ABSTRACT

Title of Thesis:	FULL SCALE STUDY OF PATHOGEN, METAL POLLUTANTS, NUTRIENTS, AND POLYBROMINATED DIPHENYL ETHERS IN CLASS A BIOSOLIDS STABILIZED BY THERMAL HYDROLYSIS AND ANAEROBIC DIGESTION PROCESSES
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Thesis Directed By:	Professor Dr. Alba Torrents Department of Civil and Environmental Engineering and Dr. Natasha Almeida Andrade Department of Civil and Environmental Engineering

Class A biosolids are solid by-product of wastewater treatment which meet Environmental Protection Agency requirements to be used as fertilizer in farms, vegetable gardens, and can be sold directly to consumers. In 2014, this study's target nutrient recovery facility adopted thermal hydrolysis pretreatment and anaerobic digestion to upgrade biosolids quality from Class B (previously lime-stabilized) to Class A. In order to certify if this newly produced material met all regulatory requirements, we performed laboratory analysis to characterize fecal coliforms, volatile solids, and metals content. In addition, we showed a baseline for nutrient

management of total nitrogen, phosphorus, and the change in levels of polybrominated diphenyl ethers (PBDEs). Samples were collected for over a year since the start of THP-AD operation. Results were compared with the Class B biosolids produced at the same facility. Based on EPA standards, Class A biosolids were produced with stable quality after March, 2015, 16 weeks after process initiation. This work suggests that THP-AD is effective in producing Class A biosolids. In general, PBDEs in biosolids decreased from 1790 ± 528 (Class B) to 720 ± 110 µg/kg d.w. Our results suggest that the total levels of PBDEs decrease, however, the impact of the THP-AD on specific congeners are complex.

FULL SCALE STUDY OF PATHOGEN, METAL POLLUTANTS, NUTRIENTS, AND POLYBROMINATED DIPHENYL ETHERS IN CLASS A BIOSOLIDS STABILIZED BY THERMAL HYDROLYSIS AND ANAEROBIC DIGESTION PROCESSES

by

Xuanzhao Wang

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Advisory Committee:
Dr. Alba Torrents, Chair
Dr. Natasha Almeida Andrade
Dr. Birthe Veno Kjellerup

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Chapter 1: Introduction

1.1 Biosolids

Biosolids are stabilized sewage sludge with high organic matter content and rich in nutrients that are produced as a result of urban wastewater treatment processes at nutrient recovery facilities (NRF), formerly known as wastewater treatment processes plants (WWTPS). Every day, within the United States, including Puerto Rico, around 130.5 million cubic meters of wastewater is treated by publicly owned NRFs. The study by Seiple et al., 2017 estimated 13.84 dry million tons of biosolids were produced yearly in the U.S. and 50% were not beneficially applied.

With the variation of solids content, biosolids can exist as liquid form, cake form, and pellet form. Liquid biosolids have high water content 94-97% and low dry solids content from 3-6%. Cake biosolids usually have a solids content of 11-40%. And pellet biosolids solids content may reach to more than 90% (Lu et al., 2012). The increase of solids contents in biosolids means the efficiently reduction of volume and weight of the sludge, lower cost in transportation and storage, easier land application handling, and more persistent but slower nutrient release (Bramryd, 2001). However, the dewatering process is usually energy intensive and associated with high treatment costs. NRFs choose different biosolids treatment strategies based on ecological, technical and economic factors. The biosolids samples from the target NRF are in cake form.

As a resource, the high organic matter and nutrients content of biosolids, their utilization in land application have both ecological and social benefits. Many studies already indicated the utilization of biosolids as fertilizer could provide significant amount of organic carbon and nutrients extractable N, P and K, optimize soil physical structure, improve soil chemical properties, and prolong the nutrients release time for better effectiveness (Alvarenga et al., 2017; Urbaniak et al., 2017; Lindsay and Logan, 1998; Basta et al., 2001; Binder et al., 2002). In addition, instead of direct combustion and landfill, the commercialization of biosolids as fertilizer is considered as the most beneficial application method that recycles this resource, saves in treatment costs, and promotes the sustainable development of the society (Wang et al., 2008).

However, with the expectation of the increase in population, wastewater treatment coverage, treatment effectiveness, and regulations, biosolids production and land application are facing rising challenges (Sanin et al., 2011). Biosolids are produced from the municipal wastewater that has a variety of highly health-risky sources, including human excreta, washing water, manufactured liquids, industrial drainage, and rainfall runoff. Several studies have shown the utilization of biosolids, especially long-term application, may bring concerns for pathogen release, nutrient pollutants, trace metals, and some toxic organic pollutants to nearby environment and agricultural production, indeed, human health (Singh and Agrawal, 2008; Marguí et al., 2016; Clarke et al., 2017; Harder et al., 2017).

Therefore, legislation and regulations were passed to improve the biosolids quality and limit the biosolids application for environment and health concerns. The development of wastewater treatment regulations in the U.S. has a long history. Following to the development in Europe, wastewater management began to gain the interests by the U.S. authorities in 1890s. Staggered in the next fifty years, no significant approach were achieved by federal legislation to regulate the disposal of wastewater, until the pass of Water Pollution Control Act of 1956 to provide federal funding for publicly owned sewage plant (Jewell and Seabrook, 1979). In 1972, the pass of Clean Water Act further required the EPA to identify and regulate pollutants in wastewater discharge and biosolids disposal (Venkatesan et al., 2015). Later in 1993, *The Standards for the Use or Disposal of Sewage Sludge* (Title 40 of the Code of Federal Rgulations (CFR) (U.S. EPA, 1993), Part 503) were published by EPA to regulate the application of biosolids.

1.2 Class A and B Biosolids Qualifications

To ensure the safety of the production and field application of biosolids, regulations were implemented to biosolids-generating facilities by federal, state, and local agencies. According to Part 503 Biosolids Rule, EPA has a classification system and considers biosolids which not only meet but also exceed the minimal requirements of pathogen reduction, metals content limits, and vector control to be of Class A "Exceptional Quality" (EQ) biosolids (U.S. EPA, 1994b).

Aiming to minimize potential for disease, biosolids are classified into two classes according to their pathogen reduction levels and vector attraction reduction: Class A and Class B. In the federal regulations, the most probable number method

(MPN) is used to statistically determine the number of bacteria per weight or volume of sample. Class A biosolids must meet at least one of the following requirements: either the density of *Salmonella* sp. must be less than 3 MPN/4g d.w. or the density of fecal coliforms must be less than 1000 MPN/g d.w. Class B biosolids contains a higher level of pathogen that requires a maximum of 2 million MPN/g d.w. of biosolids. In addition, vector attraction reduction means the Class A biosolids does not attract files, mosquitos, rodents and the other vectors to transmit diseases (U.S. EPA, 1994b).

Because Class B biosolids still contains a considerable amount of pathogens and potentially transmits to humans, Class A classification is necessary if a user wants to apply the biosolids to residential lawns, home gardens, or other unrestricted public contact areas with potential close contact with human beings (U.S. EPA, 1994b). In contrast, Class B biosolids application is limited to farmland for animal feed to avoid public area and direct food production.

In the federal rule, maximum nutrient application rates of biosolids are not defined and only nitrogen (N) is regulated based on the estimate of crop N need and biosolids N availability beings (U.S. EPA, 1994b). For phosphate (P), no federal regulation is applied, but several states introduce requirements for P for land application with the objective of protecting groundwater and surface water quality (Lu et al., 2012). For metal pollutants, the biosolids that meet the ten trace metals: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn), have to be present in concentrations below the ceiling concentration limits stipulated in the federal rule as shown in Table 1. These ten metal pollutants are toxic to environment, plants, animals, humans and the other organisms that can bioaccumulate in organic components, such as structural proteins, enzymes, nucleic acids, lipids, and etc. and affect their functions.

Table 1. Pollutant ceiling concentration limits for all biosolids and Exceptional Quality (EQ) biosolids (U.S. EPA, 1994b).

Pollutant	**Ceiling Concentration Limits for All Biosolids Applied to Land (mg/kg d.w.)**	**Pollutant Concentration Limits for EQ Biosolids (mg/kg d.w.)**
Arsenic	75	41
Cadmium	85	39
Chromium	3,000	1,200
Copper	4,300	1,500
Lead	840	300
Mercury	57	17
Molybdenum	75	——— [a]
Nickel	420	420
Selenium	100	36
Zinc	7,500	2,800
Applies to:	All biosolids that are land applied	Bulk biosolids and bagged biosolids[b]
From Part 503	Table 1, Section 503.13	Table 3, Section 503.13

[a] As a result of the February 25, 1994, Amendment to the rule, the limits for molybdenum were deleted from the Part 503 rule pending EPA reconsideration.
[b] Bagged biosolids are sold or given away in a bag or other container.

The Class A EQ biosolids meet the both requirements for metal pollutants limits in Table 1 for EQ biosolids and requirements for Class A biosolids on pathogen and vector attraction reduction. Class A EQ biosolids have higher quality and broader application range than Class B biosolids. The land application of Class A EQ biosolids is regulated as the regular fertilizer in land applications (U.S. EPA, 1994b). The improvement from Class B to A biosolids will extend the land application area into food production process and local garden area with improved economic and social value in cost saving, nutrients recycle, and sustainable development.

1.3 Thermal Hydrolysis Pretreatment and Anaerobic Digestion (THP-AD)

Anaerobic digestion (AD) is a widely recognized sludge treatment process in NRFs to stabilize biosolids. In the U.S., over 1,200 NRFs use anaerobic digesters, and about 860 plants use the biogas they produced ("Current and Potential Biogas Production," 2014). AD is a biological process running under anaerobic condition (without oxygen) that microorganisms break down complex biodegradable organic matter to biogas, which is about 50-80% methane and 30-50% of carbon dioxide (Lora Grando et al., 2017). Although AD has been known since 17^{th} century, it was not deeply studied and widely utilized in NRFs until 1980s.

Biosolids fermented by AD has the features of great mass reduction, beneficial biogas production, and improved dewatering properties (Tiehm et al., 1997). The biogas produced in this process can be used for heating, power and electricity generation, and other energy-needed services with great economic and social values. AD process has four major steps to break down large organic matters into methane and carbon dioxide, which includes hydrolysis, acidogensis, acetogenesis, and methanogenesis (Gavala et al., 2003). Hydrolysis is the initial and rate-limiting step of overall AD process in biosolids treatment that carbohydrates, lipids, and proteins are depolymerized and solubilized into soluble monomers (Angelidaki et al., 2011; Kallistova et al., 2014).

In AD process, many environmental factors, such as temperature, food sources, pH, bioconcentration, and chemicals, can affect the efficiency of biogas production. Because the microbiological digestion of sludge is a slow and complicated process, various pretreatment technologies were developed to enhance biodegradation, such as thermal pretreatment, chemical pretreatment, mechanical pretreatment, etc. (Climent et al., 2007). Pretreatment processes can reduce digester heating requirements, decrease retention time, and increase biogas production (Pilli et al., 2015, Haug et al., 1983; Li and Noike, 1992).

Thermal hydrolysis pretreatment (THP) is one of well-studied technologies that is applied in many NRFs. In the beginning THP was used to enhance biosolids dewaterability, then studies extend to improve digestibility for anaerobic digestion (Carrèreet al., 2010). With extensive studies of THP-AD process, the optimal conditions for THP are temperature of 160-180 ˚C and time in the range 30-60 min (Bougrier et al., 2008). THP has the benefits of pathogens destruction, biosolids mass reduction, dewaterability improvement, order removal, and positive energy balance (Wilson et al., 2011).

