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# Fate of 2,4-Dinitroanisole (DNAN) in Municipal Wastewater

Joel P. Neuder

*Clemson University*, [jpneuderjr@att.net](mailto:jpneuderjr@att.net)

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FATE OF 2,4-DINITROANISOLE (DNAN) IN MUNICIPAL WASTEWATER

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A Thesis  
Presented to  
the Graduate School of  
Clemson University

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In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science  
Environmental Engineering and Earth Sciences

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by  
Joel P. Neuder Jr.  
May 2019

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Accepted by:  
Dr. Kevin Finneran, Committee Chair  
Dr. Sudeep Popat  
Dr. Nicole Martinez

## **ABSTRACT**

The fate and degradation rates for the insensitive munition (IM) compound 2,4-Dinitroanisole (DNAN), and its metabolites 2-Methoxy-5-nitroaniline (MENA), 4-Methoxy-3-nitroaniline (iMENA), and 2,4-Diaminoanisole (DAAN), were investigated in municipal wastewater sludge. Data indicates that biodegradation of DNAN is a series of two electron reductions, through MENA or iMENA, to DAAN. However, data also indicate that DAAN adsorbs readily to all sludge. Two different types of sludge were used: anaerobic digester sludge and return activated sludge (RAS). Both were collected from a water resource recovery facility in Greenville, SC.

Experiments were conducted under oxic and anoxic (nitrate-reducing) conditions. Anoxic conditions were maintained with a nitrogen headspace. Experiments began with 100  $\mu$ M DNAN in unamended, iron-amended, nitrate-amended, and combination bottles. The results revealed that DNAN was biodegraded within all the environments, with rates higher at anoxic conditions. When nitrate was present, degradation was inhibited at MENA. The next experiments began with either 100  $\mu$ M MENA or DAAN added to the wastewater. Experiments in oxic conditions with MENA showed no degradation and limited removal through adsorption, while experiments with DAAN demonstrated only removal through adsorption. Experiments in anoxic conditions beginning with MENA resulted in biodegradation to DAAN with slower rates than when beginning with DNAN. Nitrate was found to inhibit MENA biodegradation in anoxic conditions as well. Experiments in anoxic conditions beginning with DAAN demonstrated no further

biodegradation of DAAN; removal was a result on adsorption with slower rates than seen in the experiments in oxic conditions.

DAAN appears to be a terminal end product of biodegradation. Both environments result in reduction of DNAN to DAAN, but once biodegradation produces DAAN, adsorption occurs at varying rates. Several experiments were conducted which show limited desorption occurs in all environments with desorption favored under oxic conditions with a higher organic content.

## **ACKNOWLEDGMENTS**

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## 1. INTRODUCTION

Explosives and insensitive munitions (IM) are a significant groundwater and industrial wastewater contaminant throughout the world due to the large quantities in which they are produced, stored, and discharged into the environment. The military and private entities use these explosives for demolition work. The overwhelming use of explosives has been associated with ammunition depots, production facilities, and live-fire training installations (Niedzwiecka and Finneran 2015; Niedzwiecka et al. 2017). After explosions, some residual remains behind which is not fully detonated or used in the reaction. Explosive wastes are poorly degraded in the natural environment and have been known to persist for many years. Explosives pose toxicity implications to humans and wildlife and the persistence within natural systems is one of the main reasons there is concern surrounding these compounds. It is imperative to understand the fate and transport of these compounds in the environment to make informed decisions on efforts to control the spread, release, and treatment of these compounds (Niedzwiecka and Finneran 2015; Niedzwiecka et al. 2017; Anguay and Ield 2016).

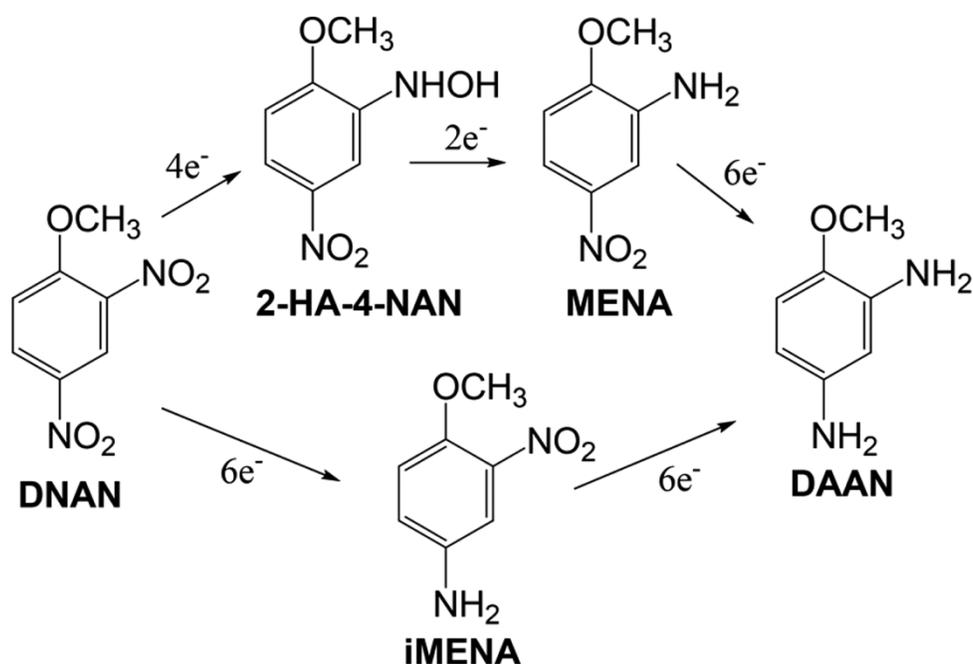
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4,6-trinitrotoluene (TNT) have been the primary explosives used by the military for many years across several shell platforms and therefore have been produced and stored in mass quantities (Trzeciński et al. 2014). RDX and TNT have been the main components in the fill for the 155mm M795 projectile and other large caliber rounds which contain explosive fillers. While these compounds are still performing as required, recent events have caused concern over the safety of rounds filled with these compounds. There has been an increasing number

unplanned explosions due to the instability of these compounds (Fung and Newland S 2015). Due to changes in the modern theaters of war, the military has been looking for a suitable replacement of TNT in its melt pour explosives that would be less sensitive to shock, citing safety of soldiers as the reason for the change. Several compounds have been considered, but 2,4-dinitroanisole (DNAN) is the most probable IM being considered for use in place of TNT. DNAN has been proposed as a less sensitive munition than TNT so melt pour explosives made from it are more stable, therefore resulting in increased stability and safety for transport, handling, and storage (Niedźwiecka et al. 2017). DNAN is insensitive to mechanical stimuli, where TNT is not. The sensitivity of these explosives is the driving factor behind the switch. However, there is an undesirable loss of energy associated with replacing TNT with DNAN, which is a drawback to this switch. Due to the loss of energy, more explosive material will need to be used to deliver the same effect (Fung and Newland S 2015; Trzciński et al. 2014). Considering both of these factors, the increase in explosives to compensate for energy loss and the toxicity of the compounds, it is possible to see more toxic compounds in the environment which necessitates effective remedial strategies.

Little is known about the environmental fate of DNAN. However, many other nitroaromatic compounds are toxic, mutagenic, and resistant to biodegradation, so it is possible DNAN will also be toxic and have similar environmental fate and transport properties. Experiments have demonstrated DNAN causes toxic effects to microorganisms, earthworms, and plants (Alsop et al. 2016). Therefore, it is a fair assumption that humans could also see toxic effects from the compounds. Since

biodegradation of DNAN within certain environments has been demonstrated, toxicology studies will need to be conducted on the metabolites of DNAN. Two of the reduced metabolites were found to have toxic effects to zebrafish embryos at 6 parts per billion (ppb), while the other metabolites were found to have toxic effects at levels above 600 ppb (Anguay and Ield 2016). The study indicates the reduced metabolites may actually be more toxic than the parent compound drawing a similarity to chlorinated solvents, where treatment can actually cause more harm. While humans may not see the same toxic effects at the same concentrations as the zebrafish, it can be assumed that there will be toxic effects from DNAN and its metabolites. Since health effects were seen in the study, understanding which of the chemicals are the most toxic ensures treatment and remediation understand which chemicals in the degradation chain are the most toxic within the environment. As IM use increases more research will be needed to understand the toxicology effects of the chemicals so effective remedial strategies can be designed (Christopher Olivares, Jidong Liang, Leif Abrell, Reyes Sierra-Alvarez 2013).

DNAN has shown promise of being readily biodegraded in groundwater systems. Within groundwater systems, degradation of DNAN down to its reduced metabolites; 2-methoxy-5-nitroaniline (MENA) and 4-methoxy-3-nitroaniline (iMENA), and 2,4-diaminoanisole (DAAN) has been demonstrated. Degradation was achieved through the use of biological and abiotic reactions within anoxic environments but was most efficient through a combination of the two. The pathways in which DNAN is reduced down to DAAN are summarized in **Error! Reference source not found.** below.



**Figure 1. Reductive Pathways of DNAN down to DAAN (Niedźwiecka et al. 2017).**

To reduce DNAN down to DAAN takes 12 electrons, but is actually two six electron steps taking DNAN to MENA or iMENA and then to DAAN (Niedźwiecka et al. 2017). Therefore, finding ways to stimulate microbial or abiotic reductions are needed. Experiments were conducted with ferrous iron and anthrahydroquinone-2,6-disulfonate (AH<sub>2</sub>QDS) in batch bottles simulating a groundwater environment. In these experiments, cells were found to degrade DNAN directly, but when amended with iron and AH<sub>2</sub>QDS the reaction rates increased. These methods would suggest an appropriate strategy for degrading DNAN within groundwater (Niedźwiecka et al. 2017; Niedzwiecka and Finneran 2015). Degradation of DNAN down to DAAN was achieved but once DAAN was reached it appeared to be the terminal end product and its fate is unknown. It has been reported to form dimers and have low solubility in water preferring to adsorb to soil

within groundwater system. Adsorption would be an effective means of removal within a groundwater environment but understanding more about the reactions DNAN undergoes in the subsurface is needed (Niedźwiecka et al. 2017; Hawari et al. 2015).

Within wastewater systems, DNAN has shown similar degradability to groundwater systems. Olivares et al, 2013 conducted experiments to test the aerobic, microaerophilic, and anaerobic biotransformation of DNAN using biomass from wastewater sludge resuspended in inoculum which was then amended with different electron donors. In anoxic wastewater systems degradation of DNAN was achieved rapidly down to MENA and DAAN. Within the anoxic wastewater system, full transformation of DNAN was achieved within 33 hours. Experiments were also conducted under microaerophilic and aerobic conditions. Within the microaerophilic conditions, 80 percent degradation of DNAN was achieved in 35 hours. Under aerobic conditions, it took the acetate amended environment almost 700 hours to degrade 87 percent of the DNAN input into the bottle (Christopher Olivares, Jidong Liang, Leif Abrell, Reyes Sierra-Alvarez 2013). While the aerobic system can achieve degradation, it is not capable of degrading DNAN in a reasonable timeframe. The different environments are comparable to what has been seen in groundwater studies with the aerobic metabolism microbes not being as suitable for reducing DNAN. When you compare the microbes to the ones which are dominant in anoxic environments, it has been demonstrated that these microbes are able to degrade DNAN more readily. Another similarity to the groundwater studies are the use of iron in anoxic wastewater systems to increase the reaction rates of DNAN degradation. The use of iron shavings within an

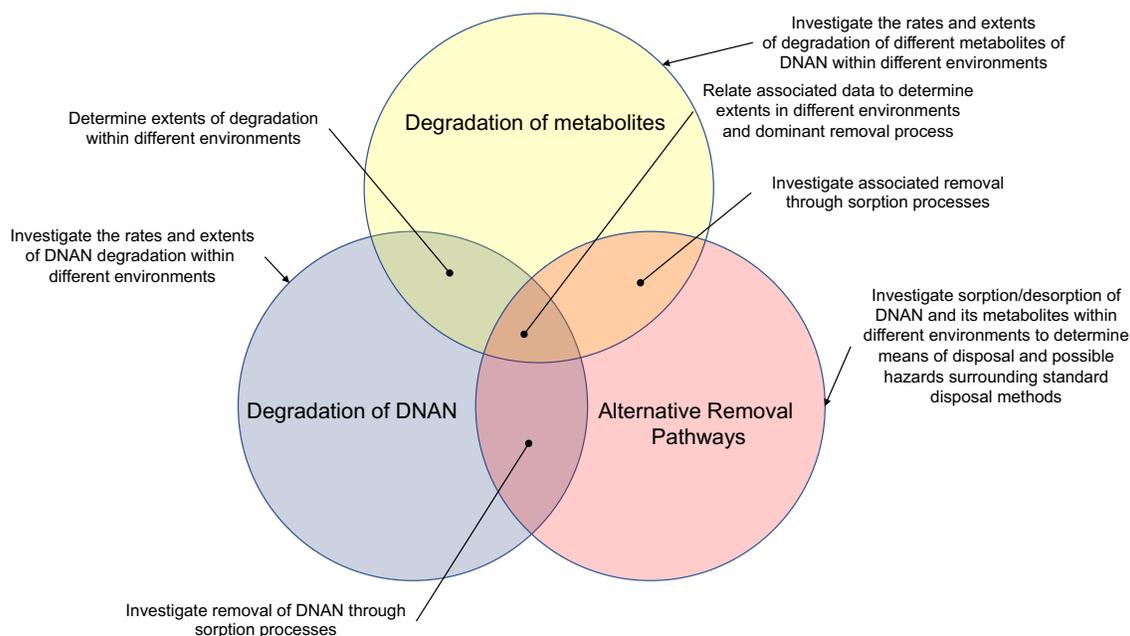
upflow anaerobic sludge blanket reactor enhanced DNAN degradation from approximately 85 percent to 99 percent and better (Ou et al. 2016). The microbial community in the anoxic environments is better suited for the degradation of DNAN and if it is stimulated with iron; it has been shown in groundwater and wastewater to increase the reduction rates. These studies have shown that many different treatment techniques and strategies can be implemented to achieve DNAN degradation. More research needs to be conducted to determine the overall fate of the metabolites produced from the degradation of DNAN in these wastewater environments.

