# THE BIODEGRADATION OF ACETAMINOPHEN IN ACTIVATED SLUDGE BATCH REACTORS

By

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THE BIODEGRADATION OF ACETAMINOPHEN IN

ACTIVATED SLUDGE BATCH REACTORS

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# CHAPTER I

# INTRODUCTION

## *Pharmaceuticals and Public Health*

Pharmaceuticals have recently been discovered in drinking water all over the world, raising concerns about public health. (CBS News, 2008) Throughout the media, discussions of the types of medications found and their related health hazards have brought this issue to the attention of the general public. Investigators have found medications ranging from antibiotics to analgesics, including azithromycin and acetaminophen. (CBS News, 2008) Acetaminophen has been found in the drinking waters of major cities such as Atlanta, Minneapolis, New York City, and Oklahoma City. (CBS News, 2008) The potential health effects of ingesting this contaminated drinking water are unknown, and researchers have been examining the acute and chronic toxicities due to these small concentrations. It is known that overdoses of acetaminophen in large quantities can lead to liver damage and necrosis. (Rumack, 1975) It is also relatively unknown whether conventional water treatment processes, such as coagulation, flocculation, sedimentation, and filtration, are successful in removing these pharmaceuticals. If the current treatment processes do not have adequate pharmaceutical removal, it will be necessary to develop new treatment trains for the water supply.

*Acetaminophen*

Acetaminophen is one of the most common medications found in the water supply. (CBS News, 2008) It is found generally in small concentrations in the µg/L range and is detected in approximately 75% of natural waters, such as rivers and lakes. (Stackelberg, 2007) Chemically, acetaminophen, also known as paracetamol or by the trade name, Tylenol, is commonly used as an analgesic for headache and other pain. It is an odorless, white compound which is soluble in water at room temperature. Structurally, acetaminophen consists of a benzene ring core, with one hydroxyl group substitution and an amide group in the *para* position. The structure is displayed in Figure 1.

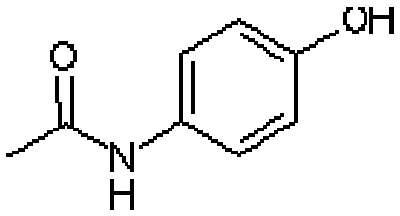


Figure 1: Chemical structure of acetaminophen
(Acros Organics, 2009)

As a commonly used analgesic, the fate of acetaminophen in the bloodstream has been studied extensively. (Rumack, 1975) Its fate in natural waters, however, has received less attention.

Acetaminophen typically degrades at room temperature with a water solvent. (Mohamed, 1997) The dominant degradation product is *p*-aminophenol, which is

commonly used as a developer in black and white film photography. *p*-Aminophenol is a highly toxic compound through inhalation, ingestion and dermal contact. Extensive exposure to *p*-aminophenol can result in necrosis, which is the premature death of cells and living tissue. (Klos, 1992) Figure 2 shows the chemical structure.

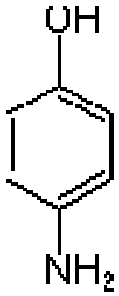


Figure 2: Chemical structure of *p*-aminophenol
(Acros Organics, 2009)

As stated earlier, p-aminophenol is the typical degradation product of acetaminophen. It is also used as the main reactant for the synthesis of acetaminophen, a reaction described in Figure 3. Here, *p*-aminophenol reacts with an acid to form acetaminophen. This reaction is also reversible, in which case acetaminophen degrades to form *p*-aminophenol. The exact degradation product of *p*-aminophenol was not found in the literature.

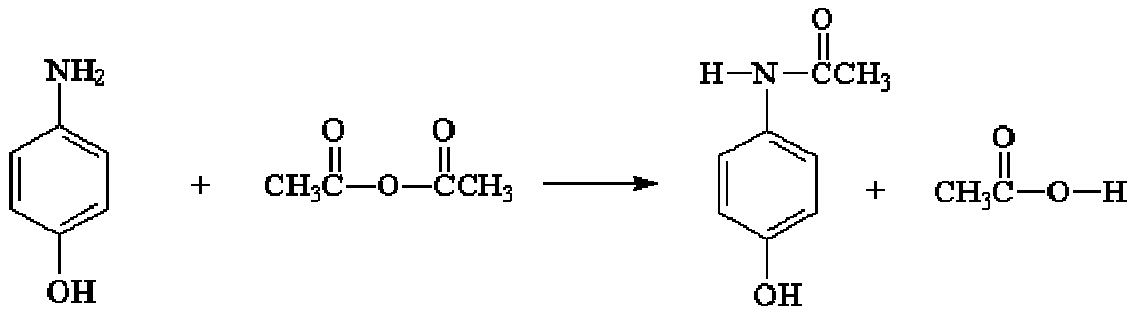

Figure 3: Synthesis of Acetaminophen from *p*-Aminophenol
(University of Missouri-St. Louis, 2009)

The toxicity of p-aminophenol and the fact that acetaminophen is found in natural water and treated waters are cause for concern. There is also an indication that acetaminophen does in fact have residual persistence, otherwise its presence would not be an issue.

*Scope of Investigation*

The purpose of this research is to investigate the persistence of acetaminophen. Specifically, the fate of acetaminophen in the water supply after treatment in a typical wastewater treatment facility will be examined. Such a facility consists of an activated sludge process, which contains aerobic biological organisms used for the degradation of the material in the influent water. Microorganisms biodegrade these compounds to use them in the general maintenance of the existing organisms and synthesis of new bacteria, which is known as metabolism. Compounds like acetaminophen are believed to be utilized in these metabolic pathways as a source of carbon. To better quantify the biodegradation of acetaminophen and *p*-aminophenol in such a treatment train, the

kinetics of the biodegradation reactions must be determined. In addition, these compounds fate in abiotic aquatic reactors was also investigated. Lastly, preliminary studies of their fate under anaerobic conditions were performed

# CHAPTER II

# REVIEW OF LITERATURE

## *Acetaminophen and Public Health*

While the chronic effects on human health through trace exposure to acetaminophen in the water is unknown, investigations regarding acute effects on other organisms have been underway. One of these studies looked at the effects of ecologically relevant concentrations of acetaminophen on *Rana pipens,* or North Leopard frog, tadpoles. (Fraker, 2004) Several tadpoles were exposed to acetaminophen concentrations of 0 $\mu gL^{-1}$, 1.0 $\mu gL^{-1}$, 10.0 $\mu gL^{-1}$, 100.0 $\mu gL^{-1}$, and 1000.0 $\mu gL^{-1}$. Their activity level was then observed as either active, moving throughout the water, or inactive, lying stationary at the bottom or top of water. The startle response was also observed for those tadpoles who were active, as well as the survivorship. (Fraker, 2004) To statistically compare each investigation, two-way ANOVAs were performed. Exposure to acetaminophen at all concentrations did not have any effect on the activity, startle response or survivorship of the tadpoles. There was also no observed effect when coupling acetaminophen and triclosan, even though triclosan alone had a major effect on the tadpoles. Acetaminophen was also coupled with caffeine, as it is a common chemical found in water. This combination did affect the activity levels of the tadpoles, even at the very low

concentrations. The reason for this is unknown. (Fraker, 2004) Overall, it is important to remediate the water from these organic pharmaceuticals.

There is information known about the toxicity of acetaminophen in large, medicinal doses. There are typically three phases to acetaminophen overdose; the first being nausea and vomiting. The second phase is when what is known as heptatonecrosis, dehydration and renal damage occurs. The third phase includes jaundice, hypoglycemia and hepatic failure. Overall, acetaminophen in large quantities can be very damaging to the liver. (Rumack, 1975)

*Abiotic Degradation of Acetaminophen*

In comparison with the acetaminophen biodegradation information, it is necessary to understand abiotic degradation of acetaminophen, which occurs without any bacterial organisms. In a study concerning the thermal degradation of acetaminophen, it was found that acetaminophen in its solid, dry form was stable at room temperature. The degradation rate for the drug in this state increases with respect to temperature. At 25°C, the degradation rate is 1.12 E -7 $hr^{-1}$, and increases to 1.78 E -6 $hr^{-1}$ at 50°C. However, with trace moisture, acetaminophen degrades more rapidly to p-aminophenol. (Gilpin, 2004) *p*-Aminophenol is a hydrolytic product of the degradation of acetaminophen, and is commonly found in acetaminophen as an impurity. It is also used for the synthesis of acetaminophen. This reaction can occur at room temperature, with water as a solvent. (Mohamed, 1997) It is toxic via inhalation and dermal exposure, causing necrosis and severe damage to the liver (Klos, 1992) and therefore vital to understand the kinetics of *p*-aminophenol. If *p*-aminophenol cannot be degraded biologically, it will not be removed from the drinking water system, and will be harmful to the public health.