1.4 Target Nutrient Recovery Facility (NRF) Operation

The target NRF is the largest advanced wastewater treatment plant in the world, which occupies about 0.6 km^2 in the Mid-Atlantic region of the U.S. The facility has a treatment capacity of around 1.4 million cubic meters per day and serves more than two million residents in the region. The treatment of wastewater in this facility generates a stream of clean water, which is discharged to the local river, and a stream of nutrient-rich biosolids. In general, the biosolids are generated after a series of treatment processes, including open-air primary sedimentation, activated sludge, and tertiary treatment (including nitrification-denitrification, filtration, and disinfection). In the past, 1200 wet tons of lime-stabilized Class B biosolids were produced daily. In an effort to improve to Class A biosolids, in November of 2014, the target NRF began operating a newly constructed Cambi™ thermal hydrolysis pretreatment combined with anaerobic digestion (THP-AD) after the thickening process to replace the lime-stabilization addition.

The THP-AD system adopted consists mainly of two parts, THP tanks and four 14.4 million liters anaerobic digesters that are designed to produce up to 450 dry tons of solids per day. A schematic of the THP-AD treatment process is shown in Fig. 1 in addition to the previous Class B biosolids production. For Cambi™ THP system adopted by the target NRF, input sludge were separated into 4 streams and each stream consists: (1) one pulper to preheat the sludge to approximately 60-99˚C with recycled steam from THP process; (2) six digester to hydrolysis under temperature around 165°C the corresponding vapor pressure around 610 kPa for about 30 minute; (3) one flash tank to decrease temperature and pressure of the sludge and flash the pressure stream back to the pulper (Armstrong et al., 2017). The high temperature of THP can significantly reduce pathogen level and help Class A biosolids generation (Oosterhuis, 2014).

The following AD process is mesophilic (about 37˚C) anaerobic digestion with about 22-day retention time. The microorganisms in the four digesters digest sludge from THP into smaller compounds and biogas. The biogas generated from AD supply THP as part of energy source. The digested solids are finally dewatered into cake form and transferred with belt conveyors to loadout. The biosolids samples for this study were collected from belt conveyors before loadout.

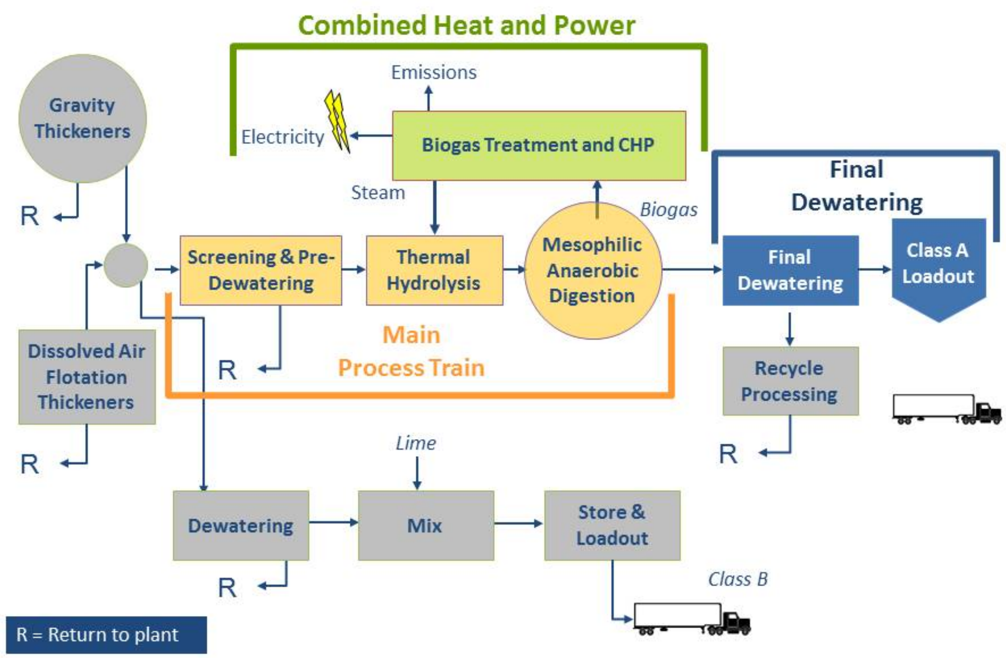


Fig. 1. Schematic of the old Class B (grey process) and the new Class A biosolids production THP-AD system (colored process) at target NRF.

Cambi^{TM} THP has been commercialized in worldwide and has the merits of increased biosolids bio-degradability and biogas production, biosolids volume reduction, 2-3 times increase in digester capacity, eliminated foaming problems, improved biosolids dewaterability and Class A biosolids production (Zhen et al., 2017). According to the data obtained from the target NRF, a great mass reduction of biosolids was observed after switch from lime-stabilized Class B biosolids production process to THP-AD for Class A biosolids production process. Around 65% of volatile solids reduction was observed for biosolids product by THP-AD treatment. Because previous lime-stabilized Class B biosolids and newly produced Class A biosolids have similar volatile solids (VS) that nearly 60%, for the same amount of organic matters in biosolids, the production of Class A biosolids will consume 1.86 times more of sludge input than the production of Class B biosolids. The data is used to correct metal pollutants concentrations and PBDEs concentrations between Class and B biosolids in this study.

Due to the large size of the facility, during the investigated period, anaerobic digesters were gradually filled with THP treated sludge in the first three month that THP-AD operation was under an unstable condition and many parameters could not reach to optimal expect. Since the biosolids samples were collected from the start of THP-AD, the period from Nov. 2014 to Feb. 2015 is named as startup stage; and the period from March 2015 to the end of last sample, Jan. 2016, is named as full-operation stage. In this study, data between startup and full-operation stages were compared to understand the impact of THP-AD on the parameters.

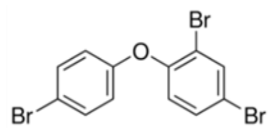
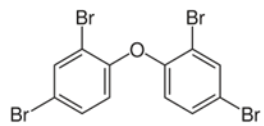
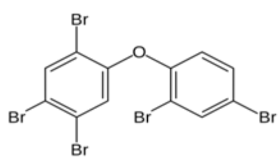
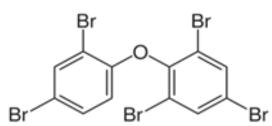
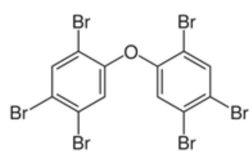
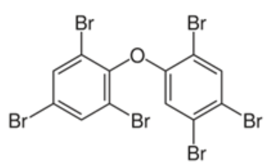
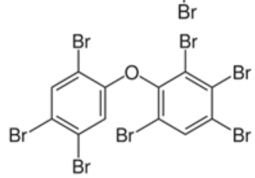
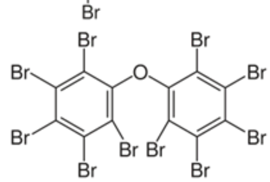
1.5 Polybrominated Diphenyl Ethers (PBDEs)

Polybrominated diphenyl ethers (PBDEs) are a group of manufactured aromatic organobromine compounds that contain a diphenyl ether skeleton and different numbers and locations of bromine atoms with 209 possible congeners (Fromme et al., 2009). Because of their remarkable flame-retardant properties, good thermal stability, and cheap cost, PBDEs were widely used as flame-resistant additive in consumer products since 1970s, in order to replacing banned flame retardants, such as polychlorinated biphenyls (PCBs) and polybrominated biphenyls (Daso et al., 2010).

Due to the directing properties of the bromine and steric hindrance, not all PBDEs congeners were manufactured for commercial use. Three major commercial formulations containing a variety of congeners, including Penta-, Octa-, and Deca-BDEs, were widely added in polymers, such as electronic components, household appliances, furniture, textiles, etc. (Krol et al., 2012). Penta-BDE formulation is a viscous liquid that contains about 41-41% of BDE-47, 44-45% of BDE-99 and -100, and 6-7% of BDE-153 and -153; Octa-BDE formulation contains mainly BDE-183; and Deca-BDE formulation consists about 97-98% of BDE-209 (Alaee, 2003). According to the study of La Guardia et al., 2006, Deca-BDE dominated 83.3% of the year 2001 PBDE global market demand, followed by Penta-BDE at 11.1% and Octa-BDE at 5.6%. In addition, BDE-28 is also present in commercial Penta-BDE as the precursors in the formation of BDE-47.

Therefore, eight congeners of PBDEs (BDE-28, -47, -99, -100, -153, -154, -183, and -209), representing the major congeners found in commercial formulations, were chosen as the targets for our analysis. PBDEs have high K_{oa}, low water solubility, high K_{ow}, and large molecular weight, which make them semi-volatile, hydrophobic and persistent in nature. The physical and chemical properties of the eight PBDEs congeners for this study were present in Table 2.

Table 2. Physical and chemical properties of the eight PBDEs congeners from the studies. K_{oa} = n-octanol/air partition coefficient; P_L = supercooled liquid vapor pressure (Pa); H = Henry's Law constant measured at 25˚C(Pa $m^3 mol^{-1}$); K_{ow} = n-octanol/water partition coefficient.

Congeners	**Structure**	**$\log K_{oa}$[a]**	**$\log P_L$[a]**	**logH[a]**	**$\log K_{OW}$**
BDE-28		9.70	-2.93	4.830	5.67[b]
BDE-47		10.34	-3.50	0.850	5.85[b]
BDE-99		11.28	-4.17	0.600	6.39[b]
BDE-100		11.40	-4.47	0.240	6.23[b]
BDE-153		12.15	-5.07	0.260	6.92[b]
BDE-154		12.18	-5.18	0.080	6.76[b]
BDE-183		12.89	-5.84	1.535	7.20[b]
BDE-209		15.73	-8.40	0.040	6.27[c]*

[a] Data reference from Xu et al., 2007.
[b] Data reference from Lebrun et al., 2014.
[c] Data reference from U.S. EPA, 2010a.

*Data obtain for Deca-BDE with over 97% of BDE-209

Similar as PCBs and polybrominated biphenyls, the characteristics of persistence, bioaccumulation, and potential carcinogenic and thyroid disturbing effects raised environmental and health concerns to PBDEs utilization (Naert et al., 2007). PBDEs are semi-volatile chemicals that can easily leach out and enter into indoor air and dust, which play as one of the major sources for exposure (Lorber, 2008). Due to the lack of binding sites on polymers, PBDEs are simply integrated into materials but not chemically bound, which means that they can easily be released from commercial products and enter the environment (Jinhui et al., 2017). A significant amount of studies have demonstrated that PBDEs' intake were toxic for plants, bacteria, and animals in organism development, thyroid hormones, neurobehaviors, and etc. (Xu et al., 2015; Talsness, 2008; Min et al., 2003). Few studies have fully understood the toxicity of PBDEs to human beings, but the bioaccumulation in body fluid, such as serum and breast milk, and the potential effects of liver toxicity, thyroid disturbance, and neurodevelopment affection have bring concerns to humans, especially pregnant mothers and infants (Herbstman et al., 2010).

Bioaccumulation and persistent property of PBDEs means PBDEs can deposit in organic matters easily and exist for a long time after phase-out in production (Lebrun et al., 2014). Many studies showed high concentrations of PBDEs exist in biosolids samples, and the application of large amounts of biosolids as fertilizer could put PBDEs into the food chain (Hale et al., 2012; Venkatesan and Halden, 2014; Stiborova et al., 2017). The study by Venkatesan and Halden, 2014, estimated about 24,000-36,000 kg/year (53,000 pound/year – 79000 pound/year) of PBDEs were released to the environment through land application of biosolids. Although PBDEs were phased out in manufacturing and commercial use in the consumer products, no regulations were applied to biosolids for PBDEs detection.

