Utilization of Aqueous Product Generated by Hydrothermal Carbonization of Waste Biomass

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Georgiy V. Vozhdayev

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Dedication

This thesis is dedicated to my Mother, Maya Vozhdayeva. Thank you for having the strength to raise two children, alone in a foreign land, and for pushing us to succeed and aspire for our dreams. Love George

Abstract

Hydrothermal carbonization (HTC) is a thermochemical treatment process that allows for the conversion of relatively dilute biomass slurries into value added products which are hydrochar and filtrate. This investigation focuses on the potential for utilization of the filtrate (aqueous by–product) created via HTC. A majority of the research to date has focused on the solid HTC product (hydrochar), however little attention has been paid to the utilization of the HTC filtrate, which makes up the larger mass fraction. Finding value added products is key to making the process a viable treatment option for waste biomass and other organic by-products.

The option of using HTC filtrate as a fertilizer replacement for agricultural crop production was evaluated through studies of soil microbial effects and impacts on seed germination and early plant growth. These studies confirmed bio-toxicity effects of HTC filtrate on agricultural soil microbes at high application rates. On the other hand, lower rates of application induced biodegradation of the phytotoxic components of the filtrate and released additional plant nutrients through N-mineralization. These effects are dependent on filtrate type, concentration, and post-treatment of the applied filtrate. Phytotoxicity effects on seed germination and seedling growth of corn *(Zea mays L.)* also showed a dependence on HTC filtrate source and concentration. Similar to the impacts observed on the soil microbes, high concentration typically inhibited seed germination and growth, but lower concentrations stimulated early corn growth. Characterization of the filtrates via a 2-dimensional gas chromatography (GC) time-of-flight mass spectrometry confirmed a very complex chemical fingerprint of the filtrates. Chemical speciation in the filtrate appeared to be a function of the feedstock. More

importantly, the simple storage of filtrate in an open container for 90 days drastically alters the chemical species composition and correspondingly the observed impact on soil microbes and plant growth, leading to the conclusion that there could be chemical inhibitors present in the filtrate that are responsible for the observed effects that are eliminated though simple volatilization or microbial mineralization during storage.

This work shows great promise for utilization of HTC filtrates as an agricultural fertilizer and the recycling of critical plant nutrients. Additional work is needed to fully characterize the chemical diversity present in these filtrates prior to the implementation of this renewable and sustainable source of agricultural fertilizers.

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CHAPTER 1

INTRODUCTION TO HTC PROCESSING

1.1 HTC Background

Increased use of biomass for energy production has the potential to offset fossil fuel use. In fact, forecasts have suggested that biomass will be capable of providing approximately 25% of the world's energy needs by 2035 (IEA, 2011). There are several thermochemical conversion technologies that are being pursued in this quest to harvest the energy contained in biomass (Yılmaz & Selim, 2013), of which, gasification, pyrolysis, and hydrothermal carbonization (HTC) are the fundamental techniques.

Gasification is a high temperature process (temp. ~ 600-1000 ^{o}C) that uses a previously dried biomass feedstock in the presence of an oxidizing agent such as oxygen, to produce syngas [carbon monoxide (CO), hydrogen (H_2), carbon dioxide (CO_2) and methane (CH_4)] for energy production or a feedstock for chemical synthesis (A. Kumar, Jones, & Hanna, 2009; Wang, Weller, Jones, & Hanna, 2008). Gasification optimizes the production of energy; therefore typically the two by-products are syngas and ash (which is a low carbon, inorganic residual). The next technique is thermal pyrolysis, which processes biomass between 400 – 600 ^{o}C in the absence of oxygen (Judy A Libra et al., 2011), and creates a carbonized solid referred to as biochar, a liquid phase referred to as bio-oil, and gases (Balat, Balat, Kırtay, & Balat, 2009; Judy A Libra et al., 2011).

The least thermally aggressive technology is HTC, which is particularly advantageous for high moisture content substrates because it eliminates costly drying operations required by other thermochemical operations such as gasification and pyrolysis (Libra et al., 2011). With HTC, biomass slurries and emulsions are subjected to

moderate temperature and typically autogenous (self-generated) pressure to achieve chemical conversion into gas, solid, and liquid products (Axel Funke & Ziegler, 2010). HTC product distributions are a function of the process temperature (180 – 250 ^{o}C), time (0.5 – 24 h), and the chemical composition of the starting material, particularly its cellulose content (Judy A. Libra et al., 2011; Lu, Pellechia, Flora, & Berge, 2013). Unlike thermal pyrolysis where the gaseous products can be >30%, gas production is a minor component in HTC (<5%). Due to the typically sealed reaction chamber during HTC (Figure 1.1), the process optimizes the formation of liquid and solid products (Judy A. Libra et al., 2011; Brandon M. Wood et al., 2013), as well as reducing gaseous product losses. This lack of gaseous losses results in higher retention of C and N in the liquid and solid products. The solid product is called *hydrochar* and is a carbonized material similar in composition to lignite coal, but with improved energy content. Hydrochar also contains a higher amount of C and has improved sorption properties compared to the original feedstock (Fuertes et al., 2010). The residual liquid phase generated by the HTC process is referred to as the *filtrate*.

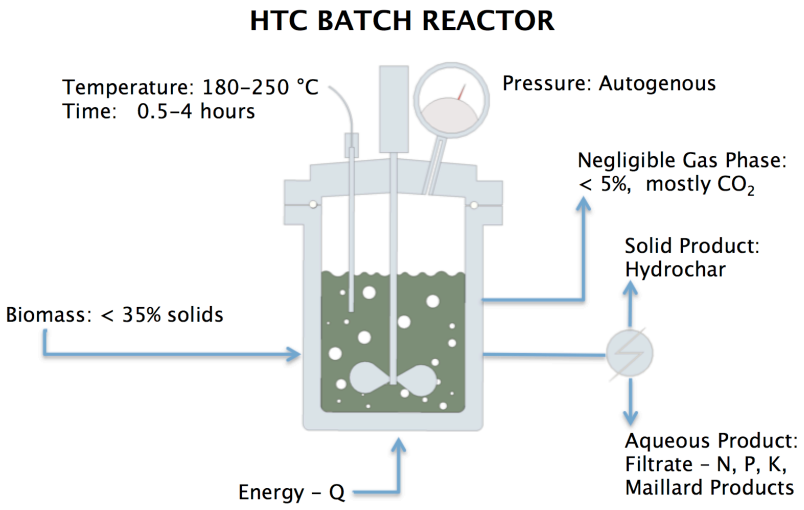


Figure 1.1 A process flow diagram of the hydrothermal carbonization process

A variety of waste streams have been processed using HTC, including human municipal waste sludge (Saetea & Tippayawong, 2013) and agricultural waste streams (i.e. cattle/dairy, swine, and poultry manures) (Cao, Ro, Chappell, Li, & Mao, 2011; Jandl et al., 2013; Judy A. Libra et al., 2011). Animal manures have been favored as a raw feedstock for soil amendments because they can increase the fertility and productivity of agricultural lands (Azevedo & Stout, 1974; Fuertes et al., 2010; Nelson, Agudelo, Yuan, & Gan, 2011; M. M. Titirici, Thomas, & Antonietti, 2007). However, HTC products may be a more favorable soil amendment because the process conserves the essential nutrient composition of the feedstock compared with other thermal pyrolysis techniques (Levine et al., 2013; Brandon M. Wood et al., 2013) and has the added advantage of sterilizing potentially pathogenic waste streams (Judy A. Libra et al., 2011). Additionally, HTC can mitigate environmental effects of antibiotics and hormones (Miyamoto, Li, Kibushi, Yamasaki, & Kasai, 2008), which have recently been observed to be transferred into the environment from manures (Khachatourians, 1998; K. Kumar, Gupta, Baidoo, Chander, & Rosen, 2005).

1.2 Liquid Products of Thermochemical Processes

The conversion of biomass by thermo-chemical processing has a very long history, dating back to the earliest period of our ancient civilizations. The Egyptians used the liquid product from pyrolysis as an embalming fluid, since it was known to slow microbial degradation reactions (Baumann, 1960; Lucas, Harris, & Harris, 1962). The collection of such liquids through pyrolysis condensate has been termed “wood vinegar”, In the modern era of science, there has been an extensive characterization and evaluation of its potential uses as a soil amendment, pesticide, antibiotic, fungicide, and plant

growth stimulant (Harada et al., 2013; Mu, Uehara, & Furuno, 2004; Xu, Chen, & Cao, 2006) (Hawley, 1926; Mac Culloch, 1814; Mu et al., 2004; Yatagai, Nishimoto, Hori, Ohira, & Shibata, 2002).

However, pyrolysis liquids do differ in chemical composition compared to HTC filtrates even for the same feedstock (Karagöz, Bhaskar, Muto, & Sakata, 2005; Mohan, Pittman, & Steele, 2006), and while much attention has been focused on generation of products derived from hydrochars, considerably less attention has been directed to the development of value-added liquid products from the HTC filtrate (Goudriaan & Peferoen, 1990; Steven M. Heilmann, Jader, Sadowsky, et al., 2011; Humphrey, 1979; Russell, Molton, & Landsman, 1980; Saetea & Tippayawong, 2013).

1.3 Phosphorus – Limited Supply, Increasing Demand

One of the variables that affect the human carrying capacity is our overall ability to maintain agricultural crop production at a rate that can sustain our growing populations. Subsequently, crop production depends on our ability to provide the essential nutrients through fertilizers, such as nitrogen, phosphorus and potassium, which are necessary for ensuring healthy plant growth (Foth & Ellis, 1997). The importance of having renewable sources for plant nutrients was first recognized in the early 1900's, as world populations began to increase due to the onset of the industrial revolution (Hall, 1910). In that time, guano as a soil amendment was the primary source of nitrogen (Clark, 1845), and as crop production increased with our first population boom, guano became a scarce commodity. The lack of this nitrogen fertilizer spurred a wave of panic and visions of doom (Clark, 1845). The Haber-Bosch process solved the issue of diminishing guano reserves, by creating a high pressure, high temperature conversion of

atmospheric N_2 into ammonium fertilizer (Erisman, Sutton, Galloway, Klimont, & Winiwarter, 2008). The invention earned Dr. Haber a Nobel Prize, and has allowed agricultural production to meet the needs of our exponentially growing global population. However, several new challenges have arisen.

One of these challenges is the source of phosphorus. Current methods for phosphorus production are not renewable, and are heavily dependent on mining of geologic phosphate rock (Van Kauwenbergh, 2010; Zerulla et al., 2001). Despite the finite nature of this commodity, our population continues to grow, and with it, the rate of phosphate consumption (Schröder, Cordell, Smit, & Rosemarin, 2010). As with all finite reserves, experts are predicting that we will run out of available phosphate rock, with estimates varying from a few decades to a few hundred years (Schröder et al., 2010). HTC has the potential to become a method for nutrient reclamation, especially phosphate, which becomes a more apparent need as we watch our population numbers continue to rise, and reserves of mineable phosphate rock diminish.

1.4 Research Overview

Hydrothermal carbonization (HTC) can be an important tool in the search for renewable phosphorus supplies (Dai et al., 2014; A Funke, Mumme, Koon, & Diakité, 2013; Judy A Libra et al., 2011). Since the aqueous phase of the HTC process is a major fraction of final products (70 – 90%), coupled with the fact that the majority of nitrogen and phosphorus of the starting material remains in the filtrate (Steven M. Heilmann, Jader, Harned, et al., 2011; Steven M. Heilmann, Jader, Sadowsky, et al., 2011), it is important that a useful and beneficial application of this by-product be developed in order to take full advantage of the benefits of HTC treatment of waste biomass. Wood (2013)

showed that HTC filtrate can be treated via anaerobic digestion for the production of methane (B. M. Wood et al., 2013). However, other products and treatment options for HTC filtrate may be more valuable in reducing the agricultural carbon footprint and moving closer to sustainable agricultural practices.

Having beneficial options for utilization of the aqueous phase generated via hydrothermal carbonization (HTC) of biomass is key to making this process economically viable. Caution must be exercised, however, as recent studies have indicated that hydrochars and their companion filtrates might possess phytotoxic compounds that have negative consequences, such as inhibition of various types of seed germination and reduced plant growth (I. Bargmann, M. C. Rillig, W. Buss, A. Kruse, & M. Kuecke, 2013a; George, Wagner, Kücke, & Rillig, 2012; Jandl et al., 2013). In addition, release of pyrolysis liquids into the environment can result in soil polycyclic aromatic hydrocarbon (PAH) contamination (Oleszczuk et al., 2014) which is a mistake we need to avoid making with HTC liquids.

This thesis deals with the question of whether the aqueous byproduct (HTC filtrate) can be used as a fertilizer for agricultural crop production. The topic is broken down into three separate critical research areas that need to be addressed prior to the utilization of HTC filtrate as an agricultural fertilizer.

The first issue (Chapter 2) examines the potential bio-toxicity effects and biodegradability of HTC filtrate by soil microbes. In this research, two different feedstocks [swine manure and corn distillers thin sillage (CDS) from a dry grind corn-ethanol plant] were tested at various direct field application levels. In addition, the effect of storage was also examined. Filtrates aged in both open and closed containers were

compared to fresh filtrates to see if there was any alteration in the response of soil microbes and seed germination.

The second topic (Chapter 3) directly assessed the impacts of the filtrates on plant growth, by first determining if the HTC filtrate is phytotoxic, and then whether there is potential to use it as an applied fertilizer by looking at plant germination and growth of corn *(Zea mays L.)* in response to three different HTC filtrate types [poultry, swine manure, and corn distillers thin sillage (CDS)]. To accomplish these objectives, filtrates were applied at varying rates to silica sand at different concentrations and different post treatment aging conditions.

The third issue (Chapter 4) addresses the complex chemical nature of the filtrate via analysis on a 2-dimensional gas chromatograph – time of flight mass spectrometry (2DGC–TOFMS), and discusses method development that was performed to better understand the complex matrix that each filtrate possesses. Such characterization is essential in order to resolve whether toxic compounds are present, and if so, in what concentrations.

Finally, there are some closing thoughts on where this research could lead and the potential for the use of HTC in treating select biomass wastes to recover an agricultural fertilizer product.

CHAPTER 2

SOIL INCUBATION: BIODEGRADABILITY OF HTC FILTRATE BY SOIL MICROBES

2.1 INTRODUCTION

Several studies to date have focused on the microbial impacts of hydrochar (the solid HTC phase) as a soil amendment, with soil incubations to determine toxicity on soil microorganisms. (Bargmann, Rillig, Kruse, Greef, & Kücke, 2014a; Rillig et al., 2010). However, only two publications have been identified that incorporated HTC filtrate into the studies; the first of which utilized HTC waters by first mixing them with the respective hydrochar and other organic raw materials, and then subjecting the mix to digestion in a compost heap prior to soil application (Busch, Stark, Kammann, & Glaser, 2013). The second, is the only study identified to date, that has attempted to determine the effect of only HTC filtrate application as well as filtrate aging on plant germination and growth (I. Bargmann, M. C. Rillig, W. Buss, A. Kruse, & M. Kuecke, 2013b). Other than those identified works, very limited research has been placed on the aqueous HTC filtrate with regards to its use as a soil amendment.

HTC filtrate is enriched with (inorganic) fertilizer constituents (N, P, K) (Steven M. Heilmann, Jader, Harned, et al., 2011; Steven M. Heilmann, Jader, Sadowsky, et al., 2011) due to the sealed nature of HTC reactors, whereas other processing techniques allow some of the contained nutrients to volatilize and escape (Reza, Lynam, Uddin, & Coronella, 2013). For this reason, there is potential for utilizing this liquid product as fertilizer for agricultural purposes. Understanding chemistry within agricultural soil in

relation to HTC filtrate application is an essential step in determining the feasibility and sustainability of this treatment option. It is imperative that filtrates are well characterized and the effects of the chemical constituents within the HTC filtrates on soil microbes and plant growth are well understood. This is required in order to avoid negative impacts on the soil microbial populations in the filtrate-amended soils.

This chapter considers how filtrate composition affects the metabolic rate of agricultural soil microbes, which is assessed through the monitoring of carbon dioxide (CO_2) production N-mineralization rates were also quantified, which occur with varying applications of filtrate concentration and type. Past research conducted with wood vinegar and hydrochar with regard to effects on soil microbial populations have shown that primary variables that affect soil bacteria are amendment type and concentration. Furthermore, it has been shown that a post treatment of hydrochar (such as aging) also diminishes toxic effects (Bargmann, Rillig, Kruse, Greef, & Kücke, 2014b). It's therefore hypothesized that an optimum HTC filtrate type and concentration will be found that will enhance microbial soil activity, and that aging of the filtrate will reduce the initial toxic impacts.

To understand how filtrate type and concentration affect the metabolic rate of agricultural soil microbes, varying concentrations of two types of HTC filtrates were used. Filtrates were generated through HTC treatment of swine manure and condensed distiller solubles (CDS) from the dry-grind ethanol industry and applied to an agricultural soil. These treatments were sealed in 125 mL serum vials. These vials were then incubated at room temperature. The headspace gas of the vials was sampled periodically, and microbial activity was gauged via analysis of levels of CO_2, O_2, nitrous oxide (N_2O),

and methane (CH_4), which are generated or consumed as filtrates undergo microbial digestion. Examination of the CO_2 production versus O_2 consumption allows for interpretation of microbial activity and overall bio-degradability of the HTC filtrates. Furthermore, tracking CH_4 & N_2O production allows for insight to overall production of these greenhouse gases (GHG's) in response to soil amendment with HTC filtrate.

Nitrous oxide, which is a highly potent GHG, is emitted when people add nitrogen to the soil through the use of synthetic fertilizers (EPA.gov). Agriculture is the largest anthropogenic source of N_2O emissions in the United States, accounting for about 75% of total U.S. N_2O emissions in 2012, (EPA.gov). Production of CH_4 is a result of the microbial process of methanogenesis, which has also been significantly increased in the past century by anthropogenic activities (A. Chan & Parkin, 2001). The emissions of these GHG's have been shown to linger in our atmosphere, where they have a 298 and 25 times greater global warming potential than CO_2, respectively (Mojeremane, 2013). It is therefore important to understand the GHG response to HTC filtrate amendment, so there are no secondary detrimental environmental impacts, such as increases in GHG emissions.

The compellation of this data will document the impact of HTC filtrate on soil microbial activity, and will benefit future research efforts, such as life cycle analyses with regards to alteration in GHG emissions that could be produced through application of HTC filtrate as a fertilizer.

2.2 MATERIALS AND METHODS

2.2.1 HTC Filtrate Preparation

Swine manure and corn distiller's solubles (CDS) were used as starting materials for a 2-hour HTC run at 225 °C. All HTC reactions were conducted in a laboratory-scale stirred stainless steel reactor fitted with a heating mantel system (1000 mL; Parr Instruments, Inc.; Moline, IL). The raw feedstock was poured into the reactor, stirred at 88 RPM, and heated to the specified temperature for the defined time, as presented in Table 4.1. No supplemental pressure was applied (autogenous).

After reaction time was reached the heating mantel was disengaged from the HTC reactor, and the unit was cooled using a fan to 40°C. At this time, the reactor was disassembled and the contents were separated via filtration (VWR Filter Paper, 415. Cat 28320-020) (Wood et al., 2013). The end result was a solid hydrochar, and the aqueous filtrate products.

2.2.2 Filtrate Characterization

To remove any particulate matter that was formed via precipitation as the filtrates cooled, filtrates were filtered through a 0.45 μm filter (Pall Acrodisc PN 4184) prior to analysis for ammonium, nitrate and phosphate via a Lachat auto analyzer (Lachat Instruments, Loveland, CO). Filtrate pH was taken in the undiluted state. Forty mL's of unfiltered filtrate was analyzed for Total Carbon (TC) via a UV-persulfate TOC analyzer (Tekmar Dohrmann - Phoenix 8000, Mason, Ohio).

2.2.3 HTC Filtrate Aging

After collecting the HTC filtrate, a portion (450 mL) was used to evaluate the impact of aging, by simulating the effect of filtrate being stored in a tank prior to use.

Half of the allocated volume was used to set up a time trial to show an effect that would be analogous to storing filtrate in an open tank, and the other half was used to set up a time trial that would be analogous to filtrate being stored in a closed tank. To minimize the confounding effects of concentration differences between open and closed treatments due to evaporation, the open treatments were topped up every week with ultrapure HPLC water (Aqua Solutions, Deer Park, TX.; W1089-4L), until the total mass (volume) of the container was the same as it was at the start of the aging process. The mass of each open container undergoing an aging period was recorded before and after each H_2O addition. The filtrates were aged in this manner for 3 months.

2.2.4 Soil Incubation Preparation

Two separate incubations were conducted to test microbial activity of agricultural soil microbes in response to application of two types of HTC filtrates at various concentrations. The first experiment analyzed metabolic activity of soil bacteria in response to an application of fresh CDS and swine HTC filtrates, and the second looked at the metabolic activity in response to the same filtrates aged for three months in closed and open containers.

Both of these experiments were conducted in the same fashion. To evaluate microbial activity, 5 grams of sieved (<2 mm) agricultural soil (Rosemont, MN; bulk surface sample 0-5 cm; Waukegan silt loam) was placed into 125 mL glass vials (Wheaton) (Figure 2.1). A dilution series of each filtrate was then applied in triplicate to the serum vials in 1 mL volumes, using 50, 20, 10, 5, and 2 fold dilutions, as well as 1 mL of undiluted filtrate. A triplicate set of deionized water (di. H_2O) was used as a control of the microbial activity with no treatment, which consisted of 1 mL of di. H_2O

applied to each of the three 5-gram soil incubation vials. The vials were then sealed and allowed to incubate at room temperature (22 °C +/- 2 °C).

Production of CO_2 and co-current consumption of O_2 within the vials were the responses assumed to be related to the overall microbial activity, and the lack of which, an indication of any potential toxicity of the filtrates to the microbial functionality (Czimczik, Trumbore, Carbone, & Winston, 2006; Elmajdoub, Barnett, & Marschner, 2014; Vasconcellos et al., 2013). N_2O and CH_4 production / consumption was also monitored.

Figure 2.1 Illustration of an example set of soil incubations from this research. The three vials in the foreground are examples of the headspace sampler vials that were used for the actual sample analysis.

2.2.5 Gas Headspace Sampling and Analysis

The vial headspace was sampled periodically via a syringe for analysis. The syringe was purged seven times with atmospheric air between each sampling. To prevent a vacuum within the incubation vials, 5 mL of laboratory air was injected into each vial, then mixed (syringe was pumped up and down 5 times). After which, 5 mL of headspace gas was collected and injected into a secondary sealed 10 mL vial (Agilent), which was previously flushed with helium for 20 seconds at a rate of 2-3 $L\text{-}min^{-1}$.

The collected samples were then loaded into the headspace autosampler (HP 7694E; Palo Alto, CA), and analyzed for O_2, N_2, CO_2, CH_4, and N_2O via gas chromatography (GC). From each sample vial, three independent gas samples were transferred into three separate columns in two GC units (HP5890; Agilent) for the analysis of O_2, N_2, CO_2 (CTR-1, Grace, TCD), CH_4 (Porapak T; FID) and N_2O [Porapak Q; ECD with a naffion dryer (Permapure)] by separate sample loops. The system was checked daily for accuracy with NIST traceable gas standards (Minneapolis Oxygen, Minneapolis, MN).

When any of the vials reached anoxic levels (< 15% O_2), all vials were de-capped for 15 minutes, and allowed to equilibrate to atmospheric levels in order to replenish oxygen to atmospheric levels (approximately 21%). To calculate cumulative gas production or consumption graphs, interval production (the amount of gas produced or consumed after venting) was either subtracted or added to the cumulative production at the time of venting, depending on whether the gas was being produced or consumed.

The first incubation ran for 8 days, and was sampled on days 1, 2, 5, 6 and 8. The venting of the vials in incubation-1 occurred after being sampled on day 5. The second incubation ran for 13 days, and was sampled on days 1, 5, 8, 10, and 13. The vials of the second incubation were vented on day 7.

2.2.6 Soil Extractions for Nutrient Analysis

Following the soil incubation trials, all incubation vials were extracted to determine the amount of inorganic N (ammonium and nitrate) present. To perform the nutrient analysis, 30 mL's of 2M KCl was added to each of the 125 mL vials that contained the 5-grams of soil, and agitated on a reciprocal shaker for 30 minutes.

Following the extraction, the supernatant was filtered through medium porosity filter paper (Whatmann, Size no.1), and subsequently analyzed on the Lachat auto analyzer (Lachat Instruments, Loveland, CO) for ammonium and nitrate. The rate of mineralization was calculated by the following formula:

$$\text{N-Mineralization Rate} = \frac{(N_{total,final} - N_{total,initial})}{(\#\ of\ days)(mass\ of\ soil)},$$

Where $N_{total,final}$ is the sum of the inorganic N at the final extraction and $N_{total,initial}$ is the sum of inorganic-N at the start of the incubation. This N-mineralization rate allows for an assessment of the observed differences between the treatments as well as the overall assessment of soil N-mineralization dynamics.

2.2.7 Statistics

Means and standard deviations were calculated for all samples. All microbial and nutrient assessments were conducted in triplicate.

Independent variables evaluated were filtrate type, concentration, and aging effects, and dependent variables which underwent statistical analyses were concentrations of produced and consumed gases (CO_2, N_2O, O_2, CH_4) as well as N-mineralization rates, which were all used as proxies for microbial soil activity. One-way analysis of variance (ANOVA) was performed using GraphPad software (GraphPad Software Inc.) to determine statistical significance between means. A P-value of $P<0.05$ was used to assess statistical significance. If a significant difference was observed in the ANOVA, then pairwise comparison using the Tukey-Kramer Multiple Comparisons Test was conducted to assess individual factors. GC data from day 8 of incubation-1 was not available due to an auto-sampler malfunction, which resulted in data being lost on the day 8 samples from incubation set #1.

2.3 RESULTS

2.3.1. Cumulative CO_2 Production

As seen in Fig. 2.3 a comparison of 5-day cumulative CO_2 production of the fresh filtrates vs. aged filtrates showed that the CO_2 response to aged filtrates was equivalent to or greater than the CO_2 production observed for the fresh filtrates. This suggests that the aging did reduce the inhibitory effects and did increase mineralization rate of the filtrate. It should be noted that visible microbial growth was observed in the fresh 1/2X CDS treatments, as well as both open and closed 1/2X CDS trials during these incubations (Figure 2.2). Visible microbial growth also developed in the 1X CDS open treatments, however the phenology of the microbes differed between the treatments, and further genetic testing to assess differences in species was not conducted. Since this growth was not seen across all treatments, it can be concluded that the filtrate did induce differential responses in the microbial community as a function of concentration in addition to the difference in the CO_2 production.

2.3.1.1. CDS filtrate: Fresh vs. Aged in Open and Closed Containers

For CDS filtrates, the greatest production occurred in the 1/2X treatments for all CDS filtrates types, whether they were fresh or aged (Figure 2.3, Figures 2.5-2.7). A comparison between filtrates aged in closed vs. open containers showed little difference between the two post-treatment options, except for in 1X CDS treatments, where CO_2 production in the CDS 1X open treatment produced 67% more CO_2 ($P < 0.001$) than the CDS 1X closed treatment (Figure 2.4). This difference between CDS 1X filtrates aged in open vs. closed containers can also be seen in the different lag times as a function of filtrate concentrations (Figures 2.6 and 2.7), where production of CO_2 in the undiluted

[1X] CDS open treatment began on day 7, whereas production of CO_2 in the closed treatment didn't begin until after day 10. This suggests that aging the filtrate in an open container reduces toxicity due to the fact that microbes were able to mineralize this filtrate earlier than filtrates aged in a closed container.

Figure 2.2 Illustration of the observed microbial growth in 1/2X CDS vials (left) and the 1X CDS vials (right). The exact cause of this growth is unknown, but clearly demonstrates that there are differences in the microbial community as a function of concentration of the filtrates added to the soil incubations.

2.3.1.2. Swine Filtrate: Fresh vs. Aged in Open and Closed Containers

Within the swine filtrate treatments, the greatest production of CO_2 occurred in the 1X concentrations for all fresh and aged filtrates (Figures 2.8 - 2.10). There were no statistically significant differences in total CO_2 produced between incubations treated with fresh swine filtrates vs. those aged in open and closed containers at day 5 (Figure 2.3). Comparison of incubations treated with filtrates aged in open vs. closed containers at day 13 also yielded no significant differences in total CO_2 production (Figure 2.4).

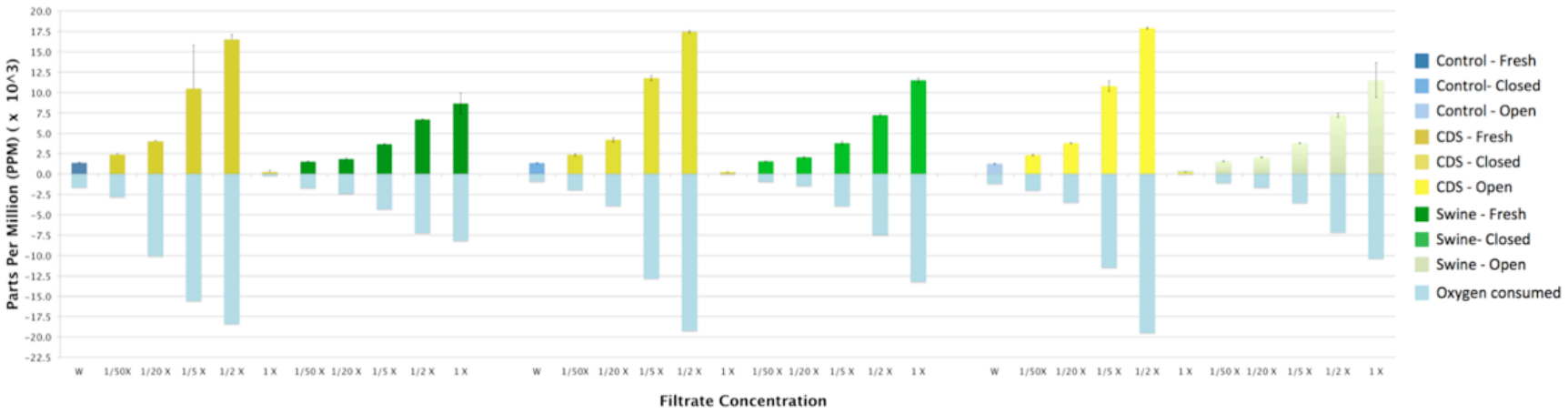


Figure 2.3 Cumulative microbial CO_2 production and O_2 consumption of soil bacteria in response to fresh and aged CDS and swine filtrates after 5 days of incubation.

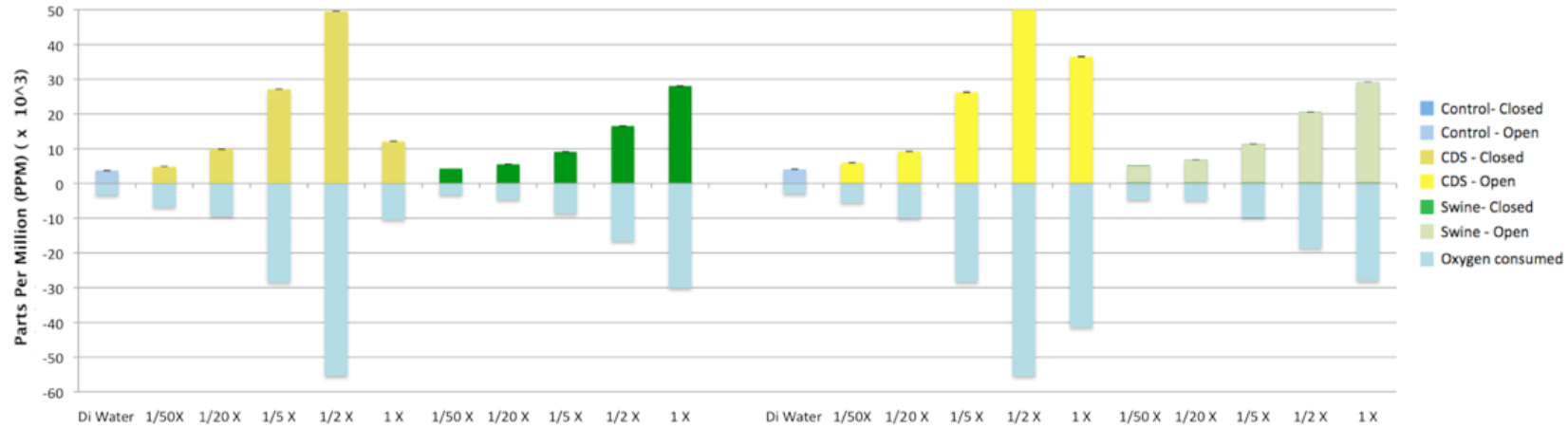


Figure 2.4 Cumulative microbial CO_2 production and O_2 consumption of soil bacteria in response to aged CDS and swine filtrates after 13 days of incubation.

Figures 2.5 – 2.7 CO2 Production – CDS: Production of atmospheric CO_2 in response to amendment with increasing concentrations of CDS HTC filtrate. Vials were vented on day 5 for fresh and day 6 for aged filtrates, after which additional data was added to the previous data point for contiguous representation. Fig 2.5 fresh filtrate, Fig. 2.6 filtrate aged in a closed container for 100 days, Fig. 2.7 filtrate aged in a open container for 100 days.

