

Cross-parameter influences of pH and heat for biosolids stabilization

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**Abstract**

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Biosolids stabilized by lime addition have high material costs and high transportation costs, and excessive ammonia volatilization. Decreasing the amount of lime added while still achieving stabilization would allow for more sustainable reuse of biosolids. This study examined the combined influence of pH and heat on fecal coliform destruction and ammonia loss in biosolids. Fecal coliform concentrations of less than 1000 MPN/g were achieved with pH as low as 10 combined with 1-hour incubation at 60 °C. Increased incubation temperatures were associated with decreased specific oxygen uptake rates, suggesting decreased readily-available carbon for microbial activity. Decreased lime additions resulted in reduced ammonia loss in the biosolids, suggesting an improved potential fertilizer benefit. The study demonstrated that optimizing combined lime and heat stabilization has the potential for increasing beneficial reuse and enhance environmental sustainability.

# Table of Contents

List of Figures .....	i
List of Tables .....	ii
Acknowledgement .....	iii
Introduction.....	1
Nutrients and beneficial re-use .....	1
Environmental Concerns for Land Application of Biosolids .....	2
Overview of Regulations for safe handling of biosolids .....	3
Stabilization of Biosolids.....	4
Lime Treatment.....	4
Heat treatment.....	5
Ammonia volatilization .....	6
Objectives and Hypothesis.....	7
Laboratory Methods.....	7
Sampling Locations .....	7
Fecal Coliform .....	8
pH.....	9
Specific Oxygen Uptake Rate (SOUR).....	9
Lime and Heat Treatment .....	10
Volatile Solids.....	10

Ammonia.....	10
Data Analysis .....	11
Results.....	11
Sample Characterization .....	11
Fecal Coliform and pH.....	12
Specific Oxygen Uptake Rate (SOUR).....	14
Volatile Solids.....	15
Ammonia.....	15
Discussion.....	17
Conclusions and Recommendations .....	19
References.....	21
Appendix A. Sampling events and Tests Performed .....	24
Appendix B. Initial Characterization of Biosolids.....	25
Appendix C. Characterization of Treated Biosolids.....	26
Appendix D. Oxygen Uptake Experimental Results used for SOUR Calculations.....	28
Appendix E. Volatile suspended solids of Polyseed Solution .....	33
Appendix F. Normalized Ammonia Concentrations .....	34

## LIST OF FIGURES

<b>Fig. 1.</b> Typical sludge flow treatment diagrams at West Point Treatment Plant, South Treatment Plant and Brightwater Treatment Plant in the King County Region, WA.....	1
<b>Fig. 2.</b> Average fecal coliform concentrations (●) and pH (◇) of biosolid amended with lime (percent dry weight of biosolids) at incubation temperatures of a) 25 °C b) 40 °C c) 50 °C and d) 60 °C. ....	13
<b>Fig. 3.</b> Specific oxygen uptake rate (SOUR) relative to ambient SOUR (0% lime and 25 °C incubation) for biosolids blended lime (shown as percent dry weight of biosolids) and incubated at varying temperatures. ....	14
<b>Fig. 4.</b> Ammonia concentrations of biosolids mixed with lime (percentage dry mass of biosolids) and incubation temperatures of a) 25 °C b) 40 °C c) 50 °C and d) 60 °C.. ....	16

## LIST OF TABLES

<b>Table 1.</b> Acid dissociation constant of ammonia at different temperatures .....	7
<b>Table 2.</b> Initial characterization of biosolids samples .....	12
<b>Table 3.</b> Volatile solids loss (%) with varying amounts of lime and incubation temperature .....	15
<b>Table 4.</b> Summary of pH and fecal coliform results .....	20

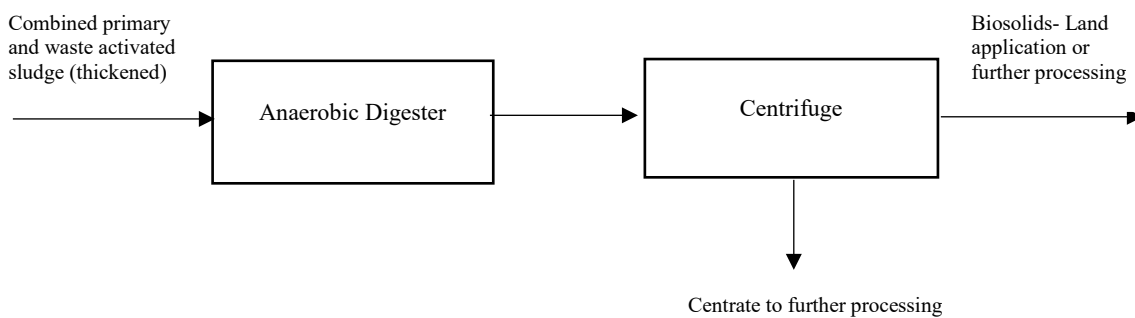
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## INTRODUCTION

Each year, an estimated  $10^8$  tons of biosolids are generated globally as a by-product of wastewater treatment (Thangarajan et al. 2013). Fig. 1 shows a typical flow diagram for solids processing during wastewater treatment. Settled sludge from primary and secondary treatment is blended and treated in an anaerobic digester to reduce the solids and volatile content. Excess water is often removed by centrifugation and the resulting product is named as biosolids.



**Fig. 1.** Typical sludge flow treatment diagrams at West Point Treatment Plant, South Treatment Plant and Brightwater Treatment Plant in the King County Region, WA. (adapted from Metcalf and Eddy (2003))

### *Nutrients and beneficial re-use*

Biosolids have the potential to alleviate pressures on the natural resources by recycling nutrients. To ensure global sustainability, it is important to recycle nutrients – nitrogen (N) and phosphorus (P) and carbon – from organic wastes back into food production (Borjesson and Katterer 2018; Pan et al. 2017). There is an increased demand for P and N based fertilizers to meet the rising global demand for food due to population growth (Irene Torri et al. 2017). Obtaining P and N from inorganic sources is not sustainable. For example, phosphate rocks are a finite nonrenewable resource, which are rapidly depleted to meet the fertilizer needs (Irene Torri et al.

2017), and production of N fertilizers using the Haber Bosch process is highly energy intensive, which contributes to greenhouse gas emissions (Woods et al. 2010).

Biosolids are a good source of nitrogen, phosphorus and micronutrients needed for plant growth (Cogger et al. 2006; Wijesekara et al. 2016). The N supplied by biosolids contains both inorganic (mainly  $\text{NH}_4^+$ ) and organic forms (Mendoza et al. 2006). Inorganic forms of nitrogen such as ammonium and nitrate are considered plant available nitrogen (PAN). Plants can also use organic nitrogen when it gets mineralized. Thus, the amount of N available for crop uptake in biosolid amended soils is dependent upon the inorganic N and the fraction of the organic N that mineralizes (Rigby et al. 2016). Additionally, biosolids can increase soil carbon content while simultaneously improving physical properties such as water holding capacity, porosity and bulk density (Brown et al. 2011; McIvor et al. 2012).

Land applications of biosolids can include soil amendments in home gardens (McIvor et al. 2012), recreational areas, agriculture lands (Zaleski 2005) and reclamation of mine sites (Brown and Chaney 2016; Wijesekara et al. 2016). Approximately 50% of the biosolids produced are land applied in the US (Wang et al. 2018). On an average 36% of biosolids are land applied in the European Union (EU).

#### *Environmental Concerns for Land Application of Biosolids*

Concerns over land application of biosolids include the presence of unwanted chemicals, and potential human exposure to pathogens. The presence of heavy metals in biosolids can limit land application due to the risk of food chain accumulation (Sharma et al. 2017). Additionally, extensive applications may result in nutrient run-off to near-by water bodies (Paramashivam et al. 2017; Shoher and Sims 2003; Wadsworth et al. 2018). There are also concerns of anthropogenic contaminants (such as antimicrobials) in biosolids which could be detrimental for

soil microbial activity (Holzem et al. 2018), or impact near-by aquatic life (Gray et al. 2017). Finally, exposure to pathogens could be detrimental to those who handle biosolids (USEPA 1994). Regulations are in place to mitigate impacts of land application of biosolids. This study focused on testing pathogenic reduction using an alternative biosolids stabilization

### *Overview of Regulations for safe handling of biosolids*

Methods for biosolid stabilization are informed by environmental and health regulations, which vary by country. Safe and responsible management is needed to prevent risks to public health and the environment and maximize the potential benefits of biosolids (Rigby et al. 2016). The US EPA published “The Standards for the Use or Disposal of Sewage Sludge” as the 40 CFR (Title 40, Code of Federal Regulations) Part 503 Biosolids rule, also known as the “503 Rule”(USEPA 1994). Biosolids used in the EU is regulated and governed by the 1986 EU Directive (Sludge Directive 86/278/EEC). The regulations establish quality requirements such as pathogen and vector attraction reduction, heavy metal concentration limits, and nutrient limits for various land application.

The pathogen reduction must be demonstrated either through measuring fecal coliform or salmonella. In the US, there are two classes of pathogen reduction- Class A and Class B. For Class A the fecal coliform density should be less than 1000 MPN per g dry solids or *Salmonella sp.* density less than 3 MPN per 4 grams of total dry solids (USEPA 2003). This designation is required for the use of biosolids on home gardens, lawns, or for sale as commercial fertilizer. Class B pathogen reduction requires that fecal coliform density be no greater than 2 million MPN per gram of dry solids (USEPA 2003). Use of this material is regulated to ensure that there is minimal human contact with pathogens. Regulations for Class B biosolids include restricting public access, limiting the type of crop growth and harvesting schedules, and limiting livestock

grazing. The EU has similar regulations for biosolids. A 2 log<sub>10</sub> reduction for fecal coliform is needed for Class B, while a 6 log<sub>10</sub> removal is needed for Class A biosolids (Rigby et al. 2016).

In addition to pathogen limits, regulations also require vector attraction reduction (VAR), which are processes that help to reduce the attractiveness of biosolids to vectors. Vectors such as rodents, flies, insects, birds have the potential to act as carriers of pathogens and transmit directly to human beings. VAR is based upon the reduction of volatile solids. To meet the 503 Rule, the volatile solids have to be reduced by at least 38% (USEPA 2003). There are 12 options that can be used to demonstrate VAR. In addition to pathogen reduction and VAR, trace elements in biosolids must meet ceiling concentrations for nine trace elements.

### *Stabilization of Biosolids*

Biosolids stabilization includes reducing pathogen levels, odor and volatile solids (Switzenbaum et al. 1997). Biosolids are stabilized by one or a combination of the following approaches (Rigby et al. 2016):

- biological processes (anaerobic, aerobic, composting);
- chemical processes (lime treatment);
- physical processes (heat treatment, thermal hydrolysis, thermal drying, air/solar drying)

This study focused on combining lime treatment and heat treatment; thus, both are discussed in more detail below.