According to a study by Stiborova et al. (2015a), biodegradation is an effective method to remove organic pollutants in biosolids. A number of studies showed the adoption of mesophilic AD in the wastewater treatment would debrominate PBDEs from high-brominated to low-brominated congeners (Huang et al., 2014; Stiborova et al., 2015b; Tokarz et al., 2008). Enhanced by THP, extensive microorganism activities are expected in anaerobic digester and the debromination of PBDEs may also be accelerated. BDE-209 is the most prevalent PBDEs congeners in the environment that generally considered as less toxic and more immobile, then less threaten to environment and human health (Stiborova et al., 2017; Liu et al., 2016). However, the possible emerging of the low-bromine PBDEs from the degradation of BDE-209 in biosolids from THP-AD system indicates BDE-209 also needs to be concerned. Therefore, both the total concentration of PBDEs and the composition of PBDEs congeners in biosolids were measured in this study for a better PBDEs toxicology understanding.

After about 20 years of PBDEs production and use since 1970s, scholars and government agencies start to realize the environmental and health risks of PBDEs and begin to restrict, phase out, and ban the utilization of PBDEs. In 1989, Germany and Netherland initiatively phased out the sale of PBDEs-containing products. Afterward, Penta-BDE was stopped production in European Union in 1997. In 2009, commercial Penta-BDE and Octa-BDE were listed in the Persistent Organic Pollutants (POPs) inventory of the Stockholm Convention. And in 2014 Deca-BDE was proposed to be restricted in the European Union. (Jinhui et al., 2017) In the U.S., the phase out of PBDEs started by the state of California in 2003 that decided to ban Penta- and Octa-BDE in 2008. Later on, several states prohibited the sale and production of PBDEs. In 2009, two major PBDE producers and the main importer committed to stop producing, import, and sale of PBDEs by the end of 2013. And in 2012, EPA proposed to phase out all PBDEs production, import and processing by the end of 2013 (U.S.EPA, 2014). Since then, no new consumer product with PBDEs addition were allowed in the U.S..

1.6 Study Objectives

In this study, two important aspects to better understand the newly produced biosolids by THP-AD were investigated: (1) would they fulfill the requirements for Class A by EPA, and (2) what is the fate of selected trace organic pollutants. The hypotheses of this study are: 1) biosolids produced from newly adopted THP-AD process at target NRF are qualified Class A biosolids based on EPA standards; 2) the total PBDEs concentration in biosolids produced from THP-AD were lower than previous Class B biosolids data from the same NRF at study by Andrade et al., 2015.

To test the hypothesis of the study, first, biosolids samples were collected at the target NRF from more than 1-year period to determine if Class A biosolids were being produced after the adoption of the new full-scale THP-AD processes. Based on EPA standards, pathogen level and metal pollutants concentrations were analyzed.

Nutrients levels are also determined to evaluate the biosolids' economic value and potential contamination of excess nutrients. In addition, metals levels in Class A biosolids were compared to the levels found in the previously produced Class B biosolids for a better understand of THP-AD process.

Second, the same biosolids samples were analyzed for PBDEs and levels were compared to the levels found in the Class B biosolids previously produced at the target NRF. The distributions of different PBDEs congeners were studies to evaluate the toxicity change of newly produced Class A biosolids. The PBDEs between startup and full-operation stages in THP-AD operation were also analyzed to have a better understanding of THP-AD system in PBDEs degradation.

Chapter 2: Full Scale Study of Class A Biosolids Produced by Thermal Hydrolysis Pretreatment and Anaerobic Digestion

Xuanzhao Wang[1]*, Jessica Shekarchi[1], Sarah J. Fischer[1], Natasha Andrade[1], Alba Torrents[1], Mark Ramirez[2]

[1] Department of Civil and Environmental Engineering, University of Maryland, College Park, MD 20742, USA
[2] DC Water, District of Columbia Water and Sewer Authority, 5000 Overlook Avenue, S.W., Washington, DC 20032, USA
*Email address: xzwang@umd.edu

Corresponding Author:
Natasha Andrade
Email: nandrade@umd.edu
Tel: 301-405-1171

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Highlight

- Qualified Class A Exceptional Quality biosolids were produced with thermal hydrolysis pretreatment and anaerobic digestion processes
- Fecal coliforms levels varied greatly during the startup stage and stabilized at very low levels in full-operation stage of THP-AD processes
- Biosolids trace metals concentrations increased due to changes in stabilization methods (from lime addition to THP-AD processes)
- Class A biosolids have high concentrations in total nitrogen and total phosphorus but low in total potassium.

2.1 Abstract

Class A biosolids is the solid by-product of wastewater treatment and contains high-organic matter and nutrient content, which can be applied to food production and gardens. In 2014, this study's target nutrient recovery facility (NRF) in the Mid-Atlantic region of the U.S. adopted thermal hydrolysis pretreatment (THP) and anaerobic digestion (AD) to increase the quality of biosolids from Class B (lime-stabilized) to Class A. According to Environmental Protection Agency (EPA) requirements, pathogen levels, nutrients levels, and metal pollutants concentrations of biosolids during one-year period were determined and compared with levels found in Class B biosolids from the same facility. After optimization and equilibrium of the process, biosolids were produced with stable quality and satisfied all Class A standards. Metal concentrations increased from Class B to Class A biosolids due to the biosolids mass reduction. In addition, Class A biosolids are rich in total nitrogen (N) and phosphorus (P), but low in potassium (K) content.

Keywords: Biosolids, Fecal Coliform, Nutrients, Metals

2.2 Introduction

A large volume of wastewater is generated every year from urban areas and is treated by nutrient recovery facilities (NRFs), formerly known as wastewater treatment plants. As population grows and urbanizes, the volume of wastewater generated generally increases and its content changes. Since the 1950s, the U.S. federal legislation has been strengthened on water pollution control (Lu et al, 2012). The Clean Water Act that passed in 1972 further required EPA to identify and regulate pollutants in wastewater discharge and biosolids disposal (Venkatesan, 2015). Thus, the quality of wastewater and the reuse of biosolids were dramatically improved. After phase separation, sedimentation, filtration, chemical and biological treatments, large quantities of high-nutrient content biosolids are produced from wastewater (Lu et al., 2012). In the U.S., approximately 50% of biosolids are applied to land as fertilizer or soil amendment for low-fertility soil improvement and degraded land reclamation (Seiple et al., 2017). Land application of biosolids can provide organic matter and nutrients, modify physical and biological properties of soils, and assist vegetation growth and ecosystem restoration (Larney and Angers, 2012; Tian et al., 2009; Scharenbroch et al., 2013). Besides, the commercialization of biosolids as fertilizer is usually considered the most beneficial disposal method with great environmental and social values (Wang et al., 2008).

Although biosolids land application has many benefits, there are some concerns that some biosolids constituents may threaten environment and the health of animals and humans (Singh and Agrawal, 2008; Smith, 2009; Marguí et al., 2016; Yergeau et al., 2016). Municipal wastewater tends to generate biosolids that are rich on pathogens, organic pollutants, and metal contents, and these levels may be harmful to environment and humans (Lu et al, 2012; Singh and Agrawal, 2008). Therefore, to ensure the safety of biosolids application, the production and field application of biosolids have to follow federal, state, and local regulations according to Part 503 Biosolids Rule by EPA (U.S. EPA, 1993) and others. EPA has a classification system and considers biosolids which not only meet but also exceed the minimal requirements of pathogen reduction, metals content limits, and vector control to be of Class A “Exceptional Quality” (EQ) biosolids (U.S. EPA, 1994b).

Aiming to minimize potential for disease and environmental risk, biosolids can be classified into Class A or Class B according to EPA standards. In the federal regulations, Class A biosolids must meet at least one of the following requirements: either the density of *Salmonella* sp. must be less than 3 MPN/4g d.w. or the density of fecal coliforms must be less than 1000 MPN/g d.w. Class B biosolids may contain a higher level of fecal coliform density, but must remain below 2 million MPN/g d.w. (U.S. EPA, 1994b). Because Class B biosolids still contain a considerable amount of pathogens, Class A classification is necessary if a user wants to apply the biosolids to food production agricultural land, residential lawns, home gardens, or other unrestricted public contact areas (U.S. EPA, 1994b).

All biosolids that are land-applied or commercialized have ten trace metals that are regulated: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn). These metals have to be present in concentrations below the ceiling concentrations, which vary according to their use and classification stipulated in the federal rule (U.S. EPA, 1994b), as shown in Table 3. In the federal rule, maximum nutrient application rate of biosolids are not defined, but several states introduce requirements for nitrogen (N) and phosphate (P) for land application with the objective of protecting groundwater and surface water quality (Lu et al., 2012).

Table 3. Pollutant ceiling concentration limits for all biosolids and Exceptional Quality (EQ) biosolids (U.S. EPA, 1994b).

Pollutant	**Ceiling Concentration Limits for All Biosolids Applied to Land (mg/kg d.w.)**	**Ceiling Concentration Limits for EQ Biosolids (mg/kg d.w.)**
Arsenic	75	41
Cadmium	85	39

Chromium	3,000	1,200
Copper	4,300	1,500
Lead	840	300
Mercury	57	17
Molybdenum	75	— [a]
Nickel	420	420
Selenium	100	36
Zinc	7,500	2,800
Applies to:	All biosolids that are land applied	Bulk biosolids and bagged biosolids[b]
Part 503 Location	Table 1, Section 503.13	Table 3, Section 503.13

[a] As a result of the February 25, 1994, Amendment to the rule, the limits for molybdenum were deleted from the Part 503 rule pending EPA reconsideration.
[b] Bagged biosolids are sold or given away in a bag or other container.

In the U.S., many NRFs adopt anaerobic sludge digestion as it is considered one of the most efficient technologies to stabilize and to produce energy from biosolids (Iranpour and Cox, 2007; Wang et al., 2008). Anaerobic digestion (AD) has great environmental and economic benefits, which include mass reduction, odor removal, pathogen reduction, and energy recovery (Pilli et al., 2015). During the digestion process, the destruction of volatile solids produces methane-rich biogas, which can be used to generate heat. Several studies also showed designed mesophilic AD could eliminate high level of pathogens and produce Class A or Class B biosolids (Forster-Carneiro et al., 2010; Rubio-Loza and Noyola, 2010; Lloret et al., 2013).

However, the microbiological digestion of sludge is a slow and complicated process and various pretreatment technologies were developed to enhance biodegradation, reduce digester heating requirements, decrease retention time, and increase biogas production (Pilli et al., 2015). Thermal hydrolysis pretreatment (THP) is one of well-studied technologies that is utilized in many NRFs (Carrèreet al., 2010). The Cambi™ THP is a pre-treatment method that generally heats sludge to around 165°C for about 30 minutes under the corresponding vapor pressure around 610 kPa. The high temperature and pressure of THP can significantly reduce pathogen level and help Class A biosolids generation (Oosterhuis, 2014).

In an effort to produce Class A biosolids, in November 2014, the target NRF in the Mid-Atlantic region of the U.S. began to operate Cambi™ THP combined with anaerobic digestion (THP-AD). The target NRF occupies about 0.6 km^2 with the treatment capacity of around 1.4 million m^3/d and serves more than two million residents in the urban area. The THP-AD system mainly consists of two parts, THP tanks and four 14.4 million liters bacterial digesters that were designed to a capability of up to 450 dry tons of solids per day. Before the introduction of THP-AD processes, Class B biosolids were produced by gravity thickening, air floating thickening, and lime addition (Fig. 1), with a final production of 1200 wet tons of lime-stabilized Class B biosolids daily.

Starting on November 29th, 2014, biosolids samples resulting from the THP-AD treatment system were collected and analyzed daily. During startup stage (from November 2014 to February 2015), four anaerobic digesters were gradually filled with seed sludge and reached optimum conditions determined by ammonia generation and total solids. During this period, the quality of biosolids was changing and experiments were conducted to monitor the biosolids quality variation. When stable quality biosolids were produced (from March 2015 until the last sampling day for this study December 29th, 2015, which we call the full-operation stage), routine experiments were conducted to verify of the produced biosolids met EPA standards.