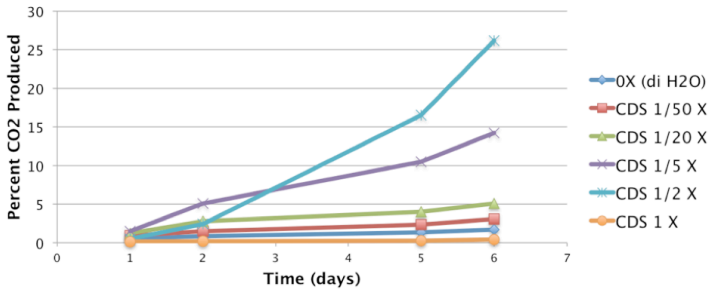


Figure 2.5 Microbial CO_2 production in response to various concentrations of fresh CDS filtrate

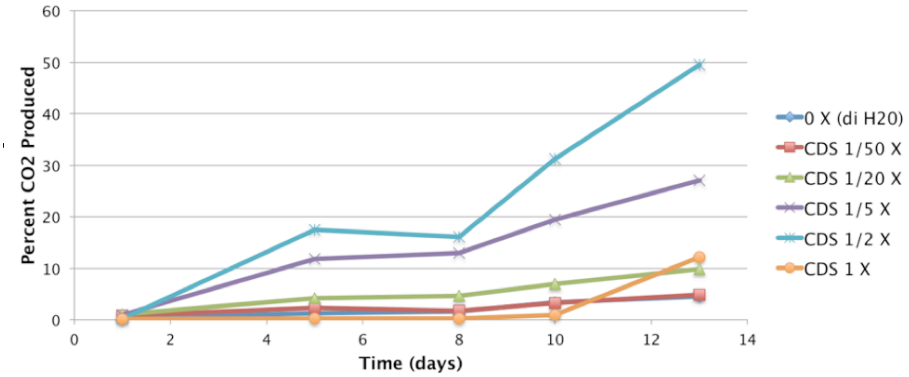


Figure 2.6 Microbial CO_2 production in response to various concentrations of CDS filtrate aged in a closed container

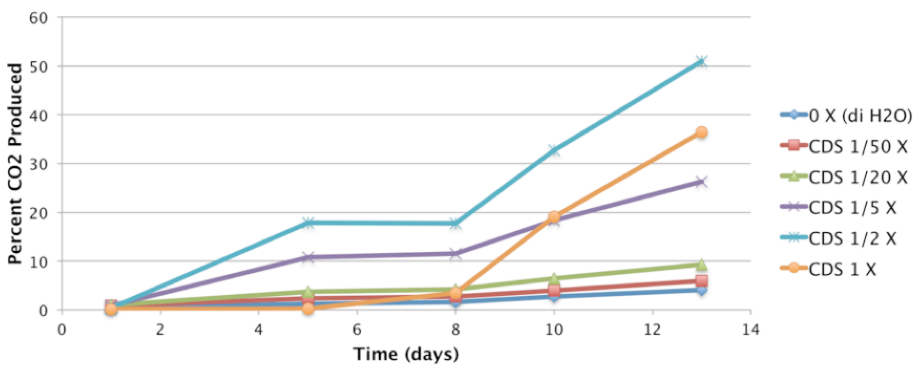


Figure 2.7 Microbial CO_2 production in response to various concentrations of CDS filtrate aged in a open container

Figures 2.8 – 2.10 CO2 Production – Swine: Production of atmospheric CO_2 in response to amendment with increasing concentrations of swine HTC filtrate. Vials were vented on day 5 for fresh and day 6 for aged filtrates, after which additional data was added to the previous data point for contiguous representation. Fig 2.8 fresh filtrate, Fig. 2.9 filtrate aged in a closed container for 100 days, Fig. 2.10 filtrate aged in a open container for 100 days.

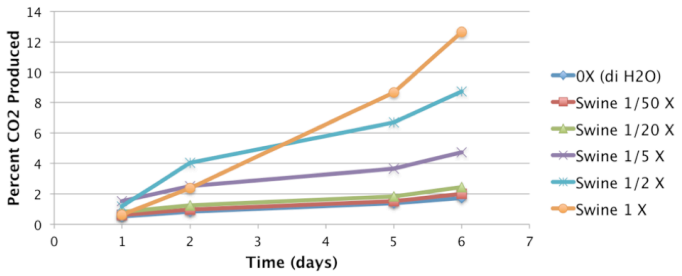


Figure 2.8 Microbial CO_2 production in response to various concentrations of fresh CDS filtrate

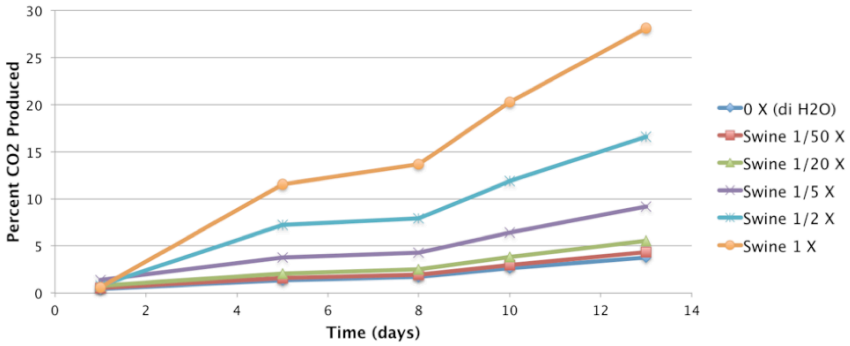


Figure 2.9 Microbial CO_2 production in response to various concentrations of swine filtrate aged in a closed container

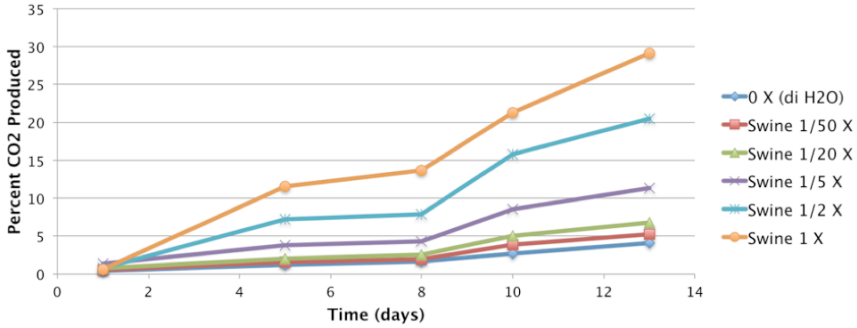


Figure 2.10 Microbial CO_2 production in response to various concentrations of swine filtrate aged in a open container

2.3.1.2. CDS Filtrate vs. Swine Filtrate

In all cases, the 1/2X CDS treatments yielded greater production of CO_2 than the undiluted (1X) swine treatments, and on average there was 33% more cumulative CO_2 produced in 1/2X CDS incubations than the 1X swine filtrates ($P < 0.001$) (Figures 2.3 and 2.4). Despite the high levels of activity observed in the 1/2X CDS treatments, applications of 1X CDS filtrate were completely inhibitory to any microbial activity for the first week in all three of the CDS treatment types, whereas the swine filtrate had significant production of CO_2 at the 1X concentrations, and thus showed no inhibition.

2.4.2. Evaluation of CO_2 Production vs. O_2 Consumption

As a secondary validation on the accuracy of CO_2 production for microbial activity, the rate of O_2 consumption was also compared for these incubations. In all cases, consumption of O_2 vs. production of CO_2 was very close to being at a 1 : 1 molar ratio, as expected theoretically (Figures 2.3 and 2.4.

2.3.3 Rates of CO_2 Production

2.3.3.1. CDS Filtrate – CO_2 Rate of Production

The rate of CO_2 production within vials treated with CDS filtrate was positively correlated to concentration up to the 1/2X dose (Figure 2.4). The highest rate of CO_2 production for CDS occurred in the 1/2X treatments, and the average for the all three CDS 1/2X treatments was (1,853 +/- 52 ug-CO_2 g-soil^{-1} d^{-1}) (Figure 2.11). There was no CO_2 production observed in the 1X vials up to the first venting (day 5). However, after the venting, there was a lower rate of production observed. CO_2 production rates between the open and closed filtrate treatments after venting were compared to production rates prior to the venting (Figure 2.12). After the venting, the rate of

production decreased in all treatments, except for the CDS 1X vials, where a drastic increase in CO_2 production was observed. Furthermore, the rate of production in the CDS 1X open (1,396 +/- 21 ug-CO_2 g-soil^{-1} d^{-1}) treatment was 28% ($P < 0.001$) greater than the rate of production in the CDS 1X closed treatment (1,006 +/- 137 ug-CO_2 g-soil^{-1} d^{-1}). The highest rate of production remained in the 1/2X CDS treatments (1,489 +/- 24 ug-CO_2 g-soil^{-1} d^{-1}). However, it was 20% lower than the rate of production before venting. There were no statistically significant differences in rates of production between open and closed treatments, other than the difference observed in the undiluted [1X] treatments.

2.3.3.2. Swine Filtrate – CO_2 Rate of Production

The rate of CO_2 production within swine incubation vials also had a positive relation to the added amount of filtrate (Figure 2.11). The highest rate of CO_2 production for swine treatments occurred in the 1X treatments, and the average for all three CDS 1/2X treatments was 1,002 +/- 156 ug-CO_2 g-soil^{-1} d^{-1} (Figure 2.11). A statistical analysis of the fresh vs. aged filtrates proved there were no significant differences between CO_2 rates. Despite having a lower interval rate of production post-venting (20-40% lower), there still were no statistically significant differences in rates of production between open and closed treatments after the vials had been vented ($P>0.05$).

2.4.3.3. CDS filtrate vs. Swine Filtrate – Comparison of CO_2 Rates of Production

Comparison of incubations treated with CDS HTC filtrates to those treated with swine HTC filtrates shows that the 1/2X CDS vials had greater rates of CO_2 production than the 1X swine filtrate. On average CDS 1/2X treatments of filtrates aged in open and closed containers had a rate of production that was 46% greater than the 1X swine

treatment in the first 5 days, and 60% greater following the venting of vials (P<0.001) (Figure 2.12).

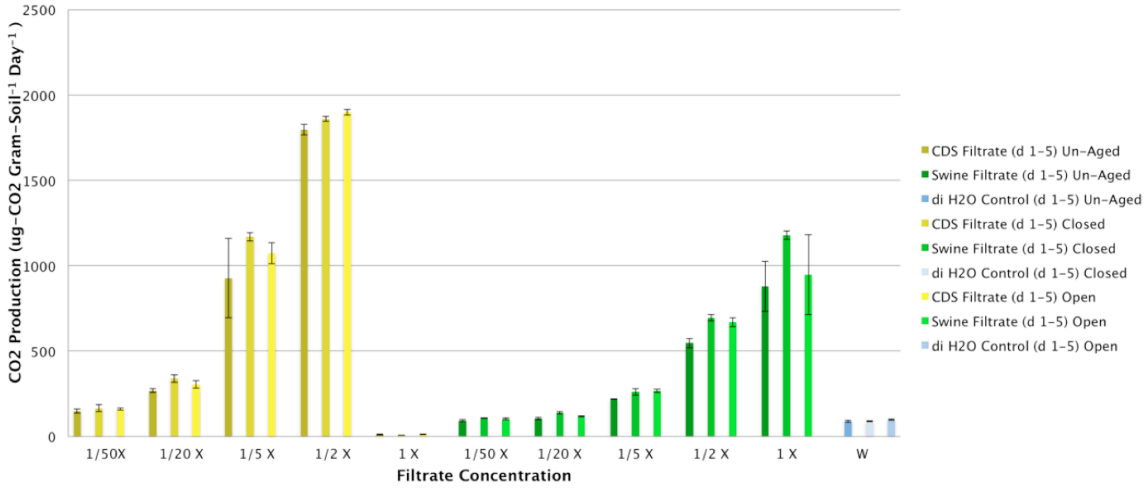


Figure 2.11 Comparison of CO_2 production rates of soil bacteria in response to increasing concentrations of CDS and swine HTC filtrates. Fresh filtrates vs. filtrates aged in close containers vs. filtrates aged in open containers after 5 days of soil incubation.

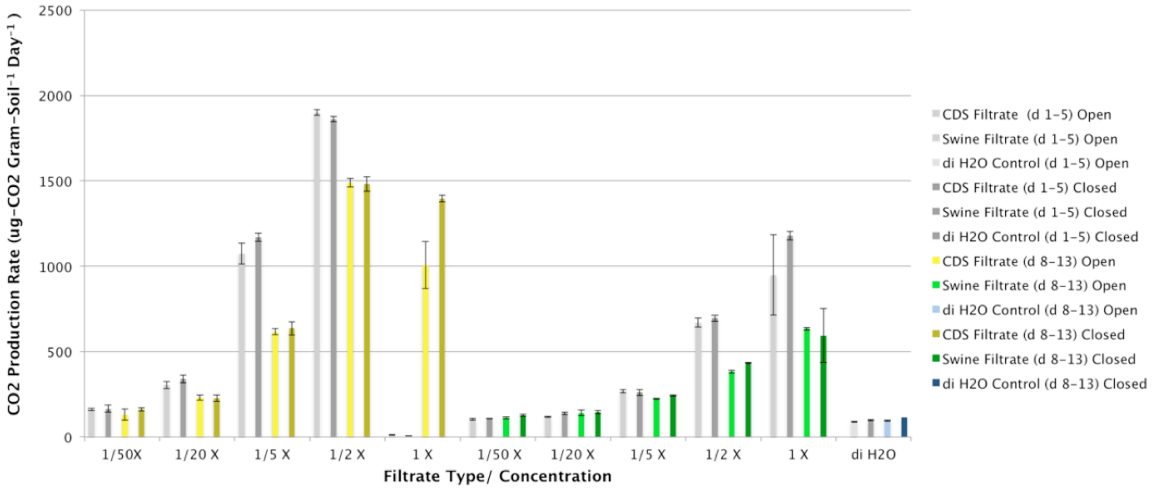


Figure 2.12 Comparison of CO_2 production rates of soil bacteria in response to increasing concentrations of aged CDS and swine HTC. Filtrates aged in close containers vs. filtrates aged in open containers. Rates of production pre vial venting (day 1 – 5) vs. rates of production post vial venting (days 8-13)

2.3.4 CH_4 Production

The production of methane (CH_4) was negligible throughout all incubation studies, therefore will not be discussed individually. The average rate of production of 0.0 ug-CH_4 g soil^{-1} d^{-1} was observed for all filtrate types at all dilutions. Furthermore, no alteration in rate of production took place for CH_4 following the venting of the vials.

Therefore, there were no major differences observed in the CH_4 production from this incubation.

2.3.5 Cumulative N_2O Production

2.3.5.1. CDS Filtrate: Fresh vs. Aged in Open and Closed Containers

The cumulative production of N_2O vs. filtrate type and concentration of fresh filtrates can be seen in Fig. 2.13. It can be seen that in comparison to the di. H_2O negative control, production of N_2O was inhibited for all CDS filtrate treatments (Figure 2.13). As seen in Fig. 2.14, the cumulative N_2O production at the end of day 5, shows that all CDS filtrates aged in an open container generally produced more N_2O than fresh filtrates or filtrates aged in a closed container (by graphical inspection). As seen in Fig. 2.16, the cumulative N_2O production at the end of the 13-day trial of aged CDS filtrates followed a similar pattern as the CO_2 production. Filtrates aged in an open container generally produced more N_2O than filtrates aged in a closed container (Fig. 2.14; graphical comparison). It should be noted that both open and closed 1/2X CDS trials developed additional microbial growth (Figure 2.2), whereas none of the swine filtrates were observed with this type of growth. Subsequently, neither of the 1/2X CDS treatments produced a substantial amount of N_2O. Filamentous microbial growth also developed in the 1X CDS open treatments, however the phenology of the microbial colony differed from that seen in the CDS 1/2X treatments (Figure 2.2). This observation was made on the same day for all treatments, and corresponds to 8 d for the open (1/2X and 1X) treatments and 7 d for the 1/2X closed treatments. Consequently, the largest difference for N_2O production was observed in CDS 1X treatments, where the CDS 1X open

treatment was approximately 34% greater than the di H_2O negative control, and approximately 74% greater than its counterpart, the CDS 1X closed treatment.

2.3.5.2. Swine Filtrate: Fresh vs. Aged in Open and Closed Containers

A comparison of cumulative N_2O for fresh swine HTC filtrate (Figure 2.13), shows that at lower concentrations of added filtrate (1/50X, 1/20X and 1/5X) there was less N_2O produced compared to the control. On the other hand, swine filtrate at the 1/2X concentration produced a cumulative N_2O amount only slightly less than that of the di. H_2O control, and the undiluted (1X) treatment surpassed the control by approximately 33%. As seen in Fig. 2.14, the cumulative N_2O production, shows that all swine filtrates aged in an open container generally produced more N_2O than fresh filtrates, or filtrates aged in a closed container (graphical comparison). Similarly, the cumulative N_2O production at the end of the 13-day trial of aged swine filtrates (Fig. 2.14), again showed that filtrates aged in an open container generally produced more N_2O than filtrates aged in a closed container (graphical comparison). A day 13 comparison of cumulative N_2O produced for aged swine filtrates (Fig. 2.15) against the control shows that swine filtrates aged in a closed container on average had a total N_2O that was less than the control at all concentrations except the 1X, whereas filtrates aged in an open container had N_2O levels that were on average greater than the control at all concentrations.

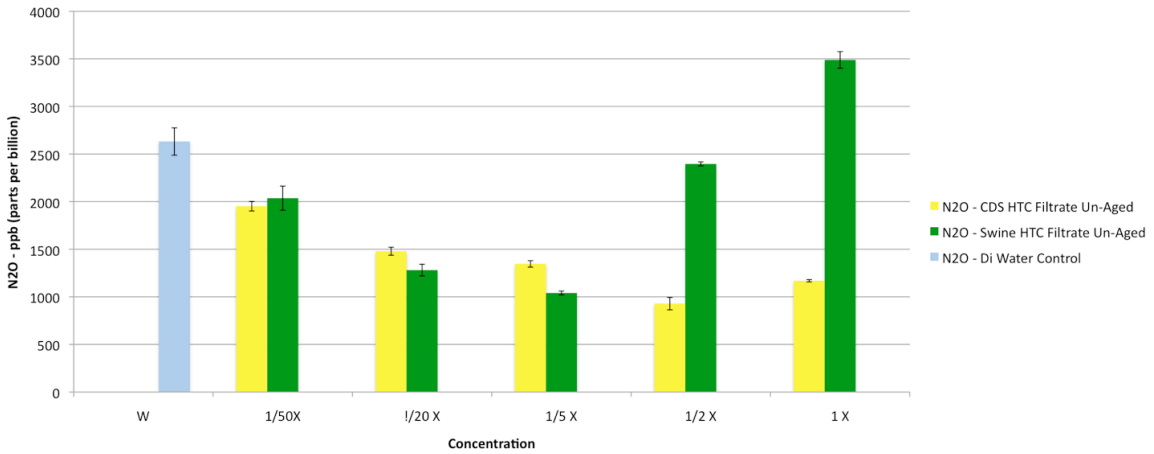


Figure 2.13 Comparison of cumulative microbial N_2O production in response to various concentrations of fresh CDS and swine HTC filtrates, after 6 days of soil incubation.

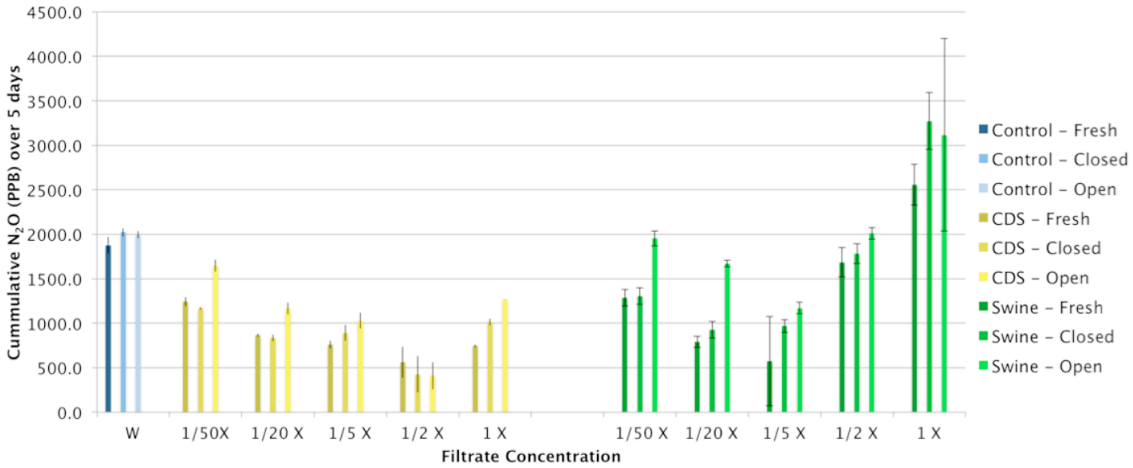


Figure 2.14 Comparison of cumulative N_2O production from soil incubations of increasing concentrations of fresh CDS and swine HTC filtrates vs. those that were aged in open and closed containers at day 5.

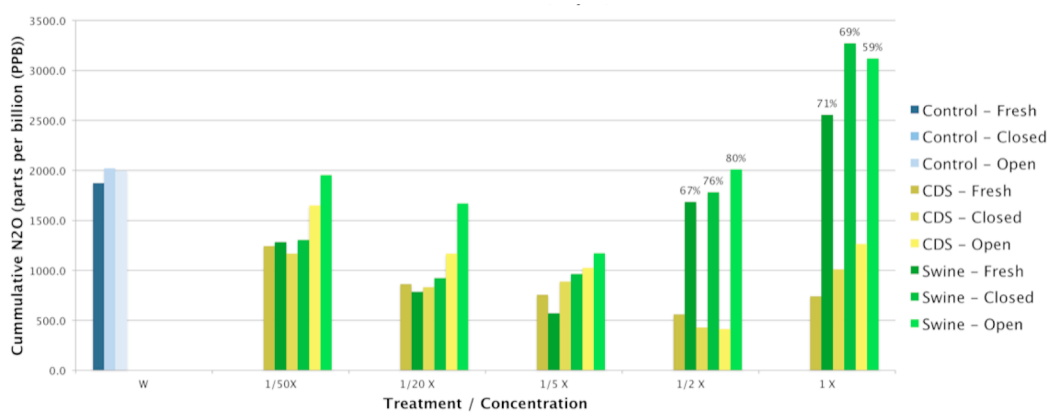


Figure 2.15 Cummulative N_2O production of CDS vs. swine filtrate: With treatments of fresh filtrate vs. filtrate aged in a closed container vs. filtrate aged in and open container (day 5)

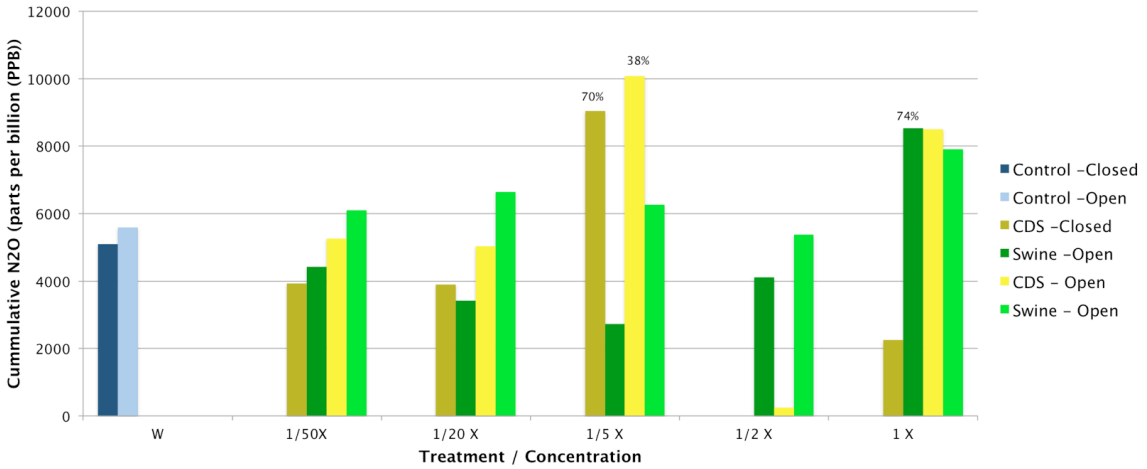


Figure 2.16 Cummulative N_2O production of CDS vs. swine filtrate: With treatments of filtrate aged in a closed container vs. filtrate aged in and open container (day 13)

2.3.5.3. CDS Filtrate vs. Swine Filtrate

Comparison of cumulative N_2O production resulting from the addition of fresh and aged CDS vs. swine filtrates (Fig. 2.15) shows that swine filtrate aged in open containers produced greater amounts of N_2O than the corresponding concentrations of fresh and aged CDS filtrates. The greatest observed difference between CDS and swine filtrates occurred within the 1/2X and 1X concentrations in comparison to the control, in the first five days, where in the 1/2X concentrations swine surpassed CDS filtrates (fresh filtrate, filtrate aged in an open container, and filtrate aged in a closed container) by 67%, 76%, and 80%, respectively. In the 1X concentration, swine filtrates surpassed CDS filtrates (fresh filtrate, filtrate aged in an open container, and filtrate aged in a closed container) by 71%, 69%, and 59%, respectively. Cumulative N_2O comparison of aged CDS and swine filtrate treatments (Fig 2.16), shows that greatest differences are observed at higher concentrations of filtrate application (1/5X, 1/2X, and 1X). At the 1/5X concentration, CDS produced a greater amount of N_2O for both closed and open treatments, which were 70% and 38% greater, respectively. Conversely, at the 1/2X both

open and closed CDS treatments produced negligible amounts of N_2O, whereas swine filtrate applications had total N_2O production that was similar to the control. At the 1X concentration, swine filtrate aged in a closed container produced approximately 74% more N_2O than the 1X CDS closed treatment, whereas the 1X open treatments had no significant differences between swine and CDS filtrate application.

2.3.6 Rates of N_2O Production

2.3.6.1. CDS Filtrate – N_2O Rate of Production

The overall trends for rate of production in CDS filtrates showed a decrease in N_2O production rate with increasing concentrations of applied filtrates. It can also be seen that filtrates aged in open containers, had a higher rate of N_2O production than that of either the fresh filtrates or the filtrates aged in a closed container, and these differences are thought to be due to the microbial growth as mentioned in the previous section. There is a very significant correlation between N_2O production and added amount of filtrate. A negative rate (consumption) is observed for all 1/2X CDS treatments, which is noteworthy, since the visible presence of microbial growth was observed in all of the 1/2X CDS vials.

Soil incubations for filtrates aged in open vs. closed containers were compared after they had been vented. As seen in Figure 2.18, the interval production rate of N_2O was different pre – and – post venting, with the most significant change occurring in CDS 1/5X open, CDS 1/5X closed, and CDS 1X open treatments, which had a 91, 92, and 92% increase in production rate after venting, and were 52, 76 and 77% greater than their corresponding di. H_2O controls, respectively.

2.3.6.2. Swine Filtrate – N_2O Rate of Production

Soil incubations that had applications of swine HTC filtrate aged in open containers also had greater rates of N_2O production than those of fresh filtrate and filtrate aged in closed containers. The greatest observed rate of N_2O production in the first five days of incubation occurred in swine 1/50X and 1X open treatments, with 14.9 (+/- 0.7) and 14.0 (+/- 9.0) ng-N_2O g_{soil}^{-1} d^{-1}, respectively. However, ANOVA analysis confirmed that these results were not statistically different than the rate of production for the control, which was 13 ng-N_2O (+/- 1 ng) g_{soil}^{-1} day^{-1}.

2.3.6.3. CDS Filtrate vs. Swine Filtrate – Comparison of N_2O Rates of Production

As seen in Fig. 2.17, the rates of production between the CDS and swine HTC filtrates under the different types of applied filtrates (fresh, aged in closed container, aged in open container) showed considerable variation, with applications of swine filtrate aged in an open container having higher rates of N_2O production compared to the CDS filtrate applications.

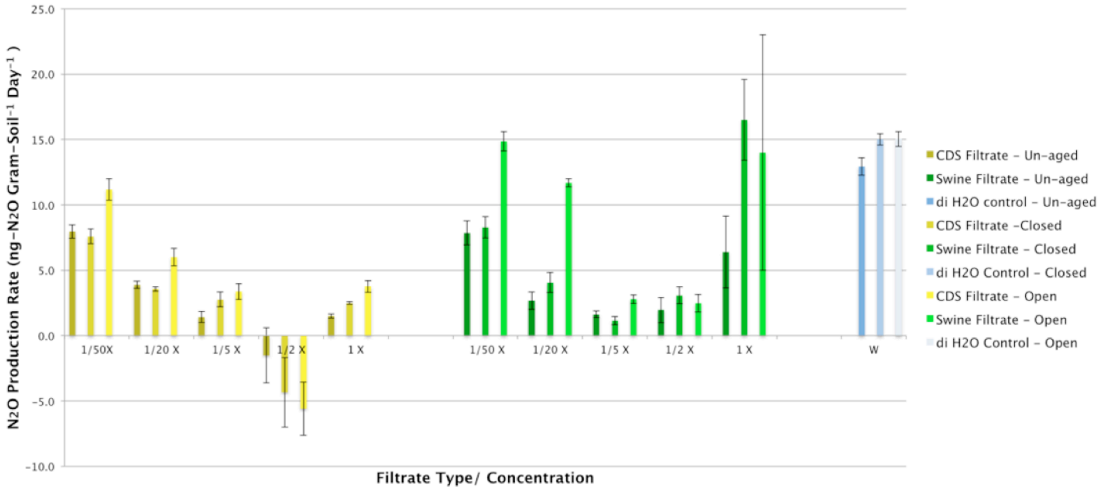


Figure 2.17 Comparison of N_2O production rates from amendments of fresh CDS and swine HTC filtrates vs. those that were aged in open and closed containers, in the first 5 days.

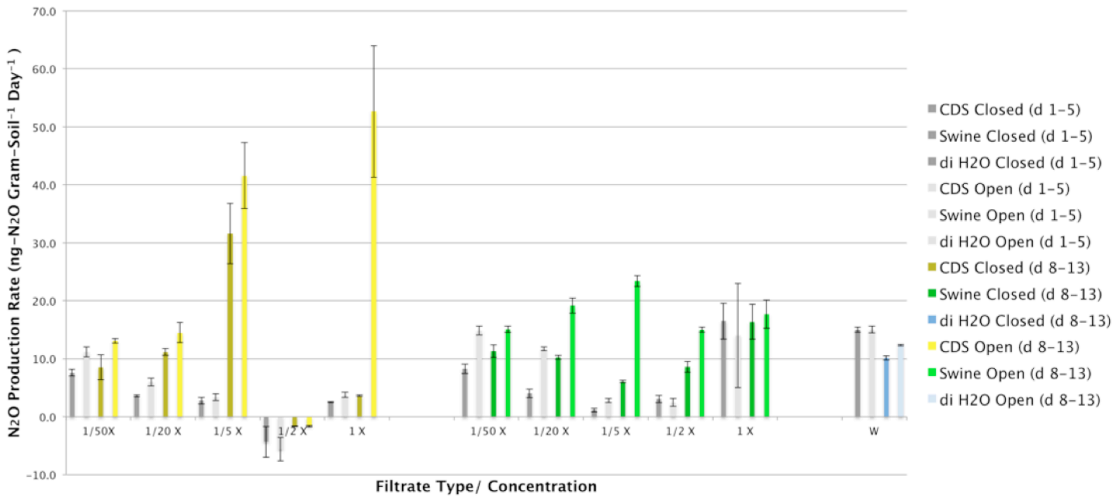


Figure 2.18 Comparison of microbial N_2O production rates of soil bacteria in response to increasing concentrations of aged CDS and swine HTC in open and closed containers. Filtrates aged in close containers vs. filtrates aged in open containers. Rates of production pre vial venting (day 1 – 5) vs. rates of production post vial venting (days 8-13).

2.3.7 Nutrient Mineralization

2.3.7.1 Initial Nutrient Concentration for Undiluted Filtrates (NH_4, and NO_3)

A nutrient analysis of the soil used for all soil incubations showed that prior to incubation, the soil contained 5.4 µg NO_3 / g_{-soil}, and 1.07 µg NH_4 / g_{-soil}

Table 2.1 Nutrient concentrations of undiluted starting filtrates of incubations 1 and 2

Soil Incubations	***Solution/ Filtrate type***	***NH_4***	***NO_3***	***PO_4***
			(mg L^{-1})	
Soil Incubation -1	CDS filtrate - fresh	3,600	< 1	5,500
	Swine filtrate - fresh	3,800	< 1	5
Soil Incubation -2	CDS filtrate - open	3,900	< 1	6,900
	Swine filtrate - open	4,500	< 1	20
Soil Incubation -2	CDS filtrate - closed	3,500	< 1	6,600
	Swine filtrate - closed	4,500	< 1	3

2.3.7.1.1 Soil Incubation-1, Fresh Filtrates

Undiluted fresh swine filtrate (~3900 mg/L +/-10%) prior to soil incubation had a slightly higher initial NH_4 concentration than undiluted CDS filtrate (~3500 mg/L+/-

10%), although the differences were within the same order of magnitude (Table 2.1). There were no detectable levels of NO_3 within either of the filtrates.

2.3.7.1.2 Soil Incubation-2, Aged Filtrates

Similarly to the fresh filtrates, NH_4 concentration of the filtrates prior to incubation had the same distributions, with undiluted swine filtrates aged in open and closed containers, both having a concentration of ~4,500 mg/L +/-10%, which were higher than the initial NH_4 concentrations of undiluted CDS filtrates aged in open and closed containers, at ~3,900 and 3,500 mg/L+/-10%, respectively. There were no detectable levels of NO_3 within any of the filtrates.

2.3.7.2 Concentrations of NH4 and NO3 of CDS Treatments Post-Soil Incubation

There was no extractable ammonium (NH_4) in soil control vials of both soil incubations, and higher levels of NH_4 with increasing concentrations of CDS filtrate, regardless of age (Figure 2.19 - 2.21). In general, nitrate levels (NO_3) were highest at the lowest treatment concentrations for CDS filtrate applications, and further decreased with increasing filtrate concentration.