The objectives of this research were determined in order to provide a more complete understanding of the degradation of DNAN and its metabolites. The objectives of this research are:

- Investigate degradation of DNAN and its metabolites in wastewater
- Determine the rates and extents of degradation
- Use native wastewater conditions seen in a typical secondary treatment process

Reduction of DNAN down to its metabolites has been demonstrated, so understanding the requirements to achieve this in wastewater is critical to being able to design effective treatment strategies as they become more prevalent in industry and in the environment.

The overall goals of this experiment are summarized in Figure 2 below.



**Figure 2. Overall Summary of Thesis work. Understanding the fate of DNAN and metabolites within different wastewater systems.**

The initial goal of this research was to determine the extent of DNAN degradation within these environments and compare the rates of degradation. Understanding whether it is biological or physical removal is a critical because this will help in the design of these systems in the future. Also understanding the extent to which DNAN is degraded in each environment will help provide a more accurate picture of what to expect in different bioreactor environments.

Following the initial experiments, it was seen that degradation of the metabolites may pose a problem in wastewater. With that, the next stage of work used the same systems, however began with the reduced metabolites. Determining which of these metabolites are biologically degradable will help to design systems that can be used for complete removal of the compounds down to harmless end products in the future.

The final goal of this research was to evaluate alternative removal processes. More specifically, there is a need to determine if adsorption is the dominant removal process for the metabolites, and if these metabolites are adsorbed to the biomass, will they ever become bioavailable again. Another consideration is if adsorption is reversible, because if so, there is a risk of them being resuspended in solution. Determining whether microbial reduction or adsorption are dominant within the different environments will help to create a more complete picture of the degradation of the compounds that could be seen within a typical water resource recovery facility.

## **2. MATERIALS AND METHODS**

### **2.1 SAMPLE COLLECTION**

All wastewater samples were collected from the Renewable Water Resources Mauldin Road Water Resource Recovery Facility located in Greenville, South Carolina. The samples were collected from two different locations within the plant. The samples were used to simulate the oxic environment were collected from the return activated sludge (RAS) lines and the samples used to an anoxic environment were collected from the anaerobic digester on site. The wastewater samples were stored in the refrigerator (4°C) until they were needed for experiments.

### **2.2 BATCH BOTTLE PREPARATION**

All degradation experiments were conducted in batch bottles. The wastewater used in each experiment was incubated overnight to allow for stabilization to the

environment prior to any addition of explosives or donors. The experiments were conducted with donors and wastewater inoculum according to Table 1 below.

**Table 1. Overview of all degradation batch experiments.**

	Wastewater Sample Used	Beginning Explosive Quantity ( $\mu\text{M}$ )	Temp.	Headspace Environment	Donor Amendments
DNAN	RAS	100	Room	Oxic	<ul style="list-style-type: none"> <li>• Unamended</li> </ul>
	Sludge	100	35°C	Anoxic ( $\text{N}_2$ )	<ul style="list-style-type: none"> <li>• Unamended</li> </ul>
	RAS	100	Room	Oxic	<ul style="list-style-type: none"> <li>• Unamended</li> <li>• 10mM Iron</li> <li>• 10mM Nitrate</li> </ul>
	Sludge	100	35°C	Anoxic ( $\text{N}_2$ )	<ul style="list-style-type: none"> <li>• Unamended</li> <li>• 10mM Iron</li> <li>• 10mM Nitrate</li> </ul>
MENA	RAS	100	Room	Oxic	<ul style="list-style-type: none"> <li>• Unamended</li> <li>• 10mM Nitrate</li> <li>• 1mM Nitrite</li> <li>• 10mM Acetate</li> </ul>
	Sludge	100	35°C	Anoxic ( $\text{N}_2$ )	<ul style="list-style-type: none"> <li>• Unamended</li> <li>• 10mM Nitrate</li> <li>• 1mM Nitrite</li> </ul>
DAAN	RAS	100	Room	Oxic	<ul style="list-style-type: none"> <li>• Unamended</li> <li>• 10mM Nitrate</li> <li>• 1mM Nitrite</li> </ul>
	Sludge	100	Room	Oxic	<ul style="list-style-type: none"> <li>• Unamended</li> <li>• 10mM Nitrate</li> <li>• 1mM Nitrite</li> </ul>
	Sludge	100	35°C	Anoxic ( $\text{N}_2$ )	<ul style="list-style-type: none"> <li>• Unamended</li> <li>• 10mM Nitrate [6]</li> <li>• 1mM Nitrite [0.6]</li> </ul>
	Sludge	100	35°C	Anoxic (95:5 $\text{N}_2:\text{H}_2$ )	<ul style="list-style-type: none"> <li>• Unamended</li> <li>• 10mM Nitrate</li> <li>• 10mM Iron</li> <li>• 10mM Sulfate</li> </ul>

Each of the batch bottles began with 60mL total volume. To reach the desired concentration of DNAN or its metabolite within each batch bottle, the volume of wastewater added was adjusted in order to reach the concentrations (Table 1). To reach the desired concentration within the unamended bottles, 54.0mL of wastewater sample was added and 6.0mL of explosive was added from a 1.0mM stock solution. The nitrate and nitrite were diluted into the solution from a 100mM stock solution. The iron was added from a 500mM iron gel stock solution. Finally, the acetate was added from a 30mM stock solution. The explosives and the amendments were added to the wastewater samples simultaneously after the wastewater had stabilized in the environment. Refer to Table 2 below for preparation guidelines for the stock solutions for DNAN and its metabolites.

**Table 2. Quantities Required to make stock solutions for experiments.**

Explosive	Desired Stock Concentration	Mass of Compound Required	Volume of Stock
DNAN	1mM	21.3mg	100mL Methanol
MENA	1mM	16.8mg	100mL Methanol
iMENA	1mM	16.8mg	100mL Methanol
DAAN	1mM	13.8mg	100mL Methanol

The oxic experiments used 125mL Erlenmeyer flasks as the glassware and used RAS as the inoculum for the experiments. The anoxic experiments were conducted using 125mL glass serum bottles with blue butyl stoppers to seal the headspace. For the anoxic experiments, the headspace of each bottle was then sparged with nitrogen to create anoxic

conditions. The experiments were incubated at different temperatures to more closely resemble the native wastewater conditions (Table 1).

### 2.3 SAMPLE ANALYSIS

Sample analysis began by extracting 1.0 mL of sample from each batch bottle at interval times designed to capture the reduction reactions of DNAN and its metabolites in each environment. The sampling was conducted until DAAN was the only metabolite left in each bottle or until the bottles remained stable for several sampling periods. The samples were then centrifuged at 11,000 revolutions per minute for 12 minutes in order to pellet the solids. After this, the liquid was decanted and filtered through a 0.2 $\mu$ m polytetrafluoroethylene filter prior to quantification. The filter was used to protect the sensitive analysis equipment from clogging. Samples were stored in the refrigerator until analysis could be done. Sample quantification was achieved through the use of a High-Performance Liquid Chromatograph (HPLC). Quantification of DNAN and its metabolites within the wastewater samples was achieved using a Thermo-Scientific Ultimate 3000 HPLC. The column used for analysis was a Thermo Fisher Scientific Acclaim Polar Advantage II C18 Column (5  $\mu$ m, 4.6 x 250 mm; Waltham, Massachusetts). The flow rate for the system was 1.0 mL/min with an eluent mixture of 40:60 Acetonitrile: Double Distilled Water. The column temperature was set to 35 degrees Celsius and the injection volume was 10  $\mu$ L. Detection of the compounds was achieved using a RS Variable Wavelength Detector with quantified DNAN at 300 nm, MENA and iMENA at 254 nm, and DAAN at 210 nm. The elution times of the compounds were 11.3, 8.8, 6, and 4.2 minutes respectively.

Concentration standards of 1.0, 6.25, 12.5, 25.0, 50.0, and 100.0  $\mu\text{M}$  were run at the beginning to determine the calibration of the machine and produce a standard curve for analysis of the environmental samples. A linear regression was fit to the standard curve and was then used to back calculate the quantity of DNAN and its metabolites found within each of the samples. Each of the batches were prepared in triplicate and error was expressed as standard deviation of the triplicate bottles.

## 2.4 ADSORPTION ASSAY

Adsorption analysis of materials was conducted at the conclusion of each experiment. These samples could only be conducted at the end due to the large sample volume required and the compromising of the experimental environment. A method was adapted from the adsorption analysis method used by Hawari et al. 2014 to quantify the explosives reversibly adsorbed to the biomass. First, enough of the wastewater was removed from the batch bottles and placed into either a 15mL or 50mL Falcon tube for digester sludge or RAS respectively in order to centrifuge the wastewater to pellet the solids and decant the water. The percentage of solids in the RAS and sludge were estimated based on the quantity of solids remaining during normal sample analysis, (Section 2.3). The remaining solids in the centrifuge tube revealed that RAS was approximately 10 percent solids and sludge was approximately 30 percent solids. Therefore, to get the 1.5mL desired volume of solids to analyze for adsorbed material, 15 mL of RAS and 5 mL of sludge had to be centrifuged. Samples were centrifuged according to the method mentioned above. Following this, the water was decanted off and disposed of. Then the solids were resuspended in an equal volume of acetonitrile to

bring the solution back to the initial volume. The goal was to use a strong organic in which DNAN and its metabolites are soluble to resuspend any material. After the resuspension, the samples were then sonicated for three hours until the solid pellets were resuspended. After this, the samples were prepared as mentioned above for quantification on the HPLC. In the event of material not able to be recovered with the method described above, it was assumed to be irreversibly adsorbed and was quantified using stoichiometric calculations.

## 2.5 TOTAL ORGANIC CARBON AND NITROGEN ANALYSIS

Determination of the total organic carbon (TOC) and total organic nitrogen (TON) portions of the RAS and sludge were determined according to the method published by Ersan et al. 2019. Analysis standards of TOC and TON were prepared to develop a standard curve which was used for sample quantification. Known concentrations of 5, 10, 25, 50, and 100 mg/L of TOC and TON were used to develop the curve. The RAS and the sludge were also diluted 1:10 and 1:50 to provide duplicate analysis of each and to ensure each fell within the known concentration standards.

## 2.6 TOTAL AND VOLATILE SUSPENDED SOLIDS ANALYSIS

Determination of the quantity of total suspended solids (TSS) and volatile suspended solids (VSS) found in the RAS and sludge samples collected was determined according to EPA Method 1684 (US EPA 2001). Glass microfiber filters were used over a vacuum pump to capture the solid portion of the RAS and the sludge. Prior to the addition of the samples, the filters were weighed. Following the weight of the filter being recorded, it was placed over the vacuum pump and 1.0mL of sludge and 5.0mL of RAS

were added to the filter. The pump was allowed to run for several minute until all of the water had been removed and only solids remained. After the addition of the samples, the filters with the sample were dried in the 103°C oven overnight to evaporate any remaining water. Then the samples were weighed again to determine to TSS concentration, with the calculation shown in equation (1 below. The samples were then placed into the 550°C furnace for twenty minutes and weighed again to determine the VSS concentration. The calculation is shown in equation  $\frac{Oven-Furnace}{Volume\ filtered} = VSS$  below. The samples were run in duplicate to provide several results for comparison.

$$\frac{Oven - Dry\ weight}{Volume\ filtered} = TSS \quad (1)$$

$$\frac{Oven - Furnace}{Volume\ filtered} = VSS \quad (2)$$

## 2.7 DESORPTION KINETICS STUDY

Desorption of adsorbed compounds was studied in batch bottles incubated at room temperature. 100µM of DAAN was added to 1L of wastewater and was given one week for adsorption to the biomass to occur. The large batch was used as a stock solution for the experiments. Samples were taken every two days over the course of a week to track the adsorption kinetics, and once complete adsorption occurred, the stock solution was extracted, centrifuged, and the water decanted only leaving the solids. These solids were taken, weighed, and added to clean 50mL falcon tubes. The goal was to place 1.0 g of solids in each of the tubes. These tubes were then filled with 50.0 mL of either a 60:40 water:acetonitrile mixture, Lake Hartwell water, 30mM Bicarbonate Buffer, or double

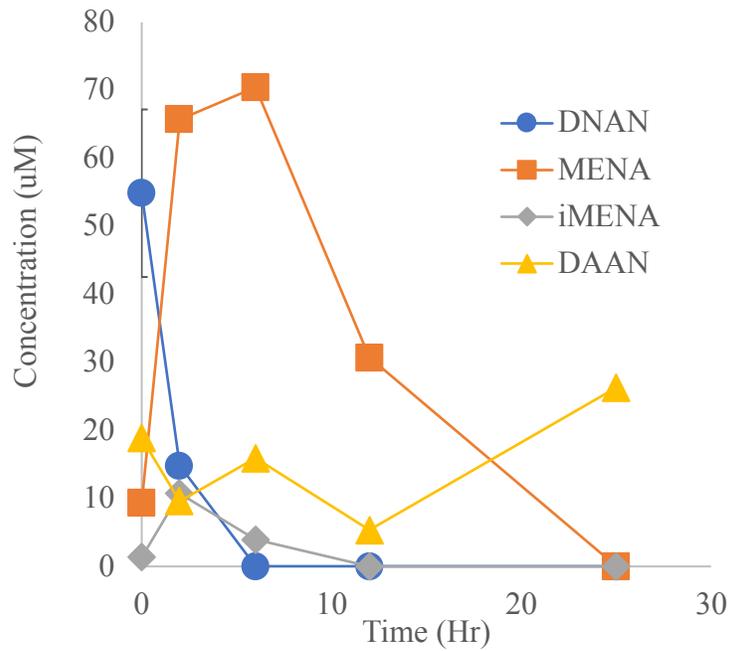
distilled water. The four buffers used were conducted in triplicate. These tubes were allowed to incubate over the course of the month and were turned periodically for limited stimulation.