*Biodegradation of Acetaminophen*

After a literature search, two studies were found that studied the biological degradation of pharmaceuticals, including acetaminophen, in municipal wastewater treatment. A classification scheme was proposed by Adriano Joss, 2006, stating that acetaminophen was biologically transformed by more than 90%, which means that this compound should be readily biodegraded by the sludge found in wastewater treatment facilities. This investigation was conducted using both conventional activate sludge and membrane bioreactor batch reactors, supplied with wastewater from the local municipality. Three experiments were run simultaneously: a control batch without sludge, a batch with diluted sludge and a batch with diluted sludge and substrate. The reactors were all spiked with 3 $\mu g L^{-1}$ acetaminophen, which is an environmentally relevant concentration. The samples were analyzed by reversed-phase liquid chromatography with detection by electrospray mass spectrometry. (Joss, 2006) The control reactors confirmed that sludge is necessary for the biodegradation of acetaminophen, and when the sludge is present, a pseudo-first-order kinetic equation is found to describe the resulting reaction: $dC/dt = k_{biol} X_{ss} S$, with $X_{ss}$ being the sludge concentration in terms of suspended solids, and S referring to the soluble compound concentration. The resulting $k_{biol}$ for acetaminophen was greater than 10 $L g_{ss}^{-1} d^{-1}$, making it a readily biodegraded chemical. (Joss, 2006) It was then concluded that certain sludge characteristics were significant in the biodegradation of acetaminophen, including the diversity of the activity of the biomass due to the differences in microbial population or the enzymatic activity, the fraction of active biomass within the total

suspended solids, and the floc size of the sludge for compounds being well degraded. (Joss, 2006)

In addition to this study, another (Yamamoto, 2009) was conducted which analyzed the concentration of acetaminophen and associated biodegradation, which determined the persistence and partitioning of the pharmaceutical in aquatic environments. The samples used were collected from rivers located in Japan, and were inoculated with 100 $\mu g L^{-1}$ acetaminophen. The resulting $k_b$, or biodegradation rate, was found to be 0.014 $hr^{-1}$, with a half life of 50 hours. The removal rate by the microorganisms was found to be 96%. (Yamamoto, 2009) These biodegradation rates are very important in determining if acetaminophen will be removed during the wastewater treatment process.

Little is known about the anaerobic biodegradation of acetaminophen. One study was found during the literature search that discussed the potential of anaerobic biodegradation for *p*-aminophenol, a derivative of acetaminophen. Using total net gas production as an indicator, 77 compounds were tested for anaerobic biodegradation potential under methanogenic conditions. (Battersby, 1988) It was observed that substituents to phenol inhibited biodegradation. Therefore, p-aminophenol, with its amine substitutient, cannot be biodegraded anaerobically. (Battersby, 1988) It is safe to say that acetaminophen will not be anaerobically biodegraded as well, because it has a larger substituent than *p*-aminophenol, and therefore any potential for anaerobic biodegradation will be inhibited.

*Removal of Acetaminophen in Drinking Water Treatment Systems*

A study by Stackelberg, 2007, observed the potential of a municipal drinking water treatment facility in the removal of acetaminophen. If acetaminophen was to leave the wastewater treatment plant, at some point it is probable that the same water would be treated in a municipal water treatment plant. The maximum concentration found in this study was 0.12 µg/L, and acetaminophen was found in 75% of the waters. After undergoing clarification, disinfection, filtration, granular activated carbon, and other processes, the finished water acetaminophen concentration was 0.0003 µg/L, a 98% removal rate. (Stackelberg, 2007) Acetaminophen can be removed to some extent through the drinking water treatment train, if not removed by the wastewater processes.

*Detection of Acetaminophen*

Acetaminophen is typically detected using spectrophotometric methods, such as high performance liquid chromatography or UV-vis. Both utilize the absorbance of photons at specific wavelengths. The HPLC method was used in one study to determine the concentrations of acetaminophen and its derivatives. (Shervington, 2000) Here, the detection wavelength was 254 nm, and retention time was recorded. It took acetaminophen 2.83 minutes, at a pump flow of 1 mL/min, to exit the column. It took the rest of the derivatives longer, approximately 3.26 minutes or more. (Shervington, 2000) This method is a efficient way of quantifying amounts of acetaminophen within a sample, as well as determining what other compounds might be present.

Acetaminophen can also be detected using UV-Vis spectrophotometry. In a study by Meola, 1978, color was added to acetaminophen so that it can be observed in the

visible spectra range. The absorbance was measured at 660 nm, and calibration provided a linear plot, allowing for precise determination of acetaminophen. (Meola, 1978) A version of this method can be done using the UV range. Overall, there are many successful methods to detecting acetaminophen

# CHAPTER III

# METHODOLOGY

Batch experiments were performed with activated sludge from a conventional activated sludge treatment facility located in Stillwater, Oklahoma. Samples of the activated sludge were inoculated with acetaminophen (reagent grade, 99% purity, Sigma-Aldrich), and *p*-aminophenol (reagent grade, 99% purity, Sigma-Aldrich) and were analyzed using ultraviolet spectrophotometry.

## *Analytical Methods*

Ultraviolet and visible (UV-Vis) spectrophotometry was used to analyze the absorbance of acetaminophen in the batch reactors. Each sample was analyzed at a wavelength of 280 nm for acetaminophen and 300 nm for *p*-aminophenol, on a Hach DR/4000U Spectrophotometer. The cells used were 2 mL quartz, and distilled water was used to zero the instrument

*Instrumentation*

There are many instruments and methods used to detect and analyze acetaminophen and *p*-aminophenol concentrations. The technique used in this investigation was ultraviolet-visible spectrum spectrophotometry. This instrument detects the absorbance of photons, or light energy, by chemical compounds at various wavelengths. Each wavelength corresponds to a certain part of the electromagnetic spectrum, and each chemical compound has a unique light absorbance pattern. Organic compounds typically absorb light in the ultraviolet (UV) region, which consist of wavelengths between 190 and 400 nm, particularly between 240 and 300 nm.

The UV-Vis spectrophotometer relays information in terms of absorbance, which can then be converted to concentration using Beer's Law. Beer's Law relates the absorbance of photon energy to the pathlength through the sample, the molar absorptivity or extinction coefficient, and the concentration of the chemical in the following equation.

$$A = \varepsilon \cdot L \cdot c \quad (1)$$

Using the Beer's Law equation, the concentration of acetaminophen can be accurately quantified.

*Experimental Design*

A calibration of the spectrophotometer was necessary to precisely determine the absorbance due to the presence of acetaminophen, and consequently calculate the concentration. Standard solutions were made by combining acetaminophen with distilled

water, and heating the mixture until the white solid dissolved. The solutions had concentrations of: 100 mg/L, 50 mg/L, 10 mg/L, 3 mg/L, and 1 mg/L, which were chosen to reflect the range of the instrument. Solutions with concentrations of 25 mg/L and 5 mg/L were produced independently and used for quality control, and the wavelength studied was 280 nm. The resulting calibration had a linearity of 0.9999, and the equation is as follows:

$$\text{Absorbance} = 0.0113(\text{concentration}) - 0.0022 \qquad (2)$$

The quality control samples reported experimental concentrations that were within 2.0% of the actual. Figure 4 below shows the resulting linear regression.

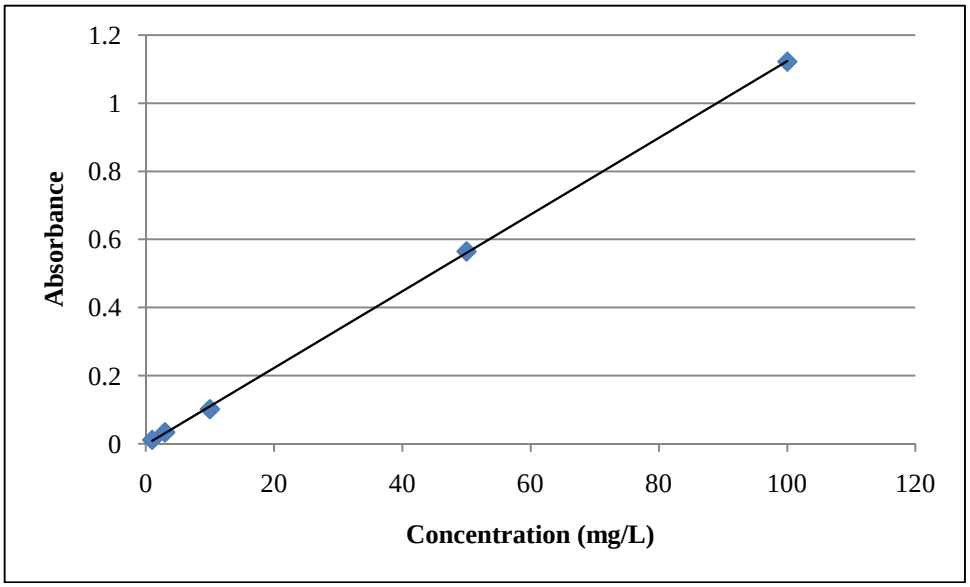


Figure 4: Calibration Data Using Acetaminophen at 280 nm

Calibration checks were conducted throughout the study using quality assurance samples, which are samples of known concentration.

In addition to the acetaminophen-based calibration, *p*-aminophenol was also used to calibrate the instrument at a wavelength of 300 nm. The wavelength of 300 nm was determined by scanning the entire UV-Vis spectrum for this solution. The peak at 290 nm shows the maximum absorbance of light by the *p*-aminophenol, and in order toincrease sensitivity, a wavelength slightly higher was employed in this investigation. Figure 5 shows the absorbance spectrum of *p*-aminophenol.