2.3.7.2.1 Fresh CDS Filtrate

As seen in Figure 2.22, the two concentrations that had the highest final NO_3 concentration for fresh CDS filtrate were the 1/50X and 1/20X treatments, which had 37 +/- 2 and 36 +/-1 ug-NH_4 - g_{soil}^{-1}, respectively. The NO_3 concentrations for these treatments were not statistically different than the control ($P>0.05$).

2.3.7.2.2 CDS Filtrate Aged in Closed Containers

As seen in Figure 2.23, the two concentrations that had the highest final NO_3 concentration for CDS filtrate aged in a closed were again the 1/50X and 1/20X treatments, which had 35 +/- 2 and 33 +/-1 ug-NH_4 - g_{soil}^{-1}, respectively. The NO_3

concentration for 1/50X treatment was 9% greater than the control ($P<0.05$), while the 1/20X treatment was not statistically different than the control ($P>0.05$).

2.3.7.2.3 CDS Filtrate Aged in Open Containers

As seen in Figure 2.24, the two concentrations that had the highest final NO_3 concentration for CDS filtrates were again the 1/50X and 1/20X treatments, which had 41 +/- 1 and 40 +/-3 ug-NH_4 - g_{soil}^{-1}, respectively. The NO_3 concentrations for these treatments were not statistically different than the control ($P>0.05$).

2.3.7.2.4 CDS - Aging in Open vs. Closed Container

Comparison of mineralization rates between filtrates aged in closed vs. open containers produced a measurable impact on N dynamics. As seen in Table 2.2 and Figure 2.25, the open container typically had a higher amount of inorganic N compared to the closed treatments at the end of the incubation. Statistically, there was no difference observed in the terminal NH_4 concentrations between the open and closed CDS treatments (Figure 2.26). However, a difference in NO_3 levels was observed between the two post treatments. The 1/50X, 1/20X and 1/5X CDS treatments had greater NO_3 mineralization in the incubations treated with filtrate aged in open containers than those aged in closed containers, and were 13% ($P<0.05$), 16% ($P<0.01$), and 50% ($P<0.001$) greater in terminal NO_3 concentration, respectively (Figure 2.25).

Table 2.2 Rates of N-mineralization observed from the varying filtrate additions. Positive values for % of total N generated, indicates N-mineralization, negative values indicate N-immobilization.

Note: * signifies that the reported value exceeded the upper threshold of the standard curve, and the value should be considered an estimate due to the uncertainty in determining the concentration above this curve

		Initial N Values			Final N Values			Rate of N Mineralization ($\mu g/g_{-soil}$/day)
Filtrate Type	**Conc.**	**NH_4 ($\mu g/g_{-soil}$)**	**NO_3 ($\mu g/g_{-soil}$)**	**Total N ($\mu g/g_{-soil}$)**	**NH_4 ($\mu g/g_{-soil}$)**	**NO_3 ($\mu g/g_{-soil}$)**	**% NO_3 of Total**	
Control di. H_20	0 X	1	5	6	1	34	98%	2.17
CDS: Fresh Filtrate	1/50X	14	< 1	21	4	37	90%	1.56
	1/20X	36	< 1	42	16	36	69%	0.70
	1/5X	144	< 1	150	96*	14	13%	-3.15
	1/2X	360	< 1	366	150*	0	0%	-16.67
	1X	720	< 1	726	217*	1	0%	-39.12
Swine: Fresh Filtrate	1/50X	15	< 1	22	4	48	92%	2.39
	1/20X	38	< 1	44	32	46	59%	2.56
	1/5X	152	< 1	158	168	20	10%	2.27
	1/2X	380	< 1	386	260*	7	3%	-9.23
	1X	760	< 1	766	329*	6	2%	-33.23
Control di. H_20	0 X	1	5	6	0	32	100%	2.00
CDS: Aged in Closed Container	1/50X	14	< 1	20	1	35	98%	1.21
	1/20X	35	< 1	41	10	33	77%	0.12
	1/5X	140	< 1	146	100	10	9%	-2.77
	1/2X	350	< 1	356	158*	0	0%	-15.26
	1X	700	< 1	706	236*	0	0%	-36.20
Swine: Aged in Closed Container	1/50X	18	< 1	24	2	42	96%	1.48
	1/20X	45	< 1	51	33	39	54%	1.60
	1/5X	180	< 1	186	179	12	6%	0.37
	1/2X	450	< 1	456	306*	2	1%	-11.40
	1X	900	< 1	906	413*	1	0%	-37.87
Control di. H_20	0 X	1	5	6	1	36	99%	2.28
CDS: Aged in Open Container	1/50X	16	< 1	22	0	41	100%	1.44
	1/20X	39	< 1	45	9	39	81%	0.24
	1/5X	156	< 1	162	93	21	18%	-3.79
	1/2X	390	< 1	396	151*	0	0%	-18.92
	1X	780	< 1	786	234*	0	0%	-42.49
Swine: Aged in Open Container	1/50X	18	< 1	24	2	43	95%	1.63
	1/20X	45	< 1	51	3	54	95%	0.44
	1/5X	180	< 1	186	107*	56	34%	-1.86
	1/2X	450	< 1	456	290*	12	4%	-11.88
	1X	900	< 1	906	363*	6	2%	-41.30

2.3.7.3 Concentrations of NH_4^+ and NO_3^- of Swine Treatments Post-Soil Incubation

There was no extractable ammonium (NH_4) in soil control vials of both soil incubations, and levels of NH_4 increased with increasing concentration of swine filtrates, regardless of age (Figure 2.19 -2.21). In general, nitrate levels (NO_3) were highest at lower treatment concentrations for swine filtrate applications, and decreased with increasing filtrate concentration.

2.3.7.2.1 Fresh Swine Filtrate

As seen in Figure 2.22, the two concentration at which final NO_3 concentration were greater than the soil control were the 1/50X and 1/20X concentrations, which at 48 +/- 1 and 46 +/-2 ug-NH_4 - g_{soil}^{-1}, had a 29% ($P < 0.001$) and 26% ($P < 0.001$) greater concentration than the control, respectively.

2.3.7.3.2 Swine Filtrate Aged in Closed Containers

As seen in Figure 2.23, the highest levels of NO_3 for applications of swine filtrate aged in a closed container were also 1/50X and 1/20X concentrations, with 42 (+/- 1) and 39 (+/- 2) ug-NH_4 - g_{soil}^{-1}, and were 23% ($P < 0.001$) and 27% ($P < 0.001$) greater than the soil control, respectively.

2.3.7.3.3 Swine Filtrate Aged in Open Containers

As seen in Figure 2.24, the highest levels of NO_3 for applications of swine filtrate aged in an open container were 1/50X, 1/20X and 1/5X concentrations, with 43 (+/- 1), 54 (+/- 1) and 56 (+/- 1) ug-NH_4 - g_{soil}^{-1}. Only the 1/20X and 1/5X swine treatments had greater NO_3 concentrations than the soil control, and were 35% ($P < 0.001$) and 36% ($P < 0.001$) greater in final concentration of the control, respectively.

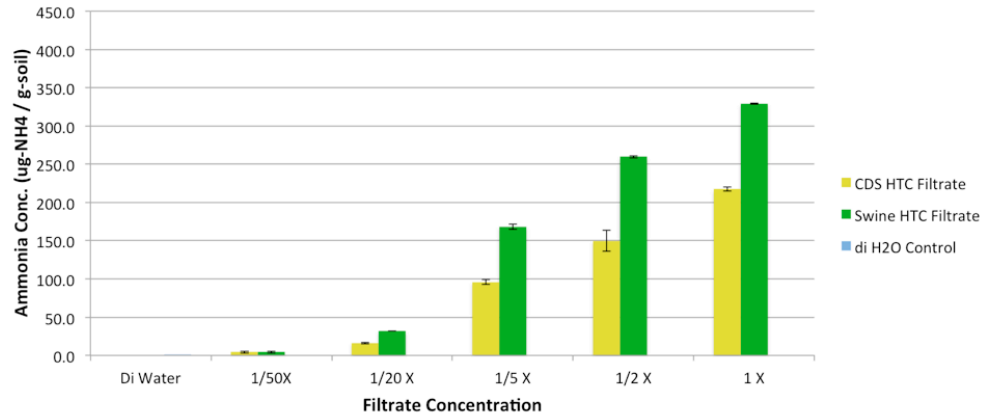


Figure 2.19 Post soil incubation NH_4 concentrations of soil treated with fresh CDS and swine HTC filtrates

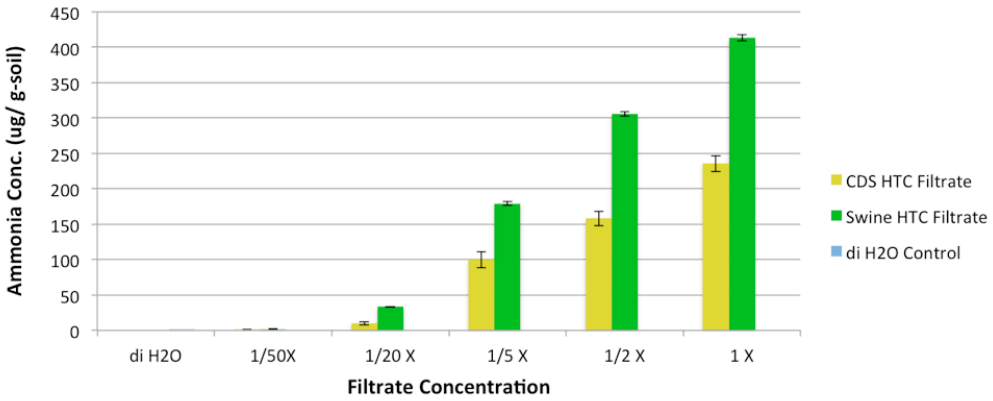


Figure 2.20 Post soil incubation NH_4 concentrations of soil treated with CDS and swine HTC filtrates aged in a closed container for 100 days.

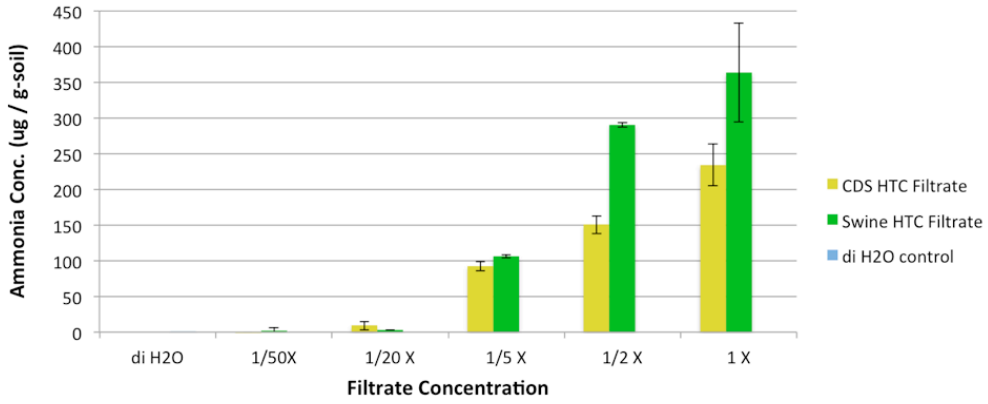


Figure 2.21 Post soil incubation NH_4 concentrations of soil treated with CDS and swine HTC filtrates aged in a open container for 100 days.

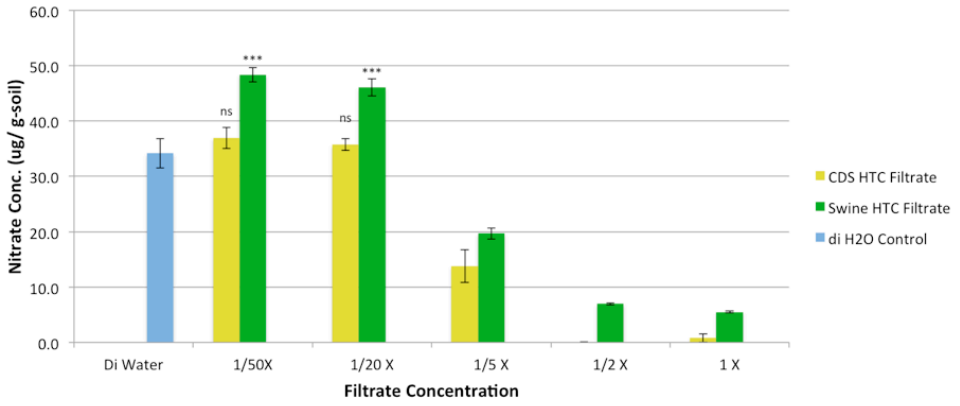


Figure 2.22 Post soil incubation NO_3 concentrations of soil treated with fresh CDS and swine HTC filtrates

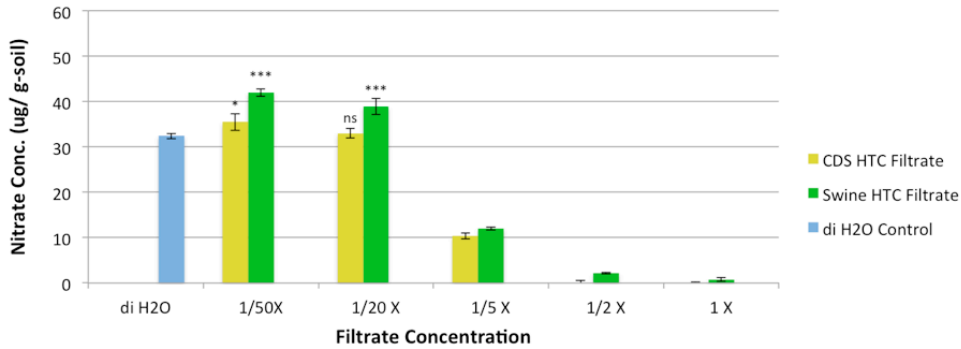


Figure 2.23 Post soil incubation NO^3 concentrations of soil treated with CDS and swine HTC filtrates aged in an open container for 100 days.

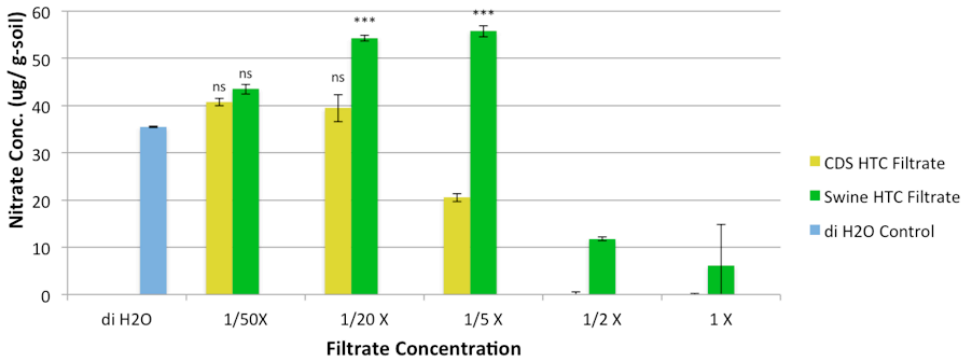


Figure 2.24 Post soil incubation NH_4 concentrations of soil treated with CDS and swine HTC filtrates aged in an open container for 100 days.

2.3.7.3.4 Swine Filtrate – Aging in Open vs. Closed Container

The type of aging of the filtrates did produce a measurable impact on inorganic-N concentrations (Figure 2.25). The only statistically different terminal NH_4 concentration between the open and closed swine treatments was observed in 1/5X treatments (Figure 2.26), where the open treatments had a 41% lower concentration ($P<0.05$) than the closed treatments. However, greater differences in NO_3 levels between the two post treatments were observed, where swine filtrate aged in an open container had 1/20X (54 +/- 1 ug-NH_4 - g_{soil}^{-1}), 1/5X (56 +/- 1 ug-NH_4 - g_{soil}^{-1}) and 1/2X (12 +/- 1 ug-NH_4 - g_{soil}^{-1}) NO_3 mineralization levels that were greater by 28% ($P<0.001$), 78% ($P<0.001$), and 81% ($P<0.05$) than the incubations treated with filtrate aged in closed containers, respectively (Figure 2.25). The results of increased N-mineralization rates suggest that the soil microbial population prefers the aged filtrate in the open container.

2.3.7.2.5 N-mineralization and immobilization of swine treatments

Swine filtrate applications had N-mineralization occurring at 1/50X, 1/20X and 1/5X concentrations of fresh filtrate applications, and filtrate aged in a closed container; and 1/50X and 1/20X concentrations for swine filtrate aged in an open container. N-immobilization was observed at the higher concentrations; 1/2X and 1X fresh filtrate applications, and filtrate aged in a closed container; and 1/5X, 1/2X and 1X concentrations for swine filtrate aged in and open container. (See Table 2.2).

2.3.7.4 Swine Filtrate vs. CDS Filtrate

As seen in Figures 2.19 – 2.21 all soil incubations treated with swine HTC filtrates contained higher terminal NH_4 levels than incubations treated with CDS filtrates. Levels of NO_3 extracted from all vials treated with swine HTC filtrate were also considerably greater than the corresponding CDS filtrate applications (Figures 2.22 -2.24)

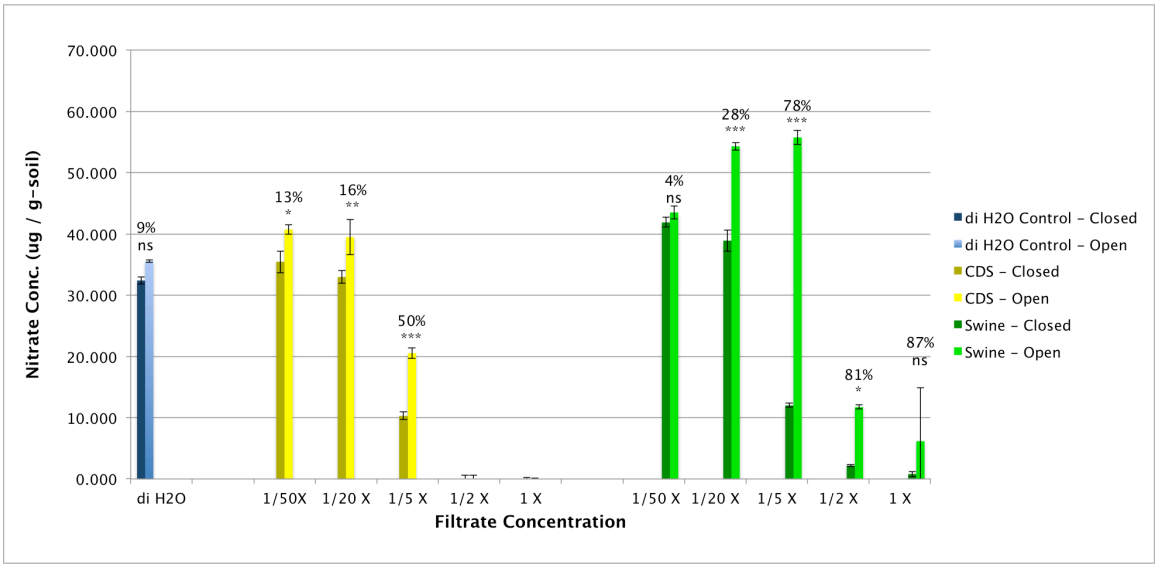


Figure 2.25 Comparison of nitrate concentrations following soil incubation in response to aged CDS and swine HTC filtrates.

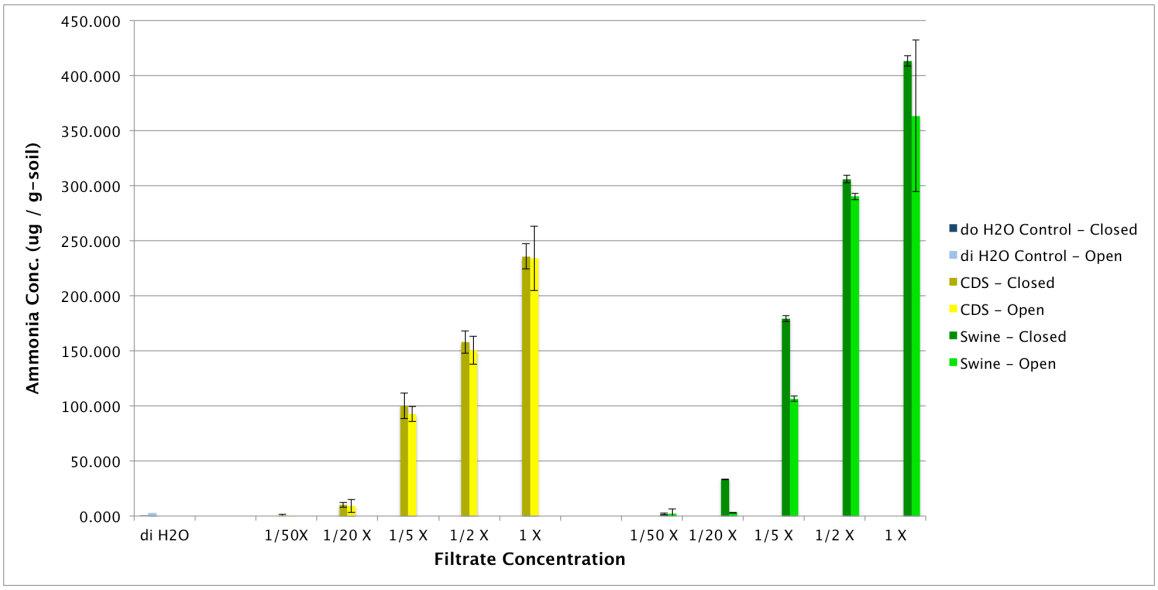


Figure 2.26 Comparison of final soil NH_4 concentrations as a function of the concentrations of aged CDS and swine HTC filtrates from open and closed containers.

2.4 DISCUSSION

Overall, there was an observable difference in microbial activity in soil incubations of both filtrate types under all three treatment conditions: fresh filtrates, and those aged in open and closed containers. CDS HTC filtrate at 1/2X proved to create an ideal environment for filamentous microbial growth (Fig. 2.2), whereas no obvious growth was seen in any of the incubations containing swine HTC filtrate. Another filamentous growth was also observed in the 1X CDS open treatment, however there was no attempt made to determine the species of organism present in any of the vials. The observed phenology of the growth seen in the 1/2X CDS vials was different than what was seen in the 1X vials. It's believed that microbial growth in the 1/2X CDS treatments may have developed due to decreased levels oxygen present at those concentrations, and the presence of which had a direct impact on the lack of N_2O production seen in the 1/2X CDS vials. The observed growth in the 1X CDS open vials could also be responsible for the increased N_2O rates observed, which may have been due to possible change in the type of microbial growth. This correlation between the presence of microbial growth in all of the 1/2X CDS filtrates, and some of the 1X treatments implies that concentrations higher than 1/2X for CDS provide optimal conditions for these microbes to proliferate. This should be further studied, as some types of microbial growth, particularly fungi, can be detrimental to crop production (Koehler & Holbert, 1930), and can also be the cause of fungi induced de-nitrification and N_2O emissions (Baggs, 2011).

Comparison of cumulative CO_2 production of CDS vs. swine treatments shows that, there was higher microbial activity in the 1/2X CDS treatments than those at any concentration of swine filtrate (Figures 2.3 and 2.4). Further comparison of cumulative

CO_2 production indicates that aging filtrate in open containers may have had a significant reduction on the inhibitory impact of both CDS and swine HTC filtrates. Since there was more CO_2 produced in aged filtrates, especially that of filtrates aged in open containers, it was assumed to indicate a higher microbial activity, and therefore reduced toxic effects of the filtrate. This effect can be seen most clearly when looking at the 1X treatments of CDS filtrate aged in open vs. closed containers. The cumulative levels of CO_2 in the CDS 1X open vials were 67% greater than those in the CDS 1X closed. Similarly, the rate of CO_2 production was 27% greater in the CDS 1X open vials than that of CDS 1X closed. This effect may be due to the presence of undetermined microbial growth in the CDS 1X open vials.

Soil extractions shed more light on the differences between filtrate types, and the impacts of aging. In general, overall terminal NH_4 concentrations for soil incubations were greater in swine filtrate treatments than CDS treatments (Figures 2.19 – 2.21, Figure 2.25). N-mineralization was greater for all soil incubation treatments containing swine filtrate, than the corresponding concentrations of CDS filtrate treatments, regardless of post-treatment type (Figures 2.22 – 2.24, Figure 2.25). Comparing the starting levels of NH_4 in both filtrate types, with CDS containing ~3,900 mg/L and swine filtrate containing ~ 3,500 mg/L, to the concentrations of NH_4 following incubations, shows that there was greater utilization of NH_4 in CDS filtrate incubations than those of swine filtrate. This information, coupled with the higher rate of CO_2 production observed in CDS filtrates points to a possible limiting factor within the swine filtrate capable of reducing microbial growth. Furthermore, it was observed that rates of CO_2 production decreased following venting, whereas the rates of N_2O production increased. This shift

in production rates implies a potential shift in microbial nutrient sources. The greater overall CO_2 production observed in incubations with CDS filtrate can be attributed to the greater total carbon (TC) present in the CDS filtrate (Table 4.1). Thus, it is feasible that the greater microbial food sources in CDS filtrate incubations created a need for more NH_4, which was possible because CDS filtrate incubations were less carbon limited than the swine filtrate incubations. This concept is also in agreement with the greater spike in N_2O production observed in CDS filtrate incubations. It is likely that the greater microbial activity in CDS filtrate incubations (omitting 1/2X vials which were dominated by fungi) caused a greater swing towards N-immobilization as available carbon was depleted.

2.5 CONCLUSION

Initial soil incubations with CDS and swine filtrates have shown that soil microbes can utilize both filtrate types. Overall, the greatest amount of microbial activity between the two filtrates occurred in the 1/2X CDS treatments, however CDS filtrate does have a threshold at which microbial activity is greatly inhibited (CDS 1X treatment - 1 mL filtrate/ 5 grams dry soil); whereas the same threshold was not reached with swine filtrate. This observed difference implies the presence of a microbial inhibitor that becomes toxic at higher concentrations of CDS filtrate. Aging of the filtrates in an open container proved to enhance nutrient mineralization of ammonium into nitrate after soil application. N_2O production stimulated by microbial degradation of the filtrates should be considered when performing life cycle analyses in regards to utilization of HTC filtrates as soil amendments. N_2O is produced at levels greater than the soil control at higher concentrations of swine HTC filtrate and 1/5X CDS aged filtrates.

Despite high metabolic activity in response to applications of filtrate with greater concentrations, lower concentration of filtrate application seem to provide the most optimal conditions for N-mineralization. These initial observations show that soil amendments with HTC filtrates do show promise. However, due to the differences observed as a function of filtrate type and storage condition, more work will have to be done to understand how repeated applications affect soil quality. Such studies should also include an evaluation of the potential salt accumulation that could occur with various filtrate types.

CHAPTER 3

RESPONSE OF MAIZE GERMINATION AND GROWTH TO HTC FILTRATE TYPE AND CONCENTRATION

3.1 INTRODUCTION

Hydrothermal carbonization (HTC) is a process that provides an option for nutrient reclamation, and more specifically, the recovery of phosphate (P). Because HTC filtrates are enriched with abundant levels of solubilized ammonium, phosphate, and potassium, they have the potential to provide a renewable source of nutrients necessary for agricultural crop production (Steven M Heilmann et al., 2010). Also, because the aqueous phase of the HTC process makes up a major fraction of final products, it is essential that a useful application of this by-product be developed in order for this treatment to become a viable option.

Several studies to date have analyzed the effect of hydrochars on plant growth, and have had mixed results with respect to hydrochar application and phyto-toxicity (I. Bargmann et al., 2013b; Bargmann et al., 2014a, 2014b; Busch, Kammann, Grunhage, & Muller, 2012; Busch et al., 2013; George et al., 2012; Rillig et al., 2010). However, only two publications have been identified that incorporated HTC filtrate into their study, the first of which utilized HTC waters by first mixing them with the respective hydrochar and other organic raw materials, and then subjecting the mixture to composting prior to soil application (Busch et al., 2013). The second is the only study identified to determine solely the effect of HTC filtrate application on plant germination and growth (I. Bargmann et al., 2013b). To better understand how filtrate type and concentration impact germination and plant growth, several studies were conducted that utilized HTC filtrates

collected from three diverse waste streams that had undergone the HTC treatment: swine and poultry manures, and condensed distiller's solubles (CDS) from the dry-grind ethanol industry. Germination studies using corn seeds (*Zea Mays L.*) were conducted to evaluate inhibition of swine and CDS filtrates, which were applied in various concentrations to blotter paper containing corn seeds and observed for appearance of the root radicle to signal germination. Also, seedling growth trials were set up to further assess the effect of filtrate type and concentration on corn growth. To eliminate sorption of compounds within the filtrate by organic substrates such as peat, the seeds were grown in washed silica sand. Aging of filtrate was also considered since several studies as well as the soil microbial results in Chapter 2 have reported that a reduced phytotoxic effect is observed when using aged or post-treated hydrochar and HTC process waters as a soil amendment (I. Bargmann et al., 2013b; Bargmann et al., 2014b; Busch et al., 2012; Busch et al., 2013). To evaluate the effect of aging, swine and poultry manure, and CDS HTC filtrates were aged for three months in open and closed containers to simulate two types of storage scenarios. Germination and growth trials were also conducted on the same filtrates after they were aged.

3.2 MATERIALS AND METHODS

3.2.1 HTC Filtrate Preparation

Swine manure, poultry manure, and condensed distillers solubles (CDS) were used as starting materials for a 2-hour HTC run at 225 °C. All HTC reactions were conducted in a laboratory-scale stirred stainless steel reactor fitted with a heating mantel system (1000 mL; Parr Instruments, Inc.; Moline, IL). The feedstock was poured into the reactor, stirred at 88 rpm, and heated to the specified temperature for the defined time

(Table 1.1). No supplemental pressure was applied (autogenous) and the system was cooled using a fan.

After reaction time was reached, the unit was allowed to cool to 40 °C. At this time, the reactor was disassembled and the contents filtered (VWR Filter Paper, 415. Cat 28320-020) (Wood et al., 2013). The end result was solid hydrochar and the aqueous filtrate products.

3.2.2 HTC Filtrate Aging

After collecting the HTC filtrate, approximately 2/3 of the volume was used to set up time trials in order to create an aging affect that would be representative of filtrate being stored in a tank prior to use. Half of the allocated volume was used to set up a time trial to show an effect that would be analogous to storing filtrate in an open tank, and the other half was used to set up a time trial that would be analogous to filtrate being stored in a closed tank. To compensate for evaporation, the open treatments were topped up every week with ultrapure HPLC water (Aqua Solutions; Deer Park, TX) fluid, until the mass of the container and fluid was the same as it was initially. The mass of each open container undergoing an aging period was recorded before and after each H_2O addition.

3.2.3 Germination Studies

Germination effects were studied by observing growth of maize radicle over the course of a week in response to various amounts of CDS and swine HTC filtrate. The experiment was set-up by evenly spreading 10 corn seeds across 3 -3/8" circular, blue blotter paper (Anchor Paper Co.; St. Paul, MN) inside a petri dish. Corn seeds were sorted for uniform size prior to placement within each petri dish, and blotter paper was saturated with 5 mL's of 0X (di. H_2O), 1/50X, 1/20X, 1/5X, 1/2X, and 1X concentrations

of CDS and swine HTC filtrates, prior to seed application. Each dilution was tested in triplicate, and two separate germination studies were performed; the first using fresh HTC filtrate and the second using the same filtrates aged in open containers for three months.

The first germination study was performed on a lab bench top exposed to window light and fluorescent lighting. There was no light, humidity or temperature control or monitoring. Visual observation for radicle formation was the only metric used to monitor response. Plates treated with CDS HTC filtrates were monitored for 7 days, and those treated with swine HTC filtrate were monitored for 6.

In order to improve environmental conditions (light equality across table top), the second germination study was initiated on a lab bench top and then transferred to a growth chamber on day 2 (Controlled Environments, Winnipeg Manitoba Canada). The light cycle in the growth chamber was set for 16-hour days and 8-hour nights. Neither humidity nor temperature was monitored. Light intensity in the growth chamber was recorded with a light sensor. Daily visual observation for radicle formation was used to monitor germination progress. In addition to the visual assessment of radicle formation, each set of seeds was weighed at the conclusion of the study to gauge overall accrued biomass.

3.2.4 Corn Seedling Growth Trials

3.2.4.1 Corn Seedling Growth Trials: Silica Based Sand Media

Growth trials were conducted in a University of Minnesota Plant Pathology growth chamber. The 6 ft. tall x 4.5 ft. wide x 8 ft. long climate controlled chamber (Controlled Environments, Winnipeg Manitoba Canada) was set to run on a 16-hour light

period, with daytime temperature of 31 °C with relative humidity of 60%, and a nighttime temperature of 26 °C and relative humidity of 50% (Table 3.1).

Washed silica sand was used as the growth media, which was rinsed again in the laboratory prior to use for 30 minutes with tap water to get rid of any previous contaminants and then air dried. The sand was pre-wetted, and lightly packed into a 4" x 4" x 4" pot containing drainage holes. There was approximately 1300 grams of sand added per container. A saucer was placed under each pot to collect any leachate in the case that drainage had occurred. Each replicate was watered with 100 mL's of autoclaved tap water every other day for the length of the 21-day growth period.