### Lime Treatment

Lime treatment uses alkaline material such as quicklime (CaO) or hydrated lime (Ca(OH)<sub>2</sub>) to increase the pH of biosolids, creating conditions that impede the survival of pathogens (Wong and Fang 2000). Cement kiln dust, fly ash, lime kiln dust, coal ash residue has also been used as

substitutes in some cases (Christie et al. 2001; Kocaer et al. 2003; Tchobanoglous et al. 2003; Wong et al. 2001). In this treatment, pH is raised to 12 using lime dose of 20-30% dry solids (Rigby et al. 2016) and maintained for a period of 2 to 72 hours depending on whether a Class B or a Class A product is required.

Fecal coliform bacteria are facultative anaerobic, gram negative, rod shaped bacteria that are found in the feces and intestines of humans and other warm-blooded animals. *Escherichia coli* is the predominant fecal coliform (Clarke et al. 2017). They are used as an indicator of other pathogens in water and biosolids (Elayse et al. 2012). The optimum pH for the growth of *E. coli* is in the near neutral range of 6-8 (Wahyuni 2015). Modifying the pH of the material can be used to destroy pathogens. A number of studies have shown that pH 12 or higher can be used effectively to reduce the fecal coliform levels to achieve Class A or Class B biosolids (Allievi et al. 1994; Bean et al. 2007; Farzadkia et al. 2009; North et al. 2008; Silva-Leal et al. 2013; Wong et al. 2001). In addition to the reduction of fecal coliform, alkaline stabilization is also capable of inactivating pathogens such as *Salmonella*, adenovirus, rotavirus, *Giardia lamblia* and ascaris eggs (Allievi et al. 1994; Bean et al. 2007; Hansen et al. 2007; Pecson et al. 2007).

### Heat treatment

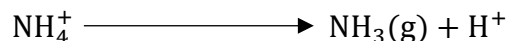
In addition to lime treatment, biosolids can be heated to 70 °C for 30 minutes to meet the Class A requirements. Fecal coliform is thermotolerant and the optimum temperature for growth is  $44.5 \pm 0.5$  °C (Elayse et al. 2012). Increasing the temperature beyond the optimum destroys pathogens. Kocaer et al. (2003) investigated the combination of quicklime-fly ash and thermal drying at 70 °C to produce Class A material from sewage sludge. Silva-Leal et al. (2013) also studied the effect of heat treatment on biosolids. In their study biosolids were heated to temperatures of 60-75 °C for 8 to 12.5 hours and fecal coliform concentrations were reduced to

less than 1000 MPN per g. Crohn et al. (1997) showed that temperatures of 60-70 °C resulted in reduction in virus concentrations.

#### *Ammonia volatilization*

In theory, increased lime incorporation decreases ammonia-nitrogen content in biosolids (North et al. 2008; Switzenbaum et al. 1997). Ammonia volatilization has been also suggested as a stability assessment technique for alkaline stabilized biosolids (Switzenbaum et al. 1997).

Ammonium ions deprotonate to ammonia when pH is increased to the alkaline range as shown below:



The acid dissociation constant for ammonium varies with temperature and the different dissociation constants have been calculated in Table 1 based on the equations developed by Bates and Pinching (1949). During lime and heat treatment ammonia gas would be theorized to volatilize, leading to loss of N. In addition to ammonia volatilization, total N is also lowered due to dilution by the large quantities of lime added (Rigby et al. 2016). This impacts the use of lime treated biosolids as fertilizers (Kocaer et al. 2003; Wijesekara et al. 2016).

**Table 1.** Acid dissociation constant of ammonia at different temperatures

<b>Temperature (°C)</b>	<b>Acid dissociation constant (pKa)</b>
25	9.24
40	8.81
50	8.54
60	8.27

## **OBJECTIVES AND HYPOTHESIS**

Traditional lime treatment results in a product with odor problems, lower total N and increased transportation costs. In this study, the hypothesis was tested that combining reduced lime addition with sustained incubation temperatures will meet fecal coliform reduction requirements. Bench scale tests were carried out using anaerobically digested and dewatered biosolids, collected from three wastewater plants in the King County region, Washington State, USA. Additionally, the project assessed the impact of altered treatment on bioavailability of carbon (using specific oxygen uptake rates as an indicator of carbon bioavailability), loss of volatile solids, and loss of free-ammonia.

## **LABORATORY METHODS**

### *Sampling Locations*

Anaerobically digested and dewatered biosolids were obtained from the following King County Treatment plants- South Plant, Renton, Washington; West Point Treatment Plant, Seattle, Washington; and Brightwater Treatment Plant, Woodinville, Washington; all in the USA. West Point treatment plant used primary settling followed by high-purity oxygen aeration tanks. The

solids from primary and secondary settlers were thickened on a gravity belt thickener with polymer to enhance flocculation. The blended and thickened sludge was digested for 28 days. The resulting product meets Class B biosolids requirements (King County, 2013). South Treatment Plant used primary settling followed by secondary air activated sludge. The solids from primary and secondary settling were blended and thickened using dissolved air flotation. The sludge was digested anaerobically for 25 days. The resulting product was dewatered using centrifuge with polymer and meets all requirements for Class B biosolids (King County, 2013). Brightwater treatment plant used enhanced primary settling followed by secondary treatment in aeration basins. Instead of using secondary clarifier, membranes were used to separate out solids. Solid treatment involved blending the primary and secondary sludge and thickened on gravity belt thickeners with polymer. Thickened sludge was anaerobically digested for 30 days and dewatered using centrifuge (King County 2011). The final product meets all the requirements for Class B biosolids. Appendix A shows the dates and locations of biosolid collection cross referenced with the tests conducted with each biosolid sample.

### *Fecal Coliform*

Fecal coliform concentrations in the biosolids were measured using US EPA method 1680 (USEPA 2010), with modifications to adjust sampling dilution. Briefly, Lauryl Tryptose Broth (LTB) medium was used as the presumptive medium and *Escherichia coli* (EC) medium was used for confirmation of fecal coliform. Five replicates were inoculated at four sample dilutions. For class B biosolids, dilutions of  $10^{-3}$  g,  $10^{-4}$  g,  $10^{-5}$  g and  $10^{-6}$  g and, for the treated biosolids dilutions of  $10^{-1}$  g,  $10^{-2}$  g,  $10^{-3}$  g and  $10^{-4}$  g were used respectively. The sample tubes were incubated at  $35 \pm 0.5$  °C. Tubes were examined at  $24 \pm 2$  hours for growth and gas production. Negative tubes were re-accessed for gas production after an additional 24 hours. EC tubes were



incubated in a water bath at  $44.5 \pm 0.5$  °C. Gas production in the EC medium was a positive test for fecal coliform. Total solids (Standard Methods of Examination of Water and Wastewater 2540G (2005)) was used to calculate the MPN/g dry weight. Fecal coliform density was reported as the MPN/g dry weight.

For quality control, the recovery was measured of a spike of known concentration of *E. coli* added to biosolids following established methods (USEPA 2010). Matrix spike recovery was 387%, which was within the accepted matrix spike percent recovery of 30-424%.

### *pH*

pH of biosolids was measured using previously published methods (North et al. 2008). Biosolids were diluted using MilliQ water in ratio of 1:2 and vortexed for 5 minutes. pH was then measured using an Orion thermo scientific ROSS Ultra pH/ATC Triode.

### *Specific Oxygen Uptake Rate (SOUR)*

Measurement of SOUR was conducted in accordance with US EPA method 1683 (USEPA 2010). Biosolids were diluted using MilliQ water at a ratio of 1:40 and aerated for 30 seconds to increase oxygen levels. The aerated sample was transferred to a BOD bottle, along with 4 ml of InterLab® Polyseed bacteria. Polyseed was prepared according to the manufacturer's instructions. The volatile suspended solids (VSS) of the polyseed was measured just prior to use using Standard Method 2540E (APHA 2005). Dissolved oxygen (DO) was monitored over time until the DO was below 1 mg/L. The oxygen consumption rate was defined graphically as the slope of the linear range of DO decline. SOUR was calculated as:

$$\text{SOUR (mg/g/h)} = \frac{\text{oxygen consumption rate (mg/L/min)}}{\text{VSS (Polyseed)}} \times 60 \frac{\text{min}}{\text{h}}$$

### *Lime and Heat Treatment*

500g of biosolids (wet) and lime were blended with an electric mixer. The amount of lime (Fisher Scientific calcium hydroxide) was varied from 5-25% of dry biosolid mass. Heat treatments was conducted at 40, 50 and 60 °C for one hour in Forma Scientific™ model 3326 incubator and compared to controls held for 1 hour at room temperature.

### *Volatile Solids*

Standard Methods for the Examination of Water and Wastewater 2540G was used to measure the volatile solids content of both untreated and treated biosolids (APHA 2005). Mass balance was used to assess if volatile solids changed during treatment:

$$VSL = \frac{VS_{in} - VS'_{out}}{VS_{in}}$$

where VSL = Volatile Solids Loss,  $VS_{in}$  = fraction of volatile solids in untreated sample and  $VS'_{out}$  = fraction of volatile solids in treated sample. The lime addition to the biosolids will not volatilize and is considered as fixed solids. Hence  $VS'_{out}$  represents the volatile solids of only the biosolids and is calculated:

$$VS'_{out} = \frac{VS_{out}(M_{bio} + M_{lime})}{M_{bio}}$$

where  $VS_{out}$  = fraction of volatile solids in lime amended biosolids,  $M_{bio}$  = dry mass of biosolids and  $M_{lime}$  = amount of lime mixed.

### *Ammonia*

Ammonia concentrations were measured using Hach AmVer™ Salicylate Test 'N Tube (high range ammonia). Biosolids were diluted in a ratio of 1:100 in Deionized Water (D.I) and 0.1 ml

of the sample was dispensed into each test tube, in accordance with manufacture instructions. D.I water was used to prepare method blanks. Salicylate and cyanurate powders were added to bring about color changes. Absorbance of the samples was measured using a Hach DR4000 Spectrophotometer after a 20-minute reaction period. The reading from the spectrophotometer was in mg/L of  $\text{NH}_3\text{-N}$ . The value was then multiplied by the dilution factor and divided by total solids, which is expressed as mg/kg dry weight. Tests were conducted in triplicate.

#### *Data Analysis*

ANOVA followed by Tukey's test were used to identify statistical differences within data sets for SOUR and ammonia concentrations.

## **RESULTS**

#### *Sample Characterization*

Table 2 shows the initial characterization of biosolid samples. pH was similar among all the sample collection locations. Total solids and volatile solids were similar among sampling events for the same sample location; however, the total solids were lower and volatile solids higher in samples collected from Brightwater Wastewater Treatment Plant. Fecal coliform concentrations were substantially below the target level for Class B biosolids but showed variation by as much as 44% from the same sample collection location (i.e. West Point Wastewater Treatment Plant). Appendix B shows daily values measured for the collected samples.