This manuscript reports on the temporal variation of fecal coliform density, metals concentrations, and nutrients levels during the full-scale startup and full-operation stages of the THP-AD system at the target NRF. Moreover, we also assessed if the stable biosolids produced after the initial adjustment period of the startup stage met EPA Class A EQ classification standards. We compared the metal content differences in the newly-produced Class A and the previously-produced Class B biosolids.

2.3 Materials and Methods

2.3.1 Sampling Location and Collection

Biosolids samples were collected daily from November 29th, 2014 to December 29th, 2015 at the target NRF from belt conveyors right after final dewatering after THP-AD and before loadout (Fig. 1). Sampling equipment and containers were sterilized in an autoclave (Hirayama Manufacturing Co. HV-50L, Japan) before use. The sampling collection and preservation followed U.S. EPA Method 1684 (U.S. EPA, 2001a).

2.3.2 Total and Volatile Solids Determination

Duplicate biosolids samples were analyzed daily to calculate total solid (TS) and volatile solid (VS) contents. Analysis followed EPA Method 1684, which dictates a minimum of 12 hours drying in an oven (Fisher Scientific, Isotemp Lab Oven) at 103°C to 105°C for TS calculation and a further minimum 2 hours ignition in the heat furnace (Neycraft Vulcan, A-550, York, PA) at 550°C for VS calculation (U.S. EPA, 2001a).

2.3.3 Fecal Coliforms Determination

The fecal coliform density analysis was conducted daily according to EPA Method 1681 (U.S. EPA, 2006). Commercial A-1 medium broth most probable number (MPN) tubes (Hach, AD12-1ED, Loveland, CO) and MPN statistical methods were used to determine fecal coliforms density daily in biosolids. Positive and negative controls were processed with *E-coli* (Kwik-Stik, 0335K, Cloud, MN) and *Enterobacter* (Kwik-Stik, 0323X, Cloud, MN) to ensure the quality of the experiments. To ensure the acceptable performance of the experiments, percent recovery of *E. coli* in spiked control and spiked matrix samples were also calculated and compared with the criteria of initial and ongoing precision and recovery (U.S. EPA, 2006). The colony forming units were counted in A-1 medium plate to calculate the spiked *E. coli* concentration and the *E. coli*-spiked sample percent recovery. The matrix spike consisted of diluted *E. coli* suspension solution added to the biosolids homogenized solution and processed with the same method as samples. In addition, the control spike consisted of diluted *E. coli* suspension solution added to commercial Milorganite^{TM} (Organic Nitrogen Fertilizer, Milwankee, WI) and processed as samples.

2.3.4 Metals Determination

Trace metals, which included As, Cd, Cr, Cu, Pb, Hg, Mo, Ni, K, Se, and Zn, were analyzed daily in Class A biosolids according to the EPA Method 200.7 (U.S. EPA, 1994a) by inductively-coupled plasma emission spectrometry (ICP-ES) (Shimadzu, ICPE-9000, Japan) from November 2014 to December 2015. In brief, biosolids samples were pre-dried and heat-digested with HNO_3, H_2O_2, and HCl to extract metal ions, and then were analyzed by ICP-ES. Class B biosolids metal contents data from Jan. 2013 to February 2015 were obtained from the target NRF.

2.3.5 Nutrients Determination

In this study, nutrient analysis consisted of the analysis of nitrate/nitrite-N (NO_3^-/NO_2^--N), ammonia nitrogen (NH_3-N), total Kjeldahl nitrogen (TKN), and total phosphorous (TP). Samples were analyzed daily from November, 2014 to February, 2015, then weekly until December, 2015. Nutrient levels in Class B biosolids from January, 2013 to February, 2015 were obtained from the target NRF.

2.3.5.1 Nitrate/nitrite-N (NO_3^-/NO_2^-–N)

The analysis of nitrate/nitrite in biosolids was based on EPA Method 1685 (U.S. EPA, 2001b). Samples were mixed with DI water to dissolve NO_3^-/NO_2^- ions. Then filtered solutions were sent to automated QuAAtro^{TM} nutrient analyzer (Seal Analytical, QuAAtro39, Mequon, WI) to determine the NO_3^-/NO_2^- – N concentration.

2.3.5.2 Ammonia Nitrogen (NH_3-N)

Based on EPA Method 1690 (U.S. EPA, 2001d), SimpleDistTM system (Environmental Express, C6000/SC100/C6002, Charleston, SC) was utilized to distill NH_3-N from the sample with anhydrous sodium tetraborate ($Na_2B_4O_7$) buffer solution under pH 9.5 at 135°C. Enhanced by the heat in the bottom and air extraction on the top, NH_3 gas was generated in the sample solution and captured by dilute sulfuric acid for 120 minutes (U.S. EPA, 2001d). The sulfuric acid solution was sent to automated QuAAtro™ nutrient analyzer (Seal Analytical, QuAAtro39, Mequon, WI) to determine the NH_3-N concentration.

2.3.5.3 Total Kjeldahl Nitrogen and Total Phosphorous (TKN and TP)

TKN is the sum of ammonia-nitrogen and organic nitrogen. The analytical methods for TKN and TP were modified from EPA Method 1688 (U.S. EPA, 2011c). Duplicate samples were prepared for TKN and TP analysis in digestion tubes (Seal Analytical Inc., AIM 600 block, USA) with 20 mL of digest acid solution each, which contained mercuric-sulfate, potassium sulfate (K_2SO_4), and sulfuric acid. Samples were digested in a block heater (Foss, Digestor 2508 autorack, China) at 180°C for an hour, at 280°C for an hour, and finally at 350°C until reach to bright yellow color , and were sent to QuAAtro™ analyzer (Seal Analytical, QuAAtro39, Mequon, WI).

2.4 Results and Discussion

2.4.1 Total Solids (TS) and Volatile Solids (VS)

2.4.1.1 Temporal Variation

From November 29th, 2014 to December 29th, 2015, daily samples were obtained daily for Class A biosolids products. The operation of the THP-AD system was separated into two parts: startup stage from November 29th, 2014 to February 28th, 2015, and full-operation stage from March 1st, 2015 to December 29th, 2015. During the startup period, the average (± standard deviation) TS was 28.12% ± 2.116% (n=199), and the average (± standard deviation) VS was 59.32% ± 1.972% (n=180). During full-operation period, the average (± standard deviation) TS was 31.39% ± 2.180% (n=344), and the average (± standard deviation) VS was 58.40% ± 4.087% (n=341). These values are similar to another biosolids stabilized with THP-AD system with VS around 50% (Pérez-Elvira and Fdz-Polanco, 2012).

From startup to the full-operation stage, a significant increase was observed in TS (p<0.0001, unpaired t-test) and significant decrease was observed in VS (p=0.0006, unpaired t-test). Due to the mass loading in the ADs and the microorganisms' population growth during startup stage, the whole THP-AD system was under an unstable condition and didn't reach to the optimal dewaterability. Therefore, more water was kept in the biosolids samples during startup stage than in the full-operation stage, explaining the higher TS in the former. In contrast, a better growth of microorganisms in AD during full-operation stage will increase biogas production, reduce mass by breakage of organic matter, and leave less organic carbon in biosolids, which decreased VS of biosolids product (McMahon et al., 2001).

2.4.1.2 Class A and B Biosolids Comparison

Before the adoption of the THP-AD system for biosolids production, a dewatering process followed by lime addition was used to stabilize and produce Class B biosolids. From Jan. 1st, 2013 to February 13th, 2015, TS and VS of the Class B biosolids were measured daily at the NRF, with TS = 33.07% ± 2.899% (*n*=735), and VS = 54.48% ± 6.159% (*n*=733). In comparison of Class A full-operation stage data, Class A biosolids had significantly lower TS (p<0.0001) but higher VS (p<0.0001), which indicates that the final biosolids cake treated by THP-AD contained a slightly higher moisture and organic matter contents than lime-stabilized Class B biosolids.

However, it should be noticed an overall 65% volatile solids reduction (VSR) was observed for biosolids by THP-AD process with consideration of total sludge input and biosolids output. On a typical day, 300 dry tons of sludge with VS 80% input into THP-AD processes produce 140 dry tons of biosolids with VS 60%. As the result of Eq.1,

$$\text{Eq. 1:}\quad VSR = \frac{(300\times 80\%)-(140\times 60\%)}{300\times 80\%} = 65\%$$

a significant part of organic matter was converted to biogas to supply the THP system.

2.4.2 Fecal Coliform Density

Fecal coliform density in biosolids is an important criteria used by EPA to ensure the quality of biosolids. Therefore, daily monitoring was conducted from November 29th, 2014 to December 29th, 2015 with duplicate measurements.

2.4.2.1 Temporal Variation

Daily fecal coliform measurements were compiled into monthly data (Fig. 2). Fecal coliforms in the Class A biosolids was high and variable during startup stage from November 2014 to February 2015. During this stage, fecal coliform density was 3915 ± 6068 MPN/g d.w. (n= 91), which is higher than EPA requirement for Class A biosolids (1000 MPN/g d.w.). In full-operation stage from March, 2015, the average fecal coliform density was consistently below 100 MPN/g d.w., at 35.85 ± 81.10 MPN/g d.w. (n= 301), achieving a relative stable condition.

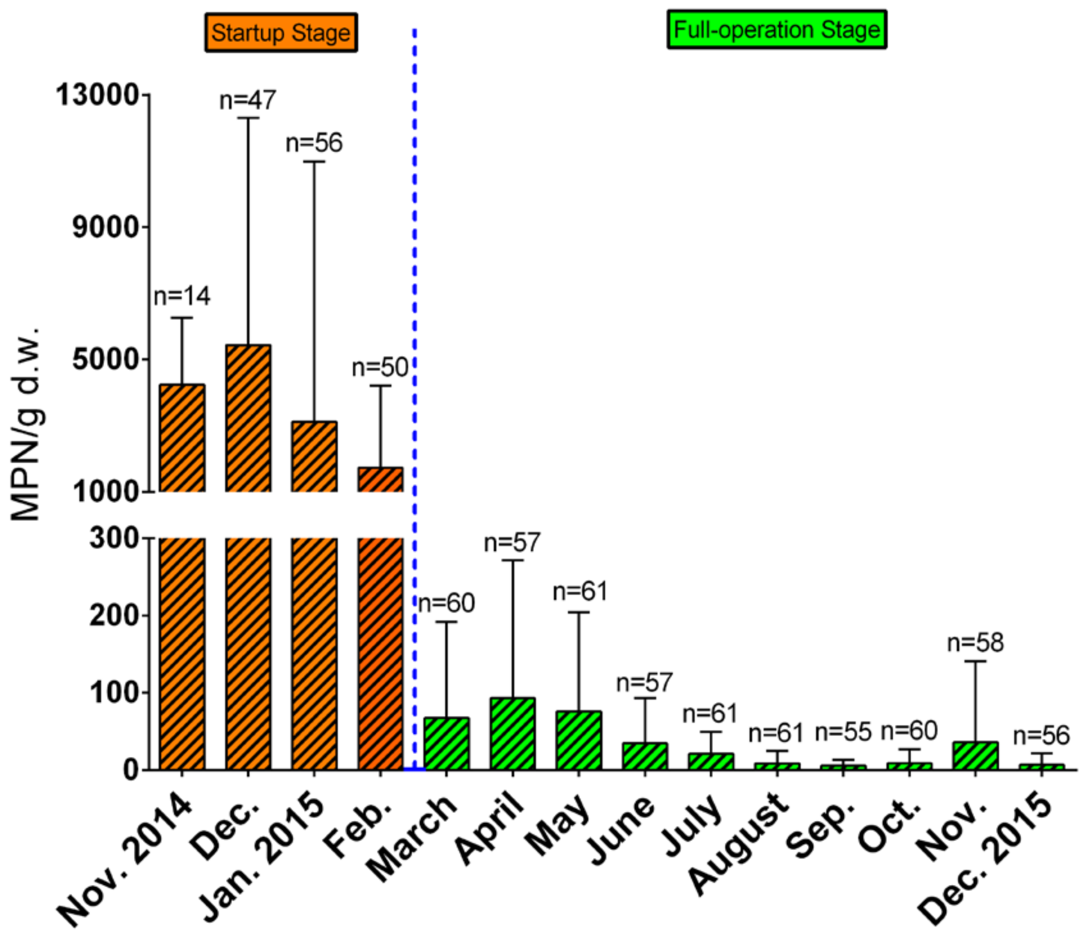


Fig. 2. Monthly averages (+ standard error) of fecal coliform density per dry mass of biosolids from November 29th, 2014 to December 29th, 2015. Number of samples used to calculate monthly averages are shown above each column.