The growth trials consisted of three types of filtrates, HTC filtrate of condensed distillers solubles (CDS) and swine manure aged in open and closed containers, as well as fresh HTC filtrate of poultry manure. To evaluate repeatability, an additional set of the same three filtrates was also run, which consisted of three filtrates that were of filtrate aged in an open container only. All filtrates were aged for a period of 3 months, regardless of whether they were aged in open or closed containers. Prior to application, filtrates were diluted to 2 and 10 fold dilutions, and then applied to the soil pots in triplicate reps with a one-time application of 100 mL of the targeted filtrate dilution per replicate. A positive control consisted of a triplicate set containing a fertilizer solution (Peat-Lite™ 20-10-20 fertilizer stock solution), which was diluted to concentration of 5 g per gallon (1.32 g/L), and 100 mL of this solution was applied per replicate. Each filtrate treatment replicate contained ~50 mL's of a partial Hoagland's solution to supply the plants with trace elements (Table 3.2). A negative control consisted of a

triplicate treatment of autoclaved tap water, which was also applied in 100 mL volumes per replicate without any supplementation with partial Hoagland's mix. After the treatments were applied, each pot was seeded with two to three corn kernels of uniform size. Upon emergence, the sprouts were thinned to one per pot, pulling the smaller sprouts. All replicates were randomized within the growth chamber, and light concentrations were measured above each pot with a light meter (FieldScout Light Sensor, Spectrum Technologies, item # 366816), which measured photo-synthetically active radiation (PAR) relating to light in the 400 to 700 nanometer wavelength, in units of μmol m^{-2} s^{-1}. Each replicate was watered with 100 mL's of autoclaved tap water every other day for a growth period of 21 days. Temperature and humidity were recorded over the course of the 21 days at 5-minute intervals with a humidity and temperature data-logger (Extech instruments, RHT10).

The seedlings were harvested on the 22nd day, and the roots were washed free of sand after which the root system was cut free from the stem at the base and both were placed into separate, pre-weighed paper bags for drying in an 85°C oven. The bags used for drying of the plant mass, had also been dried in the 85°C oven prior to being pre-weighed.

Nitrate, ammonium and phosphate nutrient analysis was performed on all of the initial filtrate dilutions using a Lachat Auto-analyzer (Lachat Instruments, Loveland CO).

Table 3.1 Corn seedling growth trials, filtrates used, and growth chamber parameters

	Growth Chamber - Trial 1 (GCT1)	**Growth Chamber - Trial 2 (GCT2)**
Growth Media Used	Silica Sand (Washed)	Silica Sand (Washed)
Average Weight of Wetted Growth Media	~ 1300 grams	~ 1300 grams
Approximate volume of initial water w/ in media	~ 116 ml's	~ 136 ml's
Plants grown in:	Growth Chamber	Growth Chamber
Daytime temp.	31° C	31° C
Daytime relative humidity	60% humidity	60% humidity
Daytime duration	16 hours	16 hours
Nighttime temp.	26° C	26° C
Nighttime relative humidity	50% humidity	50% humidity
Nighttime duration	8 hours	8 hours
Filtrate Application / Concentrations	CDS - aged in open container (1/2X & 1/10X)	CDS - aged in open container (1/2X & 1/10X)
	CDS - aged in closed container (1/10X)	N/A
	Swine manure - aged in open container (1/2X & 1/10X)	Swine manure - aged in open container (1/2X & 1/10X)
	Swine manure - aged in closed container (1/10X)	N/A
	Poultry manure - fresh (1/2X & 1/10X)	Poultry manure - aged in open container (1/2X & 1/10X)
Filtrates diluted with:	50 ml's Hoagland's solution / rep + di - H2O (Table 3.2)	50 ml's Hoagland's solution / rep + di - H2O (Table 3.2)
Replicates (n)	(n=3) per treatment , randomized	(n=3) per treatment , randomized
Volume Watered with:	100 ml's autoclaved tap water	100 ml's autoclaved tap water
Positive control	100 ml's peat-lite fertilizer (5 g/ gal) + 50 ml's Hoagland's solution / rep (Table 3.2)	100 ml's peat-lite fertilizer (5 g/ gal) + 50 ml's Hoagland's solution / rep (Table 3.2)
Negative control	100 ml's di-H2O/ rep	100 ml's di-H2O/ rep

Table 3.2 Modified Hoagland's nutrient solution used in this experiment for the corn growth trials.

Hoagland's Solution

Compound	***CONC.***	
$Ca(NO_3)2$	826.00 mg/L	Omitted
KNO_3	252.50 mg/L	Omitted
KH_2PO_4	136.10 mg/L	Omitted
$MgSO_4$	246.50 mg/L	Used
FeEDTA	1.32 mg/L	Used
Trace Elements		
1) H_3BO_3	11118.33 ng/L	Used
2) $MnCl_2.4H_2O$	144.40 ng/L	Used
3) $ZnSO_4.7H_2O$	1.23 ng/L	Used
4) $CuSO_4.5H_2O$	0.35 ng/L	Used
5) $NaMoO_4$	2.68 ng/L	Used

3.2.5 Statistics

Data presented, represents means of the triplicate samples. Standard deviations were calculated for all variables. One-way analysis of variance (ANOVA) was performed using GraphPad software (GraphPad Software, Inc.) to determine statistical significance of the experimental factors tested (n=3). If statistical significance was present, the Tukey-Kramer Multiple Comparisons Test was then used to test between means of plant height and plant mass of the treatments against means of the controls. Independent variables evaluated were light intensity, filtrate type, concentration, and aging effects. Dependent variables were plant height and plant mass. A value of $P < 0.05$ was used to assess statistical significance.

3.3 RESULTS

3.3.1 - Germination

3.3.1.1 Germination Study- Fresh Filtrates

As seen in Figure 3.1A, seeds placed on blotter paper treated with fresh swine HTC filtrate produced sprouts for 1/50, 1/20, and 1/5X at a rate that was similar to the control. The 1/2X dilution had a slower rate of germination than the 1/50, 1/20 and 1/5X dilutions. There was no growth observed for the 1X treatment over the 10 days. These assessments were performed with a visual comparison of graphs due to lack of statistically assessable data.

Seeds placed onto blotter paper treated with fresh CDS HTC filtrate produced sprouts for the 1/50X and 1/20X concentrations, at a rate that was similar to the control. The 1/5X concentration had a slower rate of germination (longer lag time before germination) than the 1/50X and 1/20X dilutions. However, for the CDS there was no germination observed during the study for the 1/2X and 1X treatments (Figure 3.1B). This data suggests that the undiluted swine and the 1/2X CDS and undiluted CDS filtrates do inhibit corn seed germination.

3.3.1.2 Filtrates Aged in Open Container

Total light intensity above the petri dishes was measured to be 115 μmol m^{-2} s^{-1}. As seen in Figure 3.2A, seeds placed on blotter paper treated with swine HTC filtrate produced sprouts for 1/50X, 1/20X, 1/5X concentrations at a rate that was similar to the control, which was determined via a visual analysis of graphs. The 1/2X concentration of the swine filtrate had a slower rate of growth (longer lag period and lower total seed germination) than the 1/50X, 1/20X and 1/5X concentrations. Unlike the germination study of fresh filtrate applications, radicle growth was observed at higher concentrations

of both the CDS and swine treatments of aged filtrate. Germination was seen on day 7 for the aged swine 1X treatment (4 of 30 seeds) and aged CDS 1/2X treatment (3 of 30 seeds). Therefore, this suggests that the inhibitory compounds may be partially lost or modified in such a way that the inhibitory effects are reduced with storage time.

Seeds placed on blotter paper treated with CDS HTC filtrate produced sprouts for 1/50X and 1/20X concentration treatments at a rate that was similar to the control. However, the 1/5X dilution had a longer lag before germination, which was not observed in the 1/50X, 1/20X, and control treatments. There was some formation of radicles seen in the 1/2X CDS treatments, and no growth observed in the 1X CDS treatments (Figure 3.2B).

As shown in Figure 3.3, the average seed mass of ten seeds from aged 1X CDS and swine HTC filtrate treatments that had no visible radicle formation was 3.67 (+/- 0.05) grams, whereas the average seed mass of all 6 negative control plates (n=6) was 5.01 (+/- 0.76) grams. Significant differences of aged filtrate applications were observed solely in the 1/2X ([swine-1/2X] – $P < 0.05$, [CDS-1/2X] – $P < 0.01$) and 1X ([swine 1X] – $P < 0.01$, [CDS-1X] – $P < 0.01$) treatments of both CDS and swine seed mass when compared to the controls (Figure 3.3). All other treatments were not significantly different from that of the controls. Statistical analysis of mass at the end of the germination trial showed that there were no significant differences between 1/50X, 1/20X and 1/5X treatments and the control. Indicating that the diluted filtrate had no observable negative impact on seed germination.

Table 3.3 Seed germination as a function of filtrate type and applied concentration for the corn seed germination trial.

Filtrate Concentration	% Of Germinated Seeds at Day-7			
	Fresh Swine Filtrate	Swine Filtrate Aged in Open Container	Fresh CDS Filtrate	CDS Filtrate Aged in Open Container
0 X (Control)	100%	100%	100%	97%
1/50 X (0.02)	100%	100%	100%	100%
1/20 X (0.05)	100%	100%	100%	100%
1/5 X (0.2)	100%	100%	73%	93%
1/2 X (0.5)	53%	80%	0%	10%
1 X (Undiluted)	0%	13%	0%	0%

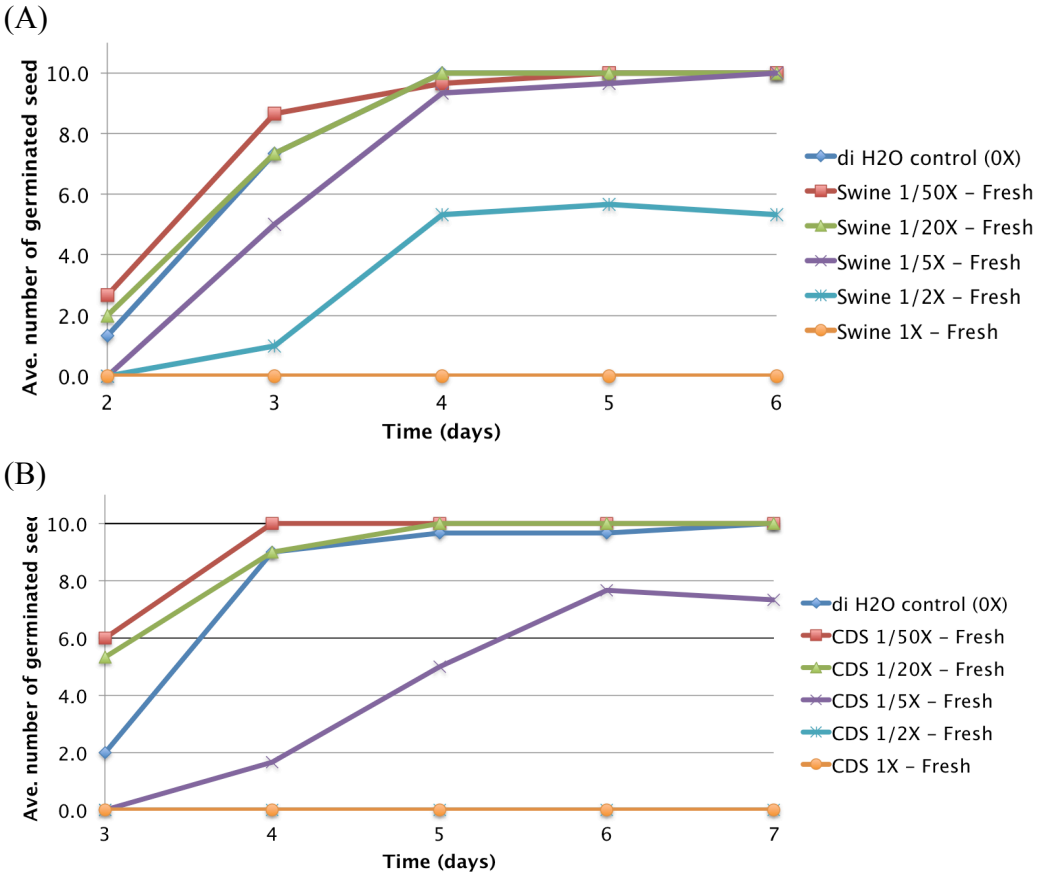


Figure 3.1 - Corn seed germination for fresh A) swine and B) CDS HTC filtrates at various dilutions out of the 10 original seeds placed on the blotter paper.

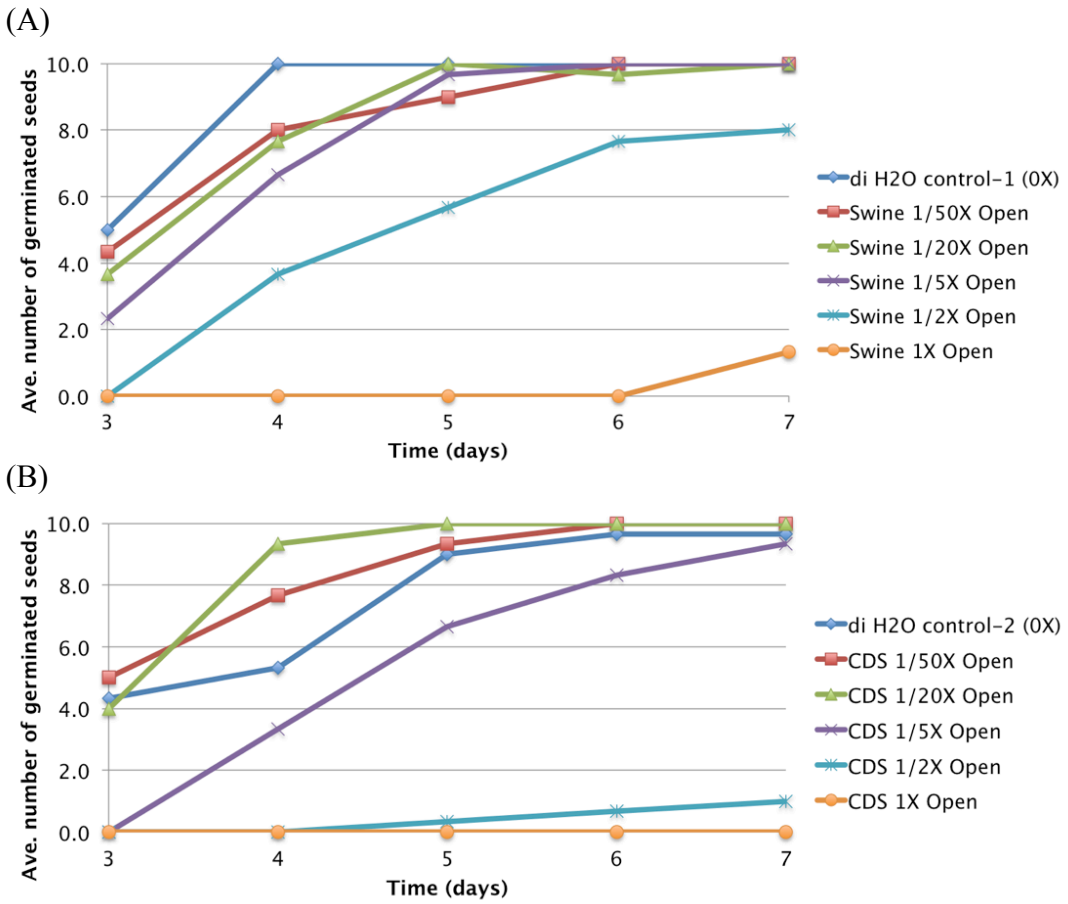


Figure 3.2 – Corn seed germination for filtrate aged in an open container for A) swine and B) CDS HTC filtrates at various concentrations out of the 10 original seeds placed on the blotter paper.

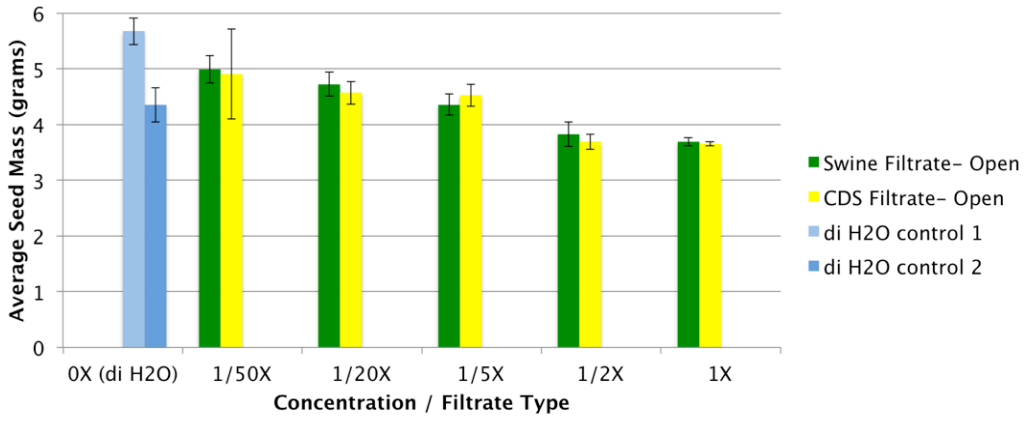


Figure 3.3 - Average mass of 10 corn seeds as a function of different concentrations of swine and CDS HTC filtrates that were aged in an open container for 3 months.

3.3.2 Germination Discussion

The data presented on the corn seed germination with varying concentration of filtrates clearly demonstrate that there are different impacts in regards to filtrate concentrations and their effect on germination. Overall trends show that application of filtrate in lower concentrations does not inhibit germination and early radicle formation. A difference in inhibition between swine and CDS filtrates becomes apparent when comparing the 1/2X treatments of the two filtrates. Swine 1/2X treatments had higher growth, whereas treatments of 1/2X CDS filtrate had no root formation with fresh filtrate, but some germination was observed with the aged CDS filtrate. Inhibition of germination was universally observed when undiluted filtrate was used, regardless of the feedstock type. This is most likely due to the complex chemistry of these filtrates (Chapter 4). Some of the compounds known to produce negative germination effects have been attributed to presence of phenols, (I. Bargmann, M. Rillig, W. Buss, A. Kruse, & M. Kuecke, 2013), polycyclic aromatic hydrocarbons (PHA's) (Rogovska, Laird, Cruse, Trabue, & Heaton, 2012) dioxins, high level of N-nutrients (Judy A Libra et al., 2011), salt stress (K. Chan, Van Zwieten, Meszaros, Downie, & Joseph, 2008), 5-hydroxymethyl-furfural-1-aldehyde (HMF), furfural, and volatile organic acids such as acetic acid (M.-M. Titirici, Antonietti, & Baccile, 2008). In particular, it has been shown that as volatile organic compounds dissipate with aging, so does the phytotoxic effect of the aged material (Busch et al., 2012). This was also observed in this study, where the aging of filtrate in an open container was more beneficial than fresh filtrate. Therefore, this confirms some of the inhibition in seedling germination could be due to the compounds that are dissipated through aging in an open container (Chapter 4).

3.3.3 Corn Growth Trials

Table 3.4 Nutrient concentrations of undiluted filtrates for corn growth trials

Growth Trials	*Solution/ Filtrate type*	*NH4*	*NO3*	*PO4*
Growth Chamber Trial 1	CDS filtrate - open	3324 mg/L	<1 mg/L	7617 mg/L
	CDS filtrate - closed	3269 mg/L	<1 mg/L	7100 mg/L
	Swine filtrate - open	3853 mg/L	<1 mg/L	53 mg/L
	Swine filtrate - closed	3269 mg/L	<1 mg/L	40 mg/L
	Poultry - fresh	2335 mg/L	<1 mg/L	48 mg/L
	Peat-Lite fertilizer	131 mg/L	175 mg/L	57 mg/L
Growth Chamber Trial 2	CDS filtrate - open	3995 mg/L	<1 mg/L	8265 mg/L
	Swine filtrate - open	3474 mg/L	<1 mg/L	50 mg/L
	Poultry - open	1971 mg/L	<1 mg/L	47 mg/L
	Peat-Lite fertilizer	127 mg/L	173 mg/L	57 mg/L

Table 3.5 Approximate NH_4 amounts applied to each plant pot

Growth Trials	*Solution/ Filtrate type*	*Initial NH4*	*1/2X Dose*	*1/10X Dose*
Growth Chamber Trial 1	CDS filtrate - open	3300 mg/L	~165 mg-NH_4/ Pot	~33 mg-NH_4/ Pot
	CDS filtrate - closed	3300 mg/L	Not Applied	~33 mg-NH_4/ Pot
	Swine filtrate - open	3900 mg/L	~195 mg-NH_4/ Pot	~39 mg-NH_4/ Pot
	Swine filtrate - closed	3300 mg/L	Not Applied	~33 mg-NH_4/ Pot
	Poultry - fresh	2300 mg/L	~115 mg-NH_4/ Pot	~23 mg-NH_4/ Pot
Growth Chamber Trial 2	CDS filtrate - open	4000 mg/L	~200 mg-NH_4/ Pot	~40 mg-NH_4/ Pot
	Swine filtrate - open	3500 mg/L	~175 mg-NH_4/ Pot	~35 mg-NH_4/ Pot
	Poultry - open	2000 mg/L	~100 mg-NH_4/ Pot	~20 mg-NH_4/ Pot

3.3.3.1 Growth Chamber Trial 1 (GCT1) – Aged Open and Closed Trials for CDS and Swine HTC Filtrates, and Fresh Poultry Manure HTC Filtrate

Figure 3.6 illustrates the various growth rates of Growth Chamber Trial 1 (GCT1) in response to different filtrate applications. It was observed that plants were growing at very different rates, which can be visually seen in the Figure by the different slopes of the

average height graph with respect to time (Table 3.3 and Figures 3.1-3.2). Measurements of the light concentrations for GCT1 showed a statistically uniform light field across all treatments, with the average light intensity of 462 +/- 9 μmol m^{-2} s^{-1} above each treatment at the time of measurement, with no statistically significant difference between treatments ($P > 0.05$).

CDS Filtrate Applications

The NH_4 of the undiluted CDS filtrate aged in an open container had a concentration of 3,300 mg/L (Table 3.4), which means that 100 mL treatment concentrations of 1/2X and 1/10X contained 165 and 33 mg-NH_4 / pot, respectively. The NH_4 concentration of the undiluted CDS filtrate aged in a closed container had a 3,300 mg/L NH_4 (Table 3.4), which corresponds to 33 mg-NH_4 / pot for the 1/10X applications (Table 3.5).

Swine Filtrate Applications

The NH_4 of the undiluted swine filtrate aged in an open container had a concentration of 3,900 mg/L NH_4, corresponding to 200 and 40 mg-NH_4 / pot for the 1/2X and 1/10X open treatments, respectively. The NH_4 concentration of the undiluted swine filtrate aged in a closed container had a 3,300 mg/L NH_4 (Table 3.4), which corresponds to 30 mg-NH_4 / pot for the 1/10X applications (Table 3.5).

Poultry Filtrate Applications

Undiluted poultry HTC filtrate contained 2,300 mg/L of NH_4 corresponds to 100 and 20 mg-NH_4 / pot for the 1/2X and 1/10X treatments, respectively (Table 3.4 - 3.5).

3.3.3.1.1 Corn Plant Height - GCT1

The growth rates between plants, and final plant height differed greatly depending on the type of treatment that was applied to the pot, as seen by the different slopes of the

seedling growth curves (Figures 3.4A). At the conclusion of the experiment, the treatments that produced the lowest amount of growth were CDS 1/2X open ($P< 0.001$), and swine 1/2X open ($P< 0.01$). Whereas poultry 1/2X, CDS 1/10X open and CDS 1/10X closed all produced plants that were taller than the fertilizer positive control, although with no statistical significance ($P > 0.05$). Measurements of the PAR light intensities for GCT1 showed that the light field was very uniform across all treatments, and not statistically different ($P> 0.05$), with an average intensity of 462 +/- 4 $\mu mol\ m^{-2}\ s^{-1}$ above each treatment at the time of measurement. Table A3.2 shows the complete set of ANOVA/ Tukey-Kramer P-Values for comparisons of GCT1 plant height.

3.3.3.1.2 Corn Plant Mass - GCT1

Average dry-weight plant mass for GCT1 is represented in Figure 3.4C. The treatments that produced plants with the lowest total dry weight were CDS 1/2X open ($P < 0.001$) and swine 1/2X open ($P < 0.001$), which were 64% and 72% lower than the fertilizer control, respectively, which had a total mass of 2.5 +/- 0.3 grams. The treatments that produced plants with total dry weights greater than the fertilizer control were CDS 1/10X open ($P < 0.05$) and CDS 1/10X closed ($P < 0.05$), which produced plants that were 40% and 35% greater in total average mass than the fertilizer control, respectively. Poultry 1/2X treatment had a lower total mass than the poultry 1/10X treatment, despite having a taller average height at the end of the trial, with 33% and 20% lower masses than the fertilizer control, respectively. Table A3.3 shows the complete set of ANOVA/ Tukey-Kramer P-Values for comparisons of GCT1 mass. Table A3.4 shows the ANOVA of just the open treatments.

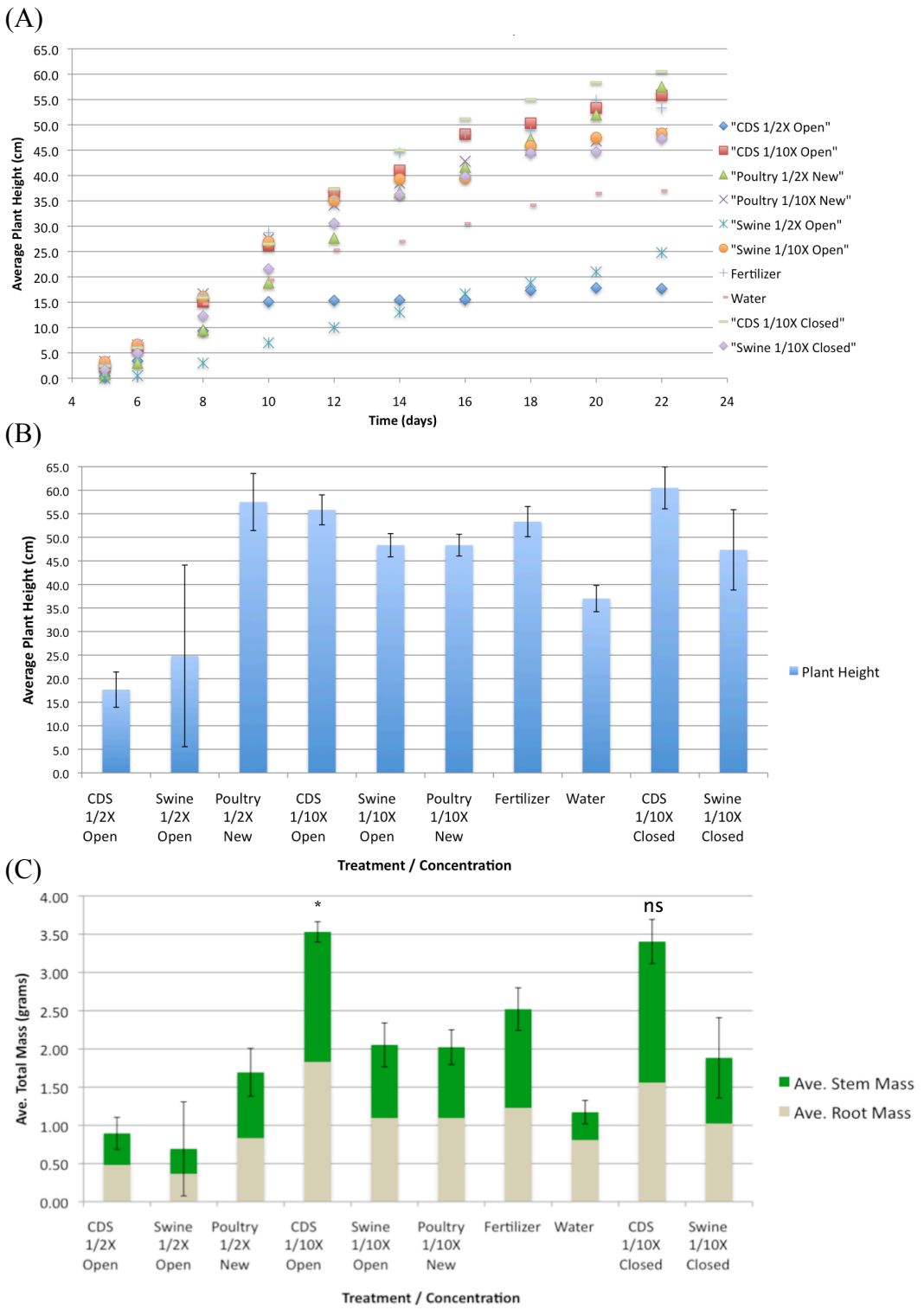


Figure 3.4 *Growth of corn seedlings* - GCT1: (A) Plant height vs. Time, (B) average plant height at the time of harvest (day 22), (C) average plant mass of shoot and root after experiment (day 22). Significance of the CDS 1/10X treatments displayed with respect to their fertilizer controls (* = $P < 0.05$, ns = not significant).

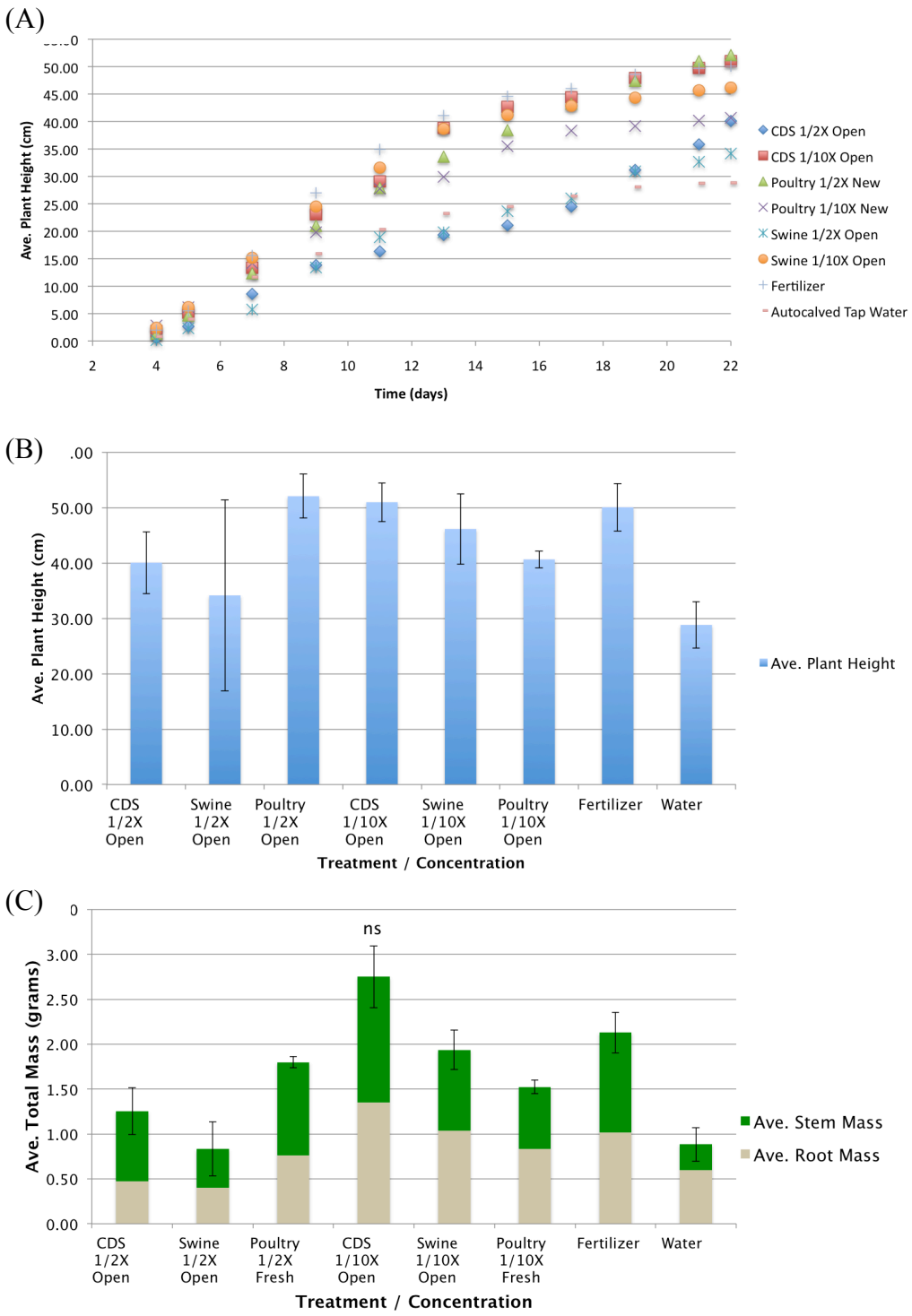


Figure 3.5 *Growth of corn seedlings* - GCT2: (A) Plant height vs. Time, (B) average plant height at the time of harvest (day 22), (C) average plant mass of shoot and root after experiment (day 22). Significance of the CDS 1/10X treatments displayed with respect to their fertilizer controls (ns = not significant).

3.3.3.2 Growth Chamber Trial 2 (GCT2) – CDS, Swine, Poultry Manure HTC Filtrates Aged in Open Containers

As seen in growth curves in Figure 3.5A, differing plant growth rates were also observed in growth chamber trial 2 (GCT2). Despite a lack of significance, there were observable differences in plant growth (different slopes of the cumulative growth lines), although these effects were not evaluated in this study. These differences could indicate that there was improved availability of nutrients at different times throughout the trial (Figure 3.4B), but these differences were not captured by the analysis of final total plant height. Measurements of the PAR light intensities for GCT2 showed that the light field was very uniform across all treatments, and not statistically different ($P > 0.05$), with an average intensity of 522 +/- 15µmol m^{-2} s^{-1} above each treatment at the time of measurement.

3.3.3.2.1 Initial NH_4 Concentrations - GCT2

CDS Filtrate Applications

The NH_4 of the undiluted CDS filtrate aged in an open container had a concentration of 4,000 mg/L (+/- 10%) (Table 3.4), which means that 100 ml treatment concentrations of 1/2X and 1/10X contained 200 and 40 mg-NH_4 / pot, respectively (Table 3.5).