An anoxic experiment was conducted with limited changes to what was described above. The anoxic study prepared the stock and the solids material to be suspended the same, except it was prepared within an anoxic glove bag to ensure no oxygen was present. The anoxic experiment also only used three solutions for the suspension; pure acetonitrile, 30mM Bicarbonate Buffer, and double distilled water. The duration of the experiment was also only 7 days as opposed to 30.

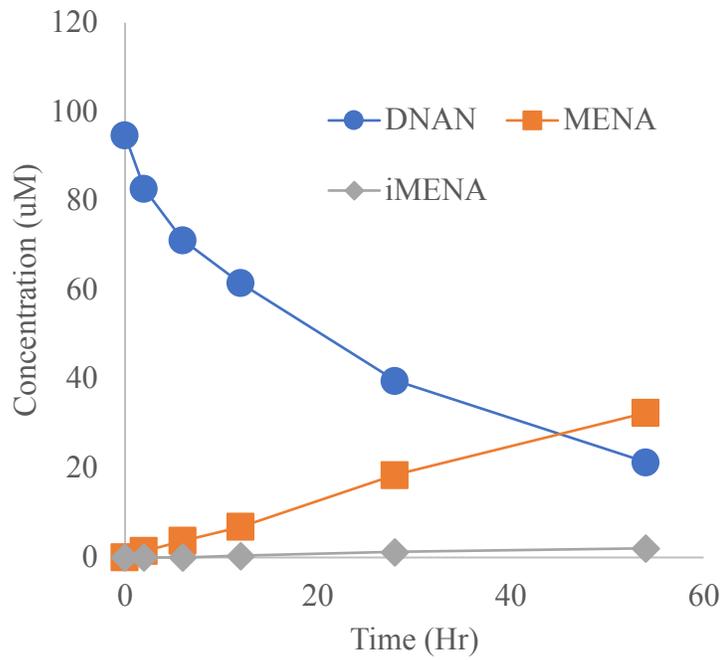
### **3. RESULTS AND DISCUSSIONS**

#### **3.1 DEGRADATION OF DNAN AND METABOLITE FORMATION**

Data from the experiments indicates that DNAN attenuation is more complex in an anoxic environment than in an oxic environment seen in Figure 3 and Figure 4 below.



**Figure 3. Anaerobic DNAN Degradation with metabolite formation in digester sludge.**



**Figure 4. Aerobic DNAN Degradation with metabolite formation in RAS**

In the anoxic environment, anaerobic degradation of DNAN achieved complete reduction of all DNAN within six hours (Figure 3). Subsequently any remaining metabolites were degraded to DAAN within 24 hours. The DAAN produced in the anoxic environment left a fraction in solution opposite the oxic environment. Aerobic degradation of DNAN is much slower than anaerobic. Aerobic degradation took 50 hours to remove approximately 80 percent of the initial concentration added (Figure 4). Aerobic degradation only half as much MENA produced overall compared to anoxic conditions. It was seen that MENA appeared to be the dominant intermediate pathway over iMENA in each environment. iMENA only appeared to be produced in limited quantities in both experiments, which is a more favorable pathway since iMENA is the more toxic intermediate (Anguay and Ield 2016). Limited amounts of iMENA were produced and seen in each experiment. In the oxic environment, approximately 25 $\mu$ M DAAN was produced and adsorbed to the biomass at rapid rates. Stoichiometric levels of DAAN were not produced in the oxic environment and this suggests there could be another possible pathway in degradation.

Additional studies were conducted which evaluated the effect of different electron acceptors on DNAN reduction. 10mM iron and nitrate were amended to batch studies to determine their effects on the degradation of DNAN. Iron and nitrate were found to have no effect on degradation of DNAN but were found to affect the metabolites. Refer to Appendix Figure 1 - Appendix Figure 12 for the relevant data. In the iron amended bottles, iron was found to have a limited effect on DNAN reduction. The rates were similar to those seen in the unamended study and there were no major inhibitions seen.

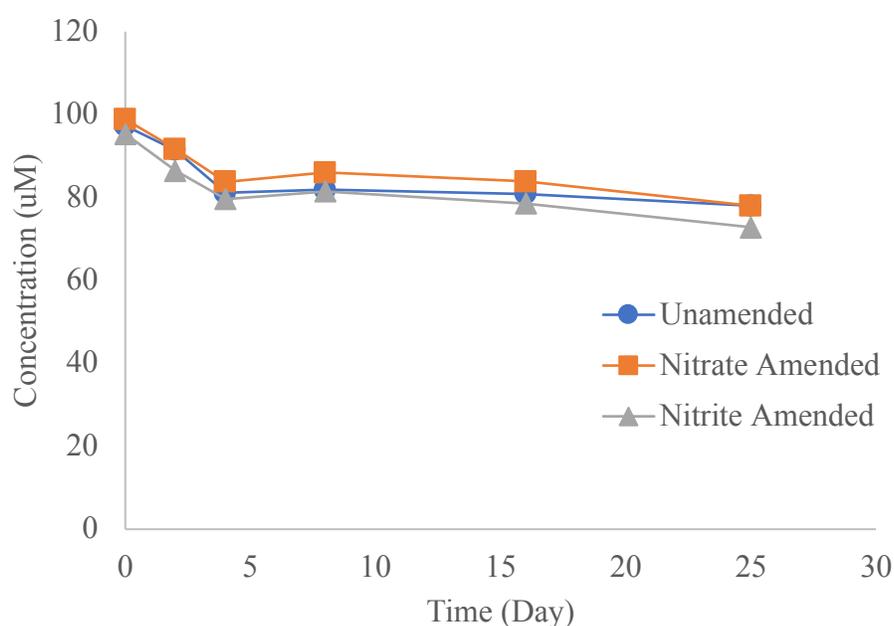
Comparing the unamended study with the nitrate amended showed a large inhibition of MENA degradation. In the oxic environment, normal reduction of DNAN to MENA was achieved, however there was inhibition up to 14 days when the nitrate is no longer detected and the reduction of MENA proceeds. The nitrate removal in the oxic bottle seems to precede MENA reduction. The initial amount of nitrate added is removed from solution by Day 7 and remains out for several sampling periods, which is when MENA reduction occurs. Then at Day 26, a large spike in the nitrate concentration appears following the removal of MENA. Refer to Appendix Figure 13 for the nitrate concentrations. The data appear to show that within an oxic environment, nitrate and MENA may be used for the same metabolic pathway, with nitrate being the preferential choice. Compared to the anoxic environment where MENA is rapidly produced and sees limited removal for the remainder of the experiment. The nitrate levels within the anoxic environment were never found to reach zero. The low levels of nitrate which remained in solution could be the reason why the MENA never underwent any further degradation. The data shows that nitrate appears to be inhibitory to the degradation of MENA in both environments.

From the data of these experiments, DNAN appears to be biologically degradable in both environments, with the rates favoring an anoxic environment. Several of the experiments revealed the metabolites may be more challenging to remove biologically so experiments were developed to test this theory.

## 3.2 DEGRADATION OF METABOLITES

### 3.2.1 MENA in an Oxic Environment

Several experiments were conducted beginning with MENA since it appears to be the dominant degradation pathway. The first experiment tracked MENA degradation within an oxic environment spiked with nitrate and nitrite as electron acceptors, seen in Figure 5 below.

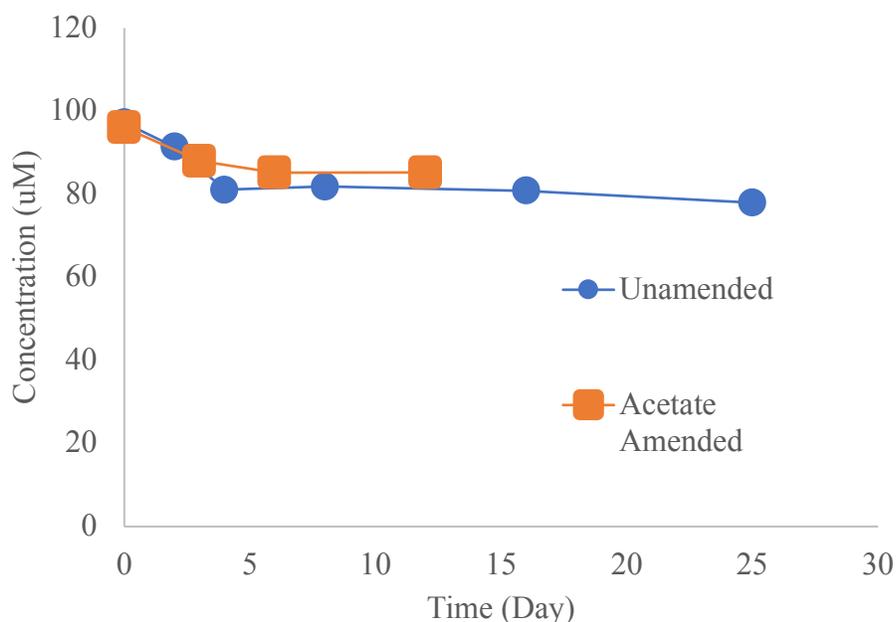


**Figure 5. Oxic RAS amended with 100 $\mu$ M MENA**

The data indicates that no degradation of MENA occurred in any of the batches. Neither electron acceptor had any effect on the degradation of MENA since all studies showed no degradation. The nitrite was very reactive and did not remain in solution long enough to cause any effect, while nitrate lingered in solution and was never removed. Neither had an effect on the adsorption kinetics, since all three batches followed similar

trends. All MENA removed from solution was recovered in the adsorption analysis. When beginning with MENA, no degradation occurs, which is different from what was seen in the DNAN experiments. When the batch studies begin with DNAN, degradation down to DAAN occurs in the oxic environment. Nitrate was found to inhibit this but once the nitrate was removed, degradation proceeded. There are two possible explanations for this; the first is that the microbial community requires DNAN to be present to activate specific pathways needed to complete aerobic degradation, or the dominant aerobic pathway for DNAN degradation down to DAAN is through iMENA, and not through MENA. Both are possible pathways which will require further investigation to determine which is the most probable pathway.

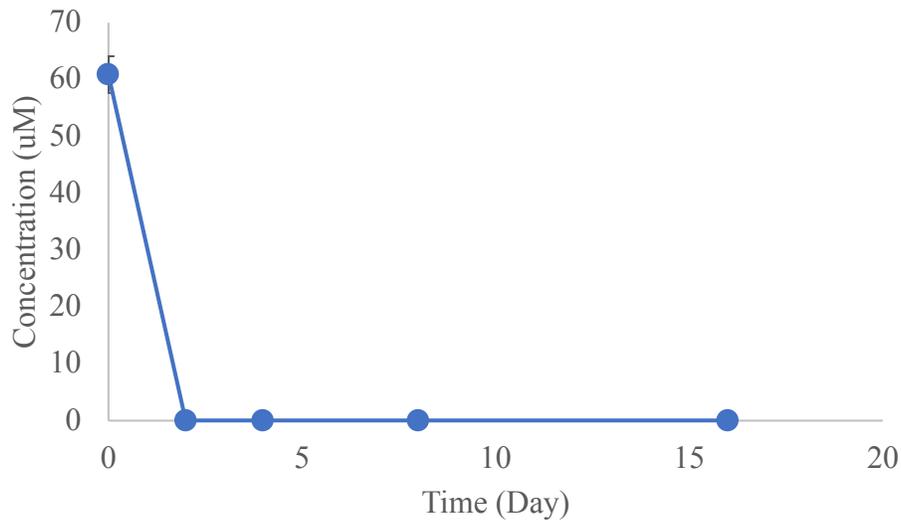
After no degradation was seen in the oxic environment beginning with MENA, experiments were run amended with acetate to see if any microbial activity could be stimulated. Similar results to all the other oxic MENA experiments were seen with no degradation of MENA within the batch study occurring. The only removal seen was through adsorption. Microbial activity was not able to be stimulated and achieve MENA degradation. Following these results, it does not appear that an oxic environment will provide reliable degradation of MENA. The results are presented in Figure 6 below.



**Figure 6. Oxic RAS beginning with 100 $\mu$ M MENA and 10mM Acetate.**

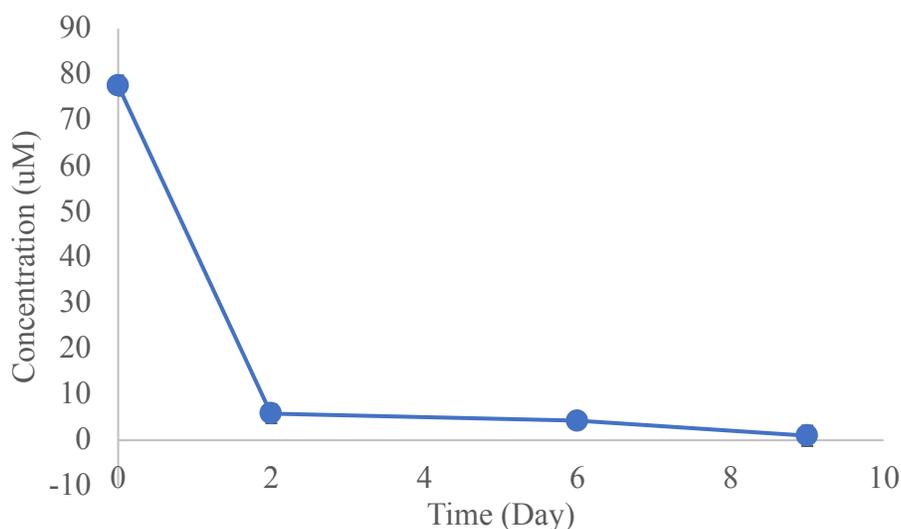
### 3.2.2 DAAN in an Oxic Environment

Since degradation of DNAN to DAAN was seen in an oxic environment in the original experiments, experiments were conducted which began with DAAN as the only metabolite added to investigate the fate and transport of it within the oxic environment. The data from the experiments demonstrated complete removal of the aqueous DAAN, but all through adsorption. The rapid rates at which DAAN is adsorbed to the biomass can be seen in the zero timepoint on Figure 7 below. The zero timepoint was taken at 10 minutes after the initial spike with DAAN and subsequently 40 percent of the initial mass added had already adsorbed to the biomass. All of the DAAN was recovered at the end of the experiment, suggesting DAAN which is adsorbed to the biomass is not biodegradable or is not bioavailable for any further degradation by the microbial community.



