H0: The difference between the means is equal to 0.

Ha: The difference between the means is different from 0.
As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.
The risk to reject the null hypothesis H0 while it is true is lower than 0.96%.

They are significantly different.

A4.2.4 T-test between 10g Types (with and without N, P) in Experiment 2 on *k*.

10g type in Experiment 2 (without N,P)		10g type in Experiment 2 (with N,P)	
Triplicate	k(d^{-1})	Triplicate	k(d^{-1})
Exper 2(without N,P) 10g 1	0.009	Exper 2(with N,P) 10g 1	0.0098
Exper 2(without N,P) 10g 2	0.0099	Exper 2(with N,P) 10g 2	0.0106
Exper 2(without N,P) 10g 3	0.01	Exper 2(with N,P) 10g 3	0.01

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Standard Deviation
k(d-1)	3	0	3	0.009	0.010	0.010	0.001
k(d-1)(2)	3	0	3	0.010	0.011	0.010	0.000

t-test for two independent samples / Two-tailed test:

95% confidence interval on the difference between the means:

] -0.002 , 0.001 [

Difference	-0.001
t (Observed value)	-1.254
|t| (Critical value)	2.776
DF	4
p-value (Two-tailed)	0.278
alpha	0.05

Test interpretation:
H0: The difference between the means is equal to 0.
Ha: The difference between the means is different from 0.
As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 27.80%.

They are not significantly different.

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