Swine Filtrate Applications

The NH_4 of the undiluted swine filtrate aged in an open container had a concentration of 3,500 mg/L NH_4 (+/- 10%) (Table 3.4), corresponding to 175 and 35 mg-NH_4 / pot for the 1/2X and 1/10X open treatments, respectively (Table 3.5).

Poultry Filtrate Applications

Undiluted poultry HTC filtrate aged in an open container had an NH_4 concentration of 2,000 mg/L (+/- 10%) of corresponds to 100 and 20 mg-NH_4 / pot for the 1/2X and 1/10X treatments, respectively (Table 3.4 – 3.5).

3.3.3.2.2 Plant Height -. GCT2:

Results for average plant height at the end of GCT2 can be seen in Figure 3.5B. The only treatments that had a significant difference in plant height were poultry 1/2X open vs. negative control ($P< 0.05$), CDS 1/10X open vs. negative control ($P< 0.05$), and fertilizer control vs. negative control ($P< 0.05$) (Figure 3.5B). CDS 1/2X open and swine 1/2X open treatments produced shorter plants with respect to the fertilizer positive control, however both treatments had average heights that were greater than the negative control, and no statistical significance was found between the 1/2X treatments and the fertilizer control. Poultry 1/2X and CDS 1/10 X open produced plants that were slightly taller than the fertilizer positive control. Table A3.5 shows the complete set of ANOVA/ Tukey-Kramer P-Values for comparisons of GCT2 plant height.

3.3.3.2.3 Plant Mass - GCT2

Results for dry total plant mass at the conclusion of GCT2 can be seen in Figure 3.5C. The treatments that produced plants with the lowest total dry weights were CDS 1/2X open (1.25 +/- 0.26 g, $P < 0.01$) and swine 1/2X open (0.83 +/- 0.30 g, $P < 0.001$), which were 41% and 61% lower than the fertilizer control (2.13 +/- 0.23 g), respectively. The only treatment that produced plants with total dry weights greater than the fertilizer control were those of CDS 1/10X open treatment (2.75 +/- 0.34 g), although this result was not statistically significant ($P > 0.05$), the average mass of this treatment was 29% greater in total average mass than the positive control. Poultry 1/2X filtrate had a higher

total plant mass than the poultry 1/10X treatment, which was in agreement with its taller average height. Statistical analysis of poultry 1/2X and poultry 1/10X treatments with respect to the fertilizer control showed that there were no significant differences for either of the poultry treatments, despite having 15% and 28% lower masses than the positive control, respectively. Table A3.6 shows the complete set of ANOVA/ Tukey-Kramer P-Values for comparisons of GCT2 plant mass.

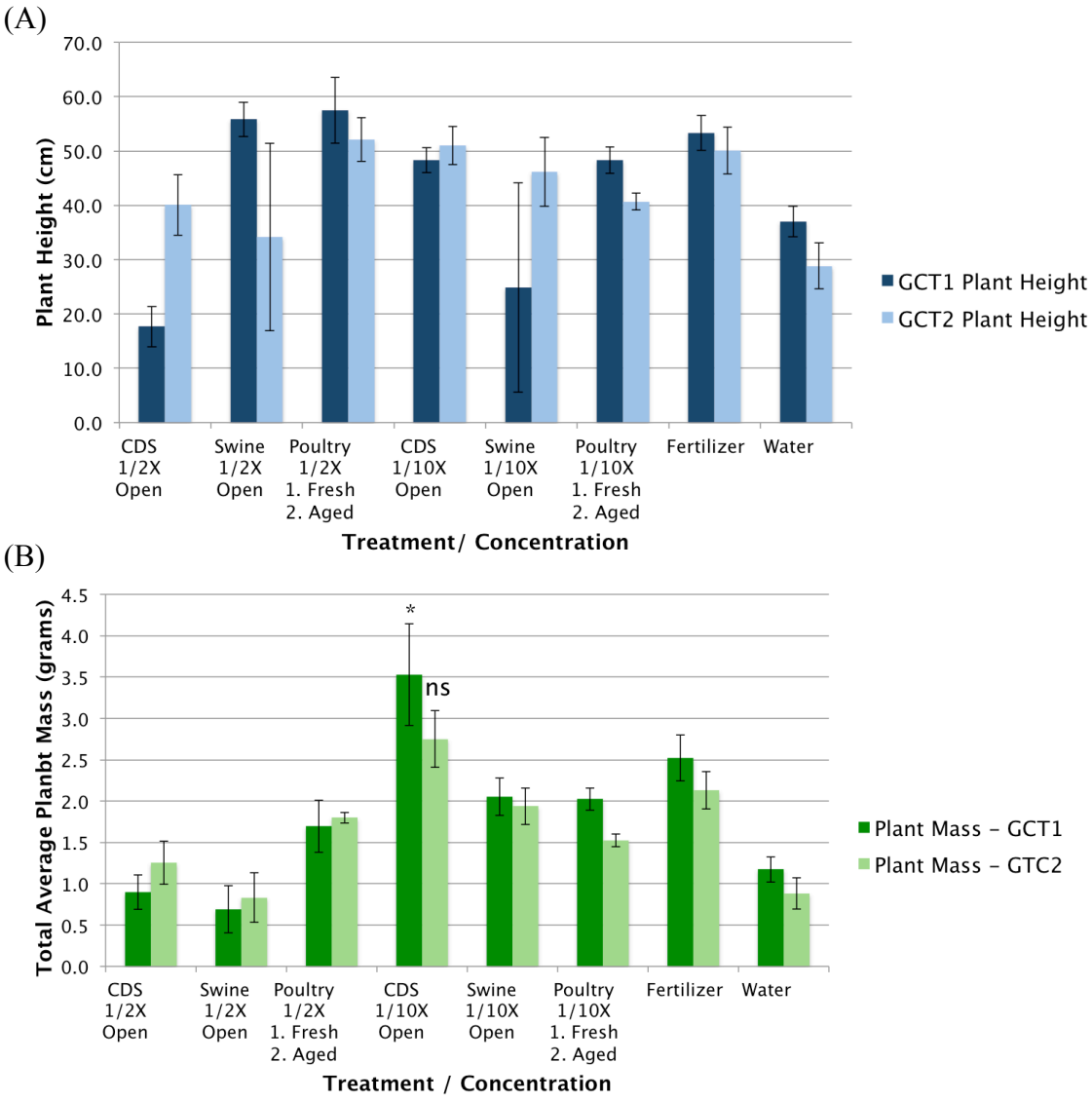


Figure 3.6 Comparison of A) average plant height and B) total average plant mass of GTC1 vs. GTC2 at the end of the experiment (day 22). All treatments were the same except poultry application, which utilized fresh filtrate for GTC1 and filtrate aged in an open container for GTC2. Significance of the CDS 1/10X treatments displayed with respect to their fertilizer controls (* = $P < 0.05$, ns = not significant)

3.3.3.3 Summary of seedling trials: GCT1 vs GCT2:

The greatest variation of height between GCT1 and GCT2 occurred for CDS 1/2X and swine 1/2X open treatments, where the treatments in GCT2 had final heights of 40.1 +/- 5.6 cm and 34.2 +/- 17.3 cm, which were 56% higher and 27% lower, respectively, than the corresponding treatments of GCT1, however, this variation was not statistically significant (Figure 3.6A). Unlike average plant heights, the corresponding total plant mass for the 1/2X treatments of CDS and swine had much less variation between GCT1 and GCT2 (Figure 3.6B). Comparison of total average plant mass of CDS 1/10X open treatments shows that this treatment in GCT1 was 22% greater than that of GCT2, and was significantly greater than it respective fertilizer control ($P < 0.05$), whereas CDS 1/10X treatment in GCT1 did not yield a statistical significance difference than its respective fertilizer control (Figure 3.6B).

3.4 CONCLUSION

Germination studies confirmed that there are optimal HTC filtrate concentrations at which inhibition of radicle formation is reduced. There is an association between filtrate type, applied rates and corn seedling phytotoxicity. The germination studies suggested that swine HTC filtrate could initially be applied at higher concentrations than CDS filtrate, without negatively impacting plant growth.

Similarly, seedling growth trial results show inhibition of seedling growth at higher applications of filtrate. The extremely high levels of NH_4 in all filtrate types is likely the greatest cause of inhibition, as it was previously shown that root toxicity has been observed at soil NH_4-N concentrations as low as 34 ppm at pH near 9. Furthermore, soil microbe stress can occur at levels greater than 200 ppm (Eno & Blue, 1957). Despite

an inhibitory effect seen in plant growth with application of higher amounts of filtrates, at lower concentrations, plants treated with CDS HTC filtrates exhibited a significant increase in plant mass after 3 weeks compared to the positive control which was treated with a one-time dose of fertilizer. Poultry and swine HTC filtrates resulted in lower total plant mass than the fertilizer control, but still showed improved growth when compared to the negative water control. It's believed that total carbon was again the limiting factor, and that the higher available carbon in CDS filtrate allowed for greater productivity at lower concentrations.

Both soil incubation studies and the plant growth studies, showed positive effects in regards to lower applications of filtrate. Therefore, a greater improvement is expected for overall plant growth, should the filtrates be applied to agricultural soil, and given a period of time for soil bacteria to digest any inhibitory compounds and mineralize NH_4 to NO_3. To have a growth trial that is more representative of real-life farm practices, a similar study in a growth chamber could be performed with agricultural soil, in which the filtrates would be allowed to undergo nitrification for a period of 2 – 4 weeks prior to planting. As it has been shown that under these conditions, microbes can help produce more available N, which would improve overall plant growth (Bargmann et al., 2014b; Busch et al., 2012; Busch et al., 2013). A study to investigate how plant N uptake is influenced by HTC filtrate addition could also be conducted, which utilizes N-15 labeled starting material to be used in an a HTC reaction for generation of N-15 labeled filtrate. Finally, studies will have to be performed in field trials to assess overall corn productivity.

CHAPTER 4

CHEMICAL CHARACTERIZATION OF HTC FILTRATES

4.1 INTRODUCTION

There are several different conversion technologies that are being pursued in an effort to harvest the energy contained in biomass (Yılmaz & Selim, 2013). However, hand in hand there is also a need for characterization of the corresponding residuals from thermochemical processing of the various biomass materials (e.g., Pedroza, Sousa, Vieira, & Bezerra, 2013). In particular, a significant requirement exists to ensure safe disposal practices to avoid repeating the environmental consequences of past biomass utilization, such as lingering soil contamination from biomass pyrolysis liquids (Chen, Wang, & Zheng, 2013; Edenborn & Severson, 2007; Hawley, 1926; Oleszczuk et al., 2014).

There have been some previous attempts at characterizing HTC filtrates using a wide-range of analytical tools. Because HTC filtrate is compositionally very complex, a wide array of analytical tools has been utilized for characterization. Various organic constituents have been determined using pyrolysis gas chromatography-mass spectrometry (py-GC/MS, Anastasakis, 2011) and high-pressure liquid chromatography (HPLC) in attempts to characterize the aqueous phase generated in the hydrothermal liquefaction of brown macro-alga (*Laminaria saccharina*). Jena and Kastner (2011) used HPLC to analyze the liquid phase produced during liquefaction of *Spirulina* algae. Eibisch (2013) utilized inductively coupled plasma (ICP) and HPLC coupled with ultraviolet (UV) and refractive index (RI) detection to separate and analyze several classes of organic compounds within the HTC filtrate of grass, straw and woodchips.

Organic compounds present in the filtrate were also examined by Poerschmann (2013), who performed solvent extraction analysis, followed by saponification and derivitization of olive mill waste (OMW) hydrochar by gas chromatography- mass spectrometry (GC-MS). Further advancements were performed by Stemann (2013), who used a combination of UV absorbance, size exclusion chromatography, organic carbon detection (LC-OCD, DOC) and combustion GC-MS to evaluate the chemical composition of poplar woodchips HTC filtrate. In addition to the organic compounds, there are also dissolved inorganic elements present in the filtrate. Heilmann (2011) used ICP to characterize the inorganic constituents within the filtrate from microalgae (*Chlamydomonas reinhardtii*). Biller (2012) examined the inorganic constituents in the process water of hydrothermal liquefaction of microalgae (*Chlorella vulgaris*) via ion-exchange chromatography and ICP-Optical Emission Spectroscopy (OES), and total phenol content by photometry. Levine et al. (2013) also demonstrated the chemical complexity of the *N. oculata* microalgae filtrate through their analysis by HPLC, GC-MS, and FT-ICR-MS.

Since HTC filtrate comprises the most abundant product stream, it is essential that studies be performed to fully evaluate the chemical composition, and potential toxicity, before agronomic utilization and/or environmental disposal. Additionally, there is a need for standardization of methodology for conducting analysis of filtrates from HTC and to examine variability over a range of substrate biomass materials. This manuscript begins to systematically characterize HTC filtrates from four diverse waste streams—swine, poultry and dairy cattle manures, and condensed distiller solubles (CDS) from the dry-grind ethanol industry.

Two methods of sample preparation were evaluated to determine their efficacy for analysis with thermal desorption (TD) of samples onto comprehensive 2-dimensional gas chromatography/time-of-flight mass spectrometry (2D-GC/TOF-MS) as a method to characterize the HTC liquid phase. The first method utilized a stir bar sorptive extraction (SBSE), which is a solventless extraction that employs sorption of analytes from aqueous solutions onto polar [polydimethylsiloxane (PDMS)] and non-polar [ethylene glycol (EG)] active-phase polymeric coatings on a magnetic stir bar, since this methodology has already demonstrated success in characterizing complex plant and other natural sample matrices (Kawaguchi et al., 2004; Peter Popp, Bauer, & Wennrich, 2001; Sandra, Tienpont, & David, 2003).

In addition, a direct evaporative method (DEM) was also utilized. Comparing chemical compositions extracted by these two coatings with those found with the DEM, provides a general assessment of the breadth of by-product organic compounds that can be formed from HTC processing. Further analyses are attempted to see how the filtrates may change with time and storage at STP conditions, since previous studies have shown that an aging affect of hydrochar is responsible for a diminished phytotoxic effect on plants (Bargmann et al., 2014b; Busch et al., 2012; Busch et al., 2013). To understand how the HTC filtrate products may change with time, filtrates generated immediately after HTC reaction (fresh) were analyzed via 2DxGC-TOFMS and compared to the same filtrates stored in and open container for 3 months (aged).

4.2 MATERIALS AND METHODS

4.2.1. Analysis of HTC filtrate

4.2.1.1. Sample Preparation

All HTC reactions were conducted in a laboratory-scale stirred stainless steel reactor (450 mL; Parr Instruments, Inc.; Moline, IL) fitted with an inductive heating system (LC Miller, Co.; Monterey Park, CA). The feedstock was poured into the reactor, stirred at 88 rpm, and heated to the specified temperature for the defined time (Table 1.1). No supplemental pressure was applied (autogenous) and the system was cooled using a fan.

After reaction time was reached, the unit was allowed to cool to 40°C. At this time, the reactor was disassembled and the contents were filtered (VWR Filter Paper, 415. Cat 28320-020) (Wood et al., 2013). The filtrate was immediately stored at 4°C until analyses could be performed. For analysis, the filtrate was removed from the refrigerator and then centrifuged for 5 min at 14000 rpm and the supernatant was then filtered through a 0.45 μm filter (Pall Acrodisc PN 4184).

4.2.1.2. Stir Bar Sorptive Extraction (SBSE)

Each sample was diluted with ultrapure water (CHROMASOLV for HPLC, Sigma-Aldrich, St. Louis, MO). Then triplicate 10 mL aliquots were extracted at room temperature and 1340 rpm for 13 h with conditioned stir bar (GERSTEL-Twister, PDMS-Silicone; Baltimore, MD). Following this 13 hr extraction, each PDMS stir bar was removed for thermal desorption analysis by 2D-GC. Each aliquot was then also extracted a second time at room temperature and 1340 rpm for 13 h with conditioned with a opposite polarity stir bars (GERSTEL-Twister, EG Silicone; Baltimore, MD). Following

extraction, each stir bar was rinsed with purified water (CHROMASOLV for HPLC, Sigma-Aldrich, St. Louis, MO) dried with a tissue (Kimwipe), and then placed into a thermal desorption tube (TDU tube) for injection into the GC system. Prior to extraction, each stir bar was analyzed as a blank to observe background peaks (contamination) associated with each stir bar. Table 4.1 provides an overview of the chemical properties for the three filtrates.

4.2.1.3. Instrumentation

A comprehensive 2-dimensional gas chromatograph–time of flight-mass spectrometer (Pegasus-4D; GCxGC-TOF-MS; LECO, St. Joseph, MO) was used, equipped with a cryogenic inlet system (CIS) injector and a thermal desorption unit (TDU) (Gerstel Inc., Baltimore, MD). The analytical column set consisted of a non-polar primary column [30 m × 0.25 mm × 0.25 μm DB-5; 95% polydimethylsiloxane, Agilent, Santa Clara, CA] and a mid-polarity secondary column [2 m × 0.10 mm × 0.1 μm BPx50 50% phenyl polysilphenylene-siloxane, SGE Analytical Science, Austin TX]. All analytical hardware was computer controlled (LECO ChromaTOF software; version 4.50). Chemical species were thermally desorbed from the stir bars by the TDU [40°C, 60° C/ min, 300°C (5min) for PDMS Twisters or 40°C, 60° C/ min, 260°C (5min) for EG Twisters] into the analytical column flow (split mode; 1:20). Injection and GC separation method was performed as published previously (Strong et al., 2014), with a total GC run time of 47 min. For peak resolution and quantification, the software integrated preprocessing tools corrected for instrumental fluctuations and noise, followed by mathematical resolution of overlapping peaks. Automated mass spectral matching

with the National Institute of Standards (NIST-2011) data library was used to assign compound identity.

4.2.1.4 Analysis Of Liquid Phase via "Direct Evaporative Method" (DEM)

Raw filtrate was prepared as described for SBSE. One µl of this dilution was placed directly into a microvial, which was then placed within the TDU tube. The liquid sample was evaporated via a TDU directly onto the 2D-GC/TOF-MS using the temperature program as described previously for the stir bars. However, in this method, the chemical species in the filtrate were directly volatilized without the aid of the extraction stir bars.

4.2.1.4. Analytical Software

LECO Statistical Compare software (LECO Stat. Compare software; version 1.6) was used for finding, identifying, and aligning peaks after data had been acquired. Gerstel Twister Calc. (v.1.0), which utilizes the log-K_{ow} of each particular species, was used to correct observed concentrations for extraction efficiencies of the Gerstel PDMS and EG stir-bars (based on manufacture recommendations).

4.2.2 HTC Filtrate Aging

After collecting the HTC filtrate, approximately 1/3 of the volume was allocated to set up time trials in order to create an aging affect that would be representative of filtrate being stored in an open tank prior to use. To compensate for evaporation during the aging process, the open treatments were topped up every week with Ultra Pure HPLC water (Aqua Solutions, Deer Park, TX. p/n W1089-4L), until the mass of the container and fluid was the same as it was initially. The mass of each open container undergoing an aging period was recorded before and after each H_2O addition.

4.2.3. Statistics

Samples were run in replicate to ensure precision in analytical methodology. Triplicates were preferred; however, maintenance procedures resulted in the loss of ~1 m of the secondary analytical column during this experiment. This difference in column length severely altered the elution retention times of the compounds and hampered direct graphical overlay comparisons. Therefore, analyses completed after this column breakage event were not used in this study. Analytes identified as column or Twister impurities were flagged if present in equal or greater concentration compared with the blank runs, or removed from compound list if found in lower concentrations. Identification of major components was verified through comparison to the spectral library (NIST) and corresponding retention time. Four compounds (phenol, acetonitrile, 4-ethylphenol, and phosphonic acid) had high standard deviations (>25% relative standard deviation), which indicated a lack of consistent detection across the replicates. These were species were removed from the top-ten compound lists since they were not quantified in all three replicates. However, these compounds were retained in the full lists of components in the Supplemental Information.

4.3 RESULTS

Overall, there was exceptional repeatability displayed by the analytical methods used here. For a majority of the identified peaks in the replicates, relative percent differences (RPD) of peak areas were below 25%. This can be seen graphically in the overlay of the reconstructed 1-D total ion chromatogram (TIC) for the triplicate runs of the three filtrates, regardless of the analytical sample prep used (Figure 4.1). Despite this repeatability for each technique, there were substantial differences in the peak

distribution and the detected peak area between the direct evaporative injections and the corresponding injections by the SBSE stir bars (Figure 1.2 and Table 1.3-1.5).

4.3.1 Filtrate Chemical Properties

The general chemical properties of the three filtrates are presented in Table 4.1. Overall, there were differences in the amount of solids in the original feedstock (10-33%), all filtrates were produced at a residence time of 2 hr. and all were at 225 ^{o}C, with the exception of the cow manure, which was heated to 250 ^{o}C (Table 4.1). All filtrates were acidic in nature (4.5 – 6.6) and were relatively high in conductivity (13 – 30 mS/cm).

Table 4.1 Summary of HTC conditions and properties for the four filtrates

	Cow manure	**CDS**	**Swine Manure**	**Poultry Manure**
Un-treated Biomass % Solids	13.2	33.3	11.1	10.0
HTC Temperature (^{o}C)	250	225	225	225
HTC Holding Time (hr.)	2	2	2	2
Filtrate pH	5.2	4.5	5.4	6.6
Filtrate Conductivity (mS/cm) at 20 °C	18.5	30.1	15.95	12.68
Filtrate Total Carbon (TC) ppm	n/a	1119.6	165.8	165.8

4.3.1 CDS filtrate: SBSE & DEM Analyses

4.3.1.1 PDMS Stir Bar Method — Fresh CDS HTC Filtrate

The PDMS-SBSE analysis of CDS filtrate was analyzed in triplicate (n=3). The top five compounds observed via this method, and their corresponding area percentages, were: 2,6-dimethylpyrazine (5.6%), 2-butanone (4.9%), trimethylpyrazine (3.8%), acetone (3.5%), and 2-methyl-2-cyclopenten-1-one (3.2%). The top ten compounds

represent a total of 48% of the detected peak area (Table 4.2). The *Stat. Compare* analysis for PDMS SBSE method showed a total of 246 matched peaks.

4.3.1.2 EG Stir Bar Method — Fresh CDS HTC Filtrate

The corresponding EG-SBSE analysis of CDS filtrate was run in triplicate (n=3). The top five compounds observed via this method, and their corresponding peak area percentages, were glycerin (5.7%), 4-ethylphenol (4.6%) 2-methoxyphenol (3.6%), 4-ethyl-2-methoxyphenol (2.9%), and N-[2-hydroxyethyl]succinimide (2.7%). The top 10 compounds observed via the EG stir bar method comprised 41% of the total peak area (Table 4.2). The *Stat. Compare* analysis for EG SBSE method showed a total of 617 matched peaks.

4.3.1.3 Direct Evaporative Method – Fresh CDS HTC Filtrate

Triplicates were attained via DEM analysis of CDS filtrate (n=3) and the top five compounds observed with this method and their corresponding area percentages of the total peak area (in parentheses) were: glycerin (29.8%), methyltartronic acid (2.6%), (S)-(+)-1,2-propanediol (1.5%), analyte 594 (0.8%), and 2,3-butanediol (0.8%). The top 10 compounds and their corresponding area integrations are presented in Table 4.5. These top 10 compounds represent 37.8% of the total detected peak area for the sample.

The *Stat. Compare* analysis for DEM of fresh CDS HTC filtrate showed total of 815 matched peaks.

4.3.1.4 Direct Evaporative Method – Aged CDS HTC Filtrate

Triplicates were attained via DEM analysis of CDS filtrate (n=3) and the top five compounds observed with this method and their corresponding area percentages of the total peak area (in parentheses) were glycerin (27.4%), 1-methyl-2,5-pyrrolidinedione,

(1.0%), 6-methyl-3-pyridinol, (0.8%), 2,3-dichloro-1-propanol, (0.8%), and 3-pyridinol (0.8%). The top 10 compounds and their corresponding percent of total area are presented in Table 4.5. These top 10 compounds represent 32.1% of the total detected peak area for the sample. The *Stat. Compare* analysis for DEM of aged CDS HTC filtrate also showed total of 815 matched peaks.

4.3.2 Swine Manure: SBSE & DEM Analyses

4.3.2.1 PDMS Stir Bar Method — Fresh Swine Manure HTC Filtrate

Duplicate samples were also run for the PDMS SBSE of swine manure filtrate (n=2). The top five compounds found with this method, and their respective area percentages, were: acetonitrile (9.8%), 2-ethyl-6-methylpyrazine (7.6%), methylpyrazine (7.0%), 2-6-dimethylpyrazine (4.9%), and 4-ethyl-2-methoxyphenol (4.1%). The top ten recognized compounds comprise a total of 48% of the detected peak area (Table 4.3). The *Stat. Compare* analysis for PDMS SBSE method showed a total of 487 matched peaks.

4.3.2.2 EG Stir Bar Method — Fresh Swine Manure HTC Filtrate

The corresponding EG-SBSE analysis of the swine filtrate was run in triplicate (n=3). The top five compounds observed via this method, and their corresponding area percentages, were: 2-methoxyphenol (6.7%), phenol (6.4%), 4-ethylphenol (5.3%), 3-ethylphenol (3.0%), and 2-methoxyphenol (2.7%). The top ten compounds comprised 38% of the detected peak area (Table 4.3). The *Stat. Compare* analysis for EG SBSE method showed a total of 1220 matched peaks.

4.3.2.3 Direct Evaporative Method – Fresh Swine Manure HTC Filtrate

Triplicates were attained via DEM analysis of swine manure filtrate (n=3), and the top five compounds with this method, and their corresponding area percentages, were butanoic acid (16.2 %), acetamide (6.3%), 2-methyl- propanoic acid, (6.3%), 3-methyl-butanoic acid, (3.6%), and methyl-pyrazine, (3.0%). The top 10 compounds observed via DEM of swine filtrate represent 42% of the detected peak area (Table 4.6). The *Stat. Compare* analysis for DEM of fresh swine HTC filtrate showed total of 750 matched peaks.

4.3.2.4 Direct Evaporative Method – Aged Swine Manure HTC Filtrate

Triplicates were attained via DEM analysis of aged swine manure filtrate (n=3), and the top five compounds with this method, and their corresponding area percentages, were: butanoic acid (13.0%), acetamide (9.4%), acetic acid, (6.1%), propanoic acid, (5.8%), and 2-methyl- propanoic acid (5.3%). The top 10 compounds observed via DEM of swine filtrate represent 53% of the detected peak area (Table 4.6). The *Stat. Compare* analysis for DEM of aged swine HTC filtrate showed total of 673 matched peaks.

4.3.3 Cow Manure HTC Filtrate: SBSE & DEM Analyses

4.3.3.1 PDMS Stir Bar Method — Fresh Cow Manure HTC Filtrate

Duplicate samples were also run for the PDMS SBSE of cow manure filtrate (n=2). The top five compounds found in PDMS SBSE of cow manure filtrate, and their corresponding area percentages, were: methylpyrazine (6.9%), 2-ethyl-5-methylpyrazine (6.6%), ethylpyrazine (4.8%), 2-butanone (4.7%) and 2,5-dimethylpyrazine (4.1%). The top 10 compounds observed via the PDMS-SBSE and their corresponding peak areas are shown in Table 4.4. The *Stat. Compare* analysis for this method showed a total of 371 matched peaks.

4.3.3.2 EG Stir Bar Method — Fresh Cow Manure HTC Filtrate

The corresponding EG SBSE of cow manure HTC filtrate was also run in duplicate samples (n=2), and the top five compounds found with these stir bars were: phenol (3.6%), 2-ethyl-6-methylpyrazine (1.9%), 3-methylphenol (1.8%), ethylpyrazine (1.6%) and methylpyrazine (1.5%). The top 10 compounds observed via EG-SBSE and their corresponding peak areas can be seen in Table 4.4. The *Stat. Compare* analysis for this method showed a total of 544 matched peaks.

4.3.3.3 Direct Evaporative Method — Fresh Cow Manure HTC Filtrate

The DEM of cow manure consisted of duplicates (n=2). The top five compounds found via DEM of the cow manure filtrate, and their corresponding area percentages, were acetic acid (27%), methylpyrazine (8%), pyrazine (4%), 2,5-dimethylpyrazine (3%), and ethylpyrazine (2%). The top 10 compounds observed via DEM of cow manure comprised a total of 50% of the detected peak area (Table 4.8). A *Stat. Compare* analysis for the DEM method showed a total of 214 matched peaks.

4.3.4 Poultry Manure HTC filtrate: DEM analyses

4.3.4.1 Direct Evaporative Method — Fresh Poultry Manure HTC Filtrate

Triplicates were attained via DEM analysis of swine manure filtrate (n=3), and the top five compounds with this method, and their corresponding area percentages, were acetic acid (14.3%), butanoic acid (6.2 %), 2-pyrrolidinone, (3.6%), propanoic acid, (4.4%), and L-lactic acid (3.2%). The top 10 compounds observed via DEM of swine filtrate represent 43% of the detected peak area (Table 4.7). The *Stat. Compare* analysis for DEM of fresh poultry HTC filtrate showed total of 492 matched peaks.

4.3.4.2 Direct Evaporative Method– Aged Poultry Manure HTC Filtrate

Triplicates were attained via DEM analysis of aged swine manure filtrate (n=3), and the top five compounds with this method, and their corresponding area percentages, were butanoic acid (8.3%), acetone (8.2%), 2-pyrrolidinone (6.2%), acetic acid, (5.2%), and pentanoic acid (3.8%). The top 10 compounds observed via DEM of swine filtrate represent 47% of the detected peak area (Table 4.7). The *Stat. Compare* analysis for DEM of aged poultry HTC filtrate showed total of 556 matched peaks.

Table 4.2 Top ten compounds detected by the two different SBSE analytical methods in the CDS filtrate

	Compound	***Average Peak Area***	***% of Total***
CDS – Filtrate: PDMS Stir-Bar (sorbes non – polar compounds) (n=3)			
1	acetonitrile	42,169,393	12.0%
2	pyrazine, 2,6-dimethyl-	19,744,079	5.6%
3	2-butanone	17,066,092	4.9%
4	pyrazine, trimethyl-	13,335,513	3.8%
5	acetone	12,215,708	3.5%
6	2-cyclopenten-1-one, 2-methyl-	11,088,445	3.2%
7	2-pentanone	10,294,879	2.9%
8	pyrazine, 2-ethyl-6-methyl-	10,140,320	2.9%
9	n-[2-hydroxyethyl]succinimide	9,584,430	2.7%
10	cyclotetrasiloxane, octamethyl-	8,975,079	2.6%
CDS – Filtrate: EG Stir-Bar (sorbes polar compounds) (n=3)			
1	phenol	92,524,614	8.2%
2	glycerin	63,619,030	5.7%
3	phenol, 4-ethyl-	51,740,778	4.6%
4	phenol	45,024,826	4.0%
5	2-butanone	41,612,615	3.7%
6	phenol, 2-methoxy-	40,121,292	3.6%
7	phenol, 4-ethyl-2-methoxy-	32,399,907	2.9%
8	n-[2-hydroxyethyl]succinimide	30,692,431	2.7%
9	2,3-butanediol	30,192,079	2.7%
10	phenylethyl alcohol	29,987,043	2.7%

Table 4.3. Top ten compounds detected by the two different SBSE analytical methods in the swine filtrate

	Compound	*Average Peak Area*	*% of Total*
Swine – Filtrate: PDMS Stir-Bar (sorbes non – polar compounds) (n=2)			
1	acetonitrile	102,088,277	9.8%
2	pyrazine, 2-ethyl-6-methyl-	78,709,426	7.6%
3	pyrazine, methyl-	72,428,935	7.0%
4	pyrazine, 2,6-dimethyl-	50,494,746	4.9%
5	phenol, 4-ethyl-2-methoxy-	42,354,755	4.1%
6	ethanone, 1-(2-thienyl)-	37,785,372	3.6%
7	pyrazine, ethyl-	37,469,073	3.6%
8	pyrazine, trimethyl-	28,436,882	2.7%
9	phenol, 2-methoxy-	27,659,590	2.7%
10	pyrazine, 3-ethyl-2,5-dimethyl-	24,186,850	2.3%
Swine – Filtrate: EG Stir-Bar (sorbes polar compounds) (n=3)			
1	phenol, 2-methoxy-	192,852,956	6.7%
2	phenol	184,813,264	6.4%
3	phenol, 4-ethyl-	151,199,048	5.3%
4	phenol, 3-ethyl-	85,697,680	3.0%
5	phenol, 2-methoxy-	76,761,592	2.7%
6	1-(2-thienyl)-1-propanone	67,157,538	2.3%
7	pyrazine, methyl-	61,962,246	2.2%
8	ethanone, 1-(3-thienyl)-	57,733,817	2.0%
9	pyrazine, 2-ethyl-5-methyl-	56,826,359	2.0%
10	phenol, 3-methyl-	52,854,350	1.8%

Table 4.4 Top ten compounds detected by the two different types of SBSE analytical methods in the cow manure filtrate

	Compound	*Average Peak Area*	*% of Total*
Cow – Filtrate: PDMS Stir-Bar (sorbes non – polar compounds) (n=2)			
1	methyl-pyrazine,	49,374,634	6.9%
2	2-ethyl-5-methylpyrazine	47,534,482	6.6%
3	ethylpyrazine	34,270,784	4.8%
4	2-butanone	33,889,471	4.7%
5	2,5-dimethyl-pyrazine	29,236,943	4.1%
6	2-methoxy-phenol	28,014,145	3.9%
7	acetone	25,265,982	3.5%
8	trimethylpyrazine,	21,588,377	3.0%
9	phenol, 4-ethyl-2-methoxy-	20,065,561	2.8%
10	2-cyclopenten-1-one, 2,3dimethyl-	17,933,910	2.5%
Cow – Filtrate: EG Stir-Bar (sorbes polar compounds) (n=2)			
1	phosphonic acid, (phydroxyphenyl)-	108,081,895	8.8%
2	phenol, 2-methoxy-	100,890,573	8.2%
3	phenol	96,124,283	7.8%
4	phenol	44,701,269	3.6%
5	pyrazine, 2-ethyl-6-methyl-	22,997,311	1.9%
6	phenol, 3-methyl-	22,575,861	1.8%
7	pyrazine, ethyl-	19,448,795	1.6%
8	pyrazine, methyl-	17,945,048	1.5%
9	pyrazine, 2,5-dimethyl-	16,598,029	1.3%
10	ethanone, 1-(3-thienyl)-	16,431,803	1.3%

Table 4.5 Top ten compounds detected by direct evaporative method in fresh CDS filtrate and filtrate aged for 3 months. Compounds in bold lettering signify species that are thought to be lost or drastically affected with aging. Concerns: SM – Saturated Mass, CB – Column Bleed, SA – Shared Apex.