Under the temperature of 165°C for about 30 minutes in the THP process, fecal coliforms in untreated sludge likely do not survive. In fact, in an analysis of a single sample collected immediately after the THP, there were no measurable levels of fecal coliforms (data not shown). However, in the ADs, bacteria are encouraged to grow, and operators need to control operating conditions to prevent re-growth of pathogens. The initial operation of the two ADs included the use of seed sludge from the other NRF. During the startup stage, two more ADs were gradually added into the treatment system and fed with sludge to the optimal volume. Although the temperature and pressure were controlled in the digesters, the initial digesting bacteria population had to acclimatize to reach optimum growth rate. Reactor configuration, microbial competition, pH, volatile fatty acid content, ammonia concentration, and biogas production all could inhibit the growth of fecal coliforms (Smith et al., 2005; Salsali et al., 2008; Orzi et al., 2015). The slow growth of methanogens in digesters during startup, or even the early full-operation period, could have allowed for lower microbial competition that allowed fecal coliform to regrow in the ADs. In addition, the initial methanogen seed from another NRF could have contained fecal coliforms, which could have thrived with the new unstable conditions offered in the startup period. Therefore, fecal coliform density was high and variable during startup period but decreased and stabilized in the full-operation period throughout the first year of the THP-AD operation.

2.4.3 Trace Metals Concentrations

EPA sets maximum limits of metal pollutants concentrations for Class A biosolids, therefore a total of 11 metals were measured daily in duplicate biosolids samples during the startup period and weekly during the full-operation period.

2.4.3.1 Temporal Variation

The average concentration of each metal species, with standard error, and number of measurements is presented in Table 4. Concentrations were highest for K and lowest for Hg and ranked from high to low concentration in the following order: K > Zn > Cu > Cr > Pb > Ni > Mo > Se > As > Cd > Hg. When compared to the concentration limits for EQ biosolids required by EPA, all metals meet the biosolids requirements.

Due to the great mass reduction from Class B to Class A biosolids production, Class A requires more sludge input than Class B sludge for the same unit weight of biosolids production. Assume all metal pollutants absorbed in the carbonic matters in biosolids, 65% VSR from Class B to Class A biosolids was used to correct the average concentrations of metal pollutants in Class B, shown Table 4. The corrected results imply the expected metal concentrations of Class B biosolids if they had the same VSR as Class A biosolids. And the differences between average concentrations in Class A and corrected average concentration in Class B can indicate the impacts of THP-AD process to the metals in biosolids.

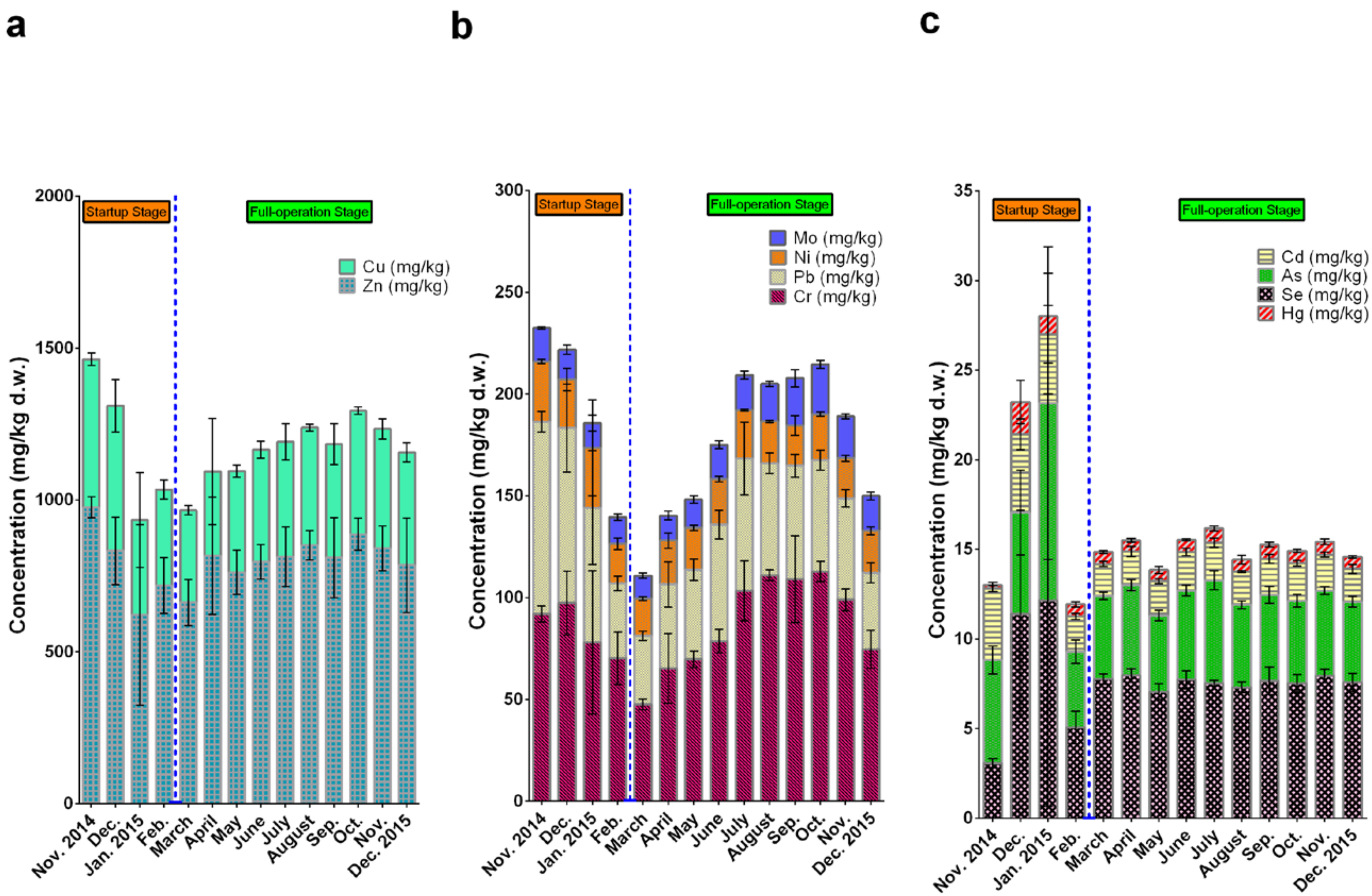
Table 4. Average concentrations of metals in Class A biosolids from November 29^{th}, 2014 to December 29^{th}, 2015, measured concentrations and corrected concentrations

of Class B biosolids from January, 2013 to February, 2015 at target NRF, and EPA concentration limits for Class A biosolids (U.S. EPA, 1994b).

Metal Pollutants	**Average Concentration in Class A (mg/kg dw)**	**Actual Average Concentration in Class B (mg/kg dw) (*n*=51)**	**Corrected Average Concentration in Class B (mg/kg dw)**	**Concentration Limits for EQ Biosolids (mg/kg dw)**
As	6.429 ± 0.4005 (*n*=141)	2.071 ± 0.05776	10.36 ± 0.2888	41
Cd	3.388 ± 0.1166 (*n*=147)	0.7602 ± 0.02526	3.801 ± 0.1263	39
Cr	88.42 ± 1.995 (*n*=148)	39.27 ± 1.251	196.4 ± 6.255	1,200
Cu	400.6 ± 9.812 (*n*=148)	128.0 ±2.314	640.0 ± 11.57	1,500
Pb	68.14 ± 2.189 (*n*=148)	17.65 ± 0.5383	88.25 ± 2.692	300
Hg	1.206 ± 0.1155 (*n*=148)	0.2902 ± 0.01448	1.451 ± 0.07240	17
Mo	14.93 ± 0.3212 (*n*=148)	7.592 ± 0.3549	37.96 ± 1.775	—*
Ni	23.81 ± 0.9109 (*n*=146)	12.56 ± 0.4906	62.80 ± 2.453	420
Se	10.02 ± 0.5726 (*n*=140)	2.616 ± 0.09719	13.08 ± 0.4860	36
Zn	778.4 ± 14.90 (*n*=148)	284.3 ± 5.453	1422 ± 27.27	2,800
K	850.2 ± 21.69 (*n*=134)	1456 ± 43.44	7280 ± 217.2	

***** As a result of the February 25, 1994, Amendment to the rule, the limits for molybdenum were deleted from the Part 503 rule pending EPA reconsideration.

Daily measurements were combined to calculate monthly average concentrations of these 11 metal species (Fig. 3). From startup to full-operation period, 9 metal species were significantly different with $p<0.05$ between startup and full-operation stages. This was not valid for Zn ($p=0.2765$) and Cr ($p=0.4764$).



a
b
c
Startup Stage
Full-operation Stage
Cu (mg/kg)
Zn (mg/kg)
Mo (mg/kg)
Ni (mg/kg)
Pb (mg/kg)
Cr (mg/kg)
Cd (mg/kg)
As (mg/kg)
Se (mg/kg)
Hg (mg/kg)
Concentration (mg/kg d.w.)
Nov. 2014
Dec.
Jan. 2015
Feb.
March
April
May
June
July
August
Sep.
Oct.
Nov.
Dec. 2015

Fig. 3. Monthly average concentration of (a) Zn and Cu, (b) Cr, Pb, Ni, and Mo, (c) Se, As, Cd, and Hg, from November 29th, 2014 to December 29th, 2015 with standard deviations.

2.4.3.2 Class A and B Biosolids Comparison

Measured average Class B biosolids metal concentrations data from January, 2013 to February, 2015 (Table 4) were compared to Class A metals concentrations. In general, Class A biosolids contained higher concentration of metals than Class B biosolids, except for K. The increase of trace metal concentrations in Class A biosolids when compared to Class B biosolids had been observed before by Wang et al. (2016), which suggested that THP might concentrate heavy metals in biosolids. This could be explained by the efficient mass reduction in Class A biosolids production. Since THP-AD removed carbon as biogas and this resulted in a 65% of volatile solids reduction, metals that remained in biosolids were concentrated.

A better comparison may be between metals concentrations in Class A and corrected metals concentrations in Class B biosolids. The metals content in Class B biosolids were corrected taking into account that the Class A biosolids had a 65% VS reduction (Table 4). With unpaired t-test, all metals (K (p<0.0001), Cu (p=0.0037), Cr (p<0.0001), Pb (p<0.0001), Ni (p<0.0001), Mo (p<0.0001), Se (p<0.0001), Cd (p<0.0001), and Hg (p=0.0025)), with the exception of Zn (p=0.1183) and As (p=0.2377), had significantly different concentrations between Class A and corrected Class B biosolids. K, Cr, Mo, and Ni were significantly higher in corrected Class B biosolids; but Cd, Cu, Pb, Hg, and Se were significantly lower in corrected Class B biosolids.