**Figure 7. Oxic RAS spiked with 100 $\mu$ M DAAN**

Due to the rapid rates of adsorption seen to RAS in the oxic experiments, experiments were conducted which used digester sludge in an oxic environment. The hope of this experiment was to stimulate microbial activity in the sludge with the addition of oxygen and achieve degradation of DAAN in the solution. The results are presented in Figure 8 below. During this experiment, no degradation of DAAN was achieved. The oxic sludge did not work as intended and the only removal was through adsorption with all DAAN recovered at the end of the experiment, like all other experiments with DAAN. The adsorption kinetics in sludge are not as rapid as what is seen in the RAS, so the removal is able to be better tracked over time.

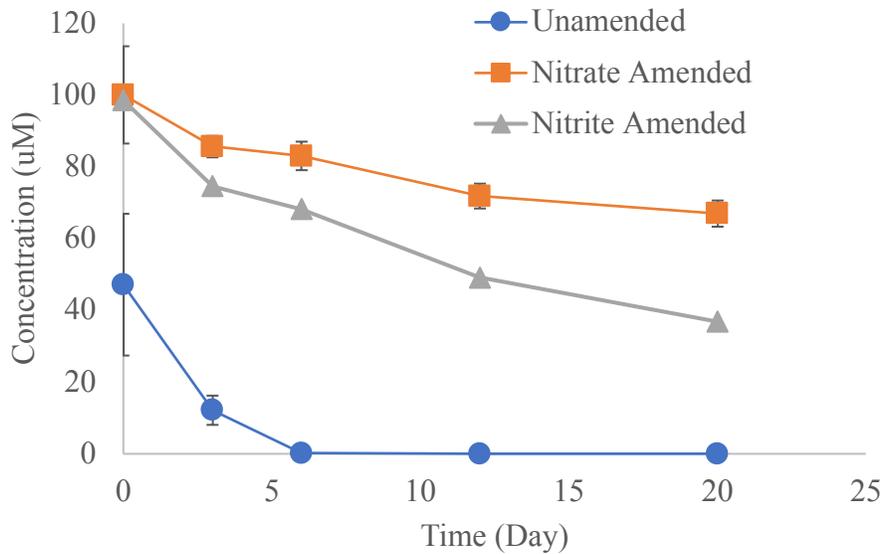


**Figure 8. Aerobic Sludge spiked with DAAN**

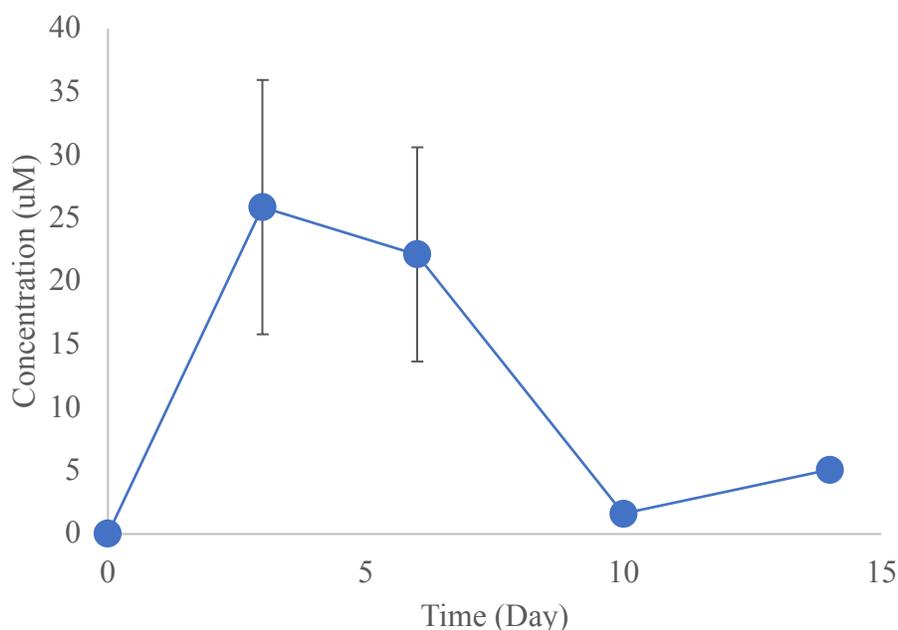
### 3.2.3 MENA in an Anoxic Environment

Experiments were conducted with MENA in the anoxic environment to determine the metabolites degradability missing the initial step in the reduction chain following the results seen in the oxic environment. The results are presented in Figure 9 below. The unamended bottle shows that MENA degradation occurs in the absence of electron donors. The MENA added to the bottle is transformed into DAAN, shown in Figure 10 below, and this DAAN is then subsequently removed from solution through adsorption. Stoichiometric levels of DAAN were recovered at the end of the experiment through adsorption analysis. When MENA was added to a sludge solution amended with nitrate, no degradation of MENA occurred. The results were similar to what was seen in the DNAN degradation experiments amended with nitrate. Nitrate appears to inhibit degradation of MENA. The nitrate remained in solution for the entire experiment, so it is unknown if nitrate disappears if MENA degradation will proceed, with the nitrate

concentrations presented in Appendix Figure 14. The only removal of MENA in the nitrate amended bottles is from adsorption, but this is not the dominant pathway and removes limited amounts. The nitrite amended batch study has partially inhibited the degradation of MENA down to DAAN. Small amounts of DAAN were recovered at the end of the experiment which were adsorbed to the biomass, but none was ever seen in solution. The adsorption kinetics appear to be faster than the rate at which MENA was being reduced to DAAN. Another interesting point about the nitrite amended batch study is no nitrite was ever detected after the initial measurements. Nitrite is a very reactive compound and appears to have affected the biomass in a way in which it decreased the rate of reduction of MENA.



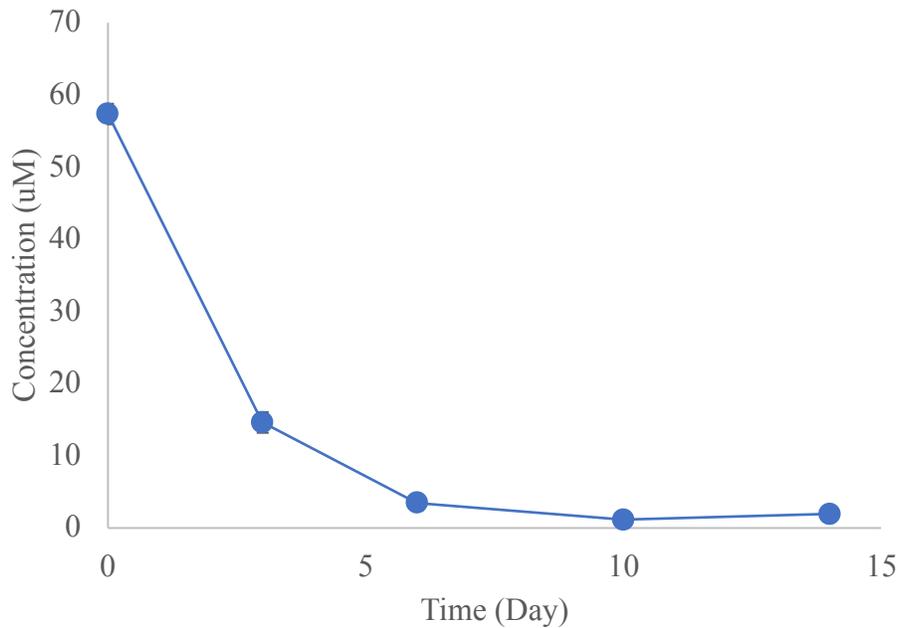
**Figure 9. Digester Sludge with Nitrogen Headspace spiked with MENA**



**Figure 10. Digester Sludge with Nitrogen Headspace Aqueous DAAN Produced from unamended MENA Degradation**

#### 3.2.4 DAAN in an Anoxic Environment

After several iterations of experiments beginning DNAN and MENA, DAAN appears to be the terminal end product and was only removed through adsorption. As seen in Figure 11, the initial mass of DAAN added to the sludge slowly s to the biomass over the next 10 days and all of the DAAN added to the bottle was recovered in the adsorption analysis. The results of this batch study mimic what was seen in all the previous experiments in which DAAN was produced. Once DAAN is produced in solution, the only way for it to be removed is through adsorption. Knowing this however will allow for proper treatment strategies to be designed in the future.



**Figure 11. Digester Sludge with Nitrogen Headspace Aqueous DAAN Concentrations**

### 3.2.5 Oxidic RAS Desorption of DAAN

Biomass storage and handling is going to be the biggest issue in biological systems designed to treat DNAN and its metabolites. Since adsorption is the only form of removal seen with DAAN in every experiment conducted, investigations into whether or not desorption would be a concern were analyzed. Several studies were conducted from the same large batch of RAS with DAAN adsorbed to it, with the results presented in Appendix Figure 15 - Appendix Figure 18. Several issues were detected with this experiment. The major issue is there was not a high enough percentage of acetonitrile used in the control batch. The RAS was only added to a 40 percent solution of acetonitrile and this did not liberate enough of the DAAN to get an accurate reading of the true amount which could be liberated over time using a strong organic solvent. It

actually liberated the least amount over the duration of the experiment which made it a poor control. However, no other values were high enough to cause concern. Over the month duration of the experiment, only one data point over  $1\mu\text{M}$  was detected for any of the solutions. Since only one data point had a spike, it appears that adsorption to RAS appears to be more irreversible, meaning that once the DAAN is adsorbed to the biomass, it will not desorb and resuspend in solution. The bicarbonate buffer had a spike at Day 21 where approximately  $4\mu\text{M}$  DAAN was detected in solution. The spike could be caused by any number of reasons and further investigation into the cause would need to be done to determine a most probable reason. Since there is no maximum contaminant level set for DAAN yet, this is not cause for concern, however in the future this could present a problem if the biomass is exposed to high levels of bicarbonate for any reason.

### *3.2.6 Oxic Sludge Desorption of DAAN*

A similar experiment to what was mentioned above was conducted for sludge in an oxic environment. The sludge was amended with DAAN and allowed to adsorb over time, and then it was added to different solutions to track any desorption kinetics. The results are presented in Appendix Figure 19 - Appendix Figure 22. The results were slightly different than what was seen in the RAS. All of the solutions had noticeable amounts of DAAN resuspend in solution, with the highest amounts around  $12\mu\text{M}$  at Day 7 being from the lake water and bicarbonate buffer. By the end of the experiment however, only the control with acetonitrile had any DAAN remaining in solution, the rest had resorbed to the biomass in all the other batch studies. Since more was desorbed in all of the sludge studies than in the RAS, it can be assumed that the adsorption kinetics in the

sludge are more reversible than what was seen in the RAS. The rate of adsorption is important to note because DAAN appears to not have as high of an affinity for the sludge biomass than for the RAS. It does not adsorb as fast to the sludge and it is more easily desorbed from it. Which could cause problems in the future for a solids handling process.

There were also interesting results seen on the HPLC in this experiment. Several of the batch studies had peaks which were eluting within several seconds of the DAAN peak during analysis. Microbial activity should have been limited during this study, however there is the possibility of some still being active. Prior to this study, no peaks were ever seen around the DAAN peak. These results lead to the thought that there could be some possible microbial reactions occurring which are unknown and producing compounds which are similar to DAAN. Further analysis needs to be done to determine if there is any validity to this thought and any possible compounds which it might be.

### *3.2.7 Anoxic Sludge Desorption of DAAN*

The same desorption experiment was conducted under anoxic conditions with the data presented in Appendix Figure 23 - Appendix Figure 25. DAAN was added to a batch bottle and given time for approximately 75 $\mu$ M to adsorb to the biomass. The biomass with adsorbed DAAN was then placed in the glove bag and the experiment continued. The biomass was then added to similar solutions as the oxic experiment with a few exceptions. Pure acetonitrile was added instead of a mixture with water to provide a better control and the lake water was not used in this study since the surface of a lake will not see anoxic conditions. There were several main differences in the anoxic desorption study from the oxic study. The first was the pure acetonitrile desorbed eight times the

amount of the bicarbonate buffer and the double distilled water. These results are what was expected since the biomass was in a pure organic solvent and more of the DAAN should have been resuspended. In the other two, neither had more than 0.35 $\mu$ M release and return to solution in the experiment. These are both drastically different from what was seen in the oxic experiments. In the oxic experiments, a larger amount of DAAN was liberated and returned to solution suggesting a difference in the biomasses. These results indicate that the anoxic sludge has a higher affinity than the oxic sludge for DAAN and will therefore adsorb more efficiently to it. There should be a difference in the biomasses since the environments have shifted, but further research should be done to help understand the differences and the role they play in the desorption kinetics of DAAN.

### 3.3 WASTEWATER CHARACTERISTICS DATA

Following the adsorption of DAAN to the biomass that was seen during the experiments, a literature search was conducted to help understand why the adsorption may be occurring. In groundwater, nitroaromatics were found to more reversibly adsorb to organic material based on their partition coefficients (Hawari et al. 2015). Even the literature is referring to a groundwater paper, some similarities can be drawn to wastewater. In wastewater where the majority of the solids are organics, it makes more sense why the medium with the more concentrated organic portion has the slower adsorption kinetics. One explanation is there is more partitioning going on between the sludge and the compounds than in the RAS. The compounds interact with the complexes more and through this interaction, there are slower adsorption kinetics and more remains

in solution longer. Several different characteristics used to help determine the organic portion of the wastewater were determined and summarized in Table 3 below.