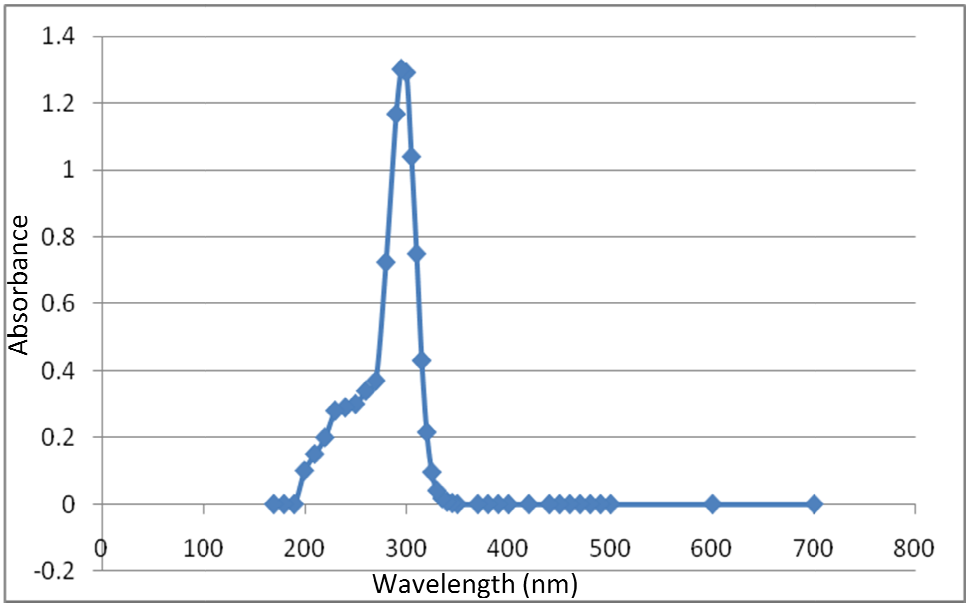


Figure 5: Absorbance Spectrum Scan of *p*-Aminophenol

An absorbance wavelength scan was taken of the unknown colored compound observed after exposing the acetaminophen solution to air, as well, for comparison to *p*-aminophenol. See Figure 6, which shows the absorbance spectrum scan. As seen above,

there was a peak at approximately 300 nm, and there was no absorbance observed in the visible spectrum.

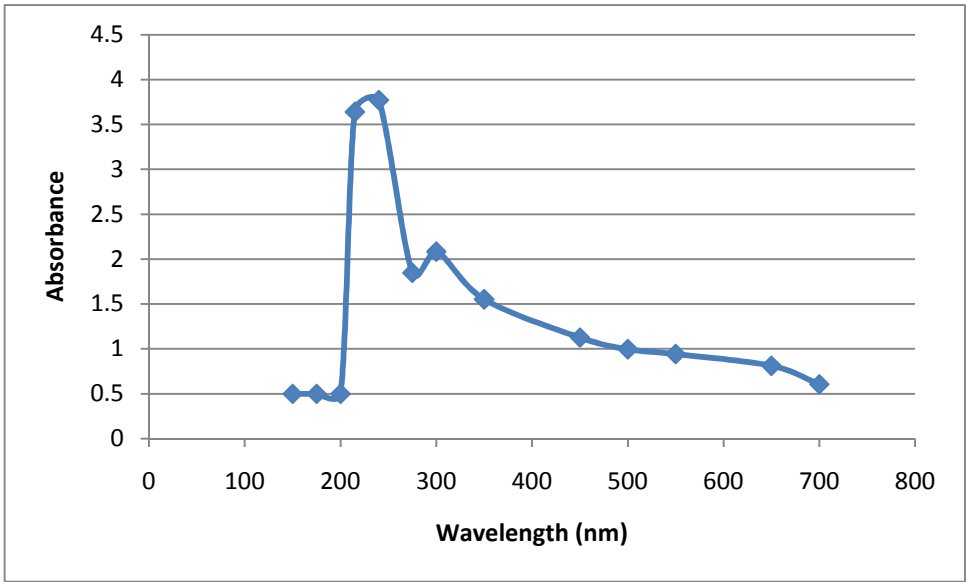


Figure 6: Absorbance Spectrum Scan of Unknown Compound

It is obvious when comparing the absorbance spectra of p-aminophenol and the unknown compound that they are in fact related. There is a peak at approximately 290 nm, which is observed in both spectra. There is some absorbance throughout the visible wavelengths, which is a further indication of the color observed. There visible spectra peak at approximately 550 nm, which is not found in the *p*-aminophenol scan.

Standard solutions were made by dissolving quantities of *p*-aminophenol in distilled water, resulting in the following concentrations: 50 mg/L, 10 mg/L, 3 mg/L, and 1 mg/L. Figure 7 shows the resulting calibration and the trend line.

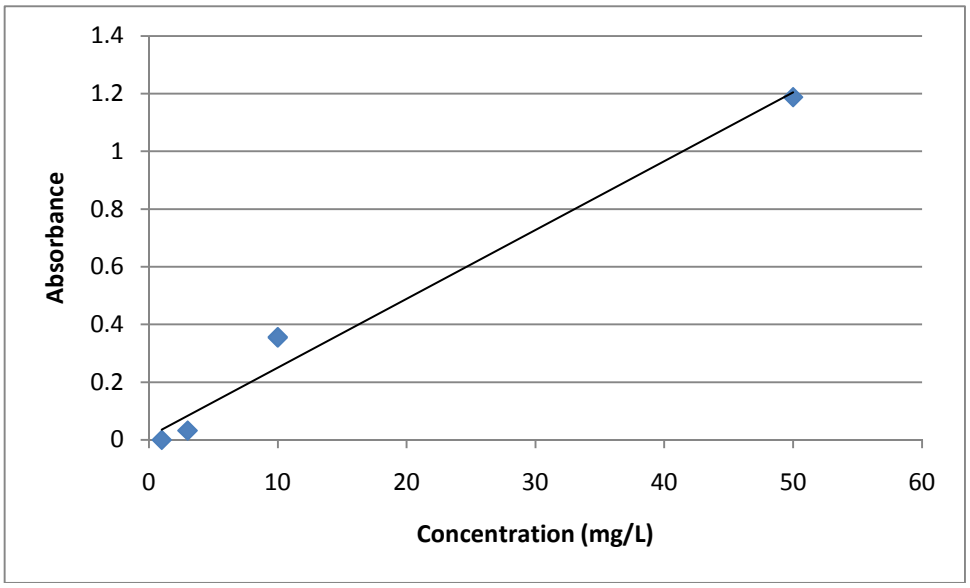

Figure 7: Calibration data using *p*-aminophenol at 300 nm

The $R^2$ value is 0.9835, proving the linearity of the calibration. This calibration is described using the linear equation of:

$$\text{Absorbance} = 0.0238(\text{Concentration}) + 0.012 \qquad (3)$$

Two quality control solutions were used, with concentrations of 25 mg/L and 5 mg/L, that were prepared independently. These samples had experimental concentrations within 4.0% of the actual.

Throughout this investigation, the dissolved oxygen concentration was also taken via a DO probe. A DO probe was inserted into the reactor and mixed with the attached propeller while the reading was taken. Over the course of a week, there was no change in the dissolved oxygen content of the abiotic reactor, which has no bacterial population. Therefore, aeration is providing the system with adequate dissolved oxygen. Figure 8 illustrates this conclusion.

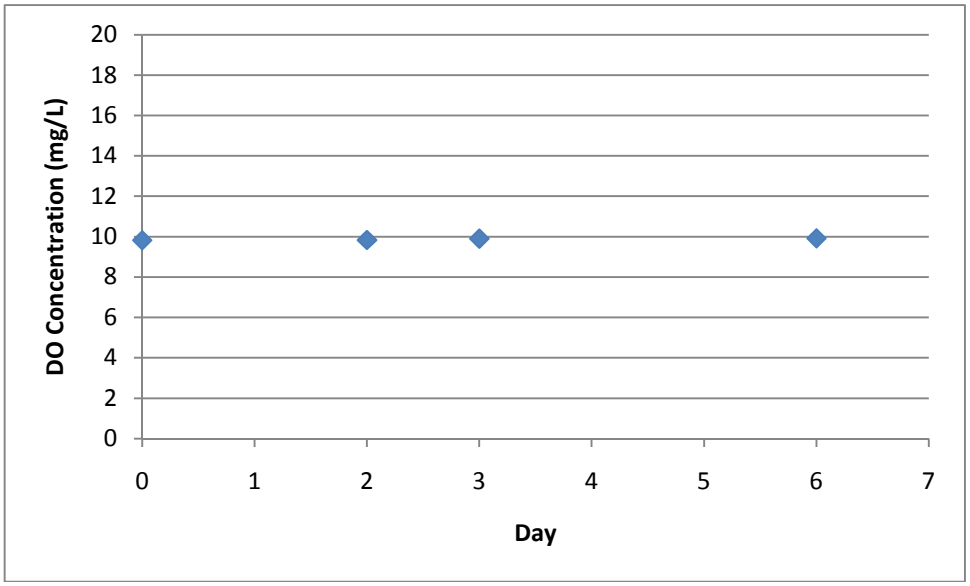

Figure 8: Dissolved oxygen concentration in abiotic reactor

In addition to the dissolved oxygen test and the abiotic controls, an abiotic control in the absence of light was observed. There was no difference between the reactor under light, and the reactor kept in the dark. Therefore, light energy does not play a role in the degradation of acetaminophen.