It is possible that Cr, Mo, and Ni were lower in Class A biosolids due to thermal treatment of THP, which could have increased the diffusivity of metal ions and released organic matter-bound metals by large molecules breakage that freed metal ions from sludge to the water phase (Appels et al., 2010). For the other metal species, which did not significantly change or increased in Class A biosolids, the high temperature of the THP might not effectively transfer those metals ions into the water phase. A further analysis of the metal concentrations in the water phase from THP-AD may aid in the understanding of the processes controlling metals concentrations in the solid phase.

2.4.4 Nutrients Concentrations

Although nutrients concentrations in biosolids are not regulated by EPA, knowing nutrients levels is helpful in evaluating the economic and agricultural benefits and environmental impacts of biosolids in field applications. In this study, NO_3^-/NO_2^-–N, TKN, TP, and NH_3-N concentrations were analyzed in Class A biosolids from November 29th, 2014 to December 29th, 2015. Daily analysis of duplicate samples for TKN and TP, and triplicate samples for NH_3-N was conducted during the startup period. In the full-operation period, nutrients concentrations were measured weekly. The results for nitrate/nitrite analysis were below the detection limit and are not reported in this study.

2.4.4.1 Temporal Variation

TKN concentrations did not change between startup and full-operation periods ($p = 0.3150$), however, for both TP ($p = 0.0256$), and NH_3-N ($p < 0,001$) we measured higher concentrations during the optimal conditions of the full-operation stage (Fig. 4). It is quite possible that the optimal growth rate of the microorganisms in the ADs could enhance the breakage of large organic molecules, which could free more P and NH_3.

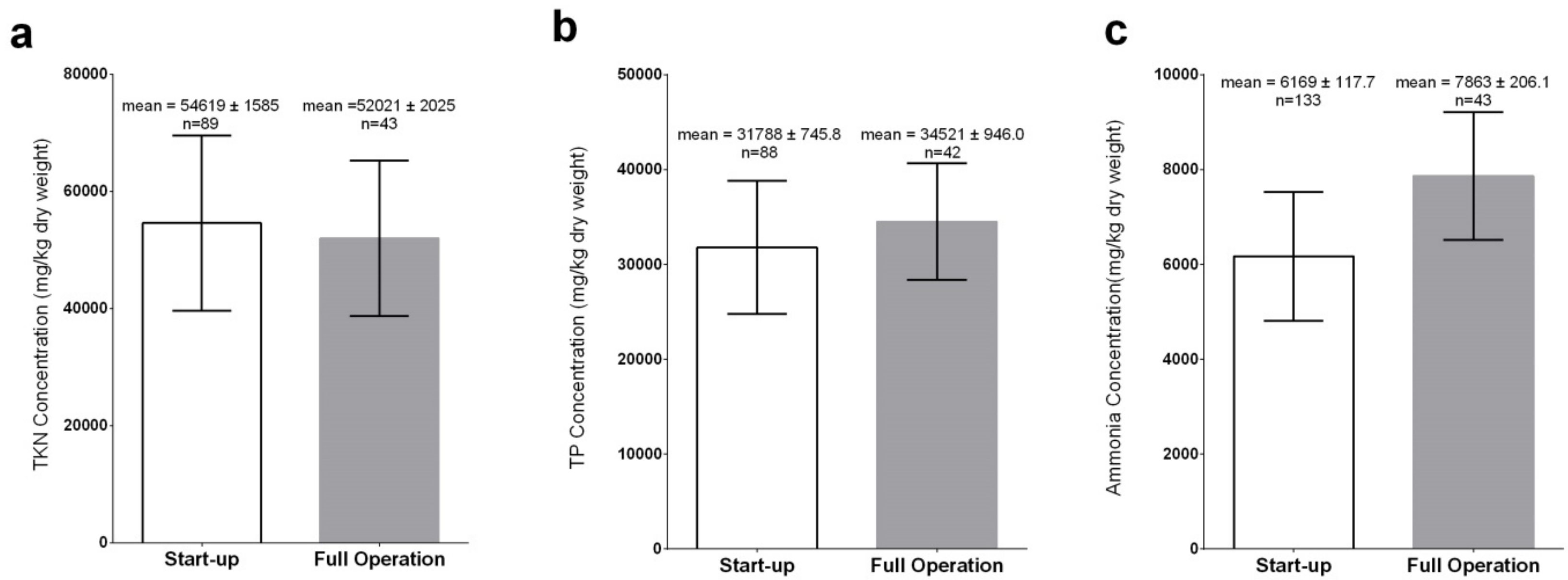


Fig. 4. Average concentrations (± standard deviation) during startup and full-operation periods for TKN (a), TP (b), and NH_3-N (c) in Class A biosolids. Number of samples shown on top of each bar.

The average concentrations for the full-operation stage for K, TKN, TP, and ammonia in Class A biosolids are presented in Table 5. In addition, the % weight (weight of nutrients/dry weight of biosolids) for each nutrient was calculated and compared with data of Class B biosolids produced from 2013-2015 at the same NRF and with data from a commercial organic fertilizer: Milorganite™ (Organic Nitrogen Fertilizer, Milwankee, WI). In nutrients study, total N is usually used to represent the nutrient level of nitrogen in fertilizer, which is the combination of NO_3^-/NO_2^-–N and TKN. Because both Class A and B biosolids have low NO_3^-/NO_2^-–N concentration that can be neglect, TKN data in biosolids is used to compare with total nitrogen of the commercial fertilizer in Table 5. In addition, K is an important component for plant growth and is generally reported in commercial fertilizers, therefore it is included in the discussion here.

Class A biosolids samples have lower total K but higher TKN, TP, and ammonia than lime-stabilized Class B biosolids from the target NRF. THP-AD seems to be effective in removing K due to the increase mobility and water dissolution, which is important to note in a product that can be used for land application. The increase of TKN, TP, and ammonia suggest an increase of organic matter breakage by the THP-AD that enhances the release of nitrogen and phosphate. Biosolids from THP-AD has lower TKN but higher TP than the commercial organic fertilizer Milorganite™. Since TKN contains both ammonia and organically bounded nitrogen, the Class A product contains a large concentration of nitrogen that has a slow release time during land application (Lu et al., 2012), making it appropriate as a fertilizer.

Table 5. Average concentrations and standard deviations (mg/kg d.w. and weight percentage) of nutrients comparison (total K, TKN, TP, and ammonia) of Class A biosolids, lime-stabilized Class B biosolids, and Milorganite^{TM} ("Specifications: Milorganite,").

	Class A biosolids		Class B Biosolids	Milorganite^{TM}
Nutrients	**mg/kg d.w.**	**% weight**	**% weight**	**% weight**
Total K	850.2 ± 21.69 (*n*=134)	0.08502 ± 0.0022	0.1456 ± 0.004344	Data not available
Total N/TKN	52021 ± 13281 (*n*=43)	5.202 ± 1.328	3.9740 ± 0.6229	6
TP	34521 ± 6131 (*n*=42)	3.445 ± 0.6131	1.2380 ± 0.1291	2
Ammonia-N	7863 ± 1352 (*n*=43)	0.7863 ± 0.1352	0.1310 ± 0.03664	Data not available

2.5 Conclusions

Since November, 2014, the target NRF replaced lime stabilization of the sludge with the new THP-AD process with the goal to change the production of Class B biosolids to Class A biosolids and reduce significantly the total mass of biosolids produced. This study focused on the first year of this full-scale system to characterize the new product. We collected samples of the biosolids produced by THP-AD and analyzed them for fecal coliforms, nitrate/nitrite, TKN, TP, ammonia, and 11 different metals. We evaluated the quality of Class A biosolids, tracked the variation of concentrations with time, and compared Class A with previously produced Class B biosolids.

Results indicated that the newly produced biosolids using the THP-AD process qualified as Class A EQ biosolids, and met all the EPA standards for pathogen density and metal concentrations during the full-operation stage of the facility. From the startup stage to full-operation stage, almost all parameters changed significantly, especially fecal coliforms density, which decreased significantly after the initial startup stage. For the comparison between Class A and measured Class B biosolids metal concentrations, most metals increased in Class A biosolids due to the great mass reduction by THP-AD process. Several metals contents in Class A biosolids were higher even after volatile solids reduction correction, which need further investigation. Furthermore, the concentrations of TKN, TP, and ammonia are high in Class A biosolids when compared to Class B biosolids from the target NRF and the commercial organic fertilizer Milorganite™, which suggests that the Class A biosolids from the target NRF may be used as a fertilizer in land application with good economic values.

Chapter 3: PBDEs in Class A Biosolids Produced from Thermal Hydrolysis and Anaerobic Digestion Processes

3.1 Abstract

Polybrominated diphenyl ethers (PBDEs) are ubiquitous in the environment and tend to accumulate in the biosolids during wastewater treatment. In this study, we measured the impact of a new biosolids stabilization method on PBDEs biosolids concentration. We investigated PBDEs levels in Class A biosolids produced from newly adopted thermal hydrolysis pretreatment and anaerobic digestion (THP-AD) at a Mid-Atlantic nutrient recovery facility (NRF). The total PBDEs concentration in Class A biosolids was 720 ± 110 μg/kg d.w. (n=21), lower than the total PBDEs concentration found in the previously produced Class B biosolids from the same facility in 2011. Among the analyzed eight congeners (BDE-28, -47, -99, -100, -153, -154, -183, and -209), BDE-47, -99, and -209 were the most common congeners and combined contributed to about 87% of the total PBDEs concentration. From Class B to Class A biosolids production, the most prevalent congener was BDE-209, and its concentration was decreased from an average 1500 μg/kg d.w. to an average 240 μg/kg d.w.. This congener also contributed less to the total PBDE concentration from 82% to 34%. In addition, despite the varied conditions of initializing a full scale anaerobic digestion system, no significant differences were observed between the startup period and the full-operation period of the THP-AD system.

3.2 Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants in consumer products. Belonging to the group of brominated flame retardants, PBDEs can prevent ignition and slow down fires in the initial phase due to their physical and chemical properties (Harrad et al., 2008). In the 1970s, in order to replace banned flame retardants, such as polychlorinated biphenyls (PCBs) and polybrominated biphenyls, PBDEs were widely added in polymers, such as electronic components, household appliances, furniture, textiles, etc. and became ubiquitous in our environment (Daso et al., 2010). Based on the International Union of Pure and Applied Chemistry (IUPAC) system, all PBDEs contain a diphenyl ether skeleton and different numbers of bromine atoms with 209 possible congeners (Fromme et al., 2009).

Three commercial formulations containing a variety of congeners have been used: Penta-, Octa-, and Deca-BDE (Krol et al., 2012). According to the study of LaGuardia et al., 2006, DecaBDE (mainly BDE-209) dominated 83.3% of the 2001 PBDE global market demand, followed by PentaBDE (mainly BDE-47, -99, -100, -153, and -154) at 11.1% and OctaBDE (mainly BDE-183) at 5.6%. Therefore, in this study, eight congeners of PBDEs (BDE-28, -47, -99, -100, -153, -154, -183, and -209), representing the major congeners found in commercial formulations, were chosen as the targets for our analysis.

Due to the lack of binding sites on polymers, PBDEs are simply integrated into materials but not chemically bound to products. PBDEs are semi-volatile and can easily leach out of the polymers and enter air, dust, soil, etc. With similar structure and metabolites as PCBs, PBDEs may have carcinogenic and thyroid disturbing effects that bring great concern to human and ecosystem health and persistent for a long time (Knoth et al., 2007; Naert et al., 2007). Therefore, commercial PentaBDE and OctaBDE were listed in the Persistent Organic Pollutants (POPs) inventory of the Stockholm Convention in 2009 and were banned or phased out in European Union and the United State. But the large compound DecaBDE, which was considered less mobile and toxic, were still widely manufactured and used in the U.S. until 2013 to be phased out (Jinhui et al., 2017).