**Table 2.** Initial characterization of biosolids samples

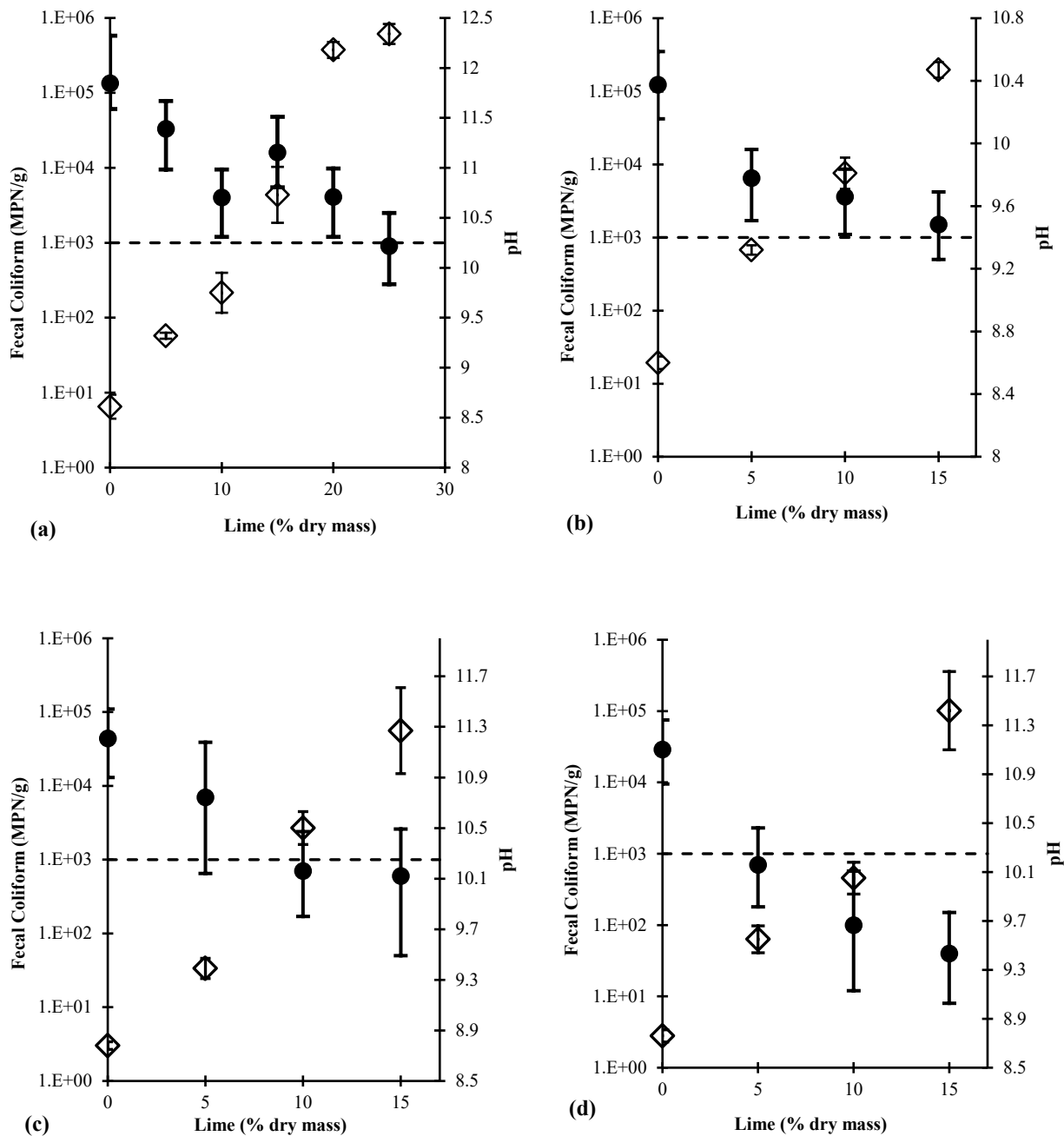
Sample Location <sup>§</sup>	<i>n</i>	Parameters			
		pH	Total Solids (%)	Volatile Solids (%)	Fecal Coliform (MPN/g)
WP	6	8.6 ± 0.2	26.5 ± 0.8	69.7 ± 0.9	160,000 ± 71,000
BW	4	8.6 ± 0.1	19.6 ± 0.6	83.8 ± 0.5	97,000 ± 19,000
SP	5	8.8 ± 0.2	21.3 ± 1.4	75.1 ± 1.6	147,000 ± 55,000

*n*, number of replicate samples; MPN – Most Probable Number

<sup>§</sup> All sample locations were part of the King County Metro Wastewater Treatment Division in Washington State. BW, Brightwater Treatment Plant; WP, West Point Treatment Plant; SP, South Treatment Plant.

### *Fecal Coliform and pH*

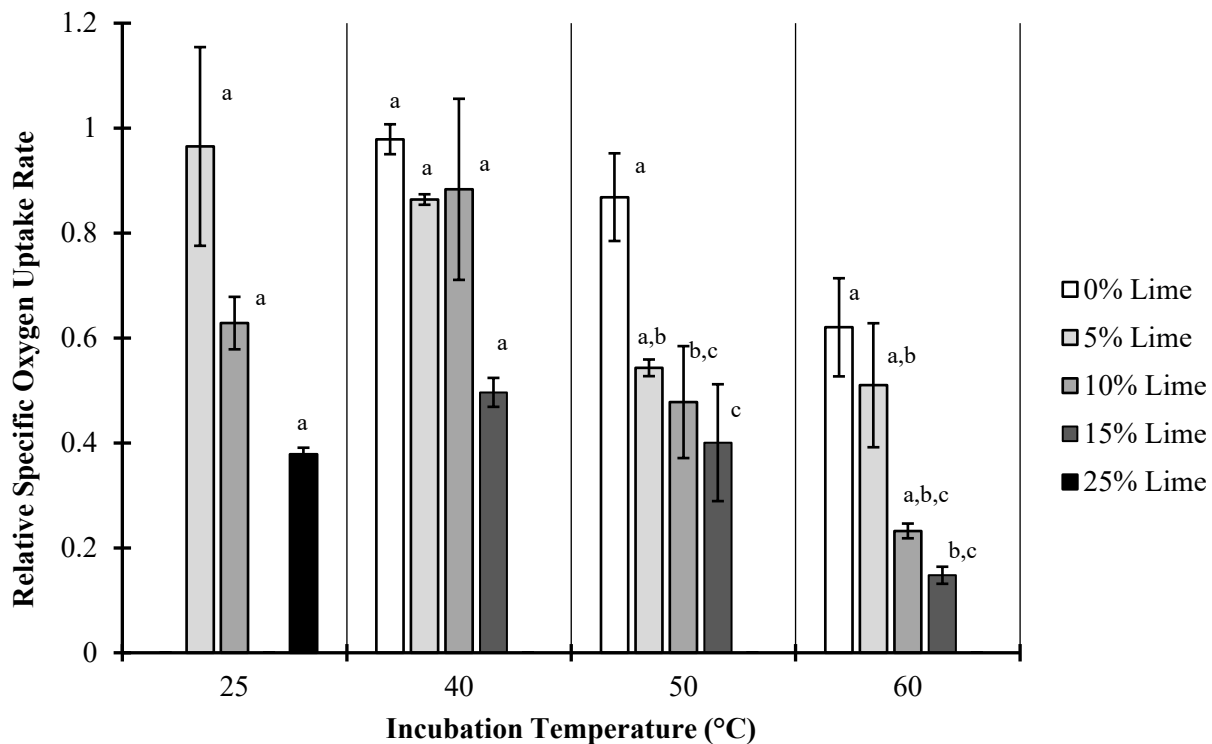
Final fecal coliform concentrations varied with differing heat and lime treatments (Fig. 2). Increased lime additions increased the pH of the biosolids, as previously documented (Bean et al. 2007; North et al. 2008). Within the ranges tested, heating or lime additions alone did not achieve the reduction of fecal coliform to the target concentration of 1000 MPN/g, as required for Class A biosolids. When no heat treatment was used (Fig. 2a), 25% lime addition (pH ~12.5) was required to reach the final target concentration. Adding heat treatment decreased the amount of lime addition needed to reach the target fecal coliform concentration. When heat treatment was at 50°C, 10% lime addition was needed (Fig. 2c). When heat treatment was at 60°C, 5% lime addition was sufficient to meet the target fecal coliform concentration (Fig. 2d). Final pH was not directly correlated to mass of lime added (data not shown), documenting that the heterogeneity of biosolids which required confirmation of pH for each test.



**Fig. 2.** Average fecal coliform concentrations (●) and pH (◇) of biosolid amended with lime (percent dry weight of biosolids) at incubation temperatures of a) 25 °C b) 40 °C c) 50 °C and d) 60 °C. The error bars represent the upper and lower 95% confidence intervals for the fecal coliform concentration and average deviation from the mean for pH. MPN, Most Probable Number. Dashed line shows the targeted fecal coliform concentrations of 1000 MPN/g.

### Specific Oxygen Uptake Rate (SOUR)

Fig. 3 shows SOUR results for differing combinations of incubation temperatures and lime additions. Dissolved oxygen measurements for each test are shown in Appendix D. The VSS for polyseed for each test is tabulated in Appendix E. SOUR compared among lime additions were not statistically different ( $p > 0.05$ ) for samples incubated at either 25 °C and 40 °C. For samples incubated at 25 °C, this was likely influenced by high heterogeneity measured for duplicates at 5% lime addition incubated. Similarly, for samples incubated at 40 °C, this was likely influenced by high heterogeneity measured for duplicates at 10% lime addition. Additional replication would be required to statistically validate these trends. The combination of 15% lime and 60 °C had the lowest SOUR value.



**Fig. 3.** Specific oxygen uptake rate (SOUR) relative to ambient SOUR (0% lime and 25 °C incubation) for biosolids blended lime (shown as percent dry weight of biosolids) and incubated at varying temperatures. Lower case letters in the same panel indicate that the data were not statistically different (ANOVA with Tukey's test,  $p=0.05$ ). The error bars show average deviation from the mean of duplicate tests.

### *Volatile Solids*

Table 3 shows the difference in volatile solids before and after heat and lime treatment. Data did not document a change in volatile solids with heat or lime treatment. The variations in VSL reflected heterogeneity of the samples.