**Table 3. Wastewater Characteristics Data**

(mg/L)	RAS	Sludge
TOC	72.2	483.3
TON	68.4	967
TSS	4300	20000
VSS	3600	17000

#### **4. CONCLUSION**

In conclusion of the experiments, several trends about the degradation of DNAN and its metabolites could be drawn which give an overall idea of what could occur at a Water Resource Recovery Facility under native conditions. DNAN can be degraded within each environment within a bioreactor. The degradation of DNAN can produce several different metabolites, but ultimately ends with DAAN. The DAAN in the bioreactor subsequently adsorbs to the biomass and enters the clarifier. The adsorbed DAAN settles to the bottom and will end up in the solids handling process where it will pose a problem for certain applications of the treated sludge. Refer to Figure 12 below for a graphical representation of the path described above.

DNAN degradation could be achieved in each environment within a bioreactor, with the rates significantly favoring the anoxic environment. The anoxic environment had only DAAN remaining after 24 hours of study while the oxic environment had 20 percent of the DNAN left at 50 hours. The dominant pathway for degradation down to DAAN

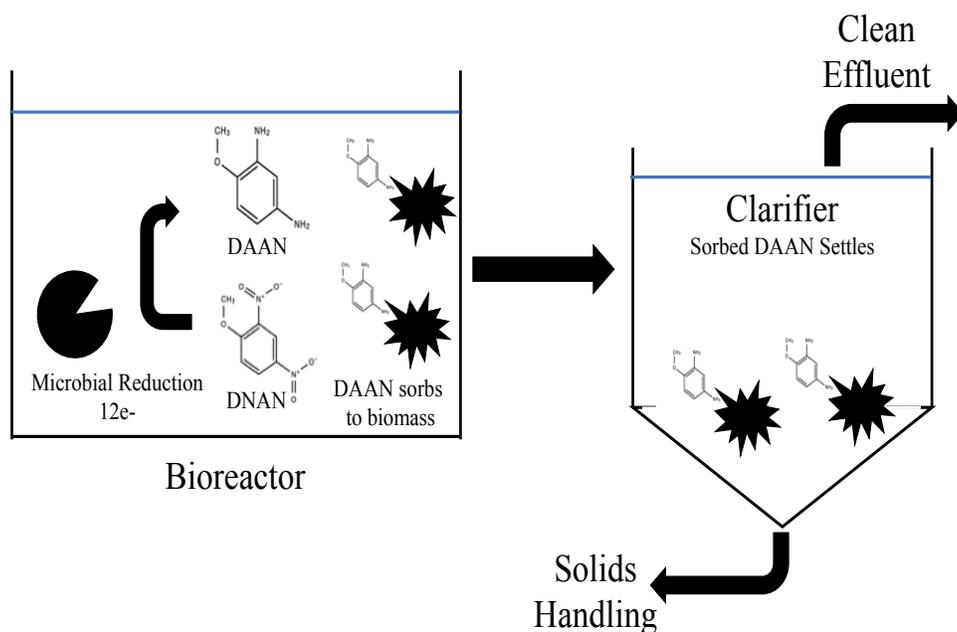
seen in these experiments appears to be through MENA, however limited amounts of iMENA were seen and need to be monitored for. The studies which began with DNAN showed no affects from any electron donors added to the system. These did not change the degradation seen within the experiments.

Since MENA appeared to be the dominant pathway for degradation of DNAN down to DAAN, experiments were conducted which began with MENA. The oxic experiments conducted showed no further degradation of MENA if it was the initial compound added. The only removal that was seen was from adsorption. Even with the addition of acetate, no degradation occurred. If an oxic treatment strategy were used, there could be a problem with the degradation of MENA and there could be a buildup of this compound. As this compound builds up in solution, it could also inhibit microbial activity. When comparing this to the anoxic environment, MENA was degraded to DAAN in an unamended tank. Nitrate was found to inhibit the degradation of MENA to DAAN, so if there is a possibility of nitrification occurring in the tank as well as looking to degrade DNAN down to DAAN, there is the possibility for inhibition.

Since DAAN was produced in both experiments that began with DNAN, experiments with DAAN were conducted in each environment. adsorption was the only means of removal seen in either environment. The oxic environment had more complete removal of the DAAN with adsorption rates favoring the oxic environment. The anoxic environment had DAAN left in solution but would eventually adsorb to the biomass if given proper time. Desorption does not appear to be a large concern for the adsorbed DAAN. The oxic sludge had the most desorb but was still less than 10 percent of the total

added to the solution, so it appears to not be a large concern. Both present unique challenges to the treatment strategies, since DAAN appears to be the terminal end product. The DAAN in solution requires further forms of treatment for the water to ensure that all the compound is removed, but the adsorbed material causes a problem for the solids handling.

Water Resource Recovery Facilities that are treating a large quantity of DNAN run the risk of handling a large portion of their solids which have DAAN adsorbed to them and this will require proper disposal. Landfilling is one of the more common methods of disposal for solids treatment at the moment, but as landfills begin to reach capacities and other strategies are needed for treatment of solids, this will need to be considered. Treating the solids for land application is a sustainable method for a way to treat the solids and dispose of them in a way that does not fill up the landfills but will not be an acceptable way of treatment if there is a large amount of DAAN adsorbed to the biomass.



**Figure 12. General overview DNAN may take in a typical treatment system**

## 5. RECOMMENDED FUTURE WORK

Several unknown trends were seen throughout the course of these experiments and have led themselves to future work on this topic. The unknown trends seen in the experiments were DAAN susceptibility to microbial oxidation, advanced oxidation process use, and alternative pathways of degradation of the compound. Experiments will need to be designed and conducted which will test the validity of each of these trends.

The first is if DAAN adsorbed to the biomass will ever become bioavailable. If experiments are given enough time, and the right microbial stimulation is provided there is the possibility of DAAN being able to biodegrade. All current knowledge of the compound is that it is the terminal end product, but more work should be done to see if this is true in an engineered system.

To test this hypothesis, several of these batch studies are already in progress, as was mentioned above. There has been no degradation seen yet in the studies, only removal through adsorption. However, as the sites fill up and the adsorption kinetics decrease due to unavailable sites, the DAAN in solution may be more susceptible to microbial degradation. Testing this theory will take a long time to fully understand. The bottles will need to be monitored for a long time to allow for a full understanding to be developed. Periodic quantification of the electron acceptors and the DAAN levels will need to be collected to allow for an understanding of what is occurring within the batch bottles. Microbial degradation may not be the best choice for oxidation of the amino compound, but several other wastewater processes may be a better choice.

The second topic is if DAAN can undergo any form of chemical oxidation and be turned into harmless end products. Research has shown that it can be done within groundwater systems using mineral oxides. Birnessite and ferrihydrite were seen to oxidize DAAN into harmless end products in the experiments conducted (Khawwaja et al. 2018). These results show promise that the compound may be susceptible to treatment. The question is if this can be recreated in a wastewater system. Several options exist for the oxidation treatment of chemicals. Chlorine is the most popular oxidant used for disinfection and if this is able to oxidize the compound, it would be an easy fix since this is a popular system in the world. If the chlorine is not a strong enough oxidant, then an advanced oxidation process would be needed, such as an ozone peroxide system or a Fenton's reaction. Since the DAAN is not typically in solution after it leaves a bioreactor, these experiments should be run with the DAAN adsorbed to the biomass. If it is possible

to treat the DAAN that is adsorbed to harmless end products, then the material leaving the advanced oxidation process could be further treated and have limited environmental effects.

Working with these advanced oxidation processes sometimes requires the adjustment of the pH of the solution. Several experiments should be conducted to determine if pH adjustment has any effect on DAAN and its reactions within the system. All of experiments discussed in the results section used the circumneutral pH of the native wastewater conditions. Since the goal was to determine the biodegradation potential of DNAN and each of its metabolites, pH adjustment was never considered for any of the experiments. The adjustment of pH poses several problems to study though. The first is it could change the adsorption kinetics surrounding DAAN and the biomass in the system. It could liberate more of the compound or force more to adsorb. Understanding this will be needed prior to the addition to an advanced oxidation process. Evaluation of the interactions of DAAN when the pH is raised back to circumneutral following the process should also be done. Since no process removes all of the contaminant of interest, some DAAN will remain in the solution so understanding where this is following the process will be needed.

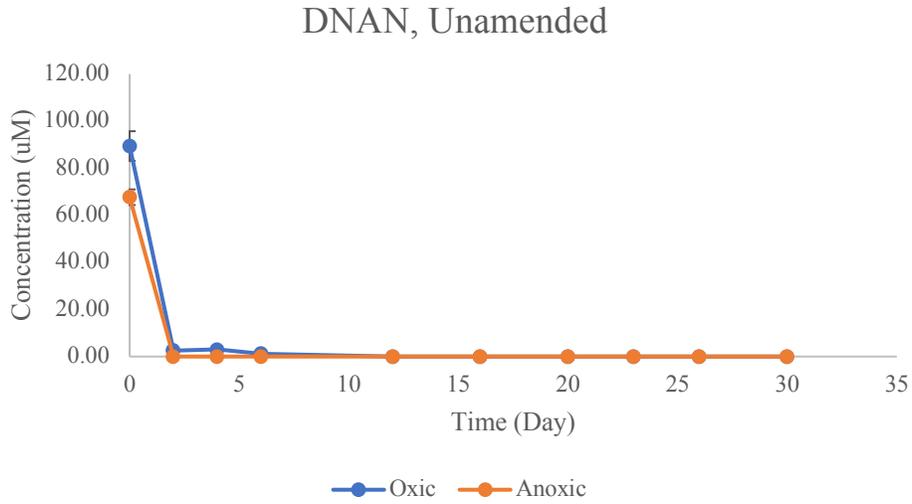
Working with these oxidants however will pose problems for quantifying the DAAN in solution using the HPLC method mentioned above. Several of these experiments were attempted, however poor results were obtained since the column used is not designed for use with these heavy oxidants being used. Finding a column which is resistant to oxidation will be one challenge surrounding this experiment. DAAN is the

hardest of the four compounds to detect, it elutes quickly and does not leave much room for error. Therefore, finding a column which can do both will be extremely difficult. Also, finding a way to quench the oxidants prior to analysis should be used. Quenching was not used in these experiments since only stoichiometric levels of the oxidants were used. It was thought that adding 1:1 ratio of each would eliminate the need for this, and it did not. The quenching will not be the most important concern with this experiment however. Ensuring the column is optimized for use with these oxidants will.

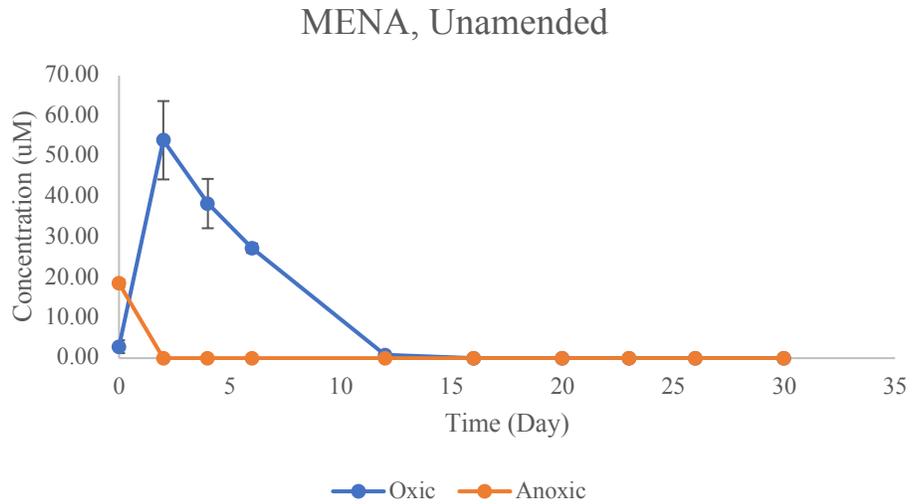
The final topic which needs to be investigated further is if DAAN undergoes any transformation within the wastewater solutions. Studies in groundwater systems have said that DAAN will form several different dimers (Hawari et al. 2015). Also studies have demonstrated that under oxic conditions DNAN can undergo transformation into a phenolic compound (Fida, T., Palamuru, S., Pandey, G., and Spain 2014). Investigations into whether or not there was alternative pathways of degradation arose from some problems seen on the HPLC with peaks much higher than expected and peaks eluting within several seconds of DAAN during the oxic experiments. Studies were done with 2,4 – Diaminophenol (Amidol) to investigate the possibility of a phenolic degradation of DAAN. More research should be done on this topic because the elution times for DAAN and Amidol are within 10 – 30 seconds of each other on the HPLC with the current method. There is a possibility of a coelution of the two compounds so investigations into where or not this is an actual pathway of degradation should be undertaken and considered.