It is also true that there was no biological growth or activity in the reactor, as indicated by the constant concentration of dissolved oxygen. If there was in fact some sort of activity, the dissolved oxygen concentration would be affected.

*Reactors*

Abiotic, aerobic and anaerobic batch reactors were used in this investigation. Distilled water was used for the abiotic reactors. The aerobic and anaerobic activated sludge reactors used wastewater taken from the Stillwater Wastewater Treatment Facility in Oklahoma. Each seed was used for 2-3 weeks. The reactors were Pyrex Erlenmeyer

flasks, with volumes of 250 mL. All but the anaerobic reactors were aerated at a steady rate. They were all kept at room temperature, 25°C. The pH was neutral; approximately 6.8. Each sample taken from the reactors was filtered with a 0.45 µm filter, using vacuum filtration in order to remove the biomass, and the absorbance was then measured on the UV-Vis spectrophotometer. All of the reactors were mixed before sampling.

# CHAPTER IV

# FINDINGS

The following is a discussion of the abiotic, aerobic and anaerobic reactor experimental results.

## *Abiotic Controls*

### **Acetaminophen**

Before the biodegradation of acetaminophen in wastewater can be characterized, it is important to run abiotic control reactors. These reactors act as a control with respect to biological activity; they are not inoculated with microbes, but they are dosed with acetaminophen. These reactors were aerated and kept at room temperature for approximately six days. Figure 9 shows the there is no degradation of acetaminophen in this abiotic environment, thus allowing that all degradation will be due to the activated sludge from the wastewater

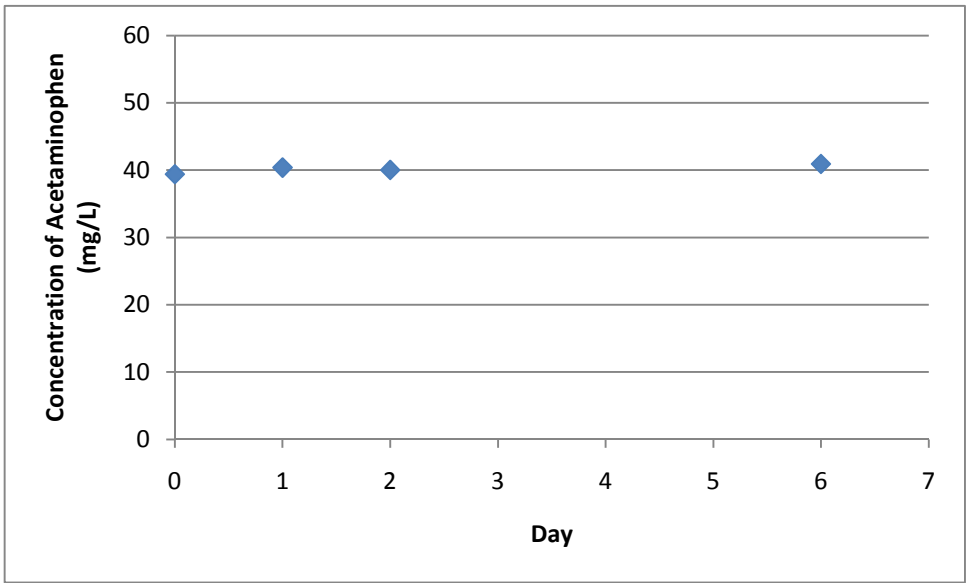

Figure 9: Abiotic reactor inoculated with 40 mg/L acetaminophen

To validate the conclusion that there was in fact no degradation of acetaminophen without biological activity, two additional abiotic reactors were run. The second reactor maintained the initial hypothesis; there was no decrease in acetaminophen concentration over the course of a week. However, in the third and final reactor, the concentration of acetaminophen dropped drastically after the third day, and was accompanied by a distinct color change from clear to brown. This brown color persisted after vacuum filtration through a 0.45 µm filter. Figure 10 below shows the concentration drop from approximately 55 mg/L to 10 mg/L. After this change, the concentration of acetaminophen continued to decrease.

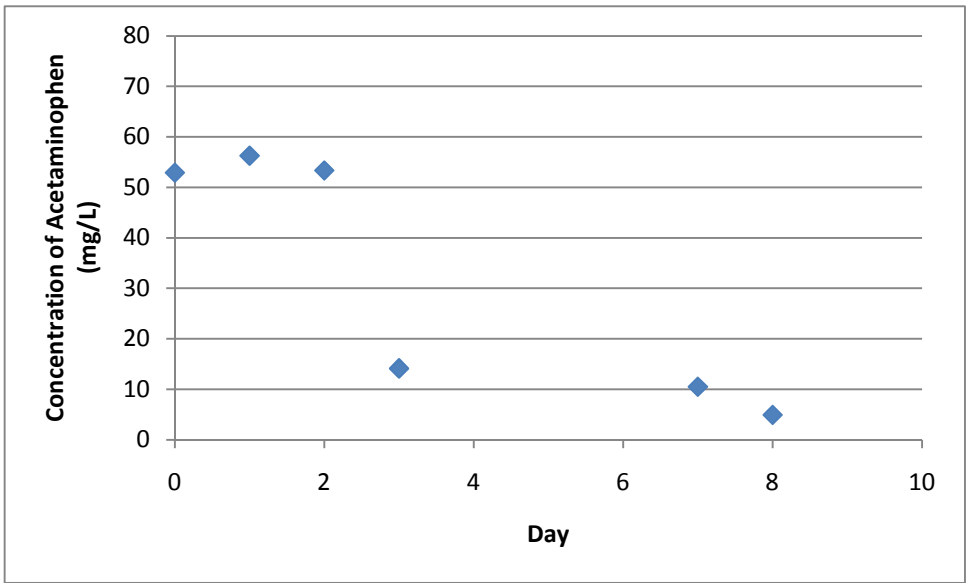

Figure 10: Abiotic reactor with degradation of acetaminophen

This phenomenon was not seen in the other two reactors, but samples left open to the atmosphere began to experience this color change after thirty days. This color change always came with a distinct change in concentration.

No further studies were completed on this reaction, because it was not observed in the biological reactors, which is confirmed by the absence of color after filtering. The unknown's color persisted after undergoing vacuum filtration. It can be explained by the degradation reaction of acetaminophen that produces *p*-aminophenol. This reaction occurs with water as a solvent, and with the presence of oxygen. (Mohamed, 1997) It also occurs much more rapidly with the presence of pure oxygen, and with a metal catalyst present. (Mohamed, 1997) It is possible that metals were present on the aeration bulb, which helped to catalyze the reaction in the final reactor. As seen later in the study, p-aminophenol quickly disappears as the colored compound appears, and due to its simple structure, it is logical that it would be degrading into the unknown.

Introduced earlier, *p*-aminophenol is the predominant degradation product of acetaminophen. Therefore, it can be deduced that as acetaminophen degrades, and the concentration decreases, the concentration of *p*-aminophenol increases. This was investigated in an abiotic reactor, by adding 50 mg/L acetaminophen and analyzing the absorbance at 280 nm and 300 nm each day. Figure 11 is the relationship between the degradation of acetaminophen and the resulting introduction of *p*-aminophenol.

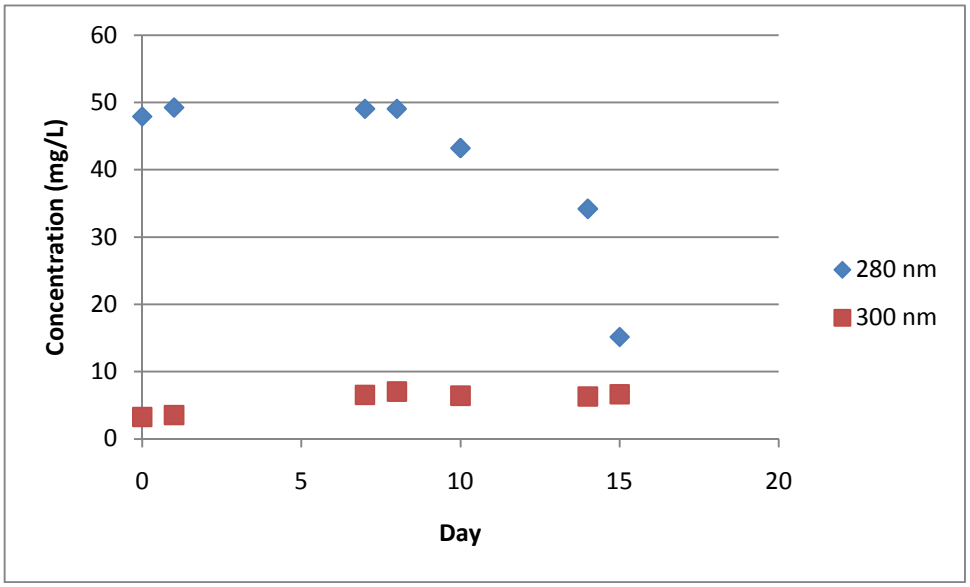


Figure 11: Degradation of Acetaminophen to *p*-Aminophenol
(280 nm: Acetaminophen, 300 nm: *p*-Aminophenol)

As seen above, the absorbance at 280 nm, from the acetaminophen, drops over time, from a concentration of approximately 50 mg/L to 15 mg/L. There is an initial concentration of *p*-aminophenol in the abiotic reactor, which is due to a slight impurity of the acetaminophen. The concentration of *p*-aminophenol does increase slightly over time; however, overall it remains steady at 6.5 mg/L. This is because *p*-aminophenol is fast

degrading at approximately the same rate as the unknown colored product is being produced.