Fresh CDS Filtrate: DEM (n=3)				**Aged CDS Filtrate: DEM (n=3)**		
	Compound Name	**Concerns**	**% of Total Area**	**Compound Name**	**Concerns**	**% of Total Area**
1	Glycerin	SM, CB	29.8%	Glycerin	SM, CB	27.4%
2	**Methyltartronic acid**	CB	2.6%	2,5-Pyrrolidinedione, 1-methyl-	SM	1.0%
3	**(S)-(+)-1,2-Propanediol**	CB	1.5%	3-Pyridinol, 6-methyl-		0.8%
4	**Analyte 594**	SM	0.8%	1-Propanol, 2,3-dichloro-		0.8%
5	**2,3-Butanediol**	SM	0.8%	3-Pyridinol	CB	0.8%
6	**2,3-Butanediol, [R-(R*,R*)]-**	SM	0.7%	Hydrogen chloride	CB	0.7%
7	**1,2-Propanediol, 3-chloro-**		0.5%	2,3-Butanediol		0.3%
8	**Acetic acid**	SM	0.4%	Acetone	SM, SA	0.1%
9	**(S)-(+)-1,2-Propanediol**		0.4%	2-Pyrrolidinone	CB	0.1%
10	**1,4-Dioxan-2-ol**		0.3%	4-Methoxycarbonyl-4-butanolide	CB	0.0%

Table 4.6 Top ten compounds detected by direct evaporative method in fresh swine filtrate and filtrate aged for 3 months. Compounds in bold lettering signify species that are thought to be lost or drastically affected with aging. Concerns: SM – Saturated Mass, CB – Column Bleed.

Fresh Swine Filtrate: DEM (n=3)				**Aged Swine Filtrate: DEM (n=3)**		
	Compound Name	**Concerns**	**% of Total Area**	**Compound Name**	**Concerns**	**% of Total Area**
1	Butanoic acid		16.2%	Butanoic acid	SM, CB	13.0%
2	Acetamide		6.3%	Acetamide		9.4%
3	Propanoic acid, 2-methyl-		4.2%	Acetic acid	SM	6.1%
4	Butanoic acid, 3-methyl-		3.6%	Propanoic acid		5.8%
5	**Pyrazine, methyl-**		3.0%	Propanoic acid, 2-methyl-		5.3%
6	**3-Pyridinol**		2.2%	Butanoic acid, 3-methyl-		4.8%
7	**Pyrazine, 2,5-dimethyl-**	SM	2.0%	Butanoic acid, 2-methyl-		2.7%
8	**Pyrazine**		1.8%	Butanamide		2.2%
9	**2,3-Epoxybutane**	SM	1.4%	Pentanoic acid		1.8%
10	Pentanoic acid		1.3%	Propanamide	SM	1.7%

Table 4.7 Top ten compounds detected by direct evaporative method in fresh poultry filtrate and filtrate aged for 3 months. Compounds in bold lettering signify species that are thought to be lost or drastically affected with aging. Concerns: SM – Saturated Mass, CB – Column Bleed.

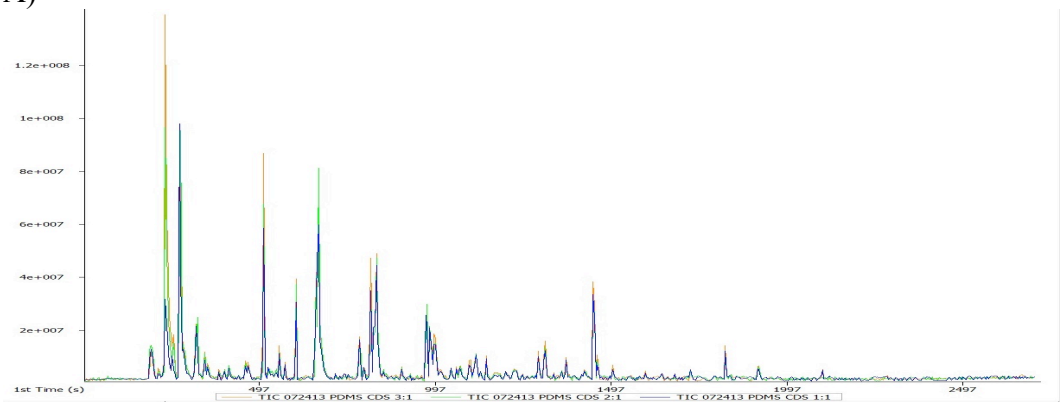
Fresh Poultry Filtrate: DEM (n=3)				**Aged Poultry Filtrate: DEM (n=3)**		
	Compound Name	**Concerns**	**% of Total Area**	**Compound Name**	**Concerns**	**% of Total Area**
1	**Acetic acid**	SM	14.3%	Butanoic acid	SM, CB	8.3%
2	Butanoic acid		6.2%	Acetone	SM	8.2%
3	2-Pyrrolidinone		4.4%	2-Pyrrolidinone	SM, CB	6.2%
4	Propanoic acid		3.2%	Acetic acid	SM, CB	5.2%
5	**L-Lactic acid**		3.1%	Pentanoic acid	CB	3.8%
6	Acetone		3.0%	Propanoic acid		3.8%
7	Acetamide		2.9%	Acetamide		3.5%
8	**Pyrazine, methyl-**		2.4%	Butanoic acid, 3-methyl-		3.4%
9	Butanoic acid, 3-methyl-		1.9%	Hydrogen azide	SM	3.1%
10	**Pyrazine, 2,5-dimethyl-**		1.6%	Isopropyl Alcohol	CB	1.9%

Table 4.8 Top ten compounds detected by direct evaporative method in cow filtrate (n=2).

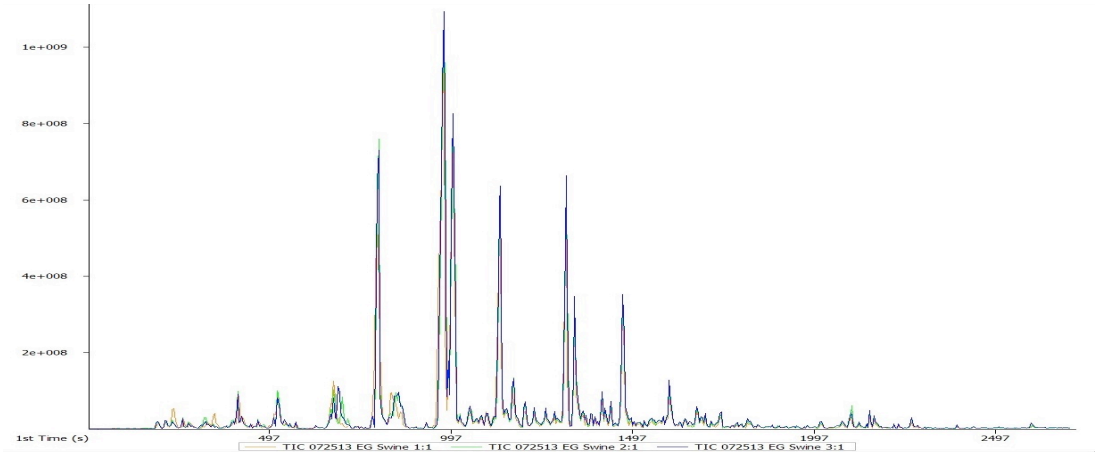
N/A – Not Apllicaple

Fresh Cow Filtrate: DEM (n=2)			
	Compound Name	**Concerns**	**% of Total Area**
1	acetic acid	N/A	14.3%
2	methylpyrazine	N/A	6.2%
3	pyrazine	N/A	4.4%
4	2,5-dimethylpyrazine,	N/A	3.2%
5	ethylpyrazine	N/A	3.1%
6	2-methoxyphenol	N/A	3.0%
7	butanoic acid	N/A	2.9%
8	3-pyridinol	N/A	2.4%
9	2,3-dimethylpyrazine,	N/A	1.9%
10	trimethylpyrazine,	N/A	1.6%

A)



B)



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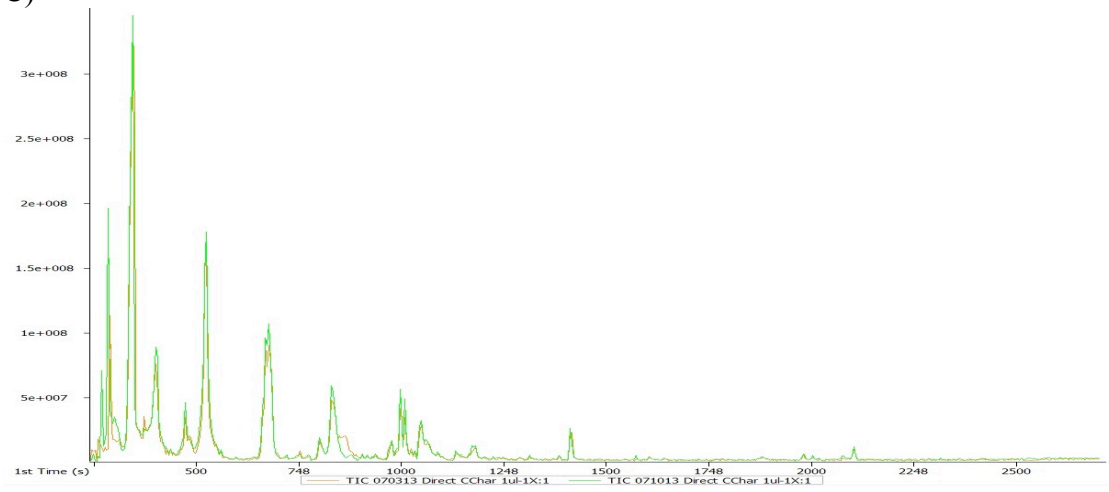
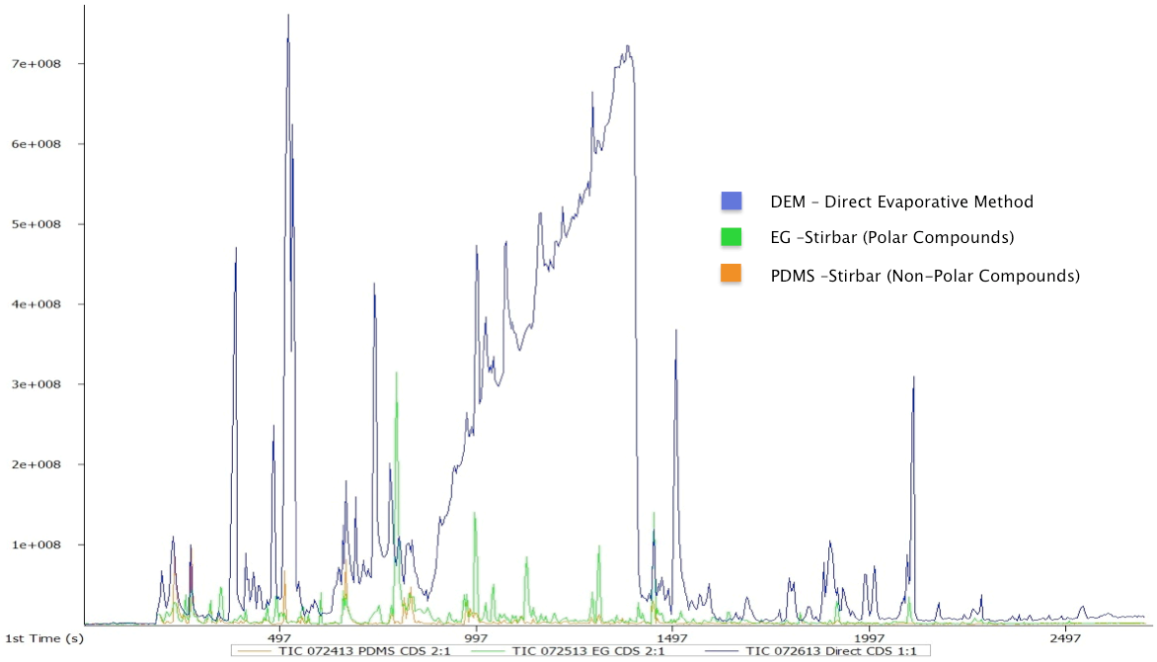


Figure 4.1 Overlay of A) PDMS triplicate analyses of the CDS filtrate, B) the triplicate analysis of the EG swine filtrate, and C) the overlay of the duplicate direct evaporative injection of the cow manure filtrate.

A)



B)

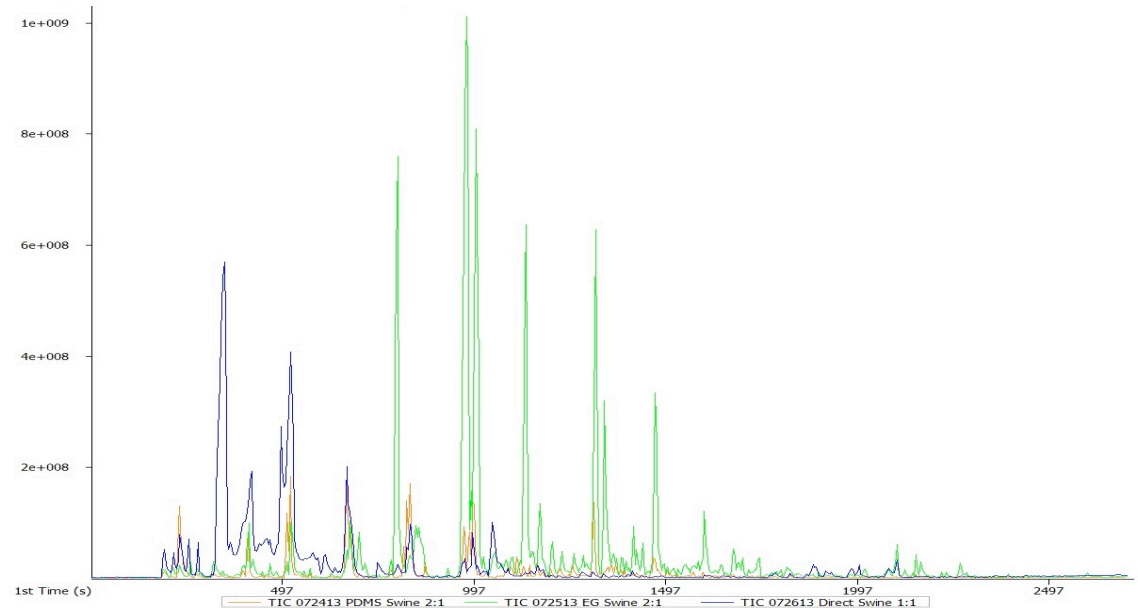


Figure 4.2 Comparison of the direct evaporative method (DEM) and solid phase extraction stir bars, (PDMS-SBSE and EG SBSE) for the A) CDS filtrate and B) swine manure filtrate.

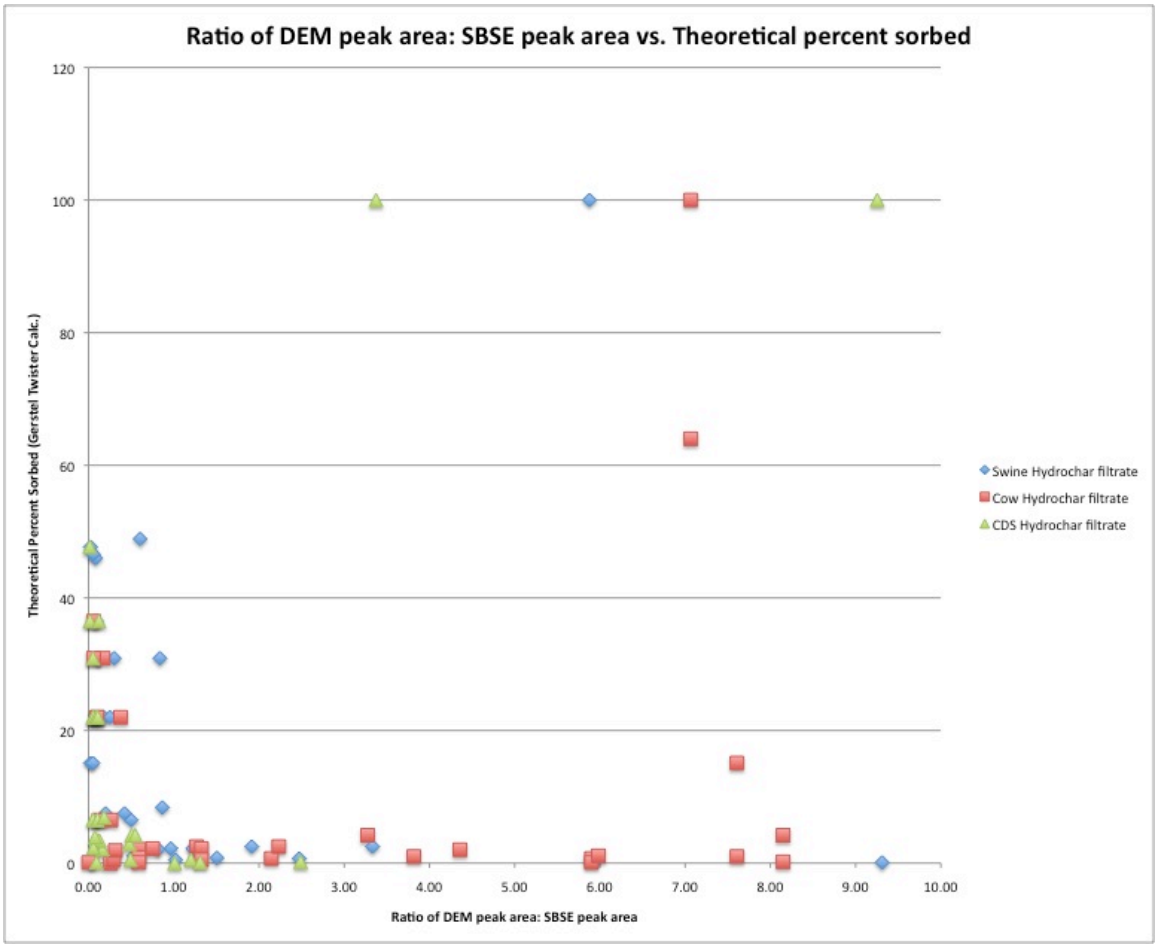
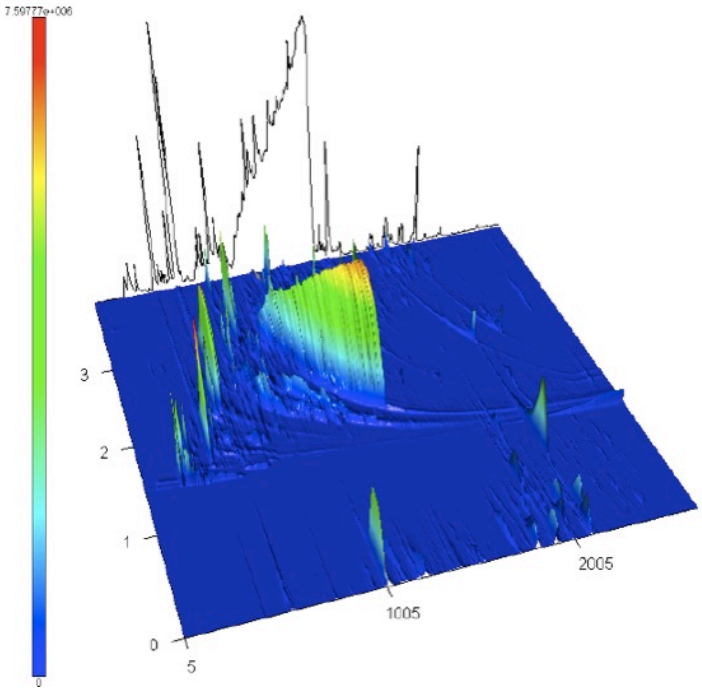


Figure 4.3 Comparison of the ratio of peak areas (DEM:SBSE) of the to 25 SBSE compounds of each stir bar, for all three filtrates, plotted against their corresponding theoretical sorption percentages (Twister Calc., V.1).

A)



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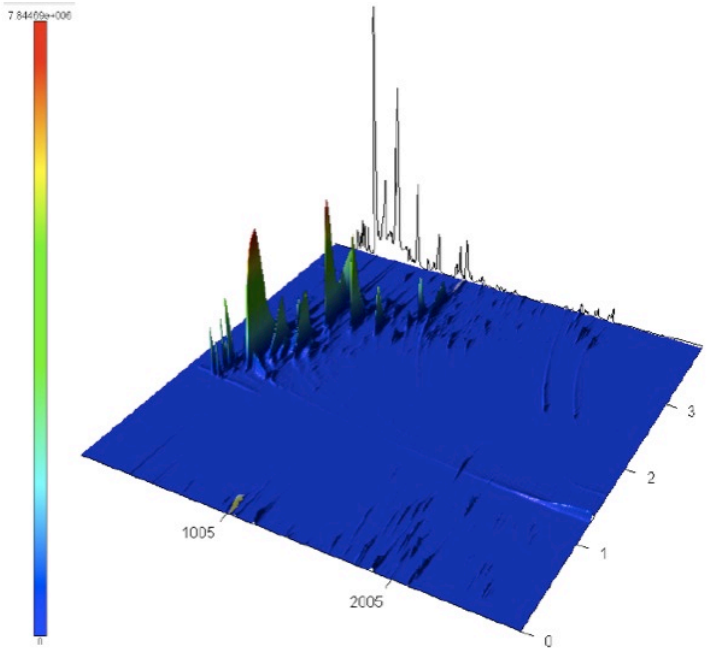

Figure 4.4 3D Plot depicting separation capability of 2DGC/ TOFMS a) CDS DEM, no dilution and b) swine DEM, 1:1 dilution.

4.4 DISCUSSION

Despite this repeatability for each respective technique, there were substantial differences in the peak distribution and the detected peak area between the direct evaporative injections and the corresponding analyses with the SBSE stir bars (Figure 4.2 and Table 4.3-4.5). Thus, due to the difference in sorptive properties and the fluctuating chemistry the stir bars would be a difficult technique, due to the lack of a representative nature of the total chemical distribution. The stir bars method would be useful for the targeted analysis of one of the strongly sorbed analytes, but not for screening of the complexity of the filtrate.

As illustrated above, there are significant differences in the quantified peaks between the SBSE stir bars and the DEM methodology. The DEM did detect common volatile fatty acids (VFAs) and other compounds that are known to be present in filtrates (i.e., aldehydes, ketones). In fact, hydrothermal treatment of an organic feedstock is known to produce large amounts of volatile fatty acids; upwards of 10% w/w acetic acid has been observed in sewage sludge HTC filtrates (Shanableh, 2000). However, when the stir bars were utilized, these VFA are not trapped due to a combination of non-targeted sorption by the stir bars as well as potential volatilization losses during stirring (Chiou, Freed, Peters, & Kohnert, 1980). This also severely hampers the use of the stir bars for screening a variety of compounds.

The sorption of compounds onto the PDMS or EG phase of the stir bars is a function of equilibrium, and for aqueous samples, the extraction of solute from the aqueous phase onto the solid phase is controlled by the partitioning coefficient of the solute between the coating and the aqueous phase. Recent studies have correlated this

partitioning coefficient with the octanol–water distribution coefficient (K_{ow}) (Kawaguchi, Ito, Saito, & Nakazawa, 2006). Although this partitioning coefficient does not fully account for all the interactions occurring with the stir bars, it does provide an indication of whether and how well a given solute can be extracted with the various coated stir bars. (Kawaguchi et al., 2006).

In addition, the chemical composition of the liquid filtrate does impact the sorption efficiency. Modification of the liquid matrix by methanol addition is known to result in higher recoveries of target compounds with sorption preparatory steps (Benijts et al., 2001). Methanol was not directly analyzed here due to the venting of the water peak from the instrument. Differences in methanol concentrations (along with other potential organic compounds) could impact the sorption equilibrium, thereby resulting in different extraction efficiencies for each filtrate based on their unique composition. To further complicate the sorption equilibrium, dissolved organic matter (DOM) has also been observed to reduce the sorption efficiency of some target compounds (P. Popp, Keil, Montero, & Rückert, 2005). However, these authors observed that some of these DOM effects could be overcome through a 20% methanol addition. This research was conducted with aqueous (river water) samples. Although the modification of the filtrate matrix with methanol additions was not attempted here, this potentially could normalize sorption behavior across different filtrates and could be a valuable method correcting the different chemical matrix effects of HTC filtrates.

It was observed that extraction efficiency of compounds from the HTC filtrate using active-phase stir bars was highly dependent on the concentration of target analyte in the liquid and the total extraction time (Kawaguchi et al., 2006). Another complicating

factor was maintaining a constant set of conditions for each stir bar. Each analytical run was an hour in duration, therefore when performing time sensitive analyses in triplicate, consideration should be made as to how the samples are loaded into the analyzer as well. With longer holding times, an early desorption of lighter compounds may occur during long hold periods after sample extraction. This could be overcome with automated sample prep (Sandra et al., 2003).

The stir bars do not pick up major components such as acetic acid and glycerin as readily, which skews the observed concentrations and product distributions. Analysis of 2D-GC data collected using the stir bar method would require a detailed assessment of the sorption behavior for each compound of interest to determine its affinity to the respective stir bar in the filtrate media. This could be achieved through co-sorption of isotopic labeled compounds (Roberts, Pollien, & Milo, 2000) or a spiked solution containing compounds of interest in different known quantities could also be employed to permit the quantification for both the PDMS and EG stir bars. These methods need to be applied in order to improve quantification of compounds within HTC filtrates.

A major issue observed with the direct evaporative analysis is that dominant compounds created excessive column loading, which in turns causes peak overload and column bleed (Deans, 1968). Although 2DGC can accommodate for some issues with the peak tailing and overlap (Ong, Shellie, & Marriott, 2001), this overloading interferes with the column's ability to resolve minor constituents and isomers of compounds. Dilution of the sample to prevent this overload leads to a reduction of trace compounds below the detectable range. This can be seen in our data with typically a lower number of total identified compounds in the DEM analysis (Table S1-S9). However, some of these

trace compounds were concentrated by the stir bars (e.g., pyridine, pyrroles, phenols). This could also be an important aspect for the use of these stir bars for HTC filtrate analyses, since pyridines, pyrroles, and pyrazines are sought after compounds for food flavoring (Knorr, Wampler, & Teutonico, 1985) as well as microbial nitrification inhibitors (Zerulla et al., 2001). The presence of pyrazines in the present investigation differs from the piperazinediones (cyclic amino acid dimers) reported elsewhere (Heilmann, et al., 2011). These fully aromatized pyrazines could have been produced from the corresponding piperazinediones by thermal dehydration during desorption from the stir bar or evaporation.

Qualitative differences between methods were observed immediately. Overlaying the chromatograms produced via DEM with PDMS and EG SBSE's show that the two methods produce significantly different results (Figure 4.2). The number of individual peaks resolved by the methods also differed substantially depending on the method and sample concentration. A ratio was produced for the observed areas of each matching compound peaks (Direct: SBSE). This ratio was then plotted against the theoretical percentage that would be sorbed onto the PDMS and EG stir bars (Figure 4.3). We hypothesized that higher theoretical sorbing percentages would lead to lower DEM:SBSE ratios, and the opposite for high ratios, yielding a negative relationship between ratio of DEM:SBSE to theoretical percent of compound sorbed onto the stir bar. As can be seen from Figure 4.3, a clear relationship was not observed and no significant relationship exists for each compound across the three filtrates. These differences could be due to the fact that the calculation does not take into account the complex interactions taking place within the filtrate and their influence on sorption equilibrium with respect to the stir bars.

This complex equilibrium hinders the direct quantification of the filtrate components by the stir bars.

As highlighted by others, there is an immense need to develop spectral libraries for the complex biological samples (e.g., Schauer et al., 2005), such as HTC filtrates. Since in some of these samples, only half of the detected compounds were actually positively identified.

We recommend time-of-flight mass spectrometry (TOF-MS) with the direct evaporative sample introduction as the analytical methodology of choice for complex HTC filtrate samples. The commonly used bench-top quadrupole MS is commonly run in the SIM (single ion monitoring) mode to maximize peak sensitivity (e.g. Mahinpey, Murugan, Mani, & Raina, 2009). However, when operated in SIM mode, the MS is unable to collect complete spectral data on the ionized fragments. This hampers the ability to identify unknown chemicals, which is paramount for complex HTC filtrates. In addition, all peaks eluting from the TOF-MS have peak widths less than 100 ms, and sometimes as small as 10ms. To properly sample such narrow peaks, detector response must be 100 Hz or more for proper peak shape characterization (Van Deursen et al., 2000). Traditional quadrupole mass spectrometers operating in full scan mode are too slow to achieve these rates, which limits the ability of detection due to the continually changing concentrations of analytes in the detector as the scan is progressing, particularly true for structural isomers. The TOF-MS operates with full-mass spectral acquisition rates exceeding 500 Hz (Čajka & Hajšlová, 2004). These attributes of the TOF-MS lead to improved mass spectral quality, which enables improved unknown compound detection due to the higher quality spectra that are obtained (Čajka & Hajšlová, 2004).

Lastly, the use of specialized software (de-convolution software), which improves the resolution of overlapping peaks with retention of the spectral signatures enables the identification/confirmation of unknown compounds and even co-eluting isomers of the same compound (Banerjee et al., 2008; Mohler et al., 2007; Wagner, Sefkow, & Kopka, 2003). Some of these benefits can be observed in Figure 1.4, where the 3D reconstruction of the peaks from the filtrate are shown, illustrating the analytical ability to resolve overlapping peaks and to resolve peak utilizing the second dimension.

DEM Characterization of fresh vs. Aged Filtrates

The data produced via DEM shows that the different filtrates have unique chemical fingerprints, and even though the chromatograph requires further improvement, the acquired data provides a starting point for further characterizations, and gives a general glimpse into the complicated chemistry of these filtrates. Note that the major alterations observed are in the abundances of compound classes, transforming from diols (double alcohol groups on the compound) in the fresh filtrate to single alcohols and ketones and cyclic-N compounds in the aged filtrate. It is important to remember that a diol can be converted to a cyclic compound through diol cyclization, which is aided by an acid catalyst (March, 1985) . The carbon–carbon bond in a vicinal (adjacent) diol (also called a glycol) can be cleaved and replaced with two carbon–oxygen double bonds resulting in either a ketone or aldehyde (Marach, 1985). These reactions along with microbial transformations could lead to these differences.

4.5 CONCLUSION

The 2DGCxTOFMS provides the sophistication needed to analyze complex matrices. The direct analysis of HTC filtrates enables one to achieve a representative view of matrix distribution rapidly due to the superior ability of the GCxGC/TOFMS to separate and resolve individual components from the matrix. However, water and high concentration matrix compounds in the HTC filtrate will degrade the analytical column, thus limiting the sustainability of this type of analysis. Overall, the SBSE method offers better resolution of the trace compounds, but the absolute quantification of these compounds is in doubt due to the lack of a predictable sorption trend across the filtrates. This would necessitate the individual calibration of each target compound in the respective filtrate when using these stir bars. It is hypothesized that the adoption of an analytical technique to fully characterize the filtrate, versus isolated organic compound classes, will rapidly increase the body of knowledge for understanding the HTC process as well as provide the information needed for the proper environmental handling of the produced filtrate. This research has shown that the direct evaporative method holds promise for this use.

FINAL STATEMENTS

Percent solids of the starting materials varied greatly for HTC, with CDS being at 33%, whereas swine and poultry manures were at 11 and 10% solids, respectively. Upon completion of the work, it becomes apparent that the common thread that ties this research together is the effect of %-solids on HTC reaction chemistry, and its subsequent effects on the number of compounds created, microbial soil kinetics, and plant germination and growth. Chemical characterization of the filtrates showed that the total carbon in CDS filtrate was more than six times greater than that of swine or poultry HTC filtrates. Similarly, analysis of the filtrates on 2D-GC confirmed that CDS filtrate produced a greater number of detectable compounds than swine or poultry HTC filtrates. Soil incubations showed that CDS filtrate at the 1/2X dose produced almost twice as much cumulative CO_2 than swine filtrate at the 1X dose. And finally, corn grown with an application of CDS 1/10X filtrate produced more biomass than that of 1/10X swine filtrate.

These effects are not strictly linear, however the trends are all the same, and point to the positive effect of total carbon on metabolic activity of soil microbes as well as overall productivity of maize. The question that arises is; how would the filtrates compare if all starting materials were the same percent solids prior to being hydrothermally carbonized?