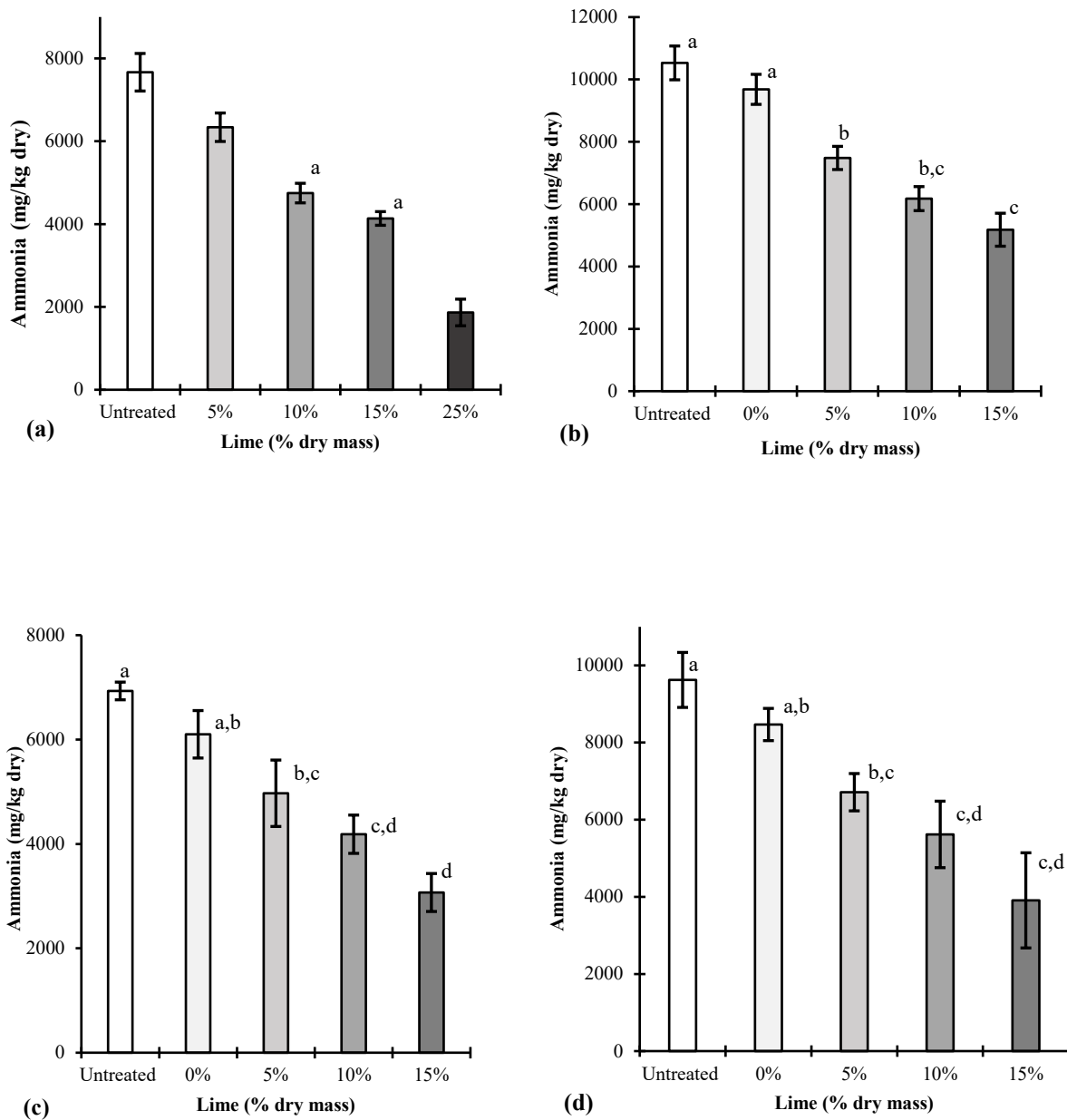
**Table 3.** Volatile solids loss (%) with varying amounts of lime and incubation temperature

Temperature (°C)	Lime Addition (% dry mass)					
	No Lime	5%	10%	15%	20%	25%
25	<i>na</i>	1.0 ± 1.1	0.1 ± 0.5	-1.7 ± 0.2	0.56 ± 6.2	2.3 ± 0.9
40	0.7 ± 1.5	0.5 ± 0.7	-1.8 ± 2.8	-4.8 ± 1.1	<i>na</i>	<i>na</i>
50	-0.03 ± 0.3	-1.7 ± 0.7	-0.3 ± 1.5	0.6 ± 0.5	<i>na</i>	<i>na</i>
60	0.04 ± 0.3	-0.04 ± 0.3	1.1 ± 0.4	-3.6 ± 3.3	<i>na</i>	<i>na</i>

*Note:* Volatile solids loss was calculated using the equation shown in the methods section.  
*na*, not analyzed

### *Ammonia*

Fig 4. shows the changes in ammonia concentrations for biosolids with differing lime and heat. Increased lime addition resulted in decreased ammonia concentrations. Reduced ammonia concentrations are consistent with the expectation that at higher pH more ammonia will be volatilized as predicted by the equilibrium equation for ammonium and ammonia (Table 1). A similar loss would be expected due to temperature influence on equilibrium constant; however, tests in this study were not designed to confirm this trend. The highest decrease in ammonia concentration was for 25% lime addition (traditional method) at room temperature.



**Fig. 4.** Ammonia concentrations of biosolids mixed with lime (percentage dry mass of biosolids) and incubation temperatures of a) 25 °C b) 40 °C c) 50 °C and d) 60 °C. Lower case letters in the same panel indicate that the data were not statistically different (ANOVA with Tukey's test,  $p=0.05$ ). Error bars represent the average deviation from the mean of triplicate tests.



## DISCUSSION

Lime stabilization and heat stabilization are common methods for treating wastewater sludge.

Raising the pH 12 has been documented to decrease pathogens, rendering the resulting biosolids safe for reuse (USEPA 1995). Typically, lime doses of 20-30% (dry solids) have been used to achieve pathogen reduction (Allievi et al. 1994; Bean et al. 2007; Farzadkia et al. 2009; Hansen et al. 2007; Rigby et al. 2016; Silva-Leal et al. 2013). Heat treatment at 60-75°C for 8-12 hours has also shown to reduce fecal coliform concentration to less than 1000 MPN/g (Silva-Leal et al. 2013). Limitations exist for both approaches including high mass additions (e.g. lime) and high energy requirements (prolonged heating).

A potential method for decreasing the amount of lime needed to meet targeted destruction of pathogens is to combine lime treatment with heat treatment. In the current study, results showed that it was possible to achieve fecal coliform concentrations below 1000 MPN/g for 50 °C and 60°C (Fig. 2). Lime addition at 25% (traditional method) met the target concentration agreeing with the prior studies (Allievi et al. 1994; Bean et al. 2007). However, statistically only two combinations met the targeted final fecal coliform concentration – 10% lime and 60 °C and, 15% lime and 60 °C. On further analysis of the controls, lime treatment had more impact on fecal coliform reduction than heat treatment. It is hypothesized that cell structure is weakened by lime treatment and destruction is completed during heat treatment. Combining lime and heat have been previously considered, which achieved Class A pathogen limits but retained the value of pH at 12 (Kocær et al. 2003; Wong et al. 2001). Here, a first demonstration was shown that fecal coliform targets could be met at lower pH when combined with heat.

Oxygen uptake rates is a potential predictor of residual carbon in the biosolids. To compensate for bacterial death during sample treatment, a Polyseed<sup>®</sup> bacterial solution was used to ensure

microbial activity in each test. SOUR decreased for those treatment combinations that met the targeted fecal coliform concentration (Fig. 1 and Fig. 3). This was contrary to the expectation that during lime and heat treatments cell lysis would release biodegradable organic matter. However, loss of carbon could have also occurred during the heat treatment, if light weight carbon molecules were volatilized. The remaining high molecular weight organic carbons are not bioavailable in the short-term (Aquino et al. 2008) which could explain the decreased observed in the less than 3-hour long SOUR tests. Additional testing would be required to determine if bioavailability of carbon differed during long-term processing, such as during composting.

Lime stabilization increases volatilization losses of ammonia (Kocaer et al. 2003; Wijesekara et al. 2016). This is caused by the shift from ammonium ion ( $\text{NH}_4^+$ ) to ammonia gas ( $\text{NH}_3$ ). At higher temperatures the pKa decreases (Table 1), resulting in more ammonia volatilization (Bates and Pinching 1949). The ammonium dissociation constant can be used to predict the concentrations of the two species for different pH values at equilibrium.

During lime treatment, the pH of the biosolids was increased beyond the pKa, which resulted in ammonia volatilization. The combination of 25 % lime addition which represents the traditional method resulted in the significant ammonia loss as shown in Fig. 4a ( $p = 2 \times 10^{-7}$ ). With reduced lime addition, decreased ammonia losses were observed (Fig. 4 b-d). However, the results did not follow the trends predicted by the dissociation constant as the loss of ammonia concentration was lower than predicted. The equation calculates concentrations at equilibrium conditions which might not be a good assumption for this system.

Theoretically, ammonia volatilization is greater at higher temperatures. This occurs because the dissociation constant of ammonium ions decreases with increased temperature (Table 1). To better understand the influence of heat treatment on data from the current study, normalized data

is presented in Appendix F. However, for this case ammonia concentrations for the same lime addition were similar regardless of incubation temperatures. This suggested that the ammonia in the biosolids did not reach equilibrium concentrations during the 1 hour heat treatment, similar to the observation at room temperature.

Lime treated biosolids have lower values of total N when compared to other stabilization methods (Rigby et al. 2016). In addition to ammonia volatilization, dilution due to addition of large quantities of lime reduces total N. Since lower lime additions were used in this study, it was possible that the effects of dilution were reduced. Tests would be needed to confirm the dilution effects on total N. In addition, to determine the fertilizer properties of biosolids, tests would be needed to quantify the organic N fraction that will mineralize when amended with soil.

## **CONCLUSIONS AND RECOMMENDATIONS**

This study showed that lower lime additions combined with short-term (1 hour) heat treatment can achieve similar pathogen indicator reductions as traditional high lime additions. Table 4 summarizes the pH and fecal coliform concentration obtained in this study. Five conditions met the target fecal coliform concentration of less than 1000 MPN/g: 10% and 15% lime addition at 50 °C and 5 to 15% lime additions at 60 °C. Evaluation of the influence of properties such as alkalinity and high volatile solids may help with future predictions of the response of materials to the combined lime and heat treatment. Additionally, testing with higher initial fecal coliform concentrations would expand knowledge about the range of biosolids for which the treatment would be successful. This information could be combined with optimization of the required heat treatment times to increase energy efficiency. To consider treatment effects on product end-use, further testing is required to understand how the resulting biosolids will respond to composting and to plant growth.