However, the characteristics of persistence in the environment and the tendency of bioaccumulation means PBDEs are likely to be detected in the environment and in wildlife for many decades after their production ceases. As PBDEs are released into wastewater from consumer products, and they are hydrophobic in nature, they can be effectively removed with organic solids during wastewater treatment processes (Andrade et al., 2010). Consequently, biosolids produced from nutrient recovery facilities (NRFs, formerly known as wastewater treatment plants) generally have a relatively high concentration of PBDEs. In the U.S., about 50% of biosolids are applied on agriculture land as a fertilizer (Seiple et al., 2017). A few studies showed high concentrations of PBDEs exist in biosolids samples, and the application of large amounts of biosolids as fertilizer could put PBDEs into the food chain (Hale et al., 2012; Venkatesan and Halden, 2014). The study by Venkatesan and Halden, 2014, estimated about 24,000-36,000 kg/year

(53,000 pound/year – 79000 pound/year) of PBDEs were released to the environment through land application of biosolids.

To ensure the safety of biosolids application in soil, the Environmental Protection Agency (EPA) has framed a classification system and considers biosolids which not only meet, but exceed the minimal requirements of pathogen reduction, metals content limits, and vector control to be of Class A “Exceptional Quality” (EQ) biosolids (U.S. EPA, 1993). In the 40 Code of Federal Regulations Part 503.32 rule (EPA, 1993), Class A biosolids must meet at least one of the following requirements: either the density of *Salmonella* sp. must be less than 3 MPN/4g d.w. or the density of fecal coliforms must be less than 1000 MPN/g dry weight. Class A classification is necessary if a user wants to apply the biosolids to residential lawns, home gardens, or other unrestricted public contact areas (U.S. EPA, 1994b). In comparison, Class B biosolids has a looser requirement that the pathogen level could reach as high as 2 million MPN/g d.w. biosolids (U.S. EPA, 1994b). For either Class A or Class B biosolids, no regulation exists for PBDEs content in biosolids in the U.S.

Since November 2014, a large Mid-Atlantic NRF put online a new stabilization treatment process for biosolids that produces Class A product. The newly adopted process includes the Cambi^{TM} Thermal Hydrolysis Pretreatment (THP) and anaerobic digestion (AD) with the intent to enhance the microbial activity to increase biogas production and decrease pathogen levels in the biosolids. As a number of past studies showed that active microbial activity could effectively biodegrade organic pollutants in biosolids, the concentrations and distribution of PBDE congeners could be affected by the THP-AD processes (Stiborova et al., 2015b , Huang et al., 2014; Stiborova et al., 2015a; Tokarz et al., 2008).

According to a study by Stiborova et al. (2015a), biodegradation is an effective method to remove organic pollutants in biosolids. A number of studies showed the adoption of mesophilic anaerobic digestion in the wastewater treatment would debrominate PBDEs from high-brominated to low-brominated congeners (Huang et al., 2014; Stiborova et al., 2015b; Tokarz et al., 2008). Since lower-brominated PBDEs are considered to be more mobile and more toxic than higher-brominated PBDEs (Liu et al., 2016), both the total concentration of PBDEs and the composition of PBDEs congeners in biosolids should be measured.

In addition, the switch from Class B biosolids production stabilization processes to THP-AD system and Class A biosolids production at the target NRF was gradually introduced. Based on the biosolids analysis results of EPA classification criteria, from November, 2014 to the end of February, 2015, the period was unstable, producing biosolids that did not meet EPA standards, and this period was named the startup stage. In this stage, THP-AD had started but not all anaerobic digesters were online and conditions were not optimized. From March, 2015 on, the period was named full-operation stage and stable quality Class A biosolids were produced. Therefore, the PBDEs comparison between startup and full-operation stages may reveal the PBDEs treatment ability of THP-AD system.

The goals of this study were to: 1) determine the PBDEs concentrations and the distribution of different congeners in Class A biosolids products; 2) compare the PBDEs concentrations in Class A biosolids and the Class B biosolids previously produced at the same location to evaluate the overall impact of the newly adopted THP-AD treatment technology on PBDEs; and 3) compare the PBDEs concentrations' variation between the startup and the full-operation stages.

3.3 Methods

Class A Biosolids samples were collected weekly from the final product belt conveyor at the target NRF from November 2014 until February 2015 and monthly from February 2015 until January 2016. Biosolids sample collection from THP-AD process started on November 26th, 2014 and due to the possible high variability in the product from the treatment operation initiation, samples were collected on a weekly basis. Samples were collected using sterile instruments and containers. After sample collection, biosolids samples were immediately transferred into 250 mL amber jars (Kimble™, 16oz, USA) and were frozen at -20^{o}C until processing.

The solids content of the samples was measured using EPA Method 1684 (U.S. EPA, 2001). The sample was dried a minimum of 12 hours in an oven (Fisher Scientific, Isotemp, USA) at 103-105 °C for total solids (TS) calculation and a minimum of 2 hours ignition in a heat furnace ((Neycraft Vulcan, A-550, York, PA) at 550 °C for volatile solids (VS) calculation (U.S. EPA, 2001).

For PBDEs analysis, the sample preparation, chemical extraction and cleaning, and chemical analysis was based on EPA method 1614 (U.S.EPA, 2010b). We utilized a modified method adopted from Deng et al., 2015, Krol et al., 2012, and Giergielewicz-Mozajska et al., 2001. Because PBDEs are photosensitive to sunlight, the glass containers, including sample jars and vials, are amber glass, and the fume hood are covered with amber color film. Before processing, biosolids samples were thawed in a refrigerator at -4 ^{o}C overnight and then allowed to reach room temperature for chemical extraction. At least duplicated analysis were prepared for each biosolids sampels. Approximated 1.5 g (±0.01 g) sample was mixed with 3.0 g of hydromatrix (Agilent Technologies, Hydromatrix, USA), and spiked with 40 ng of the surrogate polychlorinated biphenyl PCB-209 (Cambridge Isotope Laboratories, PCB-209-CS, Andover, MA) and 40 ng of the internal standard PCB-138 (Cambridge Isotope Laboratories, PCB-138-CS, Andover, MA). Samples were extracted in an Acceleration Solvent Extraction (ASE) system (Thermo Scientific, Dionex ASE 350 with Dionium Components Smartrun System & Solvent Saver System, Sunnyvale, CA). The extraction cycles were performed at a pressure of 2000 psi (13.79 MPa) and temperature of 120 ^{o}C with the mixture of solvent 4:1 of n-hexane (Fisher Scientific, n-Hexane, USA) : acetone (Fisher Scientific, Acetone, USA). After 5 minutes to heat up the oven, two static extractions of 10 min were developed at constant pressure and temperature, generating approximately 35 mL of extract into the amber vial.

Since the extract had high content of lipid and sulfur that could interfere the PBDEs analysis (Berton et al., 2016), the extract was passed through a packed glass chromatographic column (Kimble™ Kontes™ PTFE-Plugged Column, 300-mm L x 22-mm ID, USA) for clean-up. From bottom to top, the column was packed with glass wool (Acros Organics, Glass wool, New Jersey), copper powder (Fisher, Copper C434-500 Powder, USA), 1 g activated silica gel (Alfa Aesar, 150 angstroms wide pore silica gel, USA), 2 g 33% basic silica gel with NaOH (Fisher, NaOH pellets, USA), 1 g activated silica gel, 4 g 40% acid silica gel with concentrated sulfuric acid (Fisher, A300-212 Sulfuric Acid, USA), 2 g activated silica gel and 1 g anhydrous sodium sulfate (Fisher, S429-212 Sodium Sulfate Anhydrous, USA). PBDEs were eluted with n-hexane, evaporated in evaporator (Zymark, TurboVap LV Evaporator, Hopkinton, MA) under a gentle nitrogen stream, and re-dissolved with n-hexane to 1 mL for chemical analysis.

Eight PBDEs congeners (BDE-28, -47, -100, -99, -154, -153, -183, and -209) were analyzed by Agilent gas chromatography-mass spectrometry (GC-MS) 6890 N/5975. Negative chemical ionization in selected ion monitoring mode and DB-5MS capillary column were used. For each extract, helium gas is applied to push the1μL of injected extract to go through the capillary column at temperature about 300˚C for 22 minutes run. Standards of target PBDEs solution were prepared to generate calibration curve. The quantification of PBDEs was performed by monitoring the ion fragments with mass-to-charge ratio.

The recovery of PCB-209 was calculated for each sample set and the results were accepted with surrogate recovery from 90% to 110%. At least duplicate data for each biosolids sample was obtained for further analysis. For each sample batch (10 samples), a sand blank was analyzed for quality control. In addition, PBDE-spiked sand and PBDE-spiked biosolids sample (addition of 0.05 μg of BDE-28, -47, -99, -100, -153, -154, and -183, each and 0.5 μg of BDE-209) (Cambridge Isotope Laboratories, Andover, MA) were analyzed to calculate congener-specific recovery to ensure the reliability of the experimental method.

For all analysis, the average recovery of surrogate PCB-209 for sand blank was 97.80%±9.981%, and no PBDEs contamination was observed. The average and standard deviations of recoveries for PBDEs sand spiked sand and PBDEs spiked biosolids with surrogate recovery from 90% to 110% were calculated and shown in Table 5. BDE-99,-100, -153, and -154 have the best performance in recovery. To compare between spiked sand and spiked biosolids, large PBDEs congeners (BDE-153, -153, 183, and 209) had better recoveries in sand, but small PBDEs congeners (BDE-28, -47, -99, and -100) were in opposite. The reason for this situation is probably the large PBDEs congeners are harder to be extracted out from the high-organic-matter biosolids than smaller PBDEs congeners.

Table 5. Average recoveries of PBDEs spiked sand samples and biosolids samples.

	PCB209	BDE28	BDE47	BDE100	BDE99	BDE154	BDE153	BDE183	BDE209
Average PBDEs Spiked Sand Recovery	102.3%± 5.351	63.88%± 15.52	71.49%± 13.44%	85.07%± 11.29%	89.33%± 12.81%	90.79%± 10.57%	89.44%± 10.58%	85.73%± 11.567%	87.37%± 9.063%
Average PBDEs Spiked Biosolids Recovery	101.1%± 0.7233%	77.07%± 11.14	75.96%± 6.359%	91.10%± 7.910%	90.60%± 10.78%	86.26%± 6.843	87.41%± 7.528%	75.11%± 49.39%	76.286% ±0.4234 %

3.4 Results and Discussion

In our study, a total of 21 samples of Class A biosolids samples from November 2014 to January 2016 were collected and analyzed. For each sample, TS were measured to calculate PBDEs concentration in a dry weight basis. The average TS of our Class A biosolids samples was 29.3 ± 2.35% (n=21).

Among the eight PBDEs congeners analyzed, the concentrations of BDE-47, -99, and -209 were the highest. The detection limit and quantitation limit of each chemical were measured and calculated based on Carden, 1998, and the results were shown in Table 6. Due to low concentrations of BDE-28 (below detection limit), its results were not shown in this manuscript.

Table 6. Method detection limits (MDL) and quantitation limit (QL) of surrogate (PCB209) and each PBDEs congener.