## **APPENDIX**

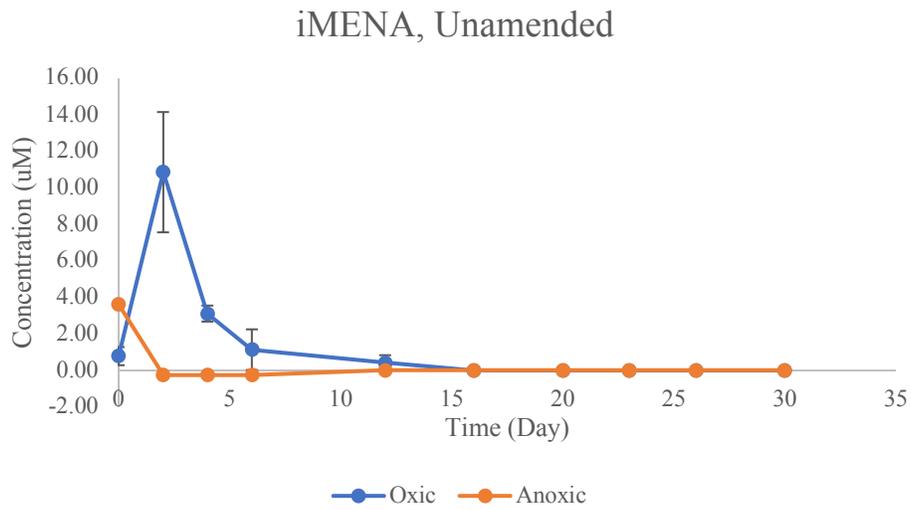
## APPENDIX



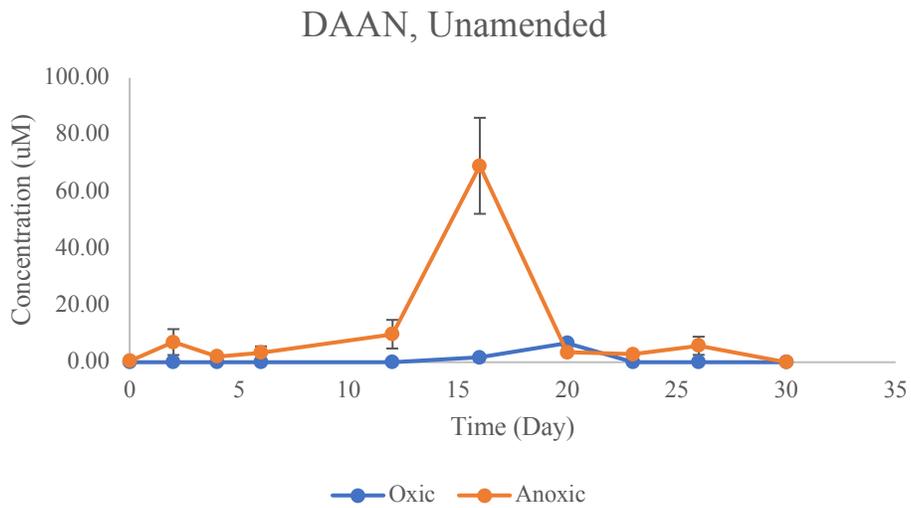
**Appendix Figure 1. Unamended Batch DNAN Degradation**



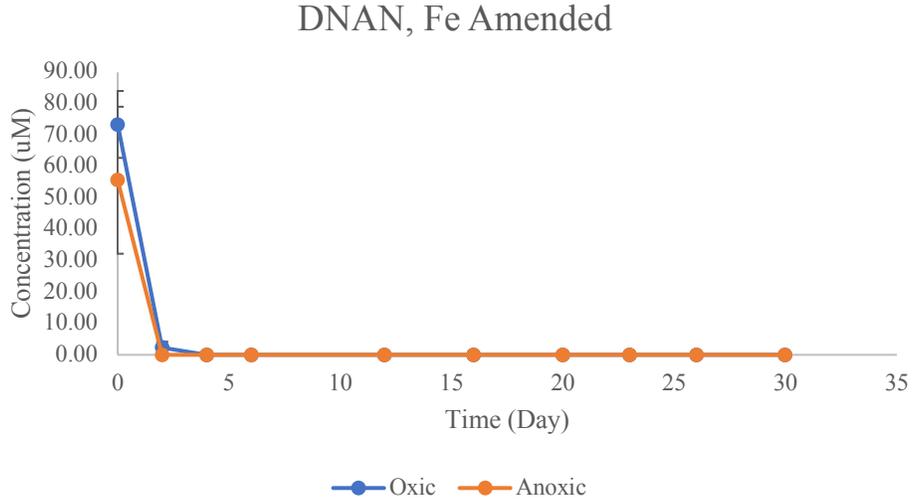
**Appendix Figure 2. Unamended Batch MENA Production from DNAN Degradation**



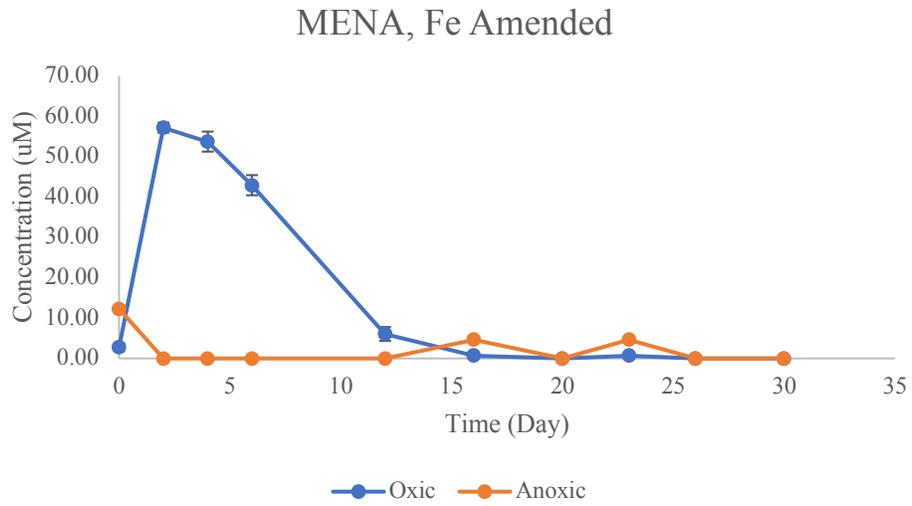
**Appendix Figure 3. Unamended Batch iMENA Production from DNAN Degradation**



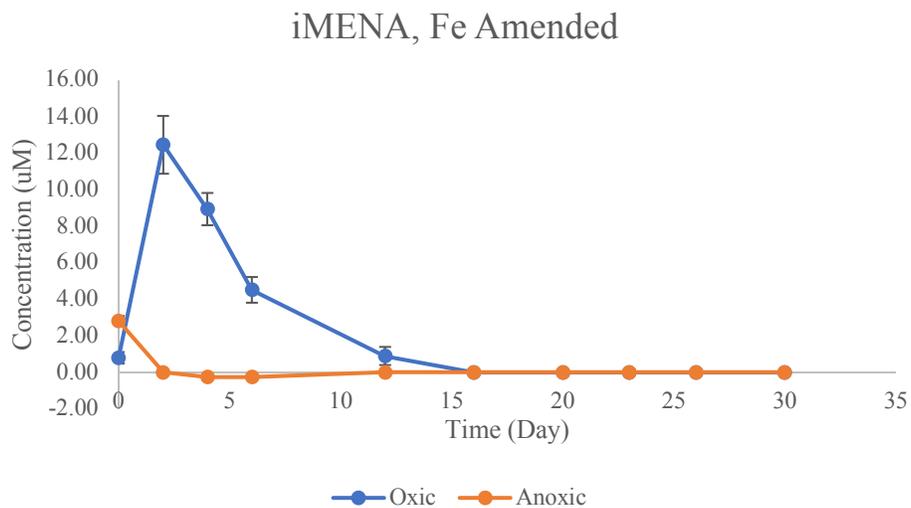
**Appendix Figure 4. Unamended Batch DAAN Production from DNAN Degradation**



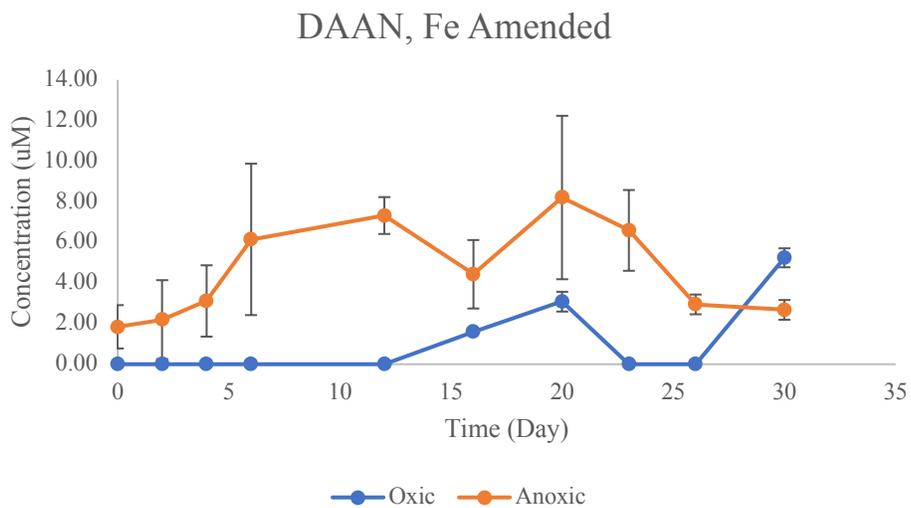
**Appendix Figure 5. Iron Amended Batch DNAN Degradation**



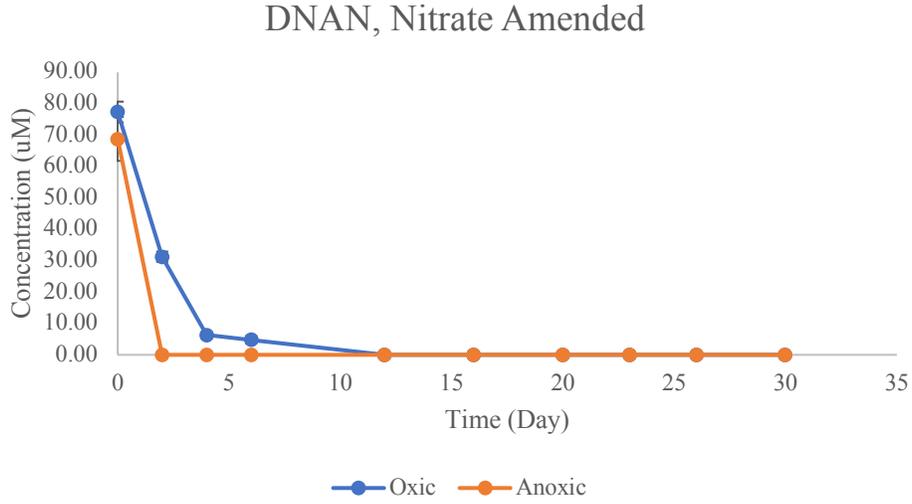
**Appendix Figure 6. Iron Amended Batch MENA Production from DNAN Degradation**



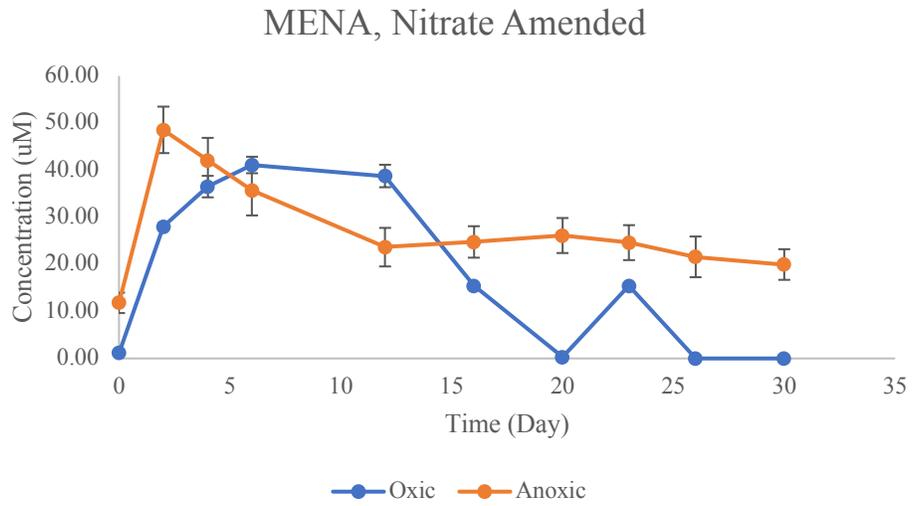
**Appendix Figure 7. Iron Amended Batch iMENA Production from DNAN Degradation**



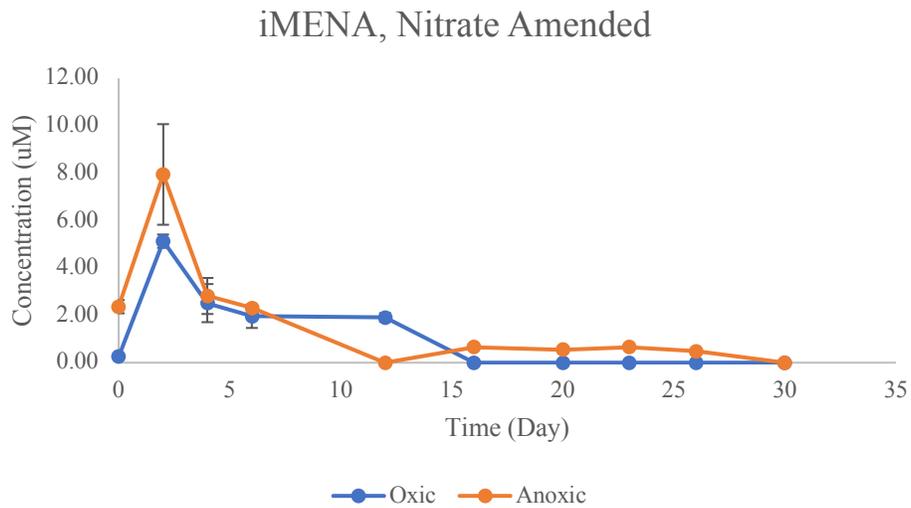
**Appendix Figure 8. Iron Amended Batch DAAN Production from DNAN Degradation**