**Table 4.** Summary of pH and fecal coliform results

		Lime Addition (dry weight of biosolids)					
		No Lime	5%	10%	15%	20%	25%
Temperature (°C)	25	pH:8.6 FC:133,000 [1][2][3]	pH: 9.3 FC:33000 [2]	pH:9.8 FC:4000 [3]	pH:10.7 FC:16000 [1]	pH:12.2 FC:4100 [1]	pH:12.3 FC:900 [2]
	40	pH:8.6 FC:123,000 [2]	pH:9.3 FC:6500 [1]	pH:9.8 FC:3600 [1]	pH:10.4 FC:1500 [1]		
	50	pH:8.8 FC:44000 [1]	pH:9.5 FC:7000 [1][2]	pH:10.5 FC:700 [1][2]	pH:11.6 FC:400 [1][2]		
	60	pH:8.8 FC:29000 [3]	pH:9.6 FC:700 [1][2]	pH:10.0 FC:100 [1][2][3]	pH:11.73 FC:15 [2][3]		

[1] – Brightwater Treatment Plant; [2] – West Point Treatment Plant; [3] – South Treatment Plant

FC – Fecal Coliform, MPN/g. MPN – Most probable number.

## REFERENCES

- Allievi, L., Colombi, A., Calcaterra, E., and Ferrari, A. (1994). "Inactivation of fecal bacteria in sewage sludge by alkaline treatment." *Bioresource Technology*, 49(1), 25-30.
- APHA (American Public Health Association). 2005. *Standard methods for the examination of water and wastewater American public health association*. 21st ed. Washington, DC: APHA.
- Bates, R. G., and Pinching, G. D. (1949). "Acidic dissociation constant of ammonium ion at 0 to 50 C, and the base strength of ammonia." *Journal of Research of the National Bureau of Standards*, 42(5), 419.
- Bean, C. L., Hansen, J. J., Margolin, A. B., Balkin, H., Batzer, G., and Widmer, G. (2007). "Class B alkaline stabilization to achieve pathogen inactivation." *International Journal of Environmental Research and Public Health*, 4(1), 53-60.
- Borjesson, G., and Katterer, T. (2018). "Soil fertility effects of repeated application of sewage sludge in two 30-year-old field experiments." *Nutrient Cycling in Agroecosystems*, 112(3), 369-385.
- Brown, S., Kurtz, K., Bary, A., and Cogger, C. (2011). "Quantifying benefits associated with land application of organic residuals in Washington state." *Environmental Science & Technology*, 45(17), 7451-7458.
- Brown, S. L., and Chaney, R. L. (2016). "Use of amendments to restore ecosystem function to metal mining-impacted sites: tools to evaluate efficacy." *Current Pollution Reports*, 2(2), 91-102.
- Christie, P., Easson, D. L., Picton, J. R., and Love, S. C. P. (2001). "Agronomic value of alkaline-stabilized sewage biosolids for spring barley." *Agronomy Journal*, 93(1), 144-151.
- Clarke, R., Peyton, D., Healy, M. G., Fenton, O., and Cummins, E. (2017). "A quantitative microbial risk assessment model for total coliforms and *E. coli* in surface runoff following application of biosolids to grassland." *Environmental Pollution*, 224, 739-750.
- Cogger, C. G., Forge, T. A., and Neilsen, G. H. (2006). "Biosolids recycling: Nitrogen management and soil ecology." *Canadian Journal of Soil Science*, 86(4), 613-620.
- Crohn, D. M., Yates, M. V., and Luker, M. (1997). "Demonstrating virus treatment efficiencies for biosolids." *Journal of Environmental Engineering*, 123(10), 1053-1059.
- Elayse, M. H., Marisa Di, B., Ana Paula, G. C., Cláudia, C. L., Solange, S. R., and Maria Inês, Z. S. (2012). "Comparison of thermotolerant coliforms and *Escherichia coli* densities in freshwater bodies." *Brazilian Journal of Microbiology*, 43(2), 675-681.
- Farzadkia, M., Jaafarzadeh, N., and Asl, L. L. (2009). "Optimization of bacteriological quality of biosolids by lime addition." *Iranian Journal of Environmental Health Science & Engineering*, 6(1), 29-34.
- Gray, J. L., Borch, T., Furlong, E. T., Davis, J. G., Yager, T. J., Yang, Y.-Y., and Kolpin, D. W. (2017). "Rainfall-runoff of anthropogenic waste indicators from agricultural fields applied with municipal biosolids." *Science of the Total Environment*, 580, 83-89.
- Hansen, J. J., Warden, P. S., and Margolin, A. B. (2007). "Inactivation of adenovirus type 5, rotavirus Wa and male specific coliphage (MS2) in biosolids by lime stabilization." *International journal of environmental research and public health*, 4(1), 61-67.
- Holzem, R. M., Stapleton, H. M., and Gunsch, C. K. (2018). "Impact of biosolids on indigenous soil denitrifying bacteria." *Journal of Environmental Engineering*, 144(9).

- Irene Torri, S., Correa, R. S., and Renella, G. (2017). "Biosolid application to agricultural land-a contribution to global phosphorus recycle: A review." *Pedosphere*, 27(1), 1-16.
- Kocaer, F. O., Alkan, U., and Baskaya, H. S. (2003). "Use of lignite fly ash as an additive in alkaline stabilisation and pasteurisation of wastewater sludge." *Waste Management & Research*, 21(5), 448-458.
- McIvor, K., Cogger, C., and Brown, S. (2012). "Effects of biosolids based soil products on soil physical and chemical properties in urban gardens." *Compost Science & Utilization*, 20(4), 199-206.
- Mendoza, C., Assadian, N. W., and Lindemann, W. (2006). "The fate of nitrogen in a moderately alkaline and calcareous soil amended with biosolids and urea." *Chemosphere*, 63(11), 1933-1941.
- North, J. M., Becker, J. G., Seagren, E. A., Ramirez, M., and Peot, C. (2008). "Methods for quantifying lime incorporation into dewatered sludge. I: Bench-scale evaluation." *Journal of Environmental Engineering*, 134(9), 750-761.
- Pan, W. L., Port, L. E., Xiao, Y., Bary, A. I., and Cogger, C. G. (2017). "Soil carbon and nitrogen fraction accumulation with long-term biosolids applications." *Soil Science Society of America Journal*, 81(6), 1381-1388.
- Paramashivam, D., Dickinson, N. M., Clough, T. J., Horswell, J., and Robinson, B. H. (2017). "Potential environmental benefits from blending biosolids with other organic amendments before application to land." *Journal of Environmental Quality*, 46(3), 481-489.
- Pecson, B. M., Barrios, J. A., Jiménez, B. E., and Nelson, K. L. (2007). "The effects of temperature, pH, and ammonia concentration on the inactivation of *Ascaris* eggs in sewage sludge." *Water Research*, 41(13), 2893-2902.
- Rigby, H., Clarke, B. O., Pritchard, D. L., Meehan, B., Beshah, F., Smith, S. R., and Porter, N. A. (2016). "A critical review of nitrogen mineralization in biosolids-amended soil, the associated fertilizer value for crop production and potential for emissions to the environment." *Science of the Total Environment*, 541, 1310-1338.
- Sharma, B., Sarkar, A., Singh, P., and Singh, R. P. (2017). "Agricultural utilization of biosolids: A review on potential effects on soil and plant grown." *Waste Management*, 64, 117-132.
- Shober, A., and Sims, J. (2003). "Phosphorus restrictions for land application of biosolids: Current status and future trends." *Journal of Environmental Quality*, 32(6), 1955-1964.
- Silva-Leal, J., Bedoya-Rios, D., and Torres-Lozada, P. (2013). "Effect of thermal drying and alkaline treatment on the microbiological and chemical characteristics of biosolids from domestic wastewater treatment plants." *Quimica Nova*, 36(2), 207-214.
- Switzenbaum, M. S., Moss, L. H., Epstein, E., Pincince, A. B., and Donovan, J. F. (1997). "Defining biosolids stability." *Journal of Environmental Engineering*, 123(12), 1178-1184.
- Tchobanoglous, G., Burton, F. L., Stensel, H. D., Metcalf, and Eddy (2003). *Wastewater engineering : treatment and reuse*, Boston : McGraw-Hill, Boston.
- Thangarajan, R., Bolan, N. S., Tian, G., Naidu, R., and Kunhikrishnan, A. (2013). "Role of organic amendment application on greenhouse gas emission from soil." *Science of The Total Environment*, 465, 72--96.
- U.S Environmental Protection Agency (USEPA). (1994). "A Plain English Guide to the EPA Part 503 Biosolids Rule." EPA/ 832/R-93/003, Washington, DC.

- U.S Environmental Protection Agency (USEPA). (2001). "Method 1683 specific oxygen uptake rate in biosolids." EPA/821/R/01/014, Washington,DC.
- U.S Environmental Protection Agency (USEPA). (2003). "Environmental regulations and technology: control of pathogens and vector attraction in sewage sludge." EPA/625/R-92/013.
- U.S Environmental Protection Agency (USEPA). (2010). "Method 1680: Fecal coliforms in sewage sludge (biosolids) by multiple-tube fermentation using lauryl tryptose broth (LTB) and EC medium." EPA/821/R/10/003, Washington, DC.
- Wadsworth, R., Hallett, S., and Sakrabani, R. (2018). "Phosphate acceptance map: A novel approach to match phosphorus content of biosolids with land and crop requirements." *Agricultural Systems*, 166, 57-69.
- Wahyuni, E. A. (2015). "The influence of pH characteristics on the occurrence of coliform bacteria in madura strait." *Basic Researches in The Tropical and Coastal Region Eco Developments*, 23, 130-135.
- Wang, X., Andrade, N., Shekarchi, J., Fischer, S. J., Torrents, A., and Ramirez, M. (2018). "Full scale study of Class A biosolids produced by thermal hydrolysis pretreatment and anaerobic digestion." *Waste Management*, 78, 43-50.
- Wijesekara, H., Bolan, N. S., Kumarathilaka, P., Geekiyanage, N., Kunhikrishnan, A., Seshadri, B., Saint, C., Surapaneni, A., and Vithanage, M. (2016). *Biosolids enhance mine site rehabilitation and revegetation*, Elsevier Academic Press Inc, San Diego, USA.
- Wong, J. W. C., and Fang, M. (2000). "Effects of lime addition on sewage sludge composting process." *Water Research*, 34(15), 3691--3698.
- Wong, J. W. C., Fang, M., and Jiang, R. F. (2001). "Persistency of bacterial indicators in biosolids stabilization with coal fly ash and lime." *Water Environment Research*, 73(5), 607-611.
- Woods, J., Williams, A., Hughes, J. K., Black, M., and Murphy, R. (2010). "Energy and the food system." *Philosophical Transactions of the Royal Society B*, 365(1554), 2991-3006.
- Zaleski, K. J., Josephson Karen L., Gerba Charles P. and Pepper Ian L. (2005). "Potential regrowth and recolonization of salmonellae and indicators in biosolids and biosolid-amended soil." *Applied and Environmental Microbiology*, 71(7), 3701-3708.