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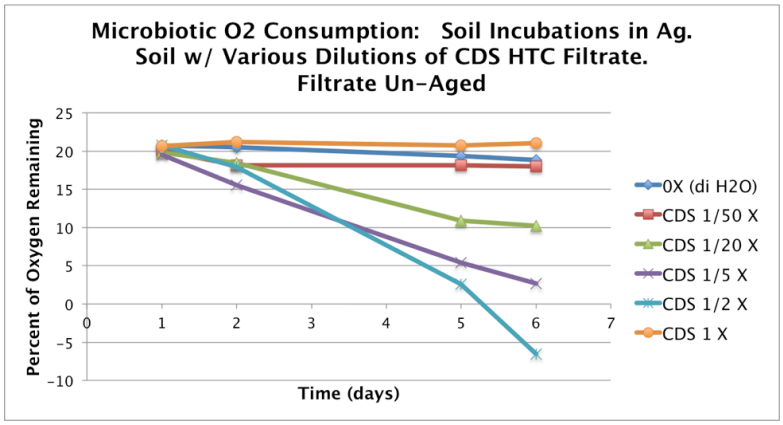
APPENDIX

Figures A2.1 – A2.3 O2 Consumption - Consumption of atmospheric oxygen in response to amendment with increasing concentrations of CDS HTC filtrate. Vials were vented on day 5 for fresh and day 6 for aged filtrates, after which additional data was subtracted from the previous data point for contiguous representation. Fig 2.7 fresh Filtrate, Fig. 2.8 filtrate aged in a closed container for 100 days, Fig. 2.9 filtrate aged in a open container for 100 days.



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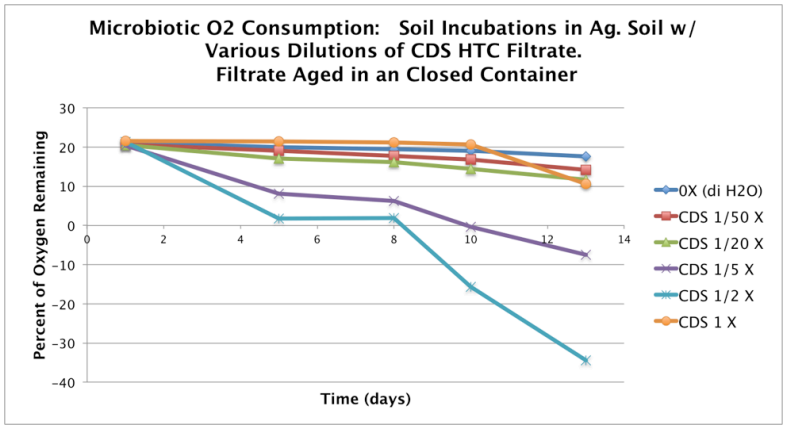

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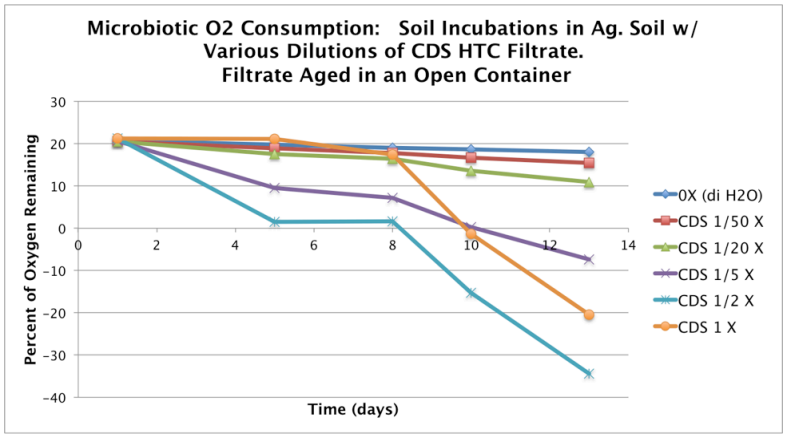
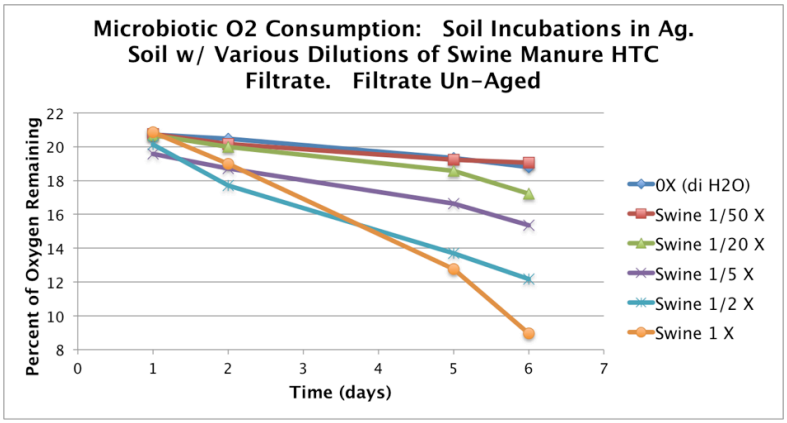


Figure A2.3

Figures A2.4 – A2.6 O2 Consumption - Consumption of atmospheric oxygen in response to amendment with increasing concentrations of swine HTC filtrate. Vials were vented on day 5 for fresh and day 6 for aged filtrates, after which additional data was subtracted from the previous data point for contiguous representation. Fig 2.11 fresh Filtrate, Fig. 2.12 filtrate aged in a closed container for 100 days, Fig. 2.13 filtrate aged in a open container for 100 days.



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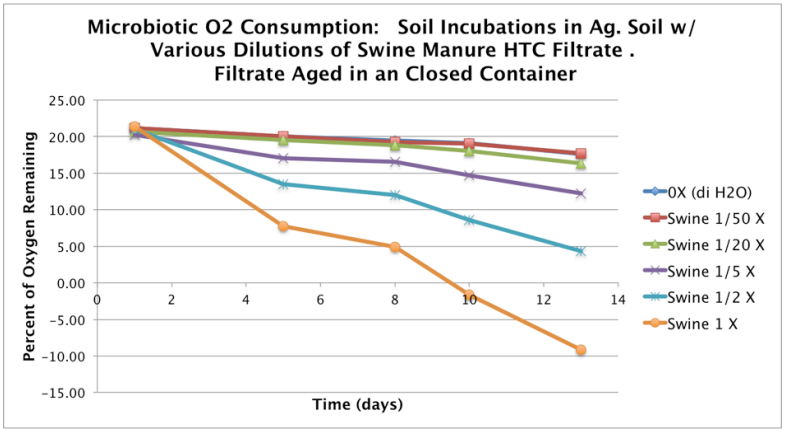

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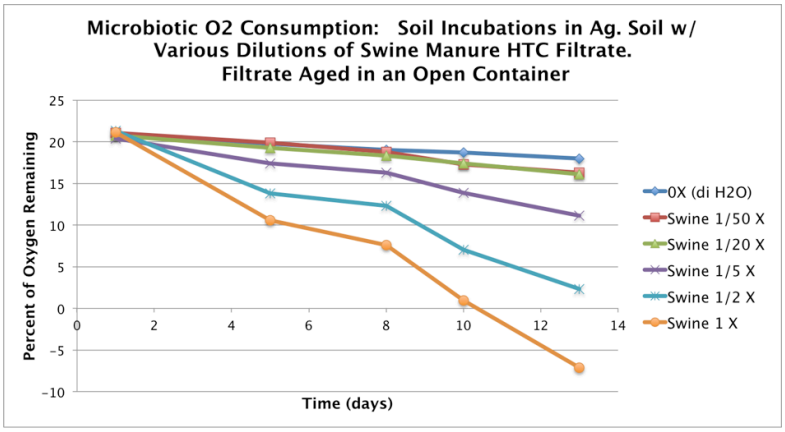
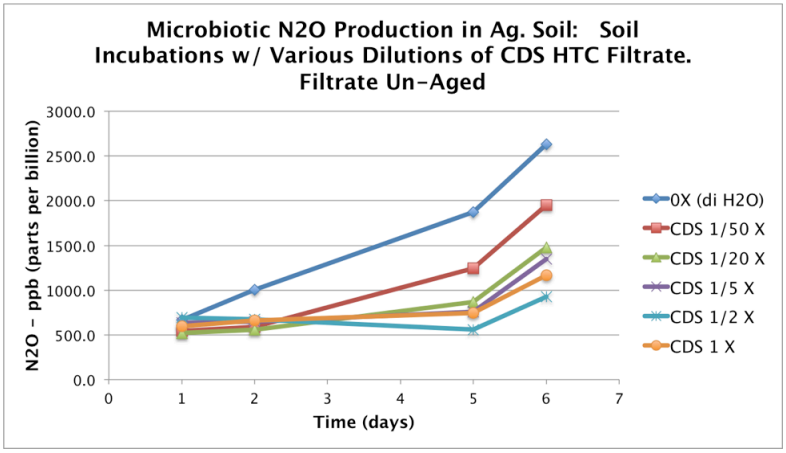


Figure A2.6

Figures A2.7 – 2.9 - N2O Production – in response to amendment with increasing concentrations of CDS HTC filtrate. Vials were vented on day 5 for fresh and day 6 for aged filtrates, after which additional data was added to the previous data point for contiguous representation. Fig 2.13 fresh Filtrate, Fig. 2.14 filtrate aged in a closed container for 100 days, Fig. 2.15 filtrate aged in a open container for 100 days.



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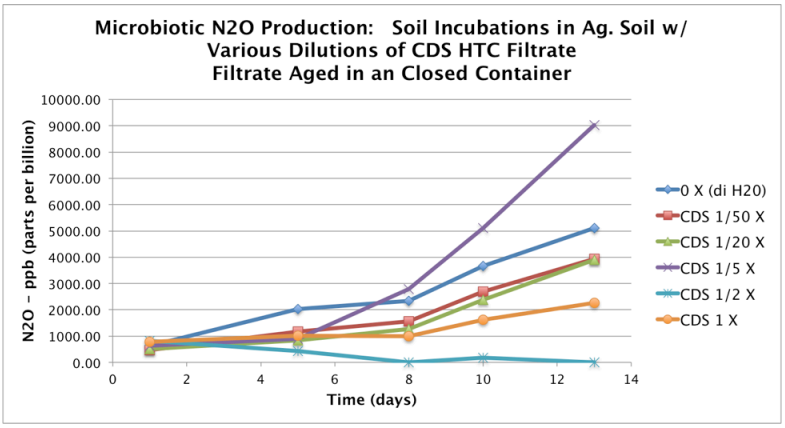

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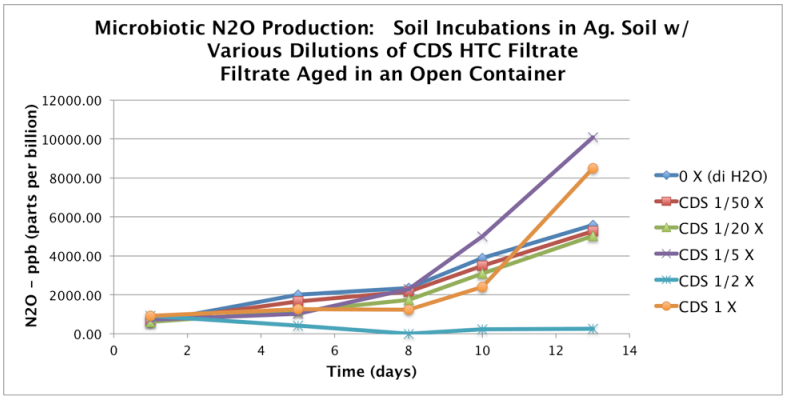
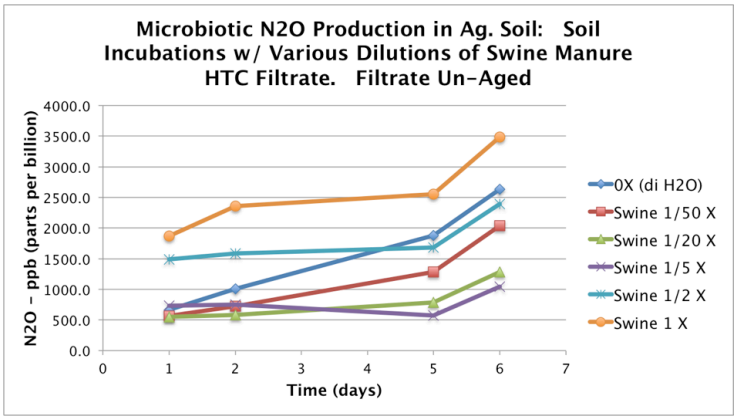


Figure A2.9

Figures A2.10 – A2.12 N2O Production - in response to amendment with increasing concentrations of swine HTC filtrate. Vials were vented on day 5 for fresh and day 6 for aged filtrates, after which additional data was added to the previous data point for contiguous representation. Fig 2.16 fresh Filtrate, Fig. 2.17 filtrate aged in a closed container for 100 days, Fig. 2.18 filtrate aged in a open container for 100 days.



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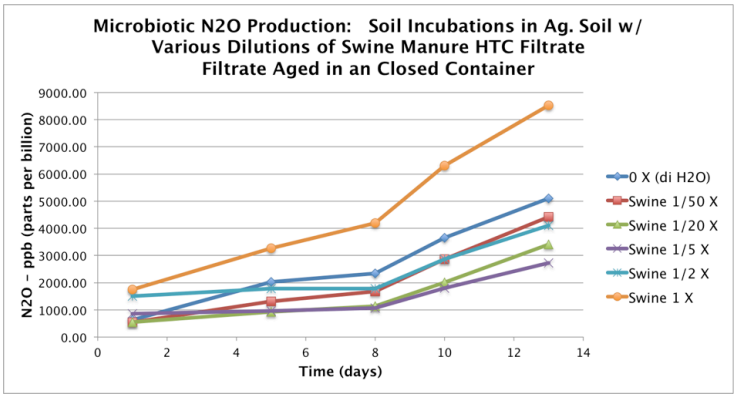

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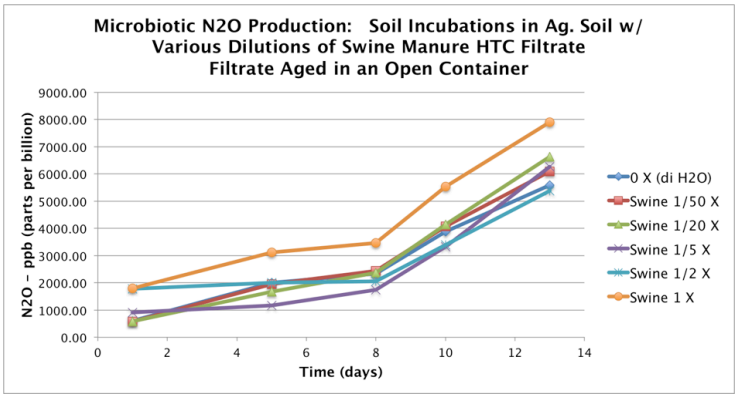

Figure A2.12

CORN GROWTH TRIALS USING SUNSHINE MVP AS GROWTH MEDIA

A3.1 MATERIALS AND METHODS

A3.1.1 Corn Growth Trials In Greenhouse

A-3.1.1.1 Growth Trial 1, 2 – Green House with Sunshine MVP soil, fresh CDS and swine Manure HTC filtrates.

Growth trial 1 was conducted in a University of Minnesota green house using Sunshine MVP starter mix as the soil media. The soil mix was pre-wetted, and lightly packed into a 4" x 4" x 4" pot containing drainage holes. A saucer was placed under each pot to collect any leachate in the case that drainage had occurred. The green house parameters were set for 16-hour days, and an approximate temperature of 75 °F. There was no temperature, or humidity monitoring for growth trials 1 and 2, and replicates were not randomized.

Growth trials 1 and 2 consisted of two filtrates, HTC filtrate of condensed distillers solubles (CDS) and swine Manure, which were diluted to 10, 20 and 50 fold dilutions, and applied to the soil pots in triplicate reps with a one time application of 100 mL's per replicate. A positive control consisted of a triplicate set containing Peat-Lite fertilizer diluted to concentration of 1 tsp. / gallon, and applied in a 100 mL volume per replicate. A negative control consisted of a triplicate treatment of deionized H_2O, which was also applied in 100 mL volumes per replicate. After the dilutions were applied, each pot was seeded with three corn seeds of uniform proportion. Upon germination, the sprouts were thinned down to one per pot, omitting the smaller sprouts. Each replicate was watered with 100 mL's of deionized H_2O every other day for a growth period of 21 days. The plants were harvested on the 22^{nd} day, and the roots were washed free of soil after which the root system was cut free from the stem at the base and both were placed

into separate, pre-weighed paper bags for drying in an 85 °C oven. The bags used for drying of the plant mass, had also been dried in the 85 °C oven prior to being pre-weighed.

Nutrient analysis was performed on all the filtrate dilutions using a Lachat Autoanalyzer (Lachat Instruments, Loveland CO). Light Concentration was measured prior to harvest using a Light Sensor (FieldScout Light Sensor, Spectrum Technologies, item # 366816).

A-3.1.1.2 Growth Trial 3 – Green House with Sunshine MVP soil

Growth trial 3 was conducted in a University of Minnesota green house using Sunshine MVP starter mix as the soil media.. The soil mix was pre-wetted, and lightly packed into 4" x 4" x 4" pots with drainage holes, with ~640 grams of mix added per container. A saucer was placed under each pot to collect any leachate in the case that drainage had occurred. The green house parameters were set for 16-hour days, and an approximate temperature of 75 °F. There was no temperature, or humidity monitoring for growth trial 3. Replicates were randomized, and light concentrations were measured with a Light Sensor (FieldScout Light Sensor, Spectrum Technologies, item # 366816) prior to planting in order to plant in an area that had more uniform light.

Growth trial 3 consisted of two filtrates, HTC filtrate of condensed distillers solubles (CDS) and swine manure, which were diluted to 2 and 10 fold dilutions, and applied to the soil pots in triplicate reps with a one time application of 100 mL's per replicate. A positive control consisted of a triplicate set containing Peat-Lite fertilizer diluted to concentration of 1 tsp. / gallon, and applied in 100 mL volume per replicate. A negative control consisted of a triplicate treatment of deionized H_2O, which was also

applied in 100 mL volumes per replicate. After the dilutions were applied, each pot was sewed with three corn seeds of uniform proportion. Upon germination, the sprouts were thinned down to one per pot, omitting the smaller sprouts. Each replicate was watered with 100 mL's of deionized H_2O every other day for a growth period of 21 days. The experiment was abandoned on day 22, and plants were not harvested due to severe white fly contamination and irregular growth due to buggy whipping.

A-3.1.2 Corn Growth Trials In Growth Chamber

A-3.1.2.1 Growth Trial 4 – Growth Chamber, Sunshine MVP soil, treated with aged HTC filtrates, 10 treatments total.

Growth trial 4 was conducted in a University of Minnesota Plant Pathology growth chamber. The 6 ft. tall x 4.5 ft. wide x 8 ft. long chamber was set to run on a 16-hour light period, with nighttime temperature of 24 °C and humidity of 0%, and a daytime temperature of 29 °C and humidity of 70%. Adjustments were made through out growth trial to adjust parameters due to faulty humidity control.

Sunshine MVP starter mix was used as the soil media. The soil mix was pre-wetted, and lightly packed into 4" x 4" x 4" pots with drainage holes, with ~ 490 grams of mix added per container. A saucer was placed under each pot to collect any leachate in the case that drainage had occurred. Replicates were randomized, and light concentrations were measured prior to planting with a Light Sensor (FieldScout Light Sensor, Spectrum Technologies, item # 366816).

Growth trial 4 consisted of three filtrates, HTC filtrate of CDS, and, swine and poultry manure, which were diluted to 2 and 10 fold dilutions, and applied to the soil pots in triplicate reps with a one time application of 100 mL's per replicate. A positive

control consisted of a triplicate set containing Peat-Lite fertilizer diluted to concentration of 5 grams / gallon, and applied in 100 mL volume per replicate. A negative control consisted of a triplicate treatment of di H_2O, which was also applied in 100 mL volumes per replicate. After the dilutions were applied, each pot was sewed with two corn seeds of uniform proportion. Upon emergence, the sprouts were thinned down to one per pot, omitting the smaller sprouts. Each replicate was watered with 100 mL's of di. -H_2O every other day for a growth period of 21 days.

The plants were harvested on the 22nd day, and the roots were washed free of soil after which the root system was cut free from the stem at the base and both were placed into separate, pre-weighed paper bags for drying in an 85 °C oven for a period of one week. The bags used for drying of the plant mass, had also been dried in the 85 °C oven prior to being pre-weighed.

Nitrate, ammonium and phosphate nutrient analysis was performed on all of the initial filtrate dilutions using a Lachat Auto-analyzer.

A-3.2 RESULTS

A-3.2.1 Corn Growth Trials In Greenhouse

Results within the greenhouse were more difficult to interpret due to the increased variability of light, temperature and humidity. Nevertheless, the work done in the greenhouse provided the crucial insight necessary to progress to the more controlled environment of the growth chamber.

A-3.2.1.1 Growth Trial 1 – Green House- Fresh CDS and Swine Filtrates, Grown in Sunshine MVP starter mix

Final data for growth trial 1 was abandoned due to a miscommunication with weekend staff, which led to a watering of the growth set with copious amounts of water over the first weekend after the initial planting, thus washing out the treatments**.**

A-3.2.1.2 Growth Trial 2 – Green House- fresh CDS and swine Filtrates, Grown in Sunshine MVP starter mix

Growth trial 2 was harvested, and the results can be seen in Figures A3.1 - 3.5. Initial results seemed to show that there were growth effects that could be attributed to the filtrate applications. However, after looking at the distribution of light in regard to the overall growth of the plants within each treatment, it can be argued that the light concentration in this growth trial was too variable, and is the explanation for the observed differences between the trials, as seen in Figure A3.5.

A-3.2.1.3 Growth Trial 3 – Green House- fresh CDS and swine Filtrates, Grown in Sunshine MVP starter mix

Despite the attempt to create a more uniform light field by rearranging the lights in the green house, final data for growth trial 3 was abandoned due to a white fly contamination that was thought to be causing a plant deforming condition called buggy whipping. Further inquiry into the cause led to the conclusion that lack of temperature control in combination with a severe winter cold snap was the more likely cause for buggy whipping, as optimal conditions for corn growth call for warmer temperatures. It was decided that the green house was unsuitable for this experiment due to lack of necessary control of light, temperature, humidity, and pests.

Green House Growth Trial 2

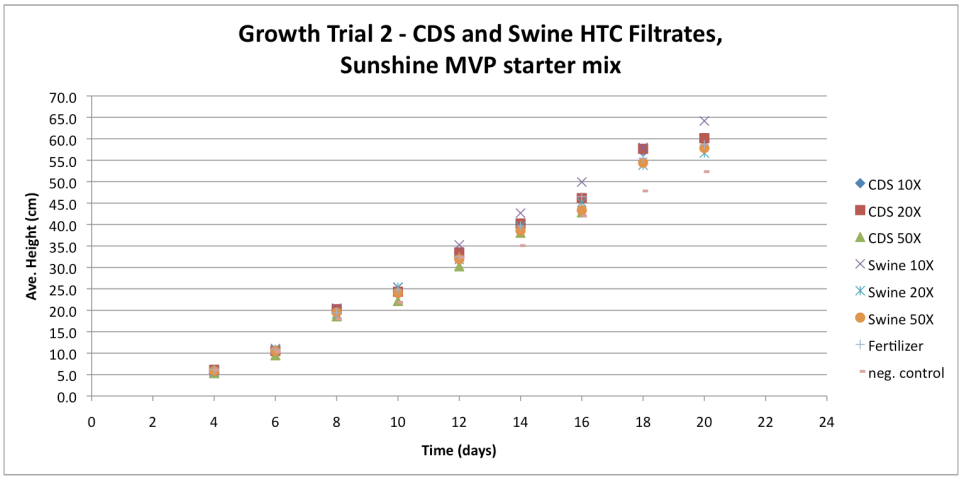

Figure A3.1 Average total plant height per treatment vs. time

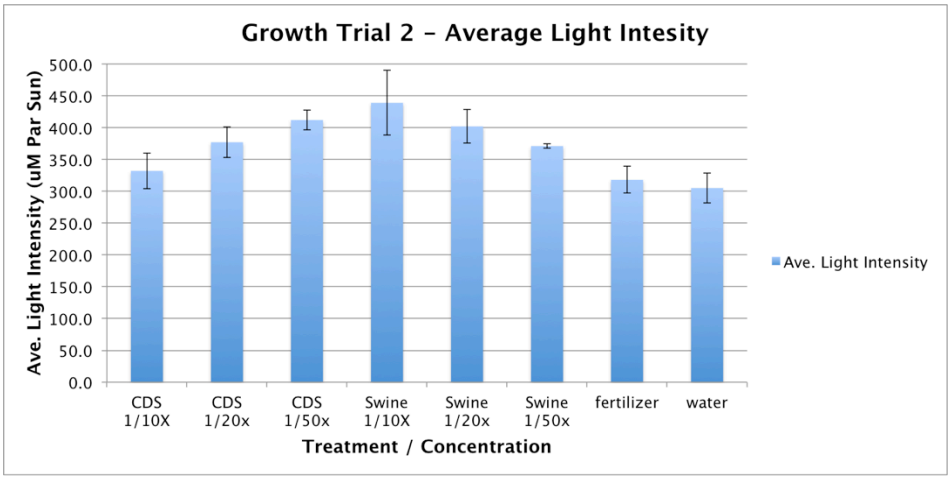

Figure A3.2 Average light intensity per treatment within green house

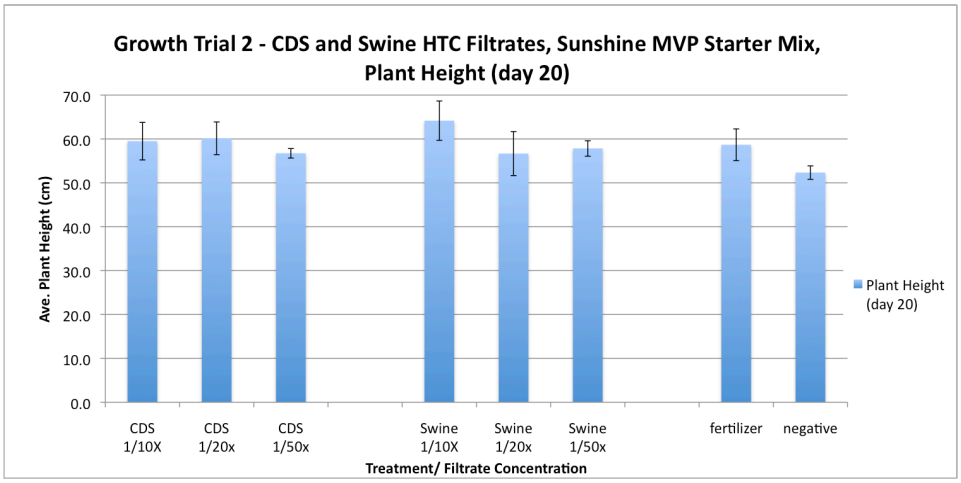

Figure A3.3 average plant height per treatment within green house

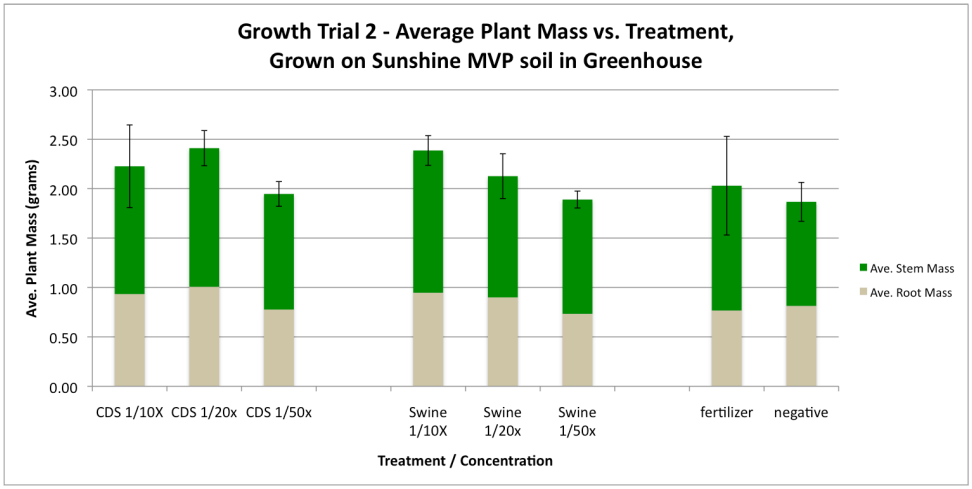

Figure A3.4 Average plant mass per treatment within green house

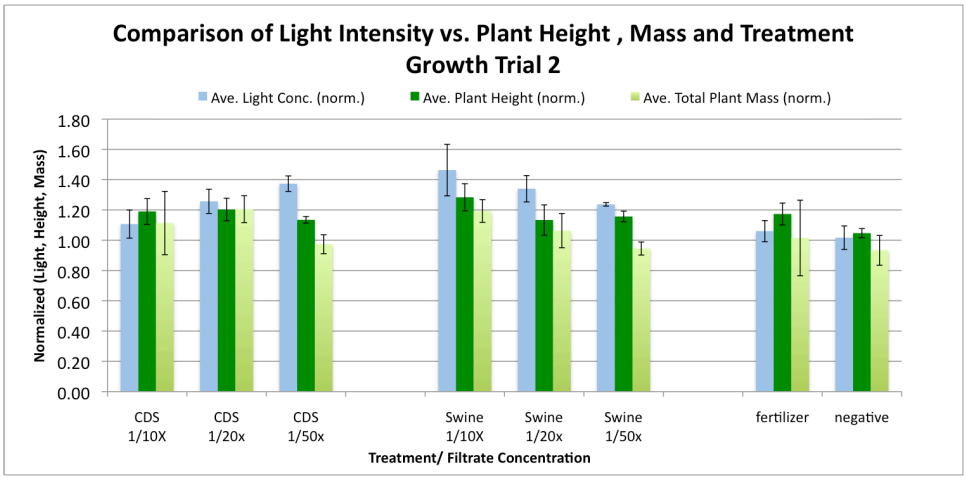

Figure A3.5 Plant Height at Time of Harvest vs. Total Harvested Plant Mass vs. Corresponding Light Intensity (Normalized).

A-3.2.2 Corn Growth Trials Growth Chamber – Growth Trial 4

A-3.2.2.1 Growth Trial 4 – Growth Chamber- – Aged open and closed, CDS and swine Filtrates, Fresh poultry manure HTC filtrate. Grown in Sunshine MVP starter mix.

Growth Trial 4 (GT4) had much better growth than the previous trials. The light concentrations within the growth chamber were very even across all the potted plants, as seen in Figure A3.6. The light concentration above each treatment at the time of measurement was on average 498-umol m^{-2} s^{-1} per pot. Each of the dilutions of applied

filtrates increase when taking into account the existing moisture content of the pre-wetted Sunshine MVP starter mix.

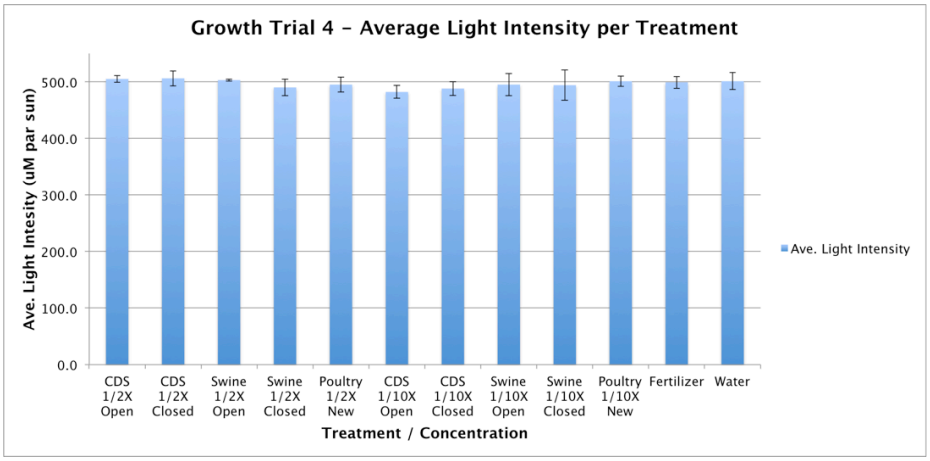

Figure A3.6 Average light intensity per treatment

A- 3.2.2.1.1 GT4 Initial NH_4 Concentrations

The "applied NH_4 concentration" was determined by the moisture content of the original soil treatment. This starting mix contained 73 % by weight H_2O, which was used to calculate the effective NH_4 concentration. (All plant pots in GT4 contained approximately 490 grams of wetted Sunshine MVP soil mix. A dry weight analysis of a 475-gram sub-sample of the wet soil mix was found to contain 346 grams of water (dried in 85 °C oven for a week). This additional volume changes the 2X dilutions to a 9X dilution, and the 10X dilutions to a 45X dilution. The starting dilutions contained ~ 4000 mg/L (+/- 10%) NH_4 (Table A3.1), which means that treatment concentrations of 1/2X and 1/10X were diluted down to ~ 440 mg/L and ~ 90 mg/L NH_4. poultry HTC filtrate contained ~2300 mg/L (+/- 10%) of NH_4, which when accounting for the dilution effect of wet media makes initial concentrations for 1/2X and 1/10X treatments equal ~260 mg/L and ~50 mg/L.

Table A3.1 – Nutrient results, analysis performed on Lachat Auto analyzer: 2M KCl Soil extractions performed for soil incubations. Filtrates used for growth trials were filtered through a 0.45 uM filter and diluted to appropriate range prior to analysis.