The same experiment was conducted with *p*-aminophenol, to see if the concentration of the unknown would increase as *p*-aminophenol decreased. Figure 12 is the relationship between these two compounds. The *p*-aminophenol concentration was measured at 300 nm, and the colored compound was measured at 550 nm.

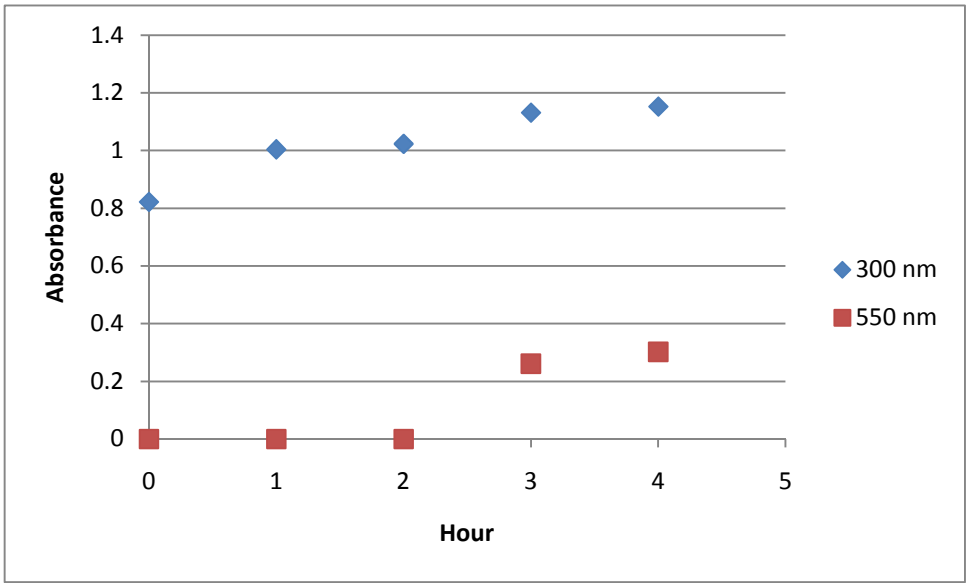


Figure 12: Degradation of *p*-Aminophenol to Unknown Compound
(300 nm: *p*-Aminophenol, 550 nm: Unknown Compound)

It is interesting how the *p*-aminophenol and the unknown's concentrations increase over time. This is because of interference occurring at 300 nm, as both compounds absorb at that wavelength. Therefore, as *p*-aminophenol degrades, the unknown concentration increases, causing a positive interference at 300 nm. UV-Vis spectrophotometry is not the best method to detect and quantify the reaction from *p*-aminophenol to the unknown. It can be stated that the unknown did form, however, because the solution began to change color after approximately four hours.

### *p*-Aminophenol

*p*-Aminophenol was analyzed in an abiotic batch reactor to observe the time needed for its degradation into the unknown, colored compound. It was found that *p*-aminophenol degraded within 24 hours. See Figure 13 below. The brown colored solution became present on day 1, as well, thus indicating that *p*-aminophenol does degrade into this unknown.

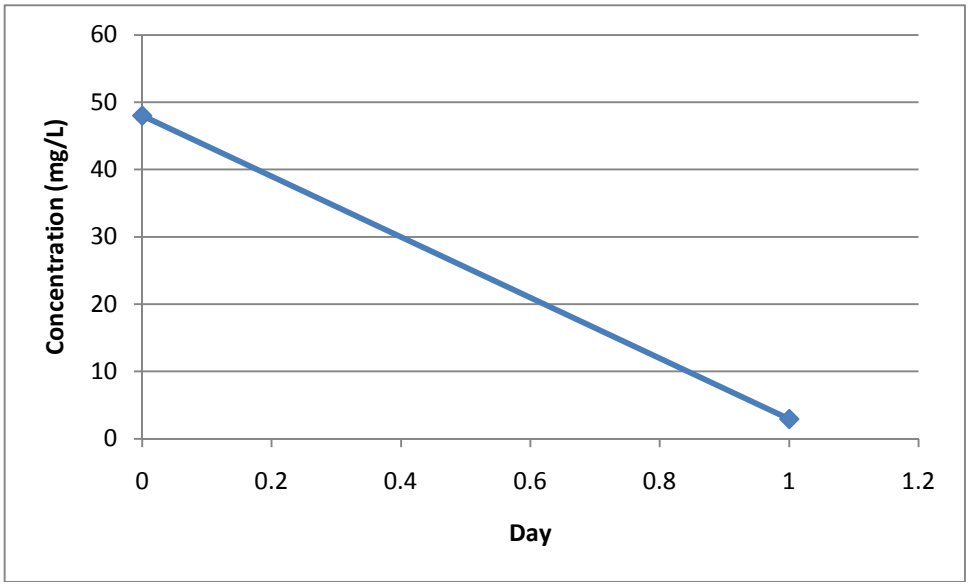


Figure 13: Abiotic Degradation of *p*-Aminophenol

*Aerobic Activated Sludge*

**Acetaminophen**

Two side-by-side aerated activated sludge batch reactors were run, one with 100 mg/L acetaminophen (reactor 1) and one with 100 mg/L acetaminophen and 100 mg/L glucose (reactor 2). Glucose was added to the second reactor as a secondary carbon source, and to see if the biodegradation rate of acetaminophen slowed with its presence. The absorbance at 280 nm was taken each day, and the resulting biodegradation trends were fit with first-order kinetic models. It should also be noted that there is a low background BOD in the reactors. Figure 14 below shows the resulting fit for the reactor without glucose.

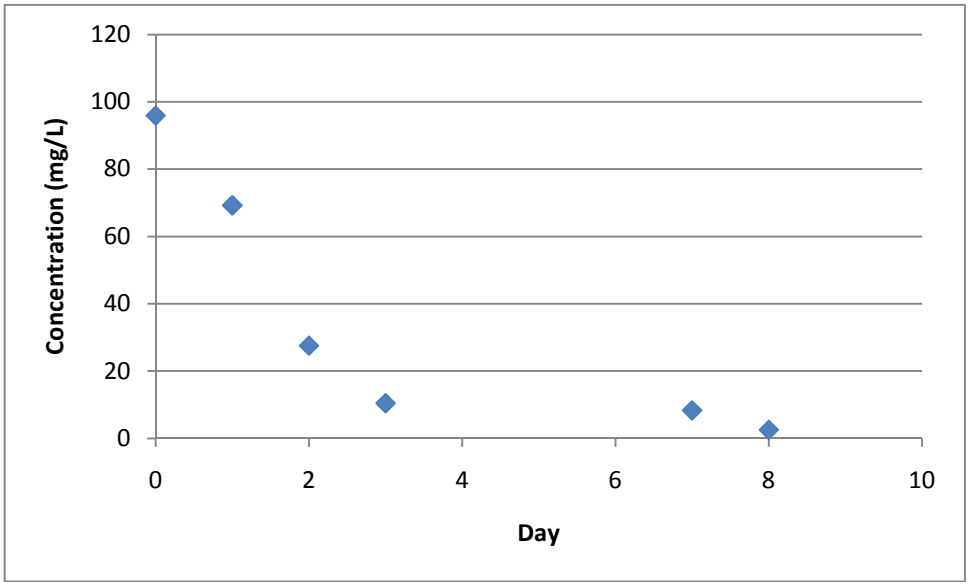


Figure 14: Exponential fit of the biodegradation of acetaminophen

Another way of analyzing first-order kinetics is to linearize the data, by taking the natural log of the concentration and plotting it against time. This results in a straight line, which is displayed in Figure 15.

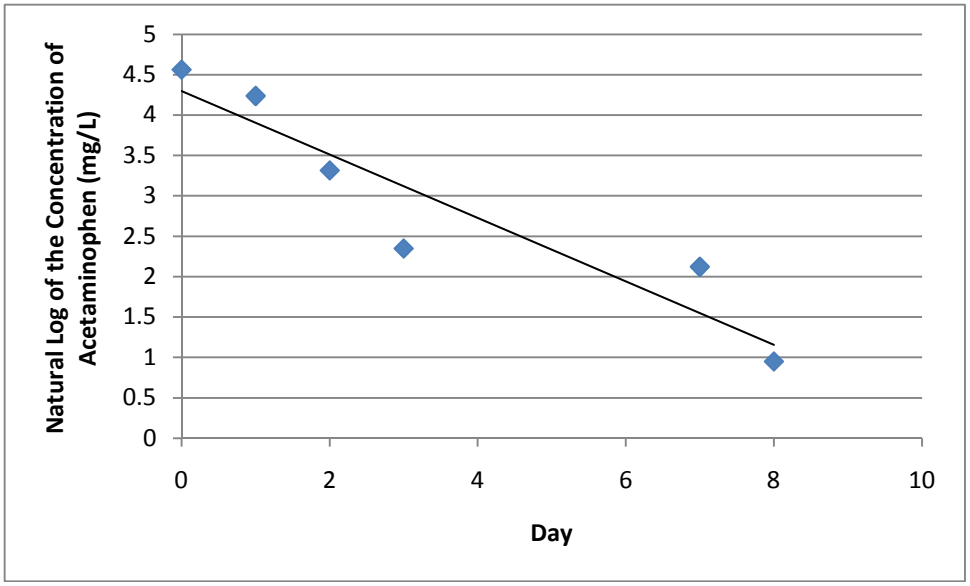

Figure 15: Linear fit of the biodegradation of acetaminophen

The resulting first-order kinetic equation for reactor 1 is as follows:

$$\text{Concentration} = 73.488e^{-0.393(\text{time})} \qquad (4)$$

The biodegradation constant is found to be 0.393 $day^{-1}$, and the half-life is 1.76 days. Acetaminophen should then be decreased by half after that time frame. For the linear plot, the $R^2$ value is 0.8745, proving that it is a relatively linear trend.