**APPENDIX A. SAMPLING EVENTS AND TESTS PERFORMED**

<b>Date*</b>	<b>Sample ID<sup>s</sup></b>	<b>Sample Location</b>	<b>pH</b>	<b>Total Solids</b>	<b>Volatile Solids</b>	<b>Fecal Coliform</b>	<b>SOUR</b>	<b>Ammonia</b>	<b>Matrix Spike</b>
26 Jun	B1	BW	X	X	X	X			
3 Jul	W1	WP	X	X	X	X			
12 Jul	S1	SP	X	X	X	X			
1 Aug	S2	SP	X	X	X	X			
7 Aug	W2	WP	X	X	X	X			
14 Aug	B2	BW	X	X	X	X			
12 Sept	S3	SP	X	X	X	X			X
26 Sept	W3	WP	X	X	X	X			
10 Oct	B3	BW	X	X	X	X	X		
17 Oct	W4	WP	X	X	X	X	X		
1 Nov	B4	BW	X	X	X	X	X	X	
7 Nov	S4	SP	X	X	X	X	X	X	
15 Nov	W5	WP	X	X	X	X	X	X	
20 Nov	S5	SP	X	X	X		X	X	
27 Nov	W6	WP	X	X	X		X	X	

MPN – Most probable number;

\* All collection dates were in 2018; <sup>s</sup>All sample locations were part of the King County Metro Wastewater Treatment Division in Washington State. BW, Brightwater Treatment Plant; WP, West Point Treatment Plant; SP, South Treatment Plant.



## APPENDIX B. INITIAL CHARACTERIZATION OF BIOSOLIDS

Sample ID	Date*	Sample Location <sup>s</sup>	pH	Total Solids (%)	Volatile Solids (%)	Fecal Coliform (MPN/g)	Fecal Coliform (MPN/g) 95% Confidence interval		Ammonia (mg/kg dry)
							Lower Limit	Upper Limit	
B1	26 Jun	BW	8.59 ± 0.02	19.31 ± 0.1	83.64 ± 0.1	112,000	35,000	340,000	NA
W1	3 Jul	WP	8.86 ± 0.02	27.59 ± 0.3	69.18 ± 0.3	87,000	28,000	280,000	NA
S1	12 Jul	SP	8.84 ± 0.03	23.50 ± 0.1	71.00 ± 0.01	194,000	66,000	510,000	NA
S2	1 Aug	SP	8.57 ± 0.03	17.79 ± 0.1	75.81 ± 0.2	73,000	18,000	175,000	NA
W2	7 Aug	WP	8.45 ± 0.1	25.08 ± 0.1	71.78 ± 1.6	197,000	67,000	510,000	NA
B2	14 Aug	BW	8.41 ± 0.1	18.87 ± 0.05	84.35 ± 0.2	73,000	19,000	180,000	NA
S3	12 Sept	SP	8.88 ± 0.1	21.52 ± 0.4	76.07 ± 0.1	209,000	41,000	370,000	NA
W3	26 Sept	WP	8.53 ± 0.1	26.99 ± 0.1	70.63 ± 0.7	89,000	28,000	282,000	NA
B3	10 Oct	BW	8.74 ± 0.04	20.48 ± 0.1	84.23 ± 0.2	83,000	23,000	225,000	NA
W4	17 Oct	WP	8.53 ± 0.1	26.32 ± 0.2	69.52 ± 0.8	300,000	94,000	720,000	NA
B4	1 Nov	BW	8.56 ± 0.02	19.89 ± 0.1	83.14 ± 0.1	120,000	38,000	384,000	NA
S4	7 Nov	SP	8.90 ± 0.1	21.76 ± 0.1	77.02 ± 0.3	110,000	35,000	350,000	6931 ± 170
W5	15 Nov	WP	8.89 ± 0.1	26.21 ± 0.1	69.11 ± 0.4	126,000	43,000	359,000	9623 ± 712
S5	20 Nov	SP	8.60 ± 0.1	21.81 ± 0.3	75.44 ± 0.2	NA	NA	NA	10530 ± 542
W6	27 Nov	WP	8.49 ± 0.1	26.48 ± 0.2	68.04 ± 0.4	NA	NA	NA	7666 ± 453

MPN – Most probable number; NA – Not analyzed

\* All collection dates were in 2018; <sup>s</sup>All sample locations were part of the King County Metro Wastewater Treatment Division in Washington State. BW, Brightwater Treatment Plant; WP, West Point Treatment Plant; SP, South Treatment Plant.

## APPENDIX C. CHARACTERIZATION OF TREATED BIOSOLIDS

Lime (%)	Temperature													
	25 °C							40 °C						
	ID	pH	TS (%)	VS (%)	FC	FC 95% Confidence interval		ID	pH	TS (%)	VS (%)	FC	FC 95% Confidence interval	
						Lower limit	Upper limit						Lower limit	Upper limit
0		NA	NA	NA	NA	NA	NA	W4	8.6 ± 0.04	26.8 ± 0.2	68.8 ± 0.7	123,000	81,000	227,000
5	W4	9.3 ± 0.05	28.2 ± 0.1	65.3 ± 0.4	33,000	23,500	45,000	B3	9.3 ± 0.1	21.5 ± 0.1	79.9 ± 0.5	6,500	4,800	9,500
10	S2	9.8 ± 0.02	19.8 ± 0.4	68.2 ± 0.2	4,000	2,800	5,500	B3	9.8 ± 0.1	21.9 ± 0.4	78.4 ± 2.3	3,600	2,500	5,000
15	B1	10.7 ± 0.3	21.2 ± 0.05	73.6 ± 0.1	16,000	10,500	32,000	B3	10.4 ± 0.05	22.3 ± 0.1	77.4 ± 0.8	1,500	1,000	2,700
20	B1	12.2 ± 0.03	22.3 ± 0.9	68.7 ± 4.7	4,100	2,900	5,700		NA	NA	NA	NA	NA	NA
25	W4	12.3 ± 0.1	31.8 ± 0.1	53.8 ± 0.1	900	600	1,600		NA		NA	NA	NA	NA

FC – Fecal Coliform (MPN/g); MPN -Most probable number; TS – Total Solids; VS – Volatile Solids; NA- not analyzed

## APPENDIX C. CHARACTERIZATION OF TREATED BIOSOLIDS

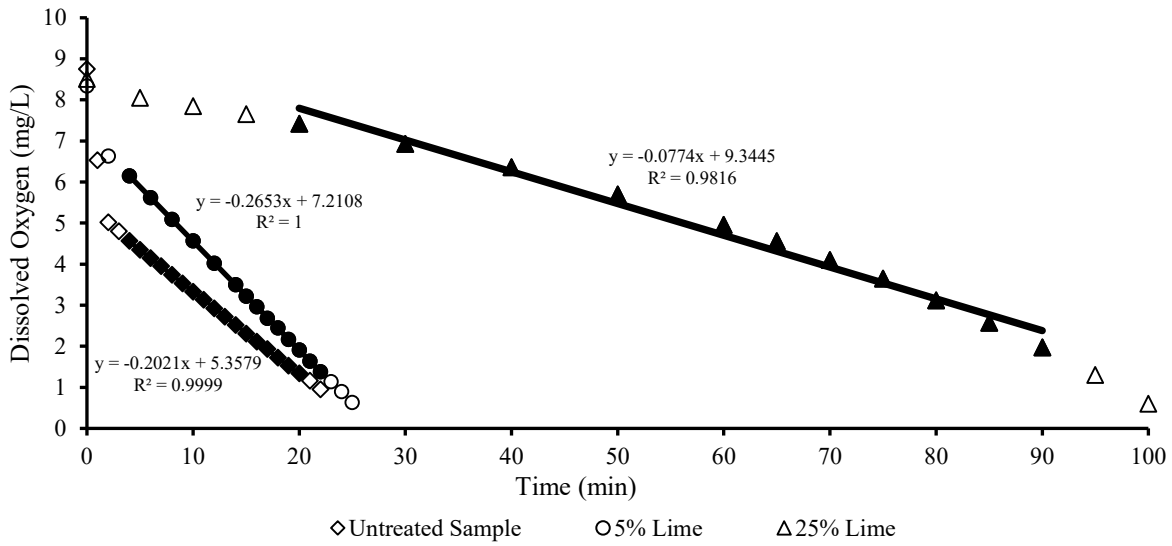
Lime (%)	Temperature													
	50 °C							60 °C						
	ID	pH	TS (%)	VS (%)	FC	FC 95% Confidence interval		ID	pH	TS (%)	VS (%)	FC	FC 95% Confidence interval	
						Lower limit	Upper limit						Lower limit	Upper limit
0	B4	8.8 ± 0.1	20.8 ± 0.4	83.2 ± 0.4	44,000	31,000	66,000	S2	8.8 ± 0.1	18.9 ± 0.6	75.7 ± 0.1	29,000	10,000	79,000
5	W2	9.5 ± 0.1	26.4 ± 0.1	69.1 ± 0.2	1,900	630	4,800	W3	9.7 ± 0.04	27.9 ± 0.6	67.6 ± 1.2	600	180	1,700
	B2	9.6 ± 0.1	19.5 ± 0.1	82.4 ± 0.2	12,000	3,900	39,000	B4	9.4 ± 0.1	21.5 ± 0.1	79.1 ± 0.1	800	200	2,300
10	W2	10.6 ± 0.1	67.4 ± 0.4	67.4 ± 2.4	600	170	1,700	S1	9.9 ± 0.1	26.1 ± 0.1	64.1 ± 0.4	50	12	120
	B2	10.4 ± 0.03	22.0 ± 0.7	74.9 ± 0.9	800	220	2,100	W3	10.2 ± 0.1	29.8 ± 0.3	62.9 ± 0.5	150	10	400
		NA	NA	NA	NA	NA	NA	B4	10.0 ± 0.1	22.2 ± 0.2	75.0 ± 1.4	200	75	570
15	B2	11.6 ± 0.02	21.9 ± 0.1	72.1 ± 0.2	400	50	850	W1	12.2 ± 0.05	30.6 ± 0.1	60.6 ± 0.8	15	1	40
	W5	10.9 ± 0.1	29.3 ± 0.4	59.8 ± 1.0	800	260	2,600	S4	11.0 ± 0.2	22.8 ± 0.1	72.2 ± 1.4	60	15	150
20		NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	NA
25		NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	NA

FC – Fecal Coliform (MPN/g); MPN -Most probable number; TS – Total Solids; VS – Volatile Solids; NA- Not analyzed