Maize Growth Trials	***Solution/ Filtrate type***	***NH4***	***NO3***	***PO4***
GT2	CDS Filtrate - fresh	3290 mg/L	0 mg/L	10126 mg/L
	swine Filtrate - fresh	3772 mg/L	0 mg/L	85 mg/L
	Peat-Lite Fertilizer	123 mg/L	231 mg/L	131 mg/L
GT4	CDS Filtrate - open	4216 mg/L	0 mg/L	6260 mg/L
	CDS Filtrate - closed	4014 mg/L	0 mg/L	5953 mg/L
	swine Filtrate - open	4364 mg/L	0 mg/L	17 mg/L
	swine Filtrate - closed	3970 mg/L	0 mg/L	19 mg/L
	poultry - fresh	2278 mg/L	0 mg/L	39 mg/L
	Peat-Lite Fertilizer	152 mg/L	199 mg/L	65 mg/L

A- 3.2.2.1.2 GT4 Plant Height

Tracking growth rates of the corn over time in response to the treatment, showed that there was difference in growth rate at 1.5 weeks, as seen in Figure A3.7, which began to dissipate as the trial went on. Referring to Figure A3.8, it can be seen that the average plant height per treatment was less of a discerning characteristic at the time of harvest.

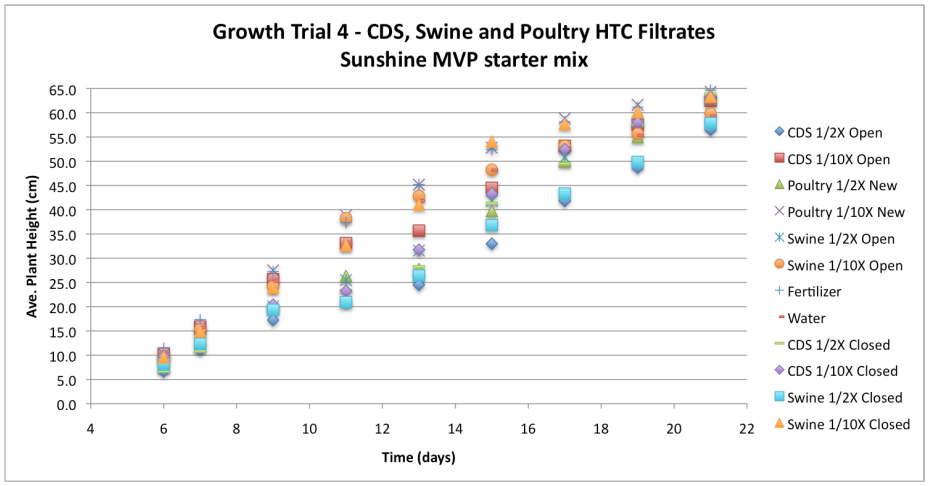

Figure A3.7 Plant Height vs. Time

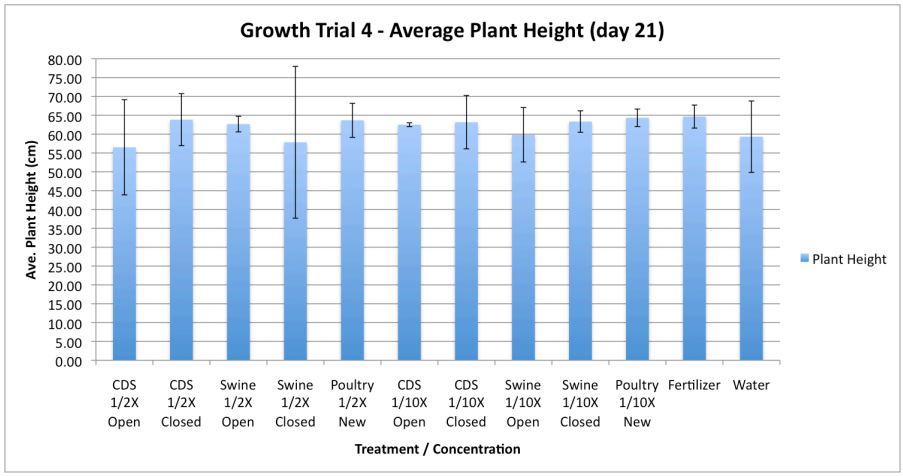

Figure A3.8 Average total plant height per treatment

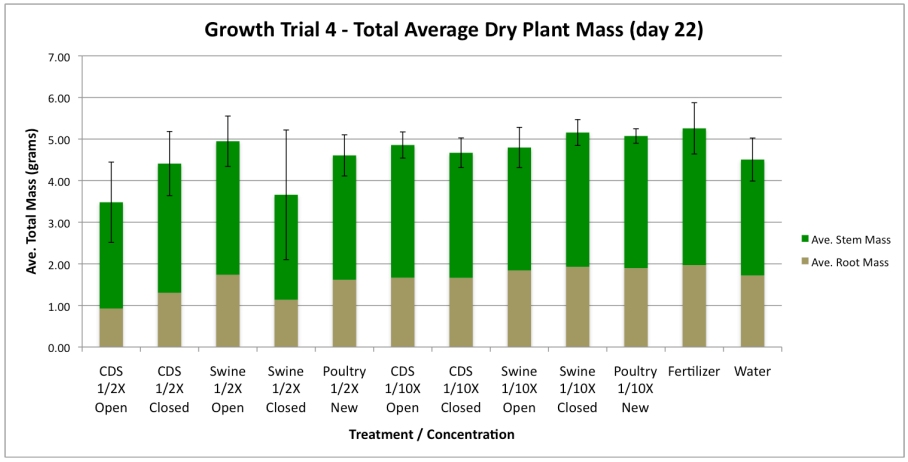

Figure A3.9 Average total plant mass per treatment

A- 3.2.2.1.3 GT4 Plant mass

Statistical comparisons of the means of dry weights of the root and stem showed no significant differences between treatments, which can be seen in Figure A3.9. It is believed that the lack of statistically significant results was due to the peat content of the Sunshine MVP soil, which has a higher sorption potential for organic compounds, thus making compounds within the HTC filtrates unavailable to act upon the roots of the plant (Rutherford, Chiou, & Kile, 1992). Another contributing factor is the great water holding capacity of Sunshine MVP mix, which had an increased diluting effect. Furthermore,

Sunshine MVP starter mix contains a charge of nutrients to promote initial growth, which may have had a contributing factor in diluting any possible effects of the HTC filtrates.

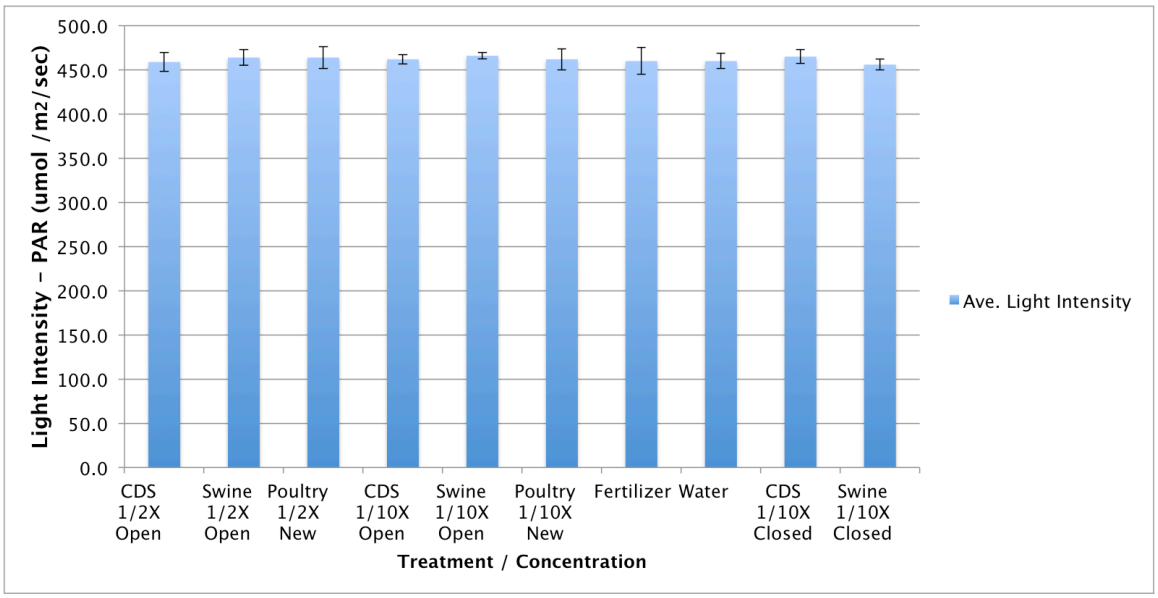

Figure A3.10 – GCT1 Average light intensity per treatment

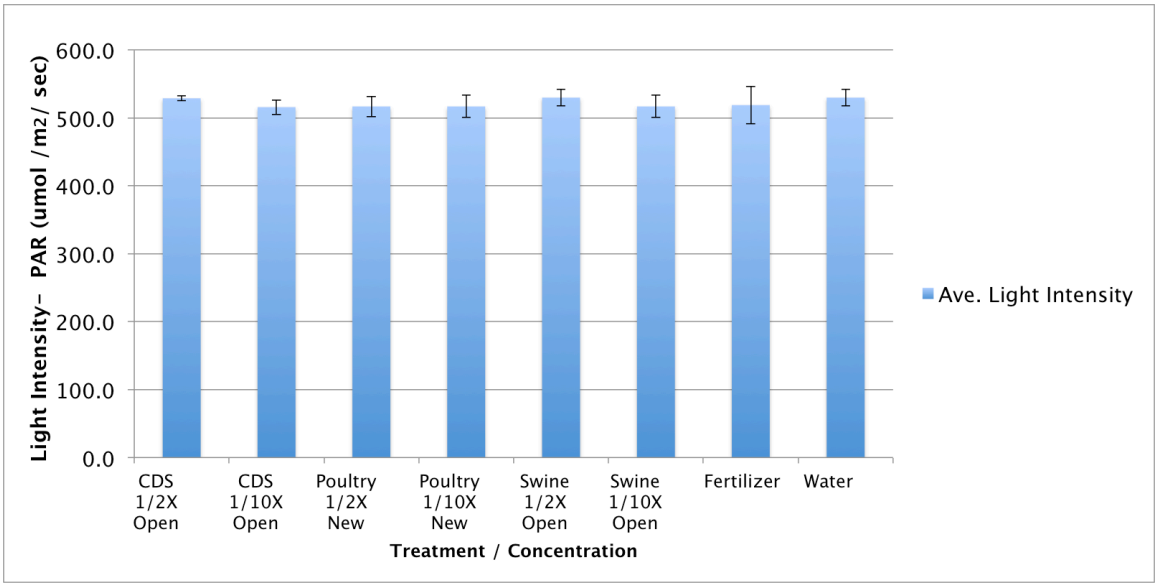

Figure A3.11 – GCT2 average light intensity per treatmen

Table A3.2 Seedling Growth GCT1 – Plant Height ANOVA – full analysis of all treatments

Comparison	Mean Difference	q	P value
CDS 1/2X Open vs Swine 1/2X Open	-7.100	1.650	ns P>0.05
CDS 1/2X Open vs Poultry 1/2X New	-39.800	9.248	*** P<0.001
CDS 1/2X Open vs CDS 1/10X Open	-38.100	8.853	*** P<0.001
CDS 1/2X Open vs Swine 1/10X Open	-30.600	7.110	** P<0.01
CDS 1/2X Open vs Poultry 1/10X New	-30.600	7.110	** P<0.01
CDS 1/2X Open vs CDS 1/10X Closed	-42.800	9.945	*** P<0.001
CDS 1/2X Open vs Swine 1/10X Closed	-29.600	6.878	** P<0.01
CDS 1/2X Open vs Fertilizer	-35.600	8.272	*** P<0.001
CDS 1/2X Open vs Water	-19.300	4.485	ns P>0.05
Swine 1/2X Open vs Poultry 1/2X New	-32.700	7.598	*** P<0.001
Swine 1/2X Open vs CDS 1/10X Open	-31.000	7.203	** P<0.01
Swine 1/2X Open vs Swine 1/10X Open	-23.500	5.461	* P<0.05
Swine 1/2X Open vs Poultry 1/10X New	-23.500	5.461	* P<0.05
Swine 1/2X Open vs CDS 1/10X Closed	-35.700	8.296	*** P<0.001
Swine 1/2X Open vs Swine 1/10X Closed	-22.500	5.228	* P<0.05
Swine 1/2X Open vs Fertilizer	-28.500	6.622	** P<0.01
Swine 1/2X Open vs Water	-12.200	2.835	ns P>0.05
Poultry 1/2X New vs CDS 1/10X Open	1.700	0.3950	ns P>0.05
Poultry 1/2X New vs Swine 1/10X Open	9.200	2.138	ns P>0.05
Poultry 1/2X New vs Poultry 1/10X New	9.200	2.138	ns P>0.05
Poultry 1/2X New vs CDS 1/10X Closed	-3.000	0.6971	ns P>0.05
Poultry 1/2X New vs Swine 1/10X Closed	10.200	2.370	ns P>0.05
Poultry 1/2X New vs Fertilizer	4.200	0.9759	ns P>0.05
Poultry 1/2X New vs Water	20.500	4.764	ns P>0.05
CDS 1/10X Open vs Swine 1/10X Open	7.500	1.743	ns P>0.05
CDS 1/10X Open vs Poultry 1/10X New	7.500	1.743	ns P>0.05
CDS 1/10X Open vs CDS 1/10X Closed	-4.700	1.092	ns P>0.05
CDS 1/10X Open vs Swine 1/10X Closed	8.500	1.975	ns P>0.05
CDS 1/10X Open vs Fertilizer	2.500	0.5809	ns P>0.05
CDS 1/10X Open vs Water	18.800	4.369	ns P>0.05
Swine 1/10X Open vs Poultry 1/10X New	0.000	0.000	ns P>0.05
Swine 1/10X Open vs CDS 1/10X Closed	-12.200	2.835	ns P>0.05
Swine 1/10X Open vs Swine 1/10X Closed	1.000	0.2324	ns P>0.05
Swine 1/10X Open vs Fertilizer	-5.000	1.162	ns P>0.05
Swine 1/10X Open vs Water	11.300	2.626	ns P>0.05
Poultry 1/10X New vs CDS 1/10X Closed	-12.200	2.835	ns P>0.05
Poultry 1/10X New vs Swine 1/10X Closed	1.000	0.2324	ns P>0.05
Poultry 1/10X New vs Fertilizer	-5.000	1.162	ns P>0.05
Poultry 1/10X New vs Water	11.300	2.626	ns P>0.05
CDS 1/10X Closed vs Swine 1/10X Closed	13.200	3.067	ns P>0.05
CDS 1/10X Closed vs Fertilizer	7.200	1.673	ns P>0.05
CDS 1/10X Closed vs Water	23.500	5.461	* P<0.05
Swine 1/10X Closed vs Fertilizer	-6.000	1.394	ns P>0.05
Swine 1/10X Closed vs Water	10.300	2.393	ns P>0.05
Fertilizer vs Water	16.300	3.788	ns P>0.05

Table A3.3 Seedling Growth GCT1 – Plant Mass ANOVA – full analysis of all treatments

Comparison	Mean Difference	q	P value
CDS 1/2X Open vs Swine 1/2X Open	0.2040	1.050	ns P>0.05
CDS 1/2X Open vs Poultry 1/2X	-0.7960	4.098	ns P>0.05
CDS 1/2X Open vs CDS 1/10X Open	-2.633	13.554	*** P<0.001
CDS 1/2X Open vs Swine 1/10X Ope	-1.156	5.951	* P<0.05
CDS 1/2X Open vs Poultry 1/10X	-1.126	5.796	* P<0.05
CDS 1/2X Open vs Fertilizer	-1.623	8.355	*** P<0.001
CDS 1/2X Open vs Water	-0.2760	1.421	ns P>0.05
CDS 1/2X Open vs CDS 1/10X Close	-2.506	12.900	*** P<0.001
CDS 1/2X Open vs Swine 1/10X Clo	-0.9860	5.076	* P<0.05
Swine 1/2X Open vs Poultry 1/2X	-1.000	5.148	* P<0.05
Swine 1/2X Open vs CDS 1/10X Open	-2.837	14.604	*** P<0.001
Swine 1/2X Open vs Swine 1/10X Ope	-1.360	7.001	** P<0.01
Swine 1/2X Open vs Poultry 1/10X	-1.330	6.847	** P<0.01
Swine 1/2X Open vs Fertilizer	-1.827	9.405	*** P<0.001
Swine 1/2X Open vs Water	-0.4800	2.471	ns P>0.05
Swine 1/2X Open vs CDS 1/10X Close	-2.710	13.950	*** P<0.001
Swine 1/2X Open vs Swine 1/10X Clo	-1.190	6.126	** P<0.01
Poultry 1/2X vs CDS 1/10X Open	-1.837	9.456	*** P<0.001
Poultry 1/2X vs Swine 1/10X Ope	-0.3600	1.853	ns P>0.05
Poultry 1/2X vs Poultry 1/10X	-0.3300	1.699	ns P>0.05
Poultry 1/2X vs Fertilizer	-0.8270	4.257	ns P>0.05
Poultry 1/2X vs Water	0.5200	2.677	ns P>0.05
Poultry 1/2X vs CDS 1/10X Close	-1.710	8.803	*** P<0.001
Poultry 1/2X vs Swine 1/10X Clo	-0.1900	0.9781	ns P>0.05
CDS 1/10X Open vs Swine 1/10X Ope	1.477	7.603	*** P<0.001
CDS 1/10X Open vs Poultry 1/10X	1.507	7.758	*** P<0.001
CDS 1/10X Open vs Fertilizer	1.010	5.199	* P<0.05
CDS 1/10X Open vs Water	2.357	12.133	*** P<0.001
CDS 1/10X Open vs CDS 1/10X Close	0.1270	0.6538	ns P>0.05
CDS 1/10X Open vs Swine 1/10X Clo	1.647	8.478	*** P<0.001
Swine 1/10X Ope vs Poultry 1/10X	0.03000	0.1544	ns P>0.05
Swine 1/10X Ope vs Fertilizer	-0.4670	2.404	ns P>0.05
Swine 1/10X Ope vs Water	0.8800	4.530	ns P>0.05
Swine 1/10X Ope vs CDS 1/10X Close	-1.350	6.949	** P<0.01
Swine 1/10X Ope vs Swine 1/10X Clo	0.1700	0.8751	ns P>0.05
Poultry 1/10X vs Fertilizer	-0.4970	2.558	ns P>0.05
Poultry 1/10X vs Water	0.8500	4.376	ns P>0.05
Poultry 1/10X vs CDS 1/10X Close	-1.380	7.104	** P<0.01
Poultry 1/10X vs Swine 1/10X Clo	0.1400	0.7207	ns P>0.05
Fertilizer vs Water	1.347	6.934	** P<0.01
Fertilizer vs CDS 1/10X Close	-0.8830	4.545	ns P>0.05
Fertilizer vs Swine 1/10X Clo	0.6370	3.279	ns P>0.05
Water vs CDS 1/10X Close	-2.230	11.480	*** P<0.001
Water vs Swine 1/10X Clo	-0.7100	3.655	ns P>0.05
CDS 1/10X Close vs Swine 1/10X Clo	1.520	7.825	*** P<0.001

Table A3.4 - Seedling Growth GCT1 – Plant Mass ANOVA- analysis of open treatments only

Comparison	Mean Difference	q	P value
CDS 1/2X Open vs Swine 1/2X Open	0.2040	1.141	ns P>0.05
CDS 1/2X Open vs Poultry 1/2X	-0.7960	4.452	ns P>0.05
CDS 1/2X Open vs CDS 1/10X Open	-2.633	14.728	*** P<0.001
CDS 1/2X Open vs Swine 1/10X Ope	-1.156	6.466	** P<0.01
CDS 1/2X Open vs Poultry 1/10X	-1.126	6.298	** P<0.01
CDS 1/2X Open vs Fertilizer	-1.623	9.078	*** P<0.001
CDS 1/2X Open vs Water	-0.2760	1.544	ns P>0.05
Swine 1/2X Open vs Poultry 1/2X	-1.000	5.593	* P<0.05
Swine 1/2X Open vs CDS 1/10X Open	-2.837	15.869	*** P<0.001
Swine 1/2X Open vs Swine 1/10X Ope	-1.360	7.607	** P<0.01
Swine 1/2X Open vs Poultry 1/10X	-1.330	7.439	** P<0.01
Swine 1/2X Open vs Fertilizer	-1.827	10.219	*** P<0.001
Swine 1/2X Open vs Water	-0.4800	2.685	ns P>0.05
Poultry 1/2X vs CDS 1/10X Open	-1.837	10.275	*** P<0.001
Poultry 1/2X vs Swine 1/10X Ope	-0.3600	2.014	ns P>0.05
Poultry 1/2X vs Poultry 1/10X	-0.3300	1.846	ns P>0.05
Poultry 1/2X vs Fertilizer	-0.8270	4.626	ns P>0.05
Poultry 1/2X vs Water	0.5200	2.909	ns P>0.05
CDS 1/10X Open vs Swine 1/10X Ope	1.477	8.262	*** P<0.001
CDS 1/10X Open vs Poultry 1/10X	1.507	8.429	*** P<0.001
CDS 1/10X Open vs Fertilizer	1.010	5.649	* P<0.05
CDS 1/10X Open vs Water	2.357	13.184	*** P<0.001
Swine 1/10X Ope vs Poultry 1/10X	0.03000	0.1678	ns P>0.05
Swine 1/10X Ope vs Fertilizer	-0.4670	2.612	ns P>0.05
Swine 1/10X Ope vs Water	0.8800	4.922	* P<0.05
Poultry 1/10X vs Fertilizer	-0.4970	2.780	ns P>0.05
Poultry 1/10X vs Water	0.8500	4.754	ns P>0.05
Fertilizer vs Water	1.347	7.534	** P<0.01

Table A3.5 - Seedling Growth GCT2 – Plant Height ANOVA – full analysis of all treatments

Comparison	Mean Difference	q		P value	
CDS 1/2X Open vs Swine 1/2X Open	5.910	1.386	ns	P>0.05	
CDS 1/2X Open vs Poultry 1/2X Open	-12.000	2.814	ns	P>0.05	
CDS 1/2X Open vs CDS 1/10X Open	-10.920	2.561	ns	P>0.05	
CDS 1/2X Open vs Swine 1/10X Open	-6.090	1.428	ns	P>0.05	
CDS 1/2X Open vs Poultry 1/10X Open	-0.5900	0.1383	ns	P>0.05	
CDS 1/2X Open vs Fertilizer	-10.000	2.345	ns	P>0.05	
CDS 1/2X Open vs Water	11.250	2.638	ns	P>0.05	
Swine 1/2X Open vs Poultry 1/2X Open	-17.910	4.200	ns	P>0.05	
Swine 1/2X Open vs CDS 1/10X Open	-16.830	3.946	ns	P>0.05	
Swine 1/2X Open vs Swine 1/10X Open	-12.000	2.814	ns	P>0.05	
Swine 1/2X Open vs Poultry 1/10X Open	-6.500	1.524	ns	P>0.05	
Swine 1/2X Open vs Fertilizer	-15.910	3.731	ns	P>0.05	
Swine 1/2X Open vs Water	5.340	1.252	ns	P>0.05	
Poultry 1/2X Open vs CDS 1/10X Open	1.080	0.2532	ns	P>0.05	
Poultry 1/2X Open vs Swine 1/10X Open	5.910	1.386	ns	P>0.05	
Poultry 1/2X Open vs Poultry 1/10X Open	11.410	2.675	ns	P>0.05	
Poultry 1/2X Open vs Fertilizer	2.000	0.4690	ns	P>0.05	
Poultry 1/2X Open vs Water	23.250	5.452	*	P<0.05	
CDS 1/10X Open vs Swine 1/10X Open	4.830	1.133	ns	P>0.05	
CDS 1/10X Open vs Poultry 1/10X Open	10.330	2.422	ns	P>0.05	
CDS 1/10X Open vs Fertilizer	0.9200	0.2157	ns	P>0.05	
CDS 1/10X Open vs Water	22.170	5.198	*	P<0.05	
Swine 1/10X Open vs Poultry 1/10X Open	5.500	1.290	ns	P>0.05	
Swine 1/10X Open vs Fertilizer	-3.910	0.9168	ns	P>0.05	
Swine 1/10X Open vs Water	17.340	4.066	ns	P>0.05	
Poultry 1/10X Open vs Fertilizer	-9.410	2.206	ns	P>0.05	
Poultry 1/10X Open vs Water	11.840	2.776	ns	P>0.05	
Fertilizer vs Water	21.250	4.983	*	P<0.05	

Table A3.6 - Seedling Growth GCT1 – Plant Mass ANOVA – full analysis of all treatments

Comparison	Mean Difference	q	P value
CDS 1/2X Open vs Swine 1/2X Open	0.4200	3.168	ns P>0.05
CDS 1/2X Open vs Poultry 1/2X	-0.5500	4.148	ns P>0.05
CDS 1/2X Open vs CDS 1/10X Open	-1.500	11.313	*** P<0.001
CDS 1/2X Open vs Swine 1/10X Ope	-0.6900	5.204	* P<0.05
CDS 1/2X Open vs Poultry 1/10X	-0.2700	2.036	ns P>0.05
CDS 1/2X Open vs Fertilizer	-0.8800	6.637	** P<0.01
CDS 1/2X Open vs Water	0.3700	2.791	ns P>0.05
Swine 1/2X Open vs Poultry 1/2X	-0.9700	7.316	** P<0.01
Swine 1/2X Open vs CDS 1/10X Open	-1.920	14.481	*** P<0.001
Swine 1/2X Open vs Swine 1/10X Ope	-1.110	8.372	*** P<0.001
Swine 1/2X Open vs Poultry 1/10X	-0.6900	5.204	* P<0.05
Swine 1/2X Open vs Fertilizer	-1.300	9.805	*** P<0.001
Swine 1/2X Open vs Water	-0.05000	0.3771	ns P>0.05
Poultry 1/2X vs CDS 1/10X Open	-0.9500	7.165	** P<0.01
Poultry 1/2X vs Swine 1/10X Ope	-0.1400	1.056	ns P>0.05
Poultry 1/2X vs Poultry 1/10X	0.2800	2.112	ns P>0.05
Poultry 1/2X vs Fertilizer	-0.3300	2.489	ns P>0.05
Poultry 1/2X vs Water	0.9200	6.939	** P<0.01
CDS 1/10X Open vs Swine 1/10X Ope	0.8100	6.109	** P<0.01
CDS 1/10X Open vs Poultry 1/10X	1.230	9.277	*** P<0.001
CDS 1/10X Open vs Fertilizer	0.6200	4.676	ns P>0.05
CDS 1/10X Open vs Water	1.870	14.104	*** P<0.001
Swine 1/10X Ope vs Poultry 1/10X	0.4200	3.168	ns P>0.05
Swine 1/10X Ope vs Fertilizer	-0.1900	1.433	ns P>0.05
Swine 1/10X Ope vs Water	1.060	7.995	*** P<0.001
Poultry 1/10X vs Fertilizer	-0.6100	4.601	ns P>0.05
Poultry 1/10X vs Water	0.6400	4.827	ns P>0.05
Fertilizer vs Water	1.250	9.428	*** P<0.001

Table A4.1- DEM of fresh CDS HTC filtrate, showing top-ten compounds, total average area, and their chromatographical concerns.

CDS Filtrate, Fresh (time –trial 3)

	Compound Name	***Average Area (n=3)***	***STDEV***	***Concerns***	***% of Total Area***
1	Glycerin	2,477,267,292	82%	SM, Column Bleed	29.8%
2	Methyltartronic acid	215,551,057	44%	Column Bleed	2.6%
3	(S)-(+)-1,2-Propanediol	121,711,913	89%	Column Bleed	1.5%
4	Analyte 594	66,312,856	86%	SM	0.8%
5	2,3-Butanediol	64,493,707	52%	SM	0.8%
6	2,3-Butanediol, [R-(R*,R*)]-	60,891,468	27%	SM	0.7%
7	1,2-Propanediol, 3-chloro-	39,918,398	7%		0.5%
8	Acetic acid	34,766,221	58%	SM	0.4%
9	(S)-(+)-1,2-Propanediol	31,512,538	34%		0.4%
10	1,4-Dioxan-2-ol	29,033,720	12%		0.3%

Table A4.2- DEM of aged CDS HTC filtrate, showing top-ten compounds, total average area, and their chromatographical concerns**.**

CDS Filtrate Aged in open Container for 3 Months (time –trial 4)

	Compound Name	***Average Area (n=3)***	***STDEV***	***Concerns***	***% of Total Area***
1	Glycerin	1,331,083,519	27%	SM, Column Bleed	27.4%
2	2,5-Pyrrolidinedione, 1-methyl-	50,271,752	2%	SM	1.0%
3	3-Pyridinol, 6-methyl-	40,741,819	8%		0.8%
4	1-Propanol, 2,3-dichloro-	39,636,003	23%		0.8%
5	3-Pyridinol	38,846,016	10%	Column Bleed	0.8%
6	Hydrogen chloride	34,625,835	87%	Column Bleed	0.7%
7	2,3-Butanediol	13,621,412	19%		0.3%
8	Acetone	5,931,410	66%	SM, Shared Apex	0.1%
9	2-Pyrrolidinone	5,931,410	66%	Column Bleed	0.1%
10	4-Methoxycarbonyl-4-butanolide	497,676	57%	Column Bleed	0.0%

Table A4.3- DEM of fresh swine manure HTC filtrate, showing top-ten compounds, total average area, and their chromatographical concerns.

Swine Filtrate, Fresh (time –trial 3)

	Compound Name	*Average Area (n=3)*	*STDEV*	*Concerns*	*% of Total Area*
1	Butanoic acid	217,390,639	4%		16.2%
2	Acetamide	84,837,949	23%		6.3%
3	Propanoic acid, 2-methyl-	56,122,414	3%		4.2%
4	Butanoic acid, 3-methyl-	47,634,584	3%		3.6%
5	Pyrazine, methyl-	40,755,566	6%		3.0%
6	3-Pyridinol	29,952,902	6%		2.2%
7	Pyrazine, 2,5-dimethyl-	26,472,993	9%	SM	2.0%
8	Pyrazine	24,744,500	8%		1.8%
9	2,3-Epoxybutane	18,504,229	10%	SM	1.4%
10	Pentanoic acid	17,157,740	6%		1.3%

Table A4.4- DEM of aged swine manure HTC filtrate, showing top-ten compounds, total average area, and their chromatographical concerns.

Swine Filtrate Aged in open Container for 3 Months (time –trial 4)

	Compound Name	*Average Area (n=3)*	*STDEV*	*Concerns*	*% of Total Area*
1	Butanoic acid	210,808,424	4%	SM, Column Bleed	13.0%
2	Acetamide	152,925,156	15%		9.4%
3	Acetic acid	99,570,868	27%	SM	6.1%
4	Propanoic acid	94,190,065	54%		5.8%
5	Propanoic acid, 2-methyl-	86,632,209	21%		5.3%
6	Butanoic acid, 3-methyl-	78,680,099	12%		4.8%
7	Butanoic acid, 2-methyl-	44,431,158	7%		2.7%
8	Butanamide	35,415,371	24%		2.2%
9	Pentanoic acid	28,493,027	8%		1.8%
10	Propanamide	26,948,330	11%	SM	1.7%

Table A4.5- DEM of fresh poultry manure HTC filtrate, showing top-ten compounds, total average area, and their chromatographical concerns.

Poultry Filtrate, Fresh

	Compound Name	***Average Area (n=3)***	***STDEV***	***Concerns***	***% of Total Area***
1	Acetic acid	87,455,945	47%	SM	14.3%
2	Butanoic acid	38,012,932	15%		6.2%
3	2-Pyrrolidinone	26,999,350	9%		4.4%
4	Propanoic acid	19,868,901	10%		3.2%
5	L-Lactic acid	18,842,499	26%		3.1%
6	Acetone	18,483,982	19%		3.0%
7	Acetamide	17,707,895	8%		2.9%
8	Pyrazine, methyl-	14,616,530	4%		2.4%
9	Butanoic acid, 3-methyl-	11,752,393	30%		1.9%
10	Pyrazine, 2,5-dimethyl-	9,625,384	14%		1.6%

Table A4.6- DEM of aged poultry manure HTC filtrate, showing top-ten compounds, total average area, and their chromatographical concerns.

Poultry Filtrate Aged in open Container for 3 Months

	Compound Name	**Average Area (n=3)**	**STDEV**	**Concerns**	**% of Total Area**
1	Butanoic acid	60,172,399	11%	Column Bleed	8.3%
2	Acetone	59,352,286	136%	SM	8.2%
3	2-Pyrrolidinone	44,830,142	11%	SM, Column Bleed	6.2%
4	Acetic acid	37,140,268	82%	SM, Column Bleed	5.2%
5	Pentanoic acid	27,095,205	54%	Column Bleed	3.8%
6	Propanoic acid	27,082,896	9%		3.8%
7	Acetamide	25,298,495	27%		3.5%
8	Butanoic acid, 3-methyl-	24,180,940	13%		3.4%
9	Hydrogen azide	22,352,061	169%	SM	3.1%
10	Isopropyl Alcohol	13,343,945	76%	Column Bleed	1.9%

Table A4.6- DEM of fresh cow manure HTC filtrate, showing top-ten compounds and total average area

Cow	***Filtrate, Fresh***		
	Compound	***Peak Area (n=1)***	***% of Total***
1	acetic acid	361,791,989	27.0%
2	methylpyrazine	105,894,912	8.0%
3	pyrazine	51,114,273	4.0%
4	2,5-dimethylpyrazine,	37,049,681	3.0%
5	ethylpyrazine	25,772,952	2.0%
6	2-methoxyphenol	17,260,122	1.0%
7	butanoic acid	17,072,435	1.0%
8	3-pyridinol	27,885,114	2.0%
9	2,3-dimethylpyrazine,	13,738,940	1.0%
10	trimethylpyrazine,	12,720,744	1.0%