It was further hypothesized that glucose would inhibit the biodegradation of acetaminophen, because glucose is a readily biodegradable compound that bacteria is acclimated to. It is also possible that the presence of glucose could promote

biodegradation of acetaminophen, because of the potential increase of biomass. Therefore, glucose was added to the second reactor along with acetaminophen to observe the effect. Figure 16 show the first-order kinetic model fit to the data from this reactor.

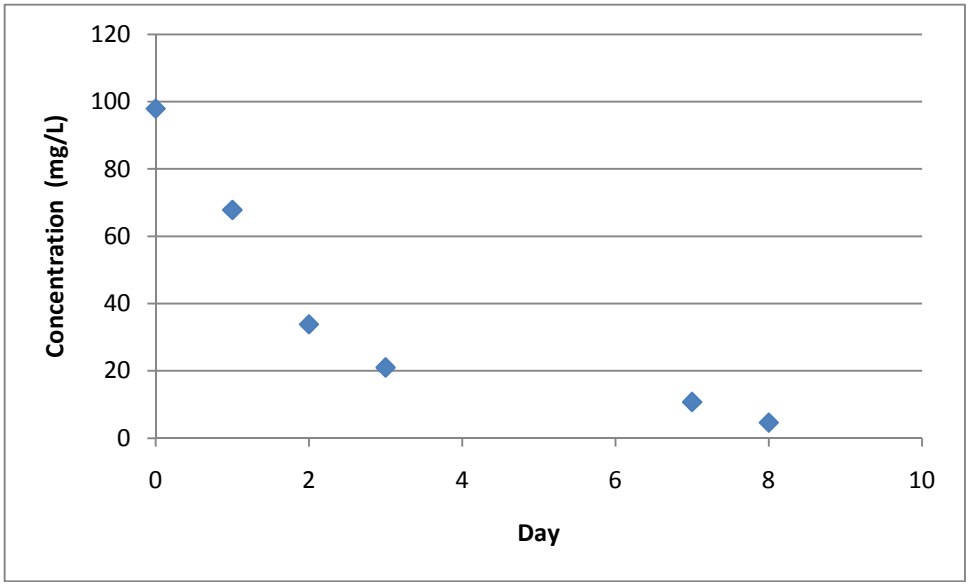


Figure 16: Exponential fit of the biodegradation of acetaminophen with glucose

The resulting first-order kinetic equation is found to be:

$$\text{Concentration} = 81.614e^{-0.34(\text{time})} \qquad (5)$$

Therefore, the half-life is 2.03 days, and the biodegradation rate is 0.34 $day^{-1}$. When comparing both reactors, there is a 13% difference in the biodegradation rates, with the reactor with glucose being faster. However, in the case of this study, it can be concluded that the rates are approximately equal. A solids test was conducted to determine the difference in solids within each reactor, which will aid in the explanation of the biodegradation rate differences. Reactors 1 and 2 both had biomass concentrations of

approximately 2000 mg/L, which is logical despite the addition of glucose. If the biomass yield was 0.5, then an addition of 100 mg/L of glucose would only yield 50 mg/L of biomass, which is too small to detect. Yamamoto also conducted a similar experiment in 2009, where he found the biodegradation rate of acetaminophen using river water inoculated with the compound. Table 1 below is the direct comparison between his data and the experimental data in this study.

Table 1: Comparison of Experimental and Literature Biodegradation Rates

| **Biodegradation Rates** | **Experimental** | **Yamamoto, 2009** |
|---|---|---|
| acetaminophen | 0.393 $day^{-1}$ | 0.336 $day^{-1}$ |
| acetaminophen with glucose | 0.34 $day^{-1}$ | - |
| | | |
| **Half-Lives** | | |
| acetaminophen | 1.76 days | 2.08 days |
| acetaminophen with glucose | 2.03 days | - |

As it turns out, Yamamoto found that the biodegradation of acetaminophen had a biodegradation rate similar to that of the reactors in this investigation. Overall, there is a 1.2% difference between reactor 2 and the literature biodegradation rates.

Another study, Joss, 2006, used pseudo-first-order kinetics to quantify the biodegradation rate. Table 2 is the comparison of the experimental biodegradation rate and Joss's, in pseudo-first-order units.

Table 2: Comparison of Acetaminophen Biodegradation Rates using Pseudo-First-Order Kinetics

| **Biodegradation Rates** | **Experimental** | **Joss, 2006** |
|---|---|---|
| acetaminophen | 0.17 L/g*day | 10 L/g*day |

There is a two-magnitude difference between the experimental biodegradation rate and that reported by Joss, 2006. One major difference between first-order and pseudo-first-order is that pseudo-first-order is dependent on the biomass concentration. Even though the exact biomass concentration is not reported in this research report, it does indicate that the sludge was diluted. A large difference in sludge concentration will cause such a difference in the rates.

When comparing abiotic and aerobic activated sludge degradation of acetaminophen, it is clear that the aerobic activated sludge does in fact biodegrade the compound. Figure 17 shows the steady abiotic acetaminophen concentration in comparison with the declining concentration of acetaminophen that was from the aerobic activated sludge reactor.

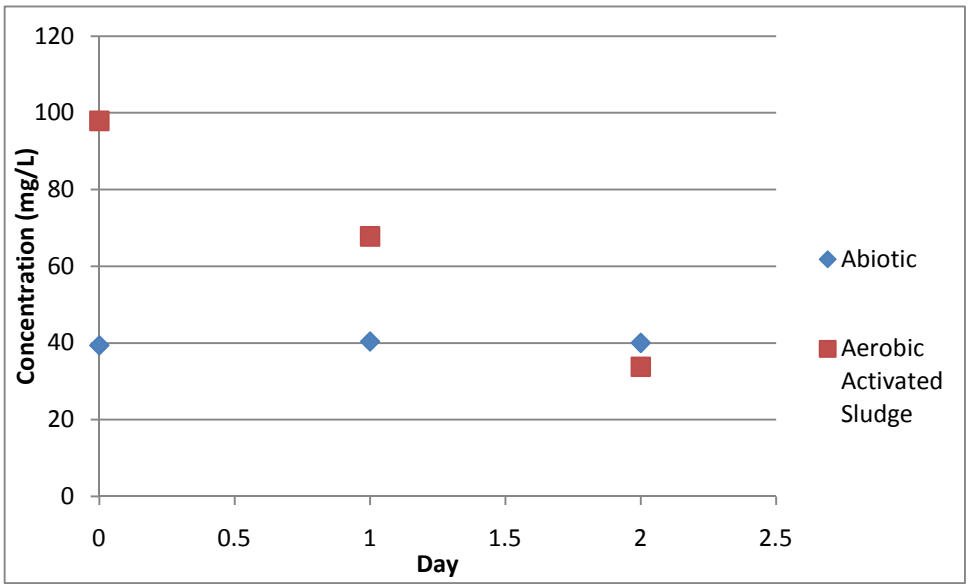


Figure 17: Comparison of Abiotic and Aerobic Activated Sludge Acetaminophen Biodegradation Trends

It is conclusive that the presence of the aerobic activated sludge must be present for acetaminophen to be biodegraded within the first few days. Acetaminophen is steady in an abiotic environment until the degradation to *p*-aminophenol reaction begins, but in the aerobic activated sludge reactor, acetaminophen is removed immediately.

**p-Aminophenol**

*p*-Aminophenol was then added to an aerated activated sludge batch reactor, and its biodegradation kinetics were observed. Upon fitting Figure 18 to first-order kinetics, the resulting equation is:

$$\text{Concentration} = 48.513e^{-1.462(\text{Time})} \qquad (6)$$

The biodegradation constant for *p*-aminophenol is 1.462 $day^{-1}$, approximately 4.3 times larger than acetaminophen. This means that *p*-aminophenol is much more readily biodegradable than acetaminophen. In other words, acetaminophen is more stable in an aerated activated sludge reactor than *p*-aminophenol.