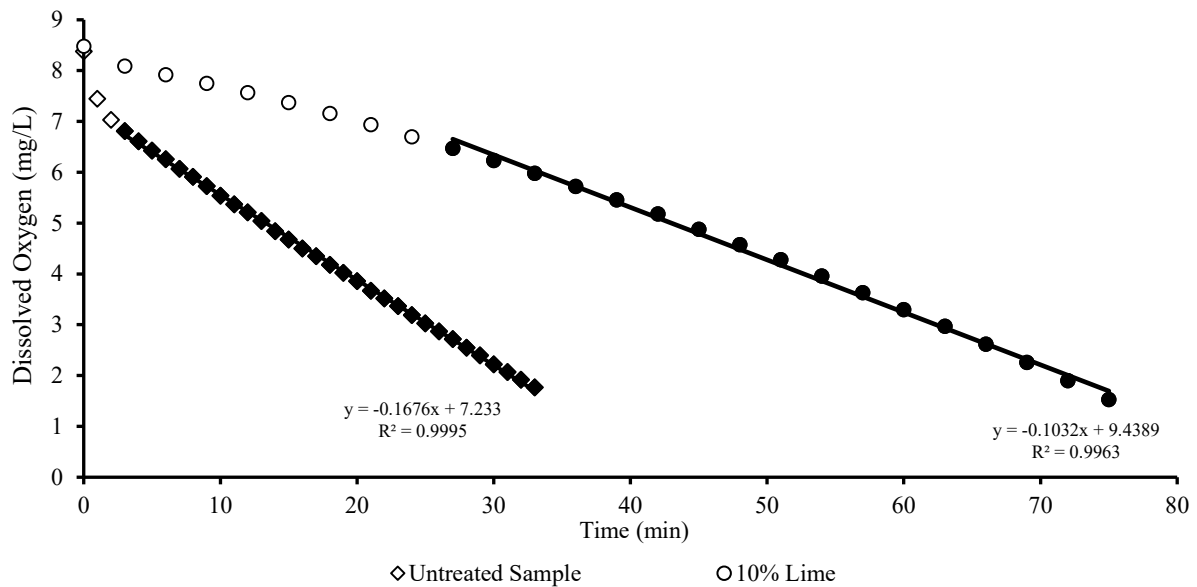
**APPENDIX D. OXYGEN UPTAKE EXPERIMENTAL RESULTS USED FOR SOUR CALCULATIONS.**

**Temperature - 25 °C**

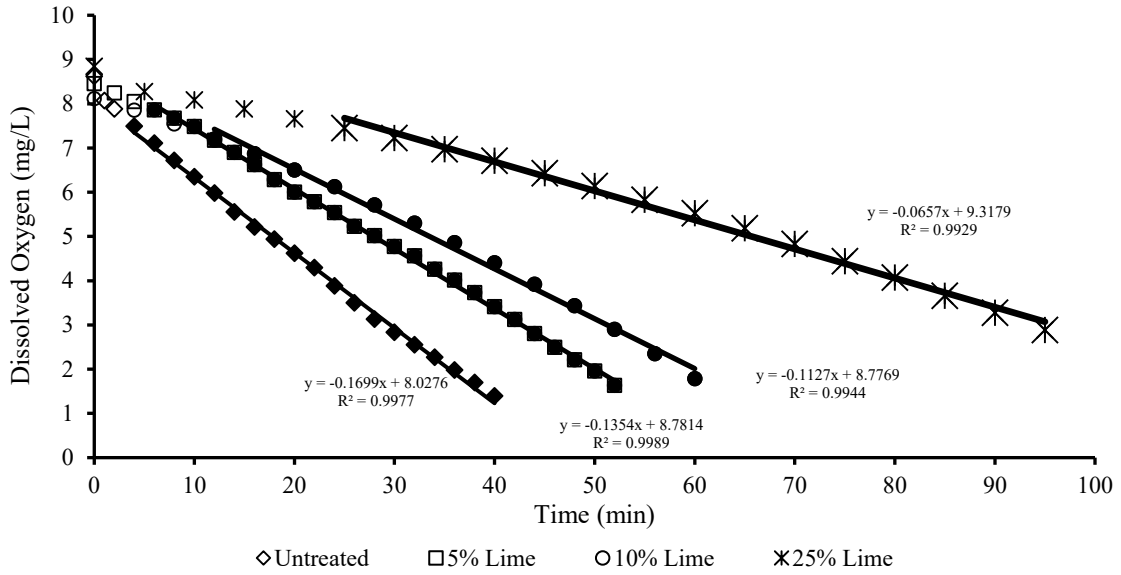
Sample ID – W4



Sample ID – W5

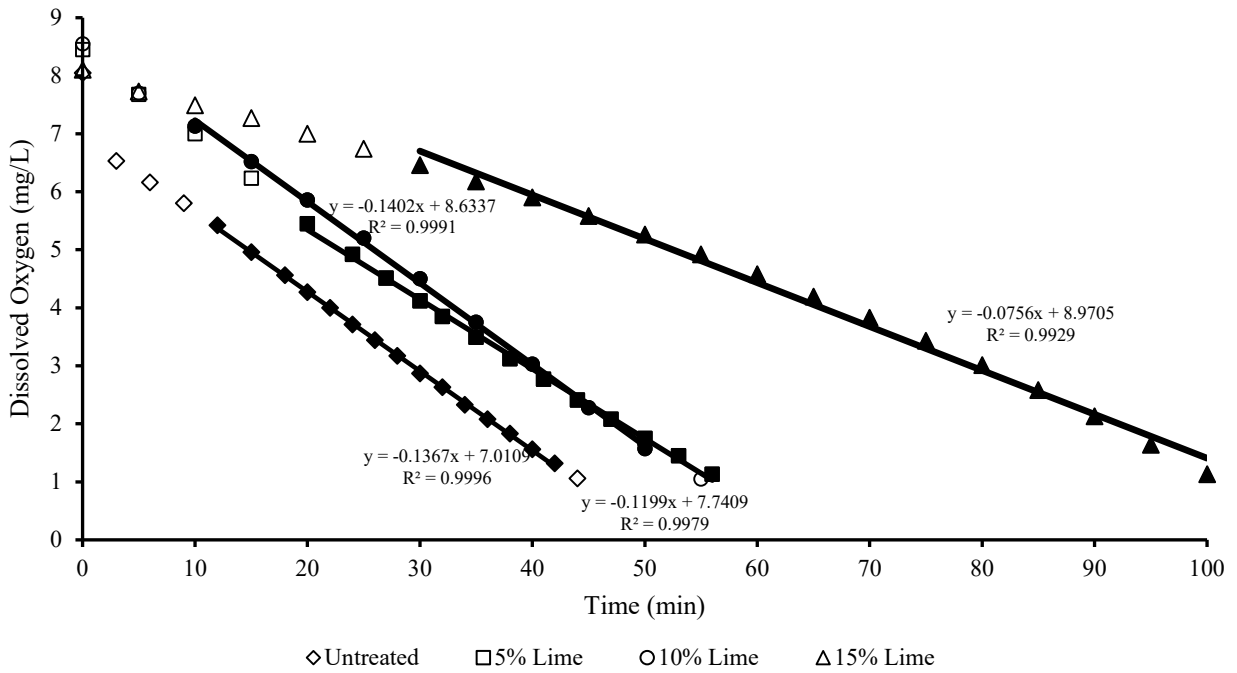


Sample ID – W6

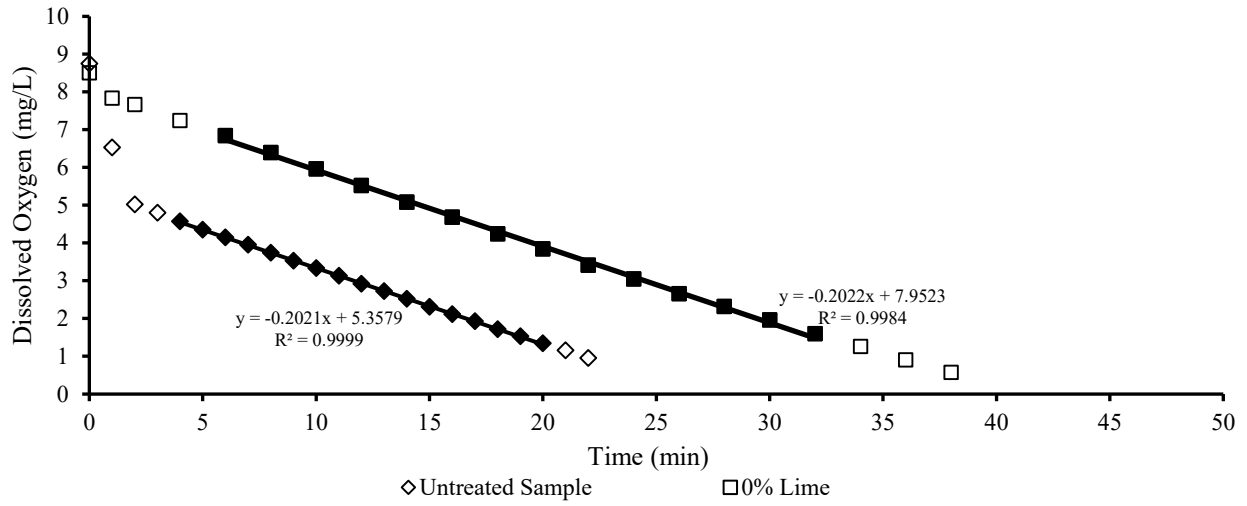


Temperature - 40 °C

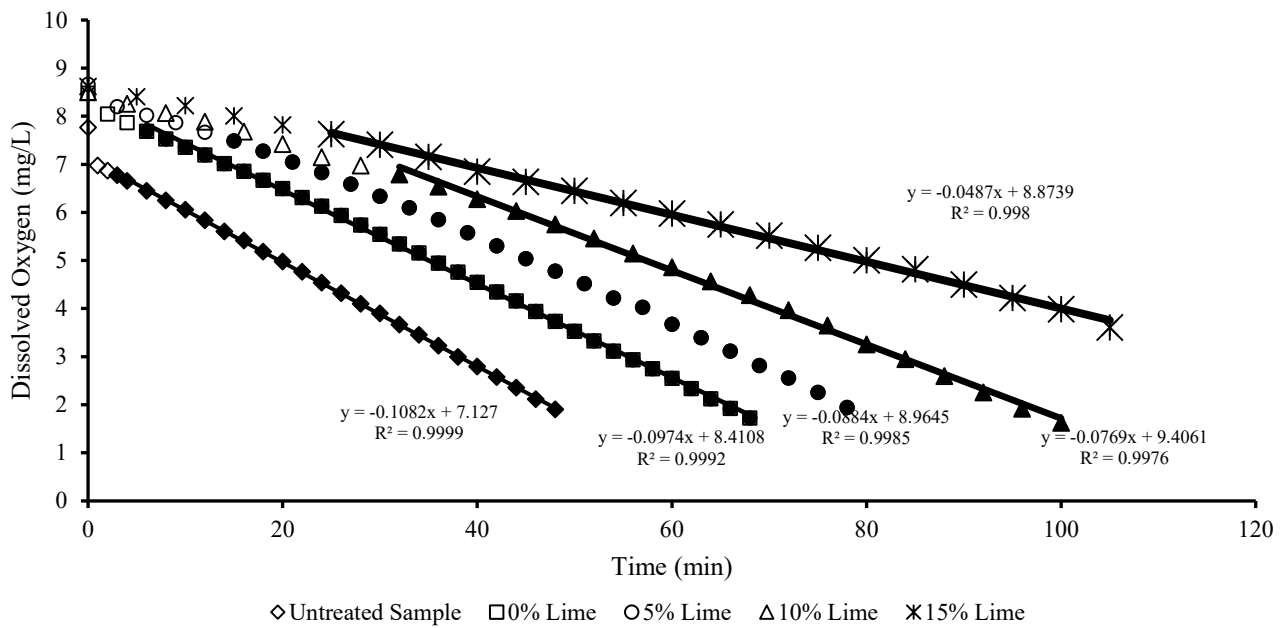
Sample ID – B3



Sample ID – W4

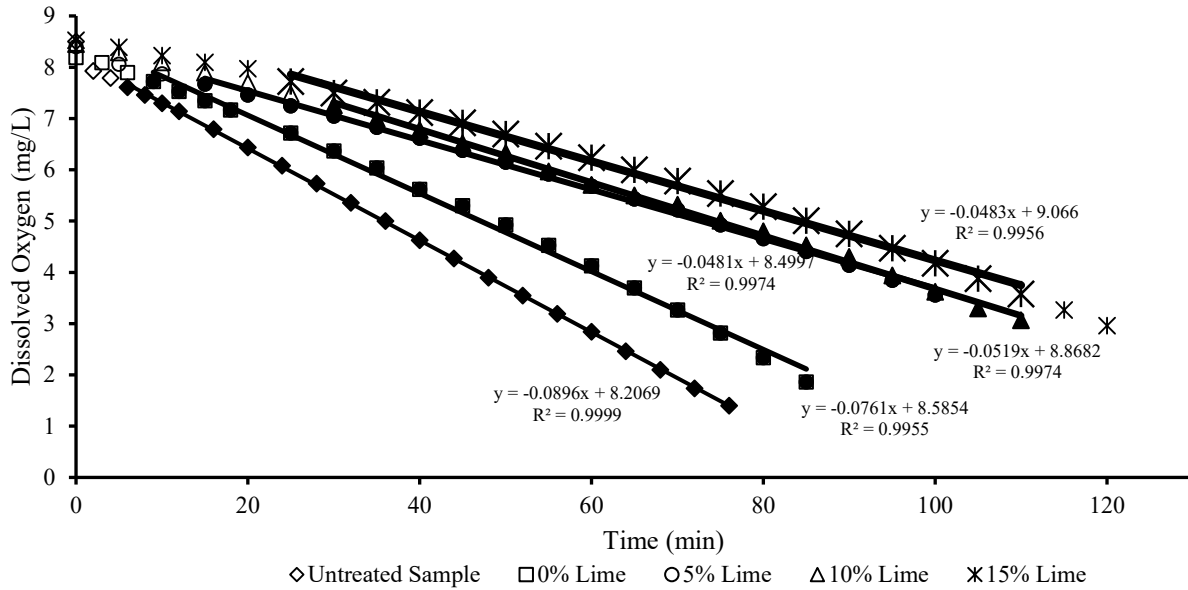


Sample ID – S5

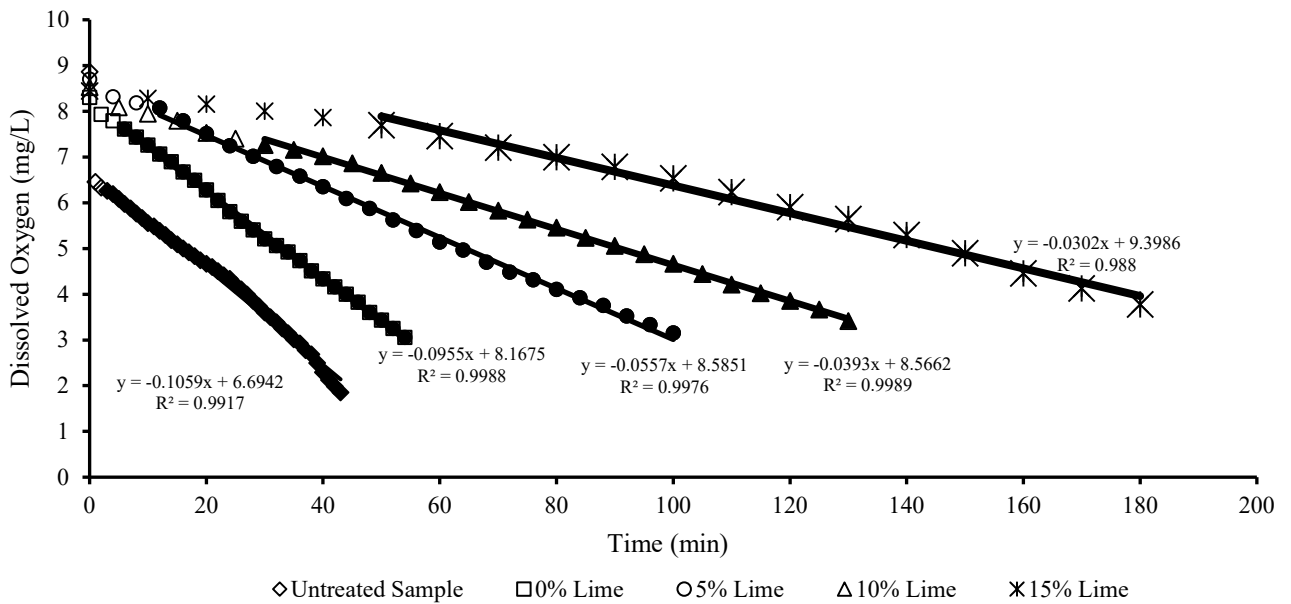