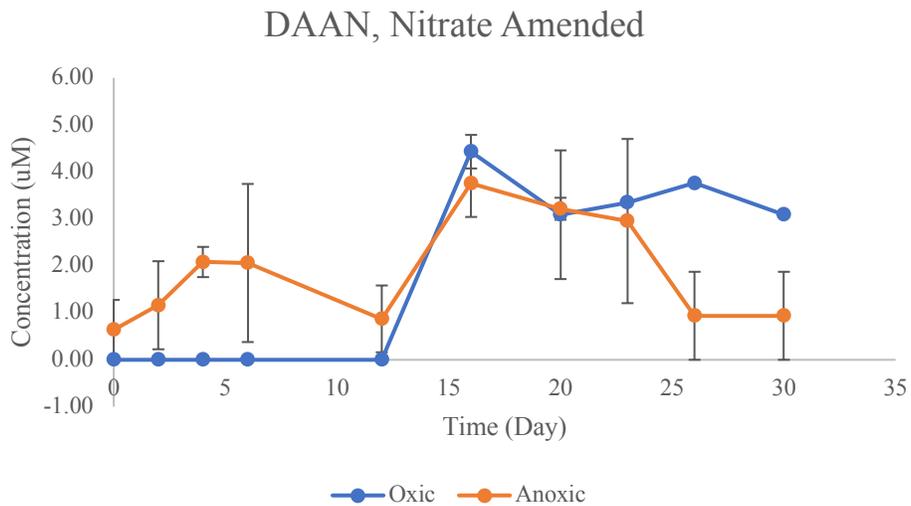
**Appendix Figure 9. Nitrate Amended Batch DNAN Degradation**



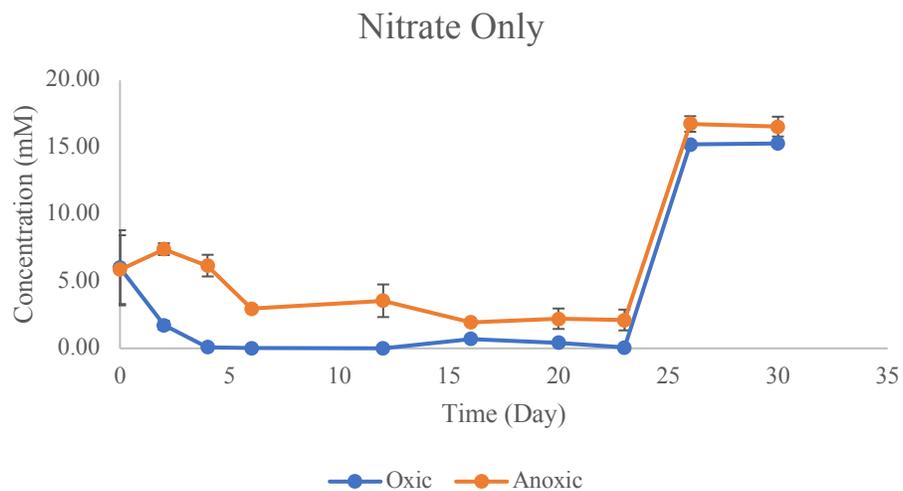
**Appendix Figure 10. Nitrate Amended Batch MENA Production from DNAN Degradation**



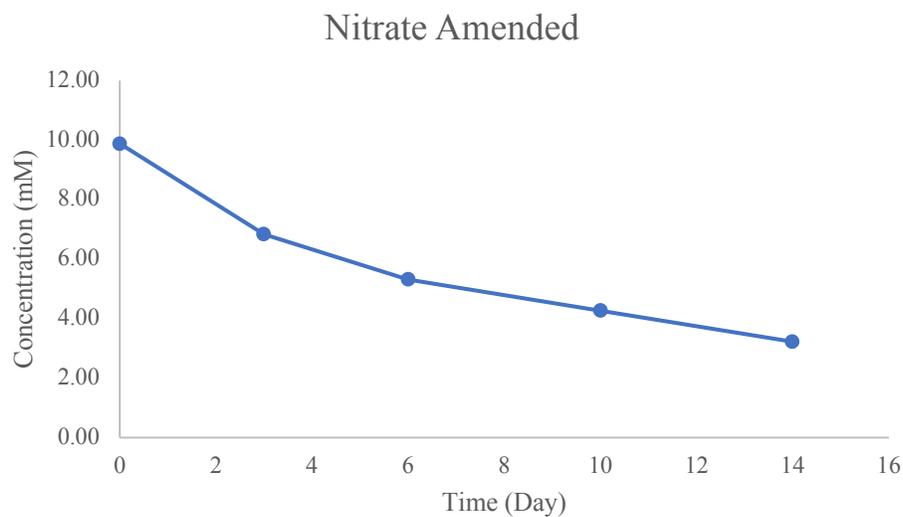
**Appendix Figure 11. Nitrate Amended Batch iMENA Production from DNAN Degradation**



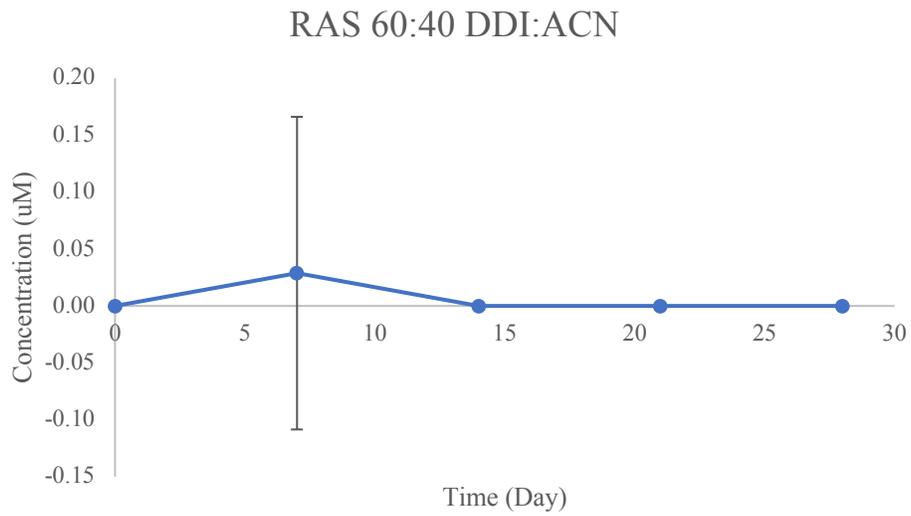
**Appendix Figure 12. Nitrate Amended Batch DAAN Production from DNAN Degradation**



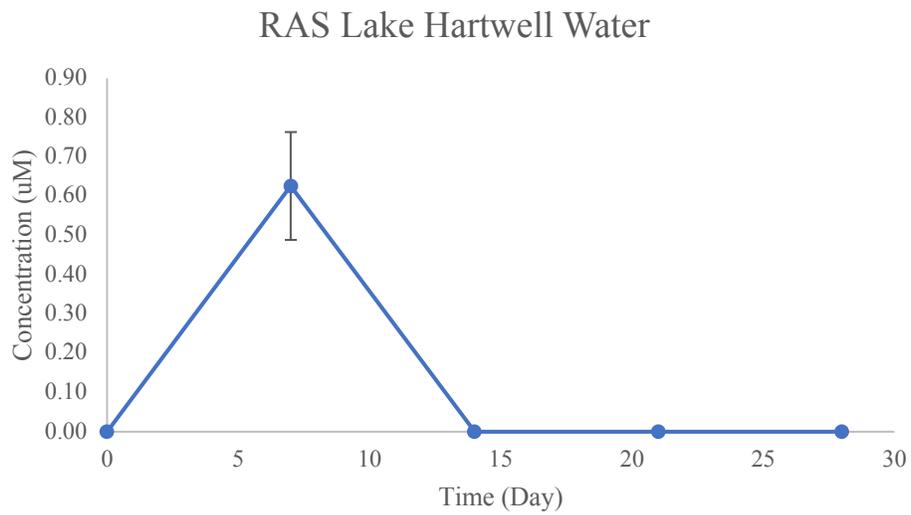
**Appendix Figure 13. Nitrate Amended Batch Aqueous Nitrate Concentrations**



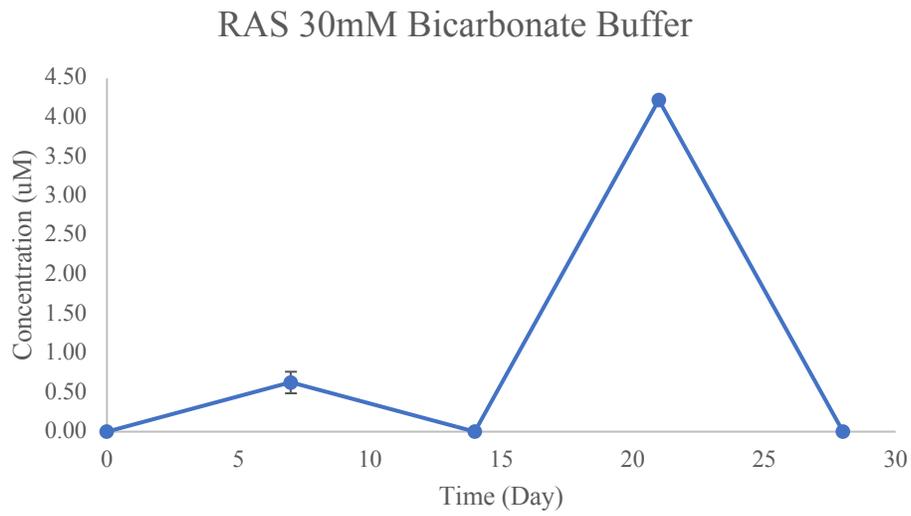
**Appendix Figure 14. Nitrate Concentrations from Anoxic MENA Amended Study**



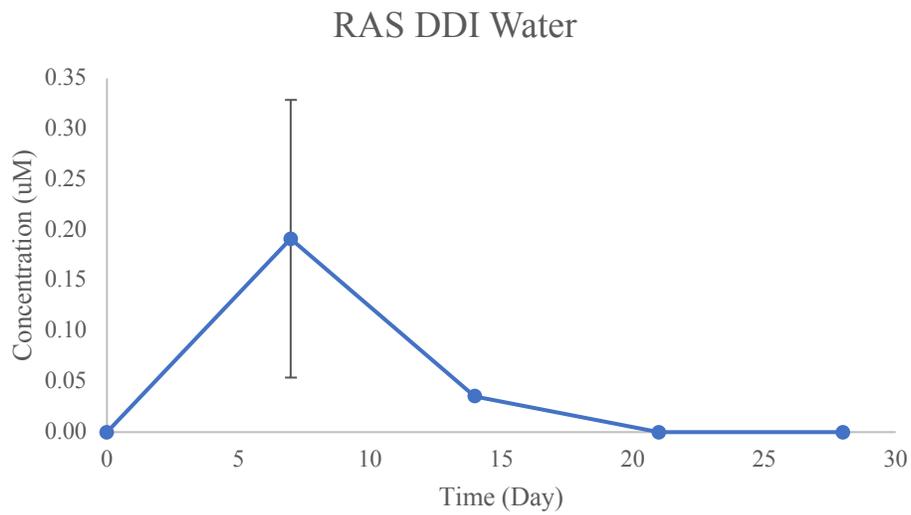
**Appendix Figure 15. RAS Oxidation Desorption DAAN Concentrations in 40 percent Acetonitrile**



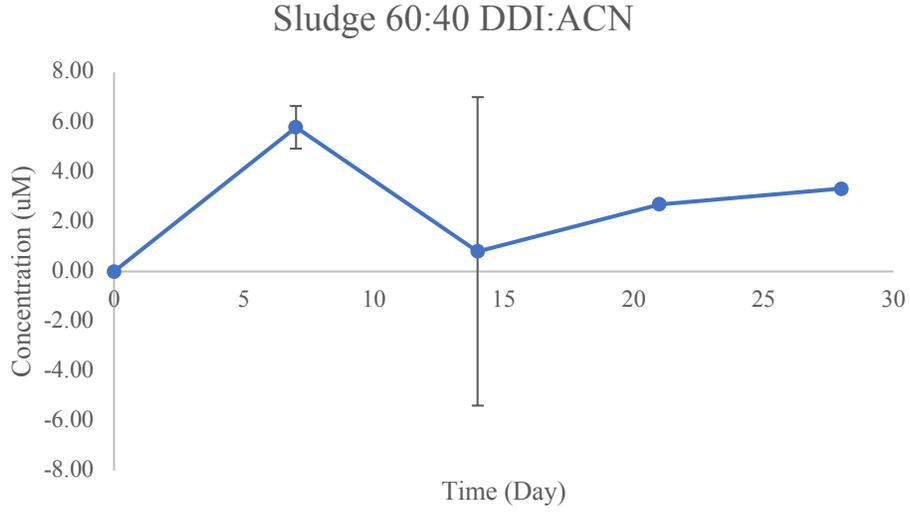
**Appendix Figure 16. RAS Oxidation Desorption DAAN Concentrations in Lake Water**



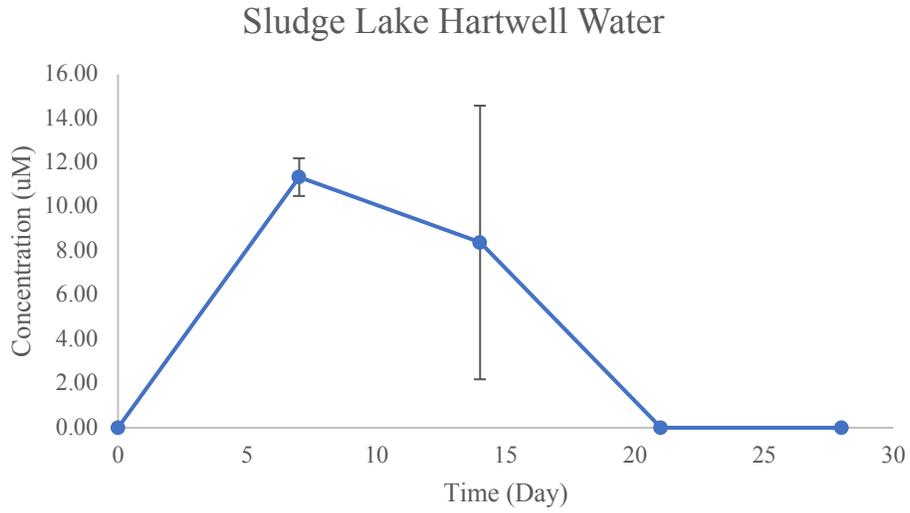
**Appendix Figure 17. RAS Oxidation DAAN Concentrations in Bicarbonate Buffer**