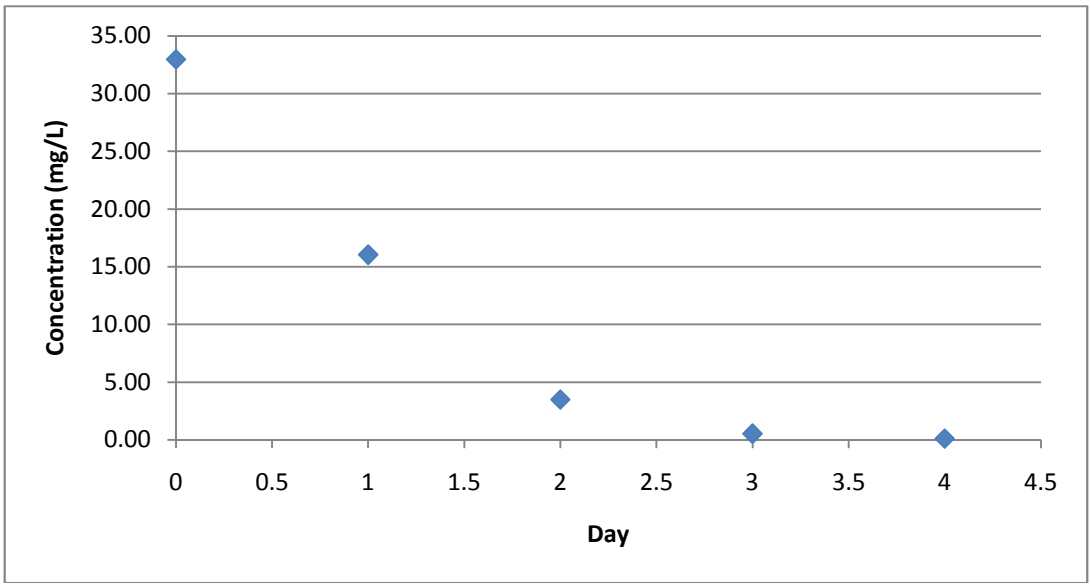

Figure 18: Exponential fit of the biodegradation of *p*-aminophenol

*Anaerobic Sludge*

Anaerobic sludge had an extremely high background at 280 nm and 300 nm, so the solution was diluted in a 4:1 ratio (4 parts water and 1 part sludge). Following dilution, 0.05 mg of each compound was added to its respective reactor, resulting in a 100 mg/L concentration.

**Acetaminophen**

It was hypothesized that the anaerobic sludge would readily biodegrade acetaminophen given its structure and biodegradable nature. However, the results proved to be the unexpected. As seen in Figure 19, the reactor was inoculated with 100 mg/L concentration of acetaminophen on day 0, but the experimental concentration was much higher, approximately 140 mg/L. It is possible that the acetaminophen reacted with other organic compounds present in the sludge to form compounds that would cause

interference. After day 0, the concentration of acetaminophen seemed to plateau. Any biodegradation that was occurring ceased completely, with acetaminophen remaining stable at a concentration of approximately 78 mg/L. There are two possible causes for this: the biodegradation is not able to be observed spectrophotomically due to interference, or the anaerobic sludge does not biodegrade acetaminophen.

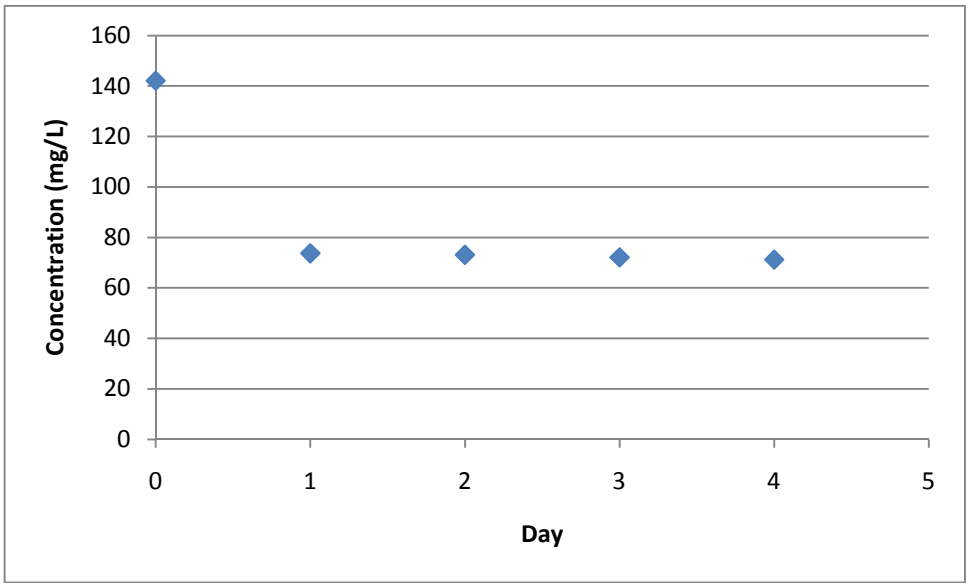


Figure 19: Biodegradation of Acetaminophen in Anaerobic Activated Sludge

To determine whether there is interference, 20 mg/L was added to the acetaminophen inoculated anaerobic reactor. The purpose was to observe whether the experimental concentration increased by 20 mg/L. If it did, then there would be no interference. Table 3 displays the data.

Table 3: Comparison of Background Acetaminophen-Inoculated Sludge and with the Additional 20 mg/L

| | **Concentration (mg/L)** |
|---|---|
| Background | 113.9 |
| With 20 mg/L Acetaminophen | 146.4 |

There is a difference of 32.5 mg/L between the two solutions, greater than the expected 20 mg/L. Therefore, it can be concluded that there is in fact some sort of positive interference occurring.

### *p*-Aminophenol

The anerobic sludge reactor inoculated with *p*-aminophenol did not prove the hypothesis, either. As seen in Figure 20, the initial experimental concentration of p-aminophenol is 17 mg/L, when really 100 mg/L was added to the reactor. This difference is probably due to a lack of solubility. The *p*-aminophenol compound may not have completely mixed with the sludge by the time the first sample was taken, even though adequate time was allowed for mixing. This would explain the spike in concentration on day 1, where the *p*-aminophenol concentration jumped to 58 mg/L.

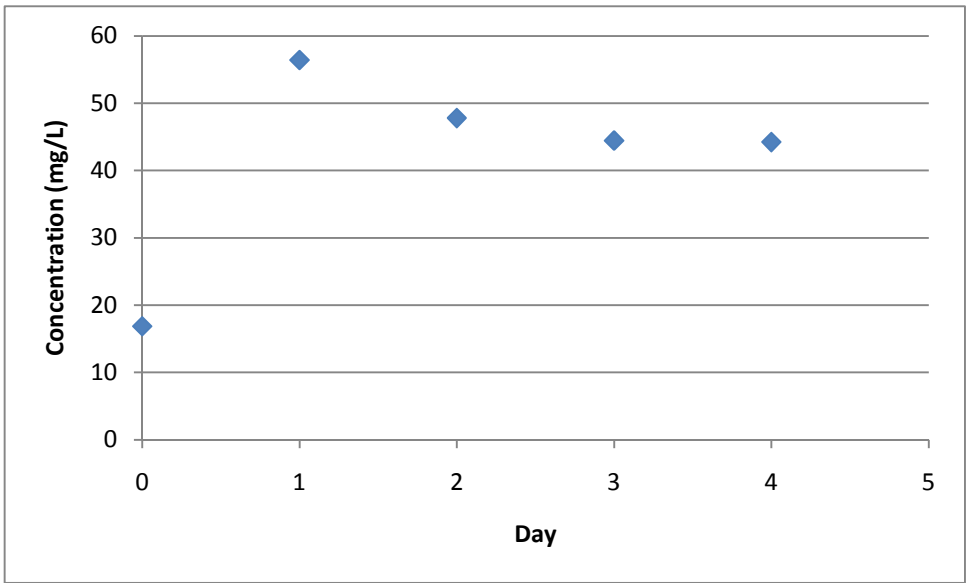

Figure 20: Biodegradation of *p*-Aminophenol in Anaerobic Activated Sludge

As seen with the acetaminophen anaerobic activated sludge reactor, there is no apparent biodegradation after day 2. The concentration of *p*-aminophenol remains relatively steady at 45 mg/L, after a slight decrease in concentration between days 1 and 2. The results are inconclusive to whether biodegradation actually occurred, or if there is an issue with the detection of *p*-aminophenol in the reactor. If it is an instrumentation issue, another method of detection will have to be employed to observe the biodegradation kinetics. However, as stated in Bettersby, 2009, *p*-aminophenol cannot be degraded anaerobically, and this conclusion can tentatively be extended to include acetaminophen

# CHAPTER V

# CONCLUSION

## *Abiotic Controls*

In an aerated, abiotic reactor, acetaminophen is found to be stable for a brief period of time, after which a two-step degradation reaction occurs, producing *p*-aminophenol, and followed by an unidentified, brown-colored compound. The second part of that reaction occurs at a much more rapid rate than the first, with *p*-aminophenol degrading much faster than acetaminophen with respect to time. This was proven by the abiotic reactor containing *p*-aminophenol, which degraded into the brown compound within 24 hours. Overall, acetaminophen was stable in abiotic environments for at least a few days.