Temperature - 50 °C

Sample ID – B4

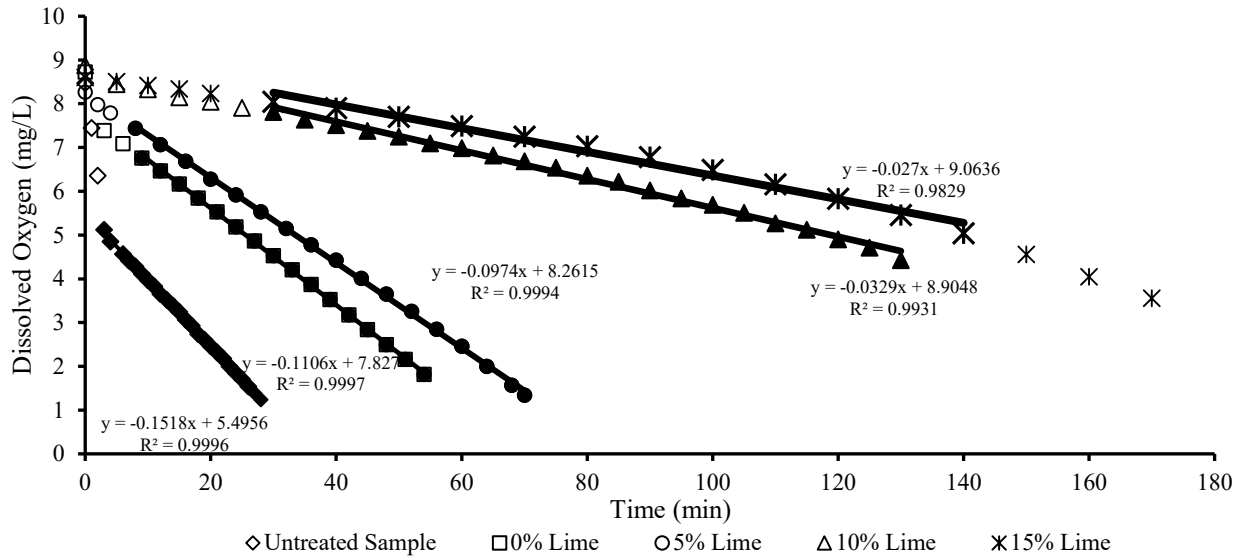


Sample ID – S5

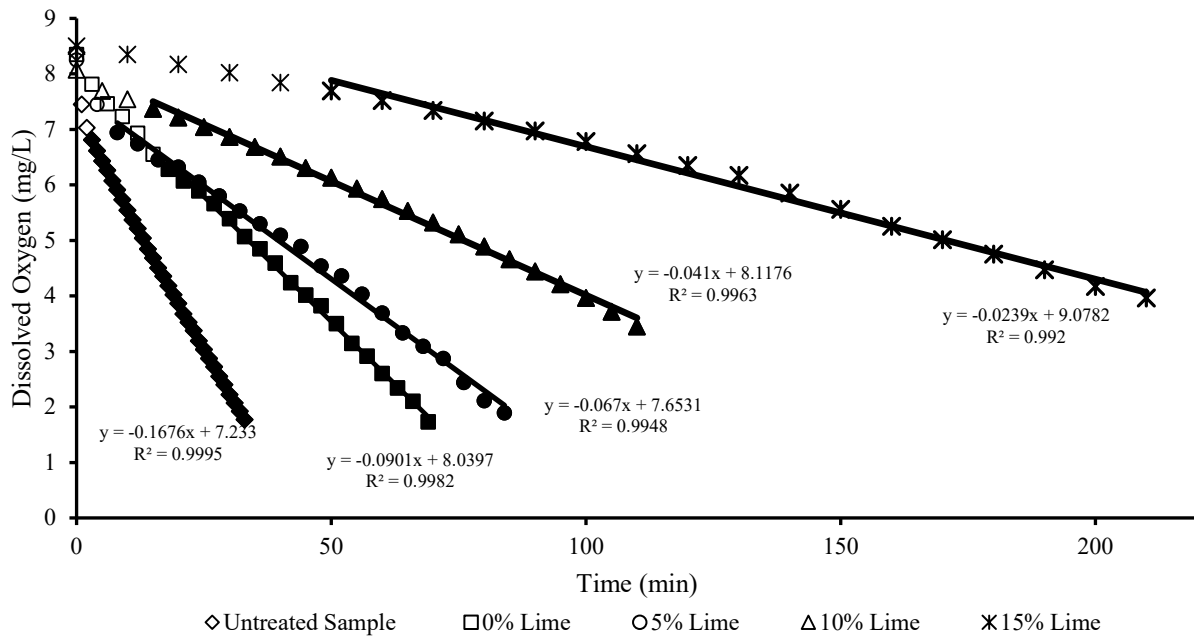


Temperature - 60 °C

Sample ID- S4



Sample ID – W5





## APPENDIX E. VOLATILE SUSPENDED SOLIDS OF POLYSEED SOLUTION

<b>SOUR Tested with Sample ID</b>	<b>Volatile suspended solids (mg/L)</b>
B3	$37.5 \pm 1.5$
W4	$39.6 \pm 2$
B4	$48.8 \pm 4$
S4	$47.0 \pm 4$
W5	$52.0 \pm 1$
S5	$40.0 \pm 4$
S5	$36.8 \pm 7.2$
W6	$62.8 \pm 4.4$

## APPENDIX F. NORMALIZED AMMONIA CONCENTRATIONS