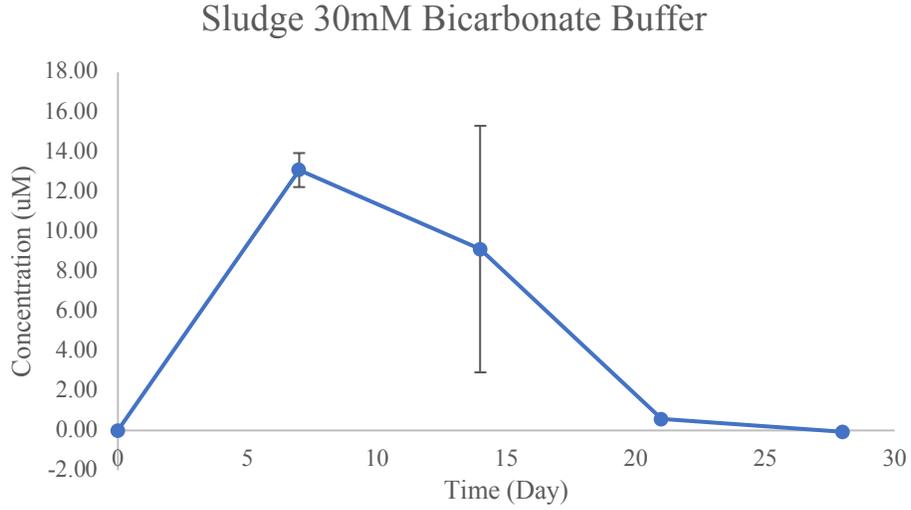
**Appendix Figure 18. RAS Oxidation DAAN Concentrations in DDI Water**



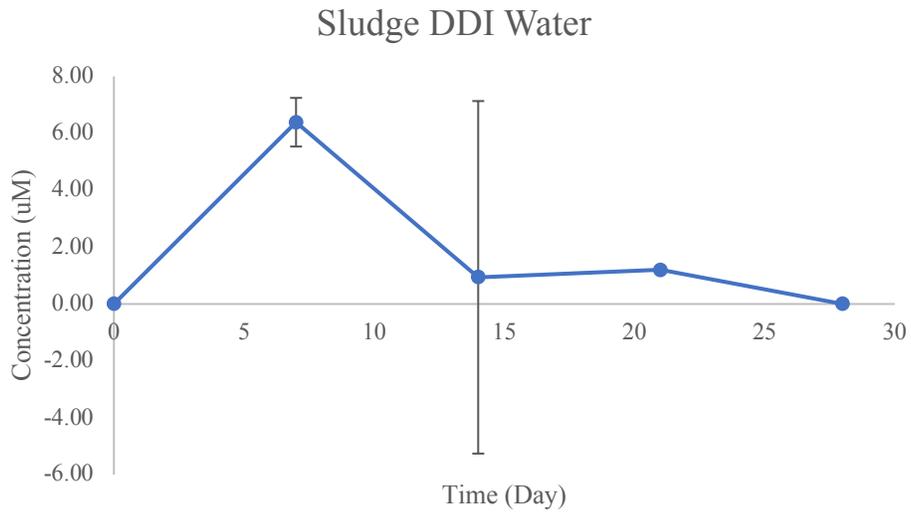
**Appendix Figure 19. Sludge Oxidation Desorption DAAN Concentrations 40 percent Acetonitrile**



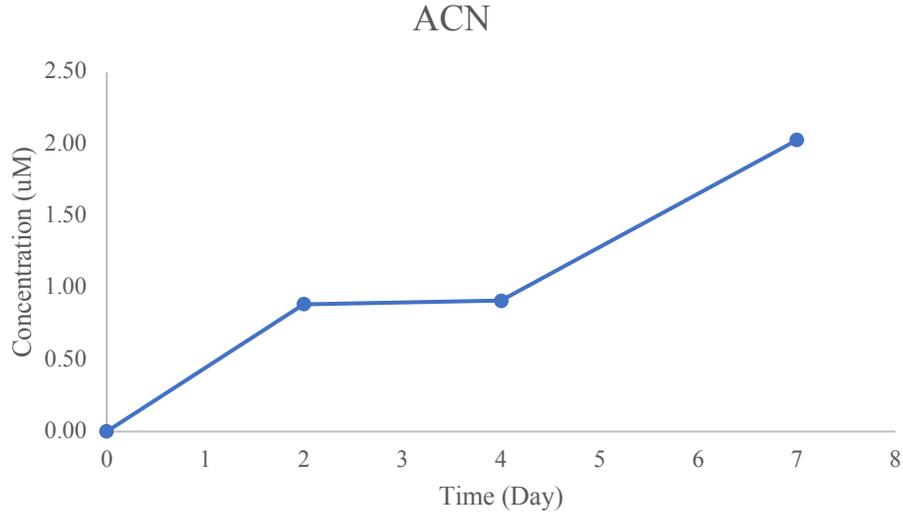
**Appendix Figure 20. Sludge Oxidation Desorption DAAN Concentrations Lake Water**



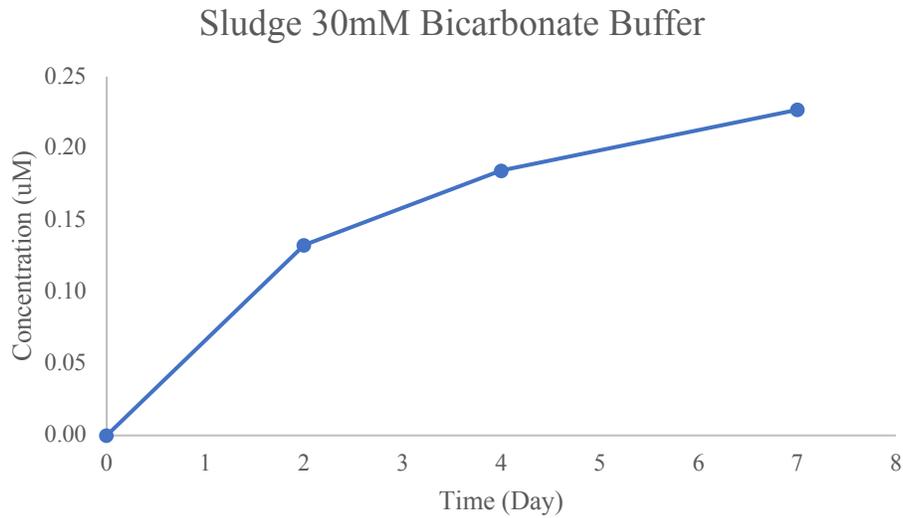
**Appendix Figure 21. Sludge Oxidation Desorption Ammonia Concentrations Bicarbonate Buffer**



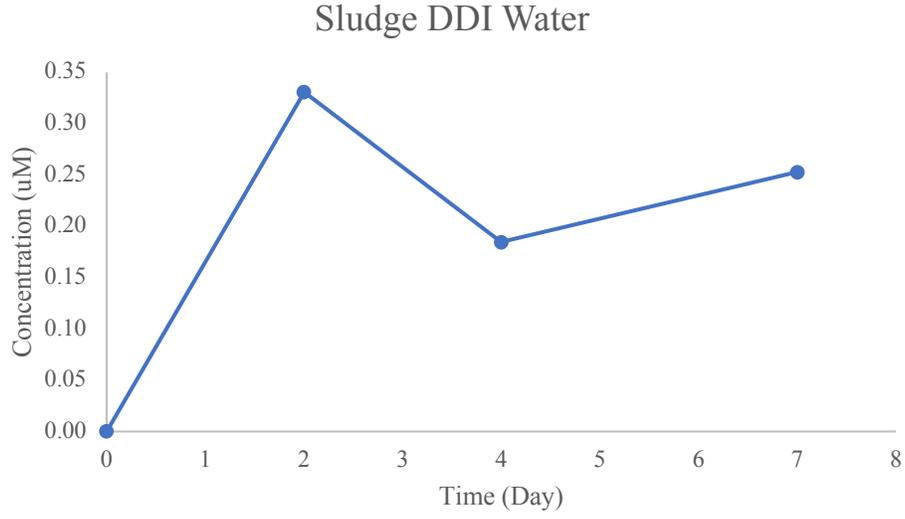
**Appendix Figure 22. Sludge Oxidation Desorption Ammonia Concentrations DDI Water**



**Appendix Figure 23. Sludge Anoxic Desorption DAAN Concentration Pure Acetonitrile**



**Appendix Figure 24. Sludge Anoxic Desorption DAAN Concentration Bicarbonate Buffer**



**Appendix Figure 25. Sludge Anoxic Desorption DAAN Concentration DDI Water**

## REFERENCES

- Alsop, Derek, Tania Y.T. Ng, M. Jasim Chowdhury, and Chris M. Wood. 2016. "Interactions of Waterborne and Dietborne Pb in Rainbow Trout, *Oncorhynchus Mykiss*: Bioaccumulation, Physiological Responses, and Chronic Toxicity." *Aquatic Toxicology* 177. Elsevier B.V.: 343–54. <https://doi.org/10.1016/j.aquatox.2016.06.007>.
- Anguay, R Obert L T, and J I M A F Ield. 2016. "ZEBRAFISH EMBRYO TOXICITY OF ANAEROBIC BIOTRANSFORMATION PRODUCTS FROM THE INSENSITIVE MUNITIONS COMPOUND 2, 4-DINITROANISOLE C HRISTOPHER I. O LIVARES, y R EYES S IERRA -A LVAREZ, y L EIF A BRELL, Zx J ON C HOROVER, z M ICHAE L S IMONICH, K" 35 (11): 2774–81. <https://doi.org/10.1002/etc.3446>.
- Christopher Olivares, Jidong Liang, Leif Abrell, Reyes Sierra-Alvarez, Jim A. Field. 2013. "Pathways of Reductive 2,4-Dinitroanisole (DNAN) Biotransformation in Sludge." *Biotechnology and Bioengineering* 110 (6): 1595–1604.
- Ersan, Mahmut S, Chao Liu, Gary Amy, and Tanju Karan. 2019. "Science of the Total Environment The Interplay between Natural Organic Matter and Bromide on Bromine Substitution" 646: 1172–81. <https://doi.org/10.1016/j.scitotenv.2018.07.384>.
- Fida, T., Palamuru, S., Pandey, G., and Spain, J. 2014. "Aerobic Biodegradation of 2,4-Dinitroanisole By" 80 (24): 7725–31. <https://doi.org/10.1128/AEM.02752-14>.
- Fung, V, and Newland S. 2015. "A Modernized IM Melt Pour Explosive Manufacturing Facility at Holston Army Ammunition Plant." *Insensitive Munitions & Energetic Materials Technology Symposium (IMEMTS)*, 1–10. <https://imemg.org/wp-content/uploads/2015/06/3B3-17261-A-modernized-IM-Melt-Pour-Explosive-Manufacturing-Facility-at-HSAAP.pdf>.
- Hawari, J, F Monteil-rivera, N N Perreault, A Halasz, L Paquet, and Z Radovic-hrapovic. 2015. "Chemosphere Environmental Fate of 2, 4-Dinitroanisole ( DNAN ) and Its Reduced Products" 119. Elsevier Ltd: 16–23. <https://doi.org/10.1016/j.chemosphere.2014.05.047>.
- Karthikeyan, Smruthi, and Jim C. Spain. 2016. "Biodegradation of 2,4-Dinitroanisole (DNAN) by *Nocardioides* Sp. JS1661 in Water, Soil and Bioreactors." *Journal of Hazardous Materials* 312. Elsevier B.V.: 37–44. <https://doi.org/10.1016/j.jhazmat.2016.03.029>.
- Khawiwada, Raju, Christopher Olivares, Leif Abrell, Robert A Root, Reyes Sierra-alvarez, James A Field, and Jon Chorover. 2018. "Chemosphere Oxidation of Reduced Daughter Products from 2, 4-Dinitroanisole ( DNAN ) by Mn ( IV ) and Fe ( III ) Oxides." *Chemosphere* 201. Elsevier Ltd: 790–98. <https://doi.org/10.1016/j.chemosphere.2018.03.020>.
- Niedźwiecka, Jolanta B., Scott R. Drew, Mark A. Schlautman, Kayleigh A. Millerick, Erin Grubbs, Nishanth Tharayil, and Kevin T. Finneran. 2017. "Iron and Electron Shuttle Mediated (Bio)Degradation of 2,4-Dinitroanisole (DNAN)." *Environmental Science and Technology* 51 (18): 10729–35. <https://doi.org/10.1021/acs.est.7b02433>.

- Niedzwiecka, Jolanta, and Kevin T Finneran. 2015. "Environmental Science Combined Biological and Abiotic Reactions with Iron and Fe(II) -Reducing Microorganisms for Remediation of Explosives and Insensitive," 34–39. <https://doi.org/10.1039/C4EW00062E>.
- Ou, Changjin, Jinyou Shen, Shuai Zhang, Yang Mu, Weiqing Han, Xiuyun Sun, Jiansheng Li, and Lianjun Wang. 2016. "Coupling of Iron Shavings into the Anaerobic System for Enhanced 2,4-Dinitroanisole Reduction in Wastewater." *Water Research* 101. Elsevier Ltd: 457–66. <https://doi.org/10.1016/j.watres.2016.06.002>.
- Trzeciński, Waldemar A., Stanisław Cudziło, Sławomir Dyjak, and Marcin Nita. 2014. "A Comparison of the Sensitivity and Performance Characteristics of Melt-Pour Explosives with TNT and DNAN Binder." *Central European Journal of Energetic Materials* 11 (3): 443–55.
- US EPA. 2001. "METHOD 1684 Total, Fixed, and Volatile Solids in Water, Solids, and Biosolids Draft January 2001 U.S. Environmental Protection Agency Office of Water Office of Science and Technology Engineering and Analysis Division (4303)." Washington, DC.