## *Aerobic Activated Sludge*

Both acetaminophen and *p*-aminophenol were found to be biodegradable by aerobic activated sludge in a batch reactor, proven with a comparison of the abiotic acetaminophen concentration stability and the decreasing acetaminophen concentration in the aerobic activated sludge reactor. Both acetaminophen and *p*-aminophenol were degraded to a concentration below the detection limit, given a particular retention time.

Acetaminophen biodegrades at a rate of 0.34 $day^{-1}$, while *p*-aminophenol degrades at a rate of 1.462 $day^{-1}$. Therefore, it can be concluded that the biodegradation of acetaminophen is the limiting step to ridding the wastewater of the compound. The reason *p*-aminophenol is never observed is due to its highly biodegradable nature, and therefore its toxicity is not an issue for public health. Despite these rapid rates of reaction, acetaminophen is still found in natural waters. This indicates that there must be substantial biomass to fully biodegrade it. However, given enough time, acetaminophen can be biodegraded and removed from the system, ensuring that the wastewater did continue to be treated without any additional steps.

*Anaerobic Sludge*

The anaerobic sludge reactors inoculated with acetaminophen and *p*-aminophenol had inconclusive biodegradation results. Both appeared to be non-biodegradable by anaerobic activated sludge, however, an issue with the instrumentation must be explored, as some positive interference was experienced. Acclimation to acetaminophen and *p*-aminophenol should also be investigated, because it might take a substantial lag time for these bacteria to "learn" how to metabolize these compounds.

*Further Comments*

Additional investigation is needed to ensure the quality of the treated wastewater as it pertains to pharmaceuticals. While it can be tentatively concluded that a typical wastewater treatment train can remove acetaminophen, it is necessary to observe the effects a municipal drinking water treatment facility will have on its removal. It is also

known that acetaminophen is found at low concentrations in natural waters, so understanding the residual concentrations leaving the treatment facilities is vital. This study should also be duplicated using more sophisticated instrumentation, using High Performance Liquid Chromatography, which will allow the use of acetaminophen concentrations commonly found in wastewater. Therefore, spiking of the samples will not be necessary, and any residual acetaminophen concentrations will be observed. Overall, this method can be replicated in the analysis of many different pharmaceuticals, including but not limited to: steroids, antibiotics and tranquilizers. It is a simple and successful method in determining the kinetics of pharmaceutical biodegradation.

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# APPENDICES

*Calibration Raw Data*

Table 1A: Acetaminophen Calibration Data

| Concentration (mg/L) | Absorbance |
|---|---|
| 100 | 1.122 |
| 50 | 0.565 |
| 10 | 0.102 |
| 3 | 0.034 |
| 1 | 0.012 |

Table 1B: *p*-Aminophenol Calibration Data

| Concentration (mg/L) | Absorbance |
|---|---|
| 100 | 1.279 |
| 50 | 1.187 |
| 10 | 0.355 |
| 3 | 0.032 |
| 1 | 0 |

*Abiotic Raw Data*

Table 2A: Abiotic Reactor with Acetaminophen

| Day | Absorbance | Concentration (mg/L) |
|---|---|---|
| 0 | 0.443 | 39.39823009 |
| 1 | 0.454 | 40.37168142 |
| 2 | 0.45 | 40.01769912 |
| 6 | 0.46 | 40.90265487 |

Table 2B: Dissolved Oxygen Data for the Abiotic Reactor with Acetaminophen

| Day | DO (mg/L) |
|---|---|
| 0 | 9.82 |
| 2 | 9.83 |
| 3 | 9.9 |
| 6 | 9.92 |

Table 2C: Second Abiotic Reactor with Acetaminophen

| Day | Absorbance | Concentration (mg/L) |
|---|---|---|
| 0 | 0.6 | 52.90265487 |
| 1 | 0.638 | 56.26548673 |
| 2 | 0.605 | 53.34513274 |
| 3 | 0.162 | 14.14159292 |
| 7 | 0.121 | 10.51327434 |
| 8 | 0.058 | 4.938053097 |

Table 2D: Abiotic Reactor with p-Aminophenol

| Day | Absorbance | Concentration (mg/L) |
|---|---|---|
| 0 | 1.154 | 47.98319328 |
| 1 | 0.082 | 2.941176471 |

Table 2E: Degradation of Acetaminophen to *p*-Aminophenol

| Day | Absorbance at 280 nm | Absorbance at 300 nm | Concentration (mg/L) at 280 nm | Concentration (mg/L) at 300 nm |
|---|---|---|---|---|
| 0 | 0.539 | 0.09 | 47.89380531 | 3.277310924 |
| 1 | 0.554 | 0.097 | 49.22123894 | 3.571428571 |
| 7 | 0.552 | 0.168 | 49.04424779 | 6.554621849 |
| 8 | 0.552 | 0.18 | 49.04424779 | 7.058823529 |
| 10 | 0.486 | 0.165 | 43.20353982 | 6.428571429 |
| 14 | 0.384 | 0.162 | 34.17699115 | 6.302521008 |
| 15 | 0.169 | 0.17 | 15.15044248 | 6.638655462 |

Table 2F: Degradation of p-Aminophenol to Unknown

| Hour | Absorbance at 300 nm | Absorbance at 550 nm |
|---|---|---|
| 0 | 0.822 | 0 |
| 1 | 1.004 | 0 |
| 2 | 1.023 | 0 |
| 3 | 1.131 | 0.261 |
| 4 | 1.152 | 0.302 |

*Aerobic Activated Sludge Raw Data*

Table 3A: Aerobic Activated Sludge Reactor with Acetaminophen

| Day | Absorbance | Absorbance (minues blank) | Concentration (mg/L) |
|---|---|---|---|
| 0 | 1.245 | 1.081 | 95.85840708 |
| 1 | 0.944 | 0.78 | 69.22123894 |
| 2 | 0.473 | 0.309 | 27.53982301 |
| 3 | 0.28 | 0.116 | 10.46017699 |
| 7 | 0.256 | 0.092 | 8.336283186 |
| 8 | 0.191 | 0.027 | 2.584070796 |

Table 3B: Aerated Activated Sludge Reactor with Acetaminophen and Glucose

| Day | Absorbance | Absorbance (minus blank) | Concentration (mg/L) |
|---|---|---|---|
| 0 | 1.268 | 1.104 | 97.89380531 |
| 1 | 0.928 | 0.764 | 67.80530973 |
| 2 | 0.544 | 0.38 | 33.82300885 |
| 3 | 0.399 | 0.235 | 20.99115044 |
| 7 | 0.283 | 0.119 | 10.72566372 |
| 8 | 0.214 | 0.05 | 4.619469027 |

Table 3C: Aerated Activated Sludge Reactor with *p*-Aminophenol

| Day | Absorbance | Absorbance (minus background) | Concentration (mg/L) |
|---|---|---|---|
| 0 | 1.16 | 0.802 | 32.96 |
| 1 | 0.771 | 0.413 | 16.04 |
| 2 | 0.453 | 0.095 | 3.5 |
| 3 | 0.383 | 0.025 | 0.54 |
| 4 | 0.37 | 0.012 | 0.12 |

*Anaerobic Activated Sludge*

Table 4A: Anaerobic Activated Sludge Reactor with Acetaminophen

| Day | Absorbance | Absorbance (minus background) | Concentration (mg/L) |
|---|---|---|---|
| 0 | 2.16 | 1.603 | 142.0530973 |
| 1 | 1.388 | 0.831 | 73.73451327 |
| 2 | 1.381 | 0.824 | 73.11504425 |
| 3 | 1.37 | 0.813 | 72.14159292 |
| 4 | 1.36 | 0.803 | 71.25663717 |

Table 4B: Anaerobic Activated Sludge Reactor with *p*-Aminophenol

| Day | Absorbance | Absorbance (minus background) | Concentration (mg/L) |
|---|---|---|---|
| 0 | 0.771 | 0.344 | 16.86956522 |
| 1 | 1.68 | 1.253 | 56.39130435 |
| 2 | 1.482 | 1.055 | 47.7826087 |
| 3 | 1.405 | 0.978 | 44.43478261 |
| 4 | 1.4 | 0.973 | 44.2173913 |

VITA

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Scope and Method of Study:

The purpose of this research is to determine if acetaminophen with biodegrade in traditional activated sludge batch reactors. Abiotic, aerated activated sludge and anaerobic sludge reactors were investigated, and were inoculated with acetaminophen or *p*-aminophenol, which is a common degradation product of acetaminophen. Each sample was filtered to remove biomass, and UV-Vis spectrophotometry was used to detect analyte concentrations. The biodegradation results were fit with first-order kinetic models.

Findings and Conclusions:

It was found that acetaminophen degrades abiotically to *p*-aminophenol, and finally to an unknown colored compound under certain conditions. However, without the presence of oxygen and a catalyst, the reaction was not observed. Aeraobic activated sludge was successful in biodegrading both acetaminophen and *p*-aminophenol, with a biodegradation rate of 0.34 $day^{-1}$ and 1.462 $day^{-1}$, respectively. The anaerobic activated sludge proved inconclusive in the biodegradation of these two compounds. There was positive interference observed, as well as an absence of any biodegradation.

Further study needs to be done with a different method of instrumentation to investigate concentrations commonly found in the environment. Additional studies with the anaerobic activated sludge should be completed to determine the biodegradation kinetics.

ADVISER'S APPROVAL: Dr. Gregory Wilber