	PCB209	BDE28	BDE47	BDE100	BDE99	BDE154	BDE153	BDE183	BDE209
MDL (µg/kg d.w.)	6.026	1.431	1.172	1.006	1.087	0.911	0.666	0.996	5.731
QL (µg/kg d.w.)	18.077	4.292	3.515	3.017	3.261	2.733	1.998	2.988	17.192

The average concentrations and standard deviations of BDE-47, -99, -100, -153, -154, -183, -209, and total PBDEs were calculated (Table 7). BDE-209 concentrations were at similar levels to BDE-47 and BDE-99. Due to chemical partition and commercial production, BDE-209 is generally observed in significantly higher levels than other PBDEs congeners in biosolids samples (Venkatesan and Halden, 2014). In a previous study by Andrade et al. 2015, that reported PBDE concentrations in the Class B product produced at the same facility from July 2005 until June 2011, as shown in Table 7, BDE-47+BDE-99 concentrations hovered around 250 µg/kg d.w.. The sum of the same two congeners in the Class A biosolids would be around 390 µg/kg d.w., indicating an increase in the concentration of these two common congeners. Similar situation were applied to the rest small PBDEs congeners, except BDE-209, that the concentrations of PBDEs increased from Class B to Class A. Moreover, the concentration of BDE-209, the most abundant PBDE congener, in the previously-produced Class B material hovered around 1500 µg/kg d.w., which was much higher than values observed in the Class A material at around 240 µg/kg d.w..

Table 7. Mean PBDEs concentrations and standard deviations in Class A biosolids samples and previously-produced Class B biosolids and corrected Class B biosolids data at the target NRF.

	Mean ± SD (µg/kg d.w.)		
Congeners	**Class A**	**Class B***	**Corrected Class B**
BDE47	190 ± 34	121 ± 36.1	346 ±103
BDE100	44 ± 6.9	20.5 ± 11.1	58.6 ± 31.7
BDE99	200 ± 27	134 ± 42.1	383 ± 120
BDE154	18 ± 2.6	7.97 ± 0	22.8 ± 0
BDE153	22 ± 4.0	8.23 ± 0	23.5 ± 0
BDE183	7.5 ± 1.6	6.64 ± 0	19.0 ± 0
BDE209	240 ± 72	1490 ± 503	4260 ± 1440
Total	720 ±110	1790 ± 528	5110 ± 1510

*Data obtained from Andrade, et al. 2015

According to the data obtained at the target NRF, the volatile solids reduction from Class B to Class A biosolids is about 65%. Because THP-AD process has a great mass reduction effect in biosolids production, the concentrations of PBDEs in Class B biosolids were corrected for the same amount of organic matter input in the sludge as the Class A biosolids production, as showed in Table 7. After correction, all PBDEs congeners' concentrations in Class B biosolids were higher than in Class A biosolids. The PBDEs in newly produced Class A biosolids from Nov. 2014 to Jan. 2016 was much lower than the average PBDEs concentration in Class B biosolids at the same NRF from July 2005 to June 2011.

Based on data in Table 7, a clear PBDEs concentration comparison between Class A and Class B for different PBDEs congeners was present in Fig. 5. Except BDE-209, the small PBDEs congeners' concentrations in Class A biosolids were from 1.1-2.7 times of the congeners' concentrations in Class B biosolids. In contrast, concentration of BDE-209 were extremely decreased that Class A only had one-sixth as the concentrations in Class B biosolids. Because small PBDEs congeners are more mobile and more toxic, the increase of small PBDEs congeners may bring concerns in environmental and health risk when apply the Class A biosolids into food production land and dense population area.

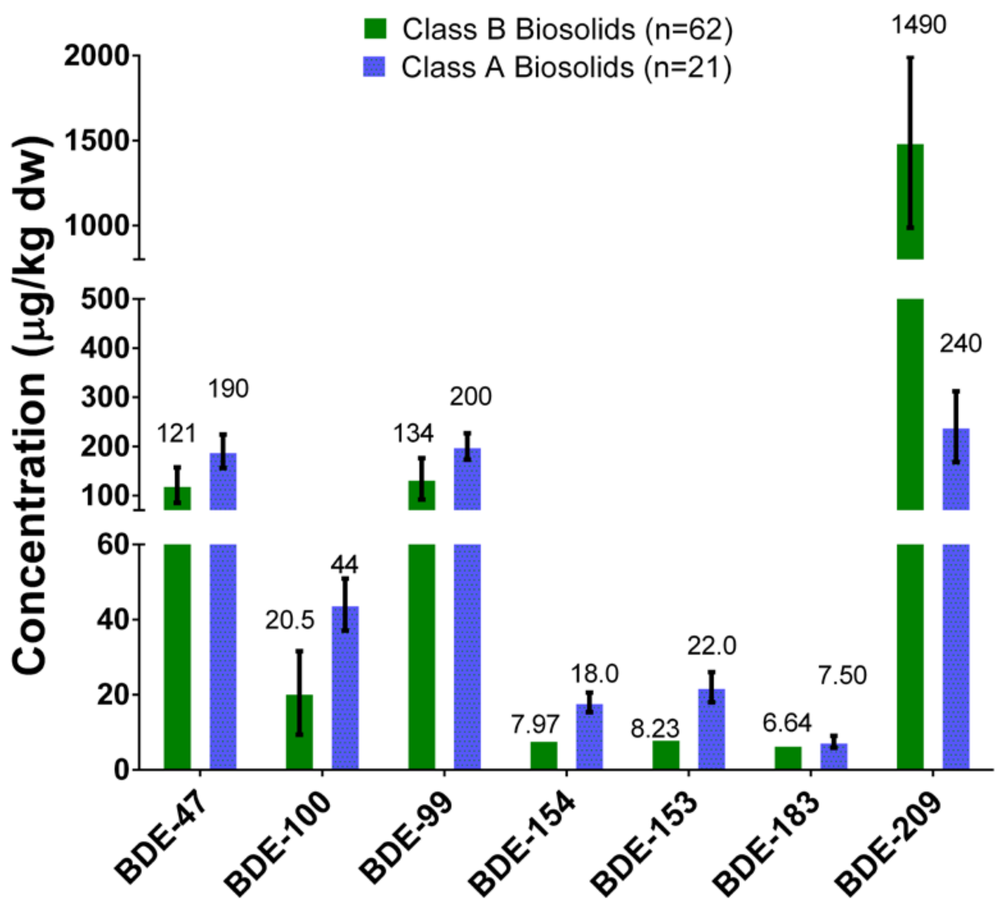


Fig. 5. Average concentrations of seven PBDEs congeners in Class A and Class B biosolids.

Although in the most studies, BDE-209 was extremely high (> 90%) in the distribution of the total PBDEs concentration (Kim et al., 2017; Aigars et al., 2017; Eljarrat et al., 2011), BDE-209 in the Class A biosolids from THP-AD had a unique distribution form. As shown in Fig. 6, the eight PBDEs congeners' distribution to the total concentration between Class A and Class B biosolids products were calculated. In the Class A biosolids, BDE-47, -99, and -209 totally contributed to about 87% of total PBDEs while in the Class B biosolids, these three congeners contributed to over 97% of total PBDEs. Most significantly, the percent distribution of BDE-209 was decreased from 82 ± 5.9% (*n*=62) to 34 ± 6.9% (*n*=21) from Class B to Class A biosolids (Andrade et al., 2015). In addition, the rest PBDEs congeners had increased distribution in Class A biosolids.

A probable explanation to this situation is the THP-AD system could enhance removal of BDE-209, perhaps by the removal of bromines, efficiently debrominating the larger congener and generating more of the lower-brominated compounds. Several studies had demonstrated that anaerobic microorganisms could efficiently remove bromines from the diphenyl ether skeleton that degrade large PBDEs congeners into smaller congeners (Tokarz et al., 2008; Eljarrat et al., 2011; Stiborova et al., 2015). In the study by Tokarz et al., 2008, the debromination pathways of BDE-209 by microorganism were present in Fig. 7. The most target small PBDEs congeners in our study may be generated from the debromination of the large congeners. Besides the great mass reduction, the increase concentrations and distributions of small PBDEs congeners may be also contributed by the debronmination of large PBDEs, and lead to the decreased concentration and contribution of the largest congener, BDE-209. Since microorganisms can debrominate the most prevalent BDE-209 into more mobile and toxic small congeners, the extensive microorganisms' activities by THP-AD may increase the toxicity of biosolids in PBDEs aspect, and need to be concerned.

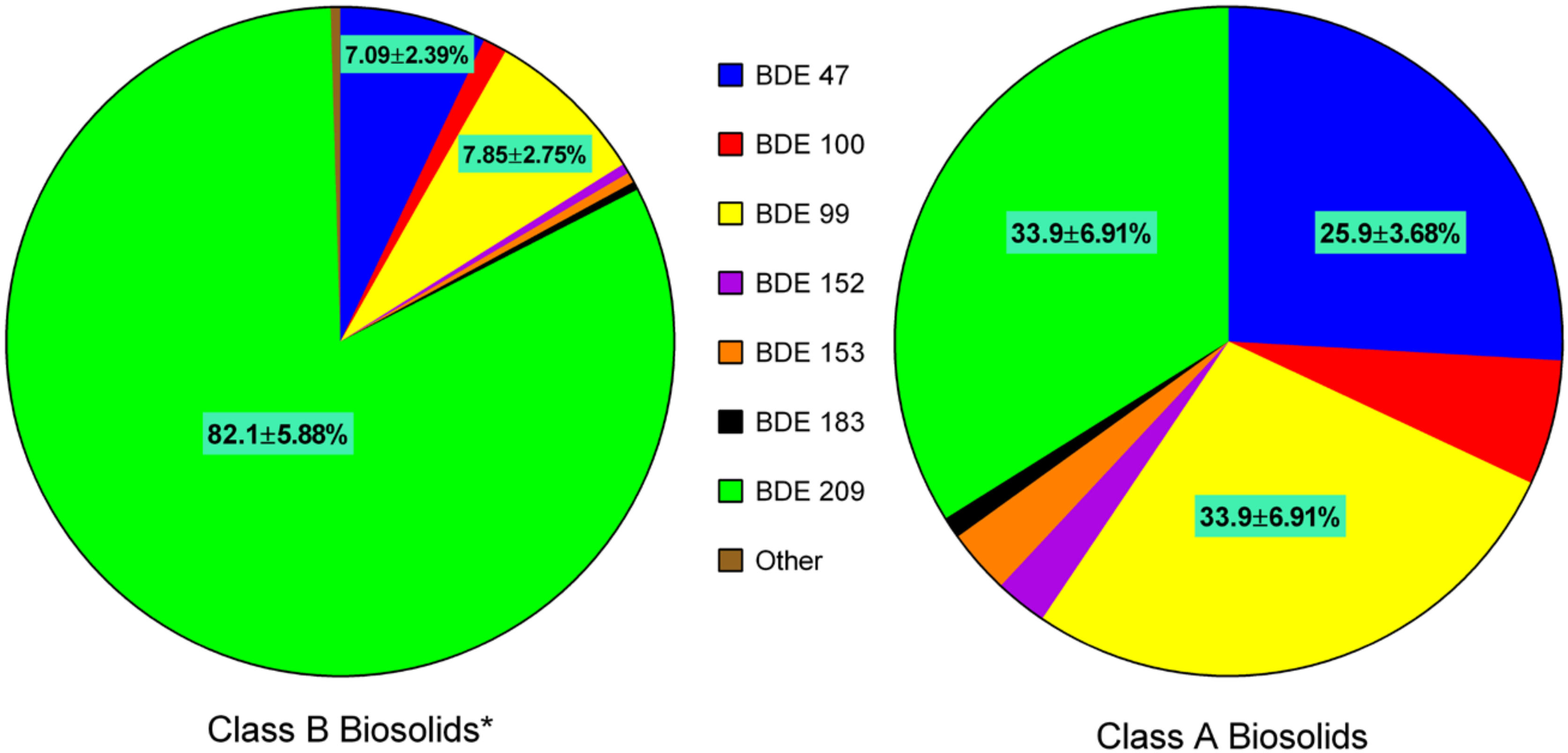


Fig. 6. Average PBDEs congeners' distribution to the total PBDEs concentration in Class A and Class B biosolids produced by the same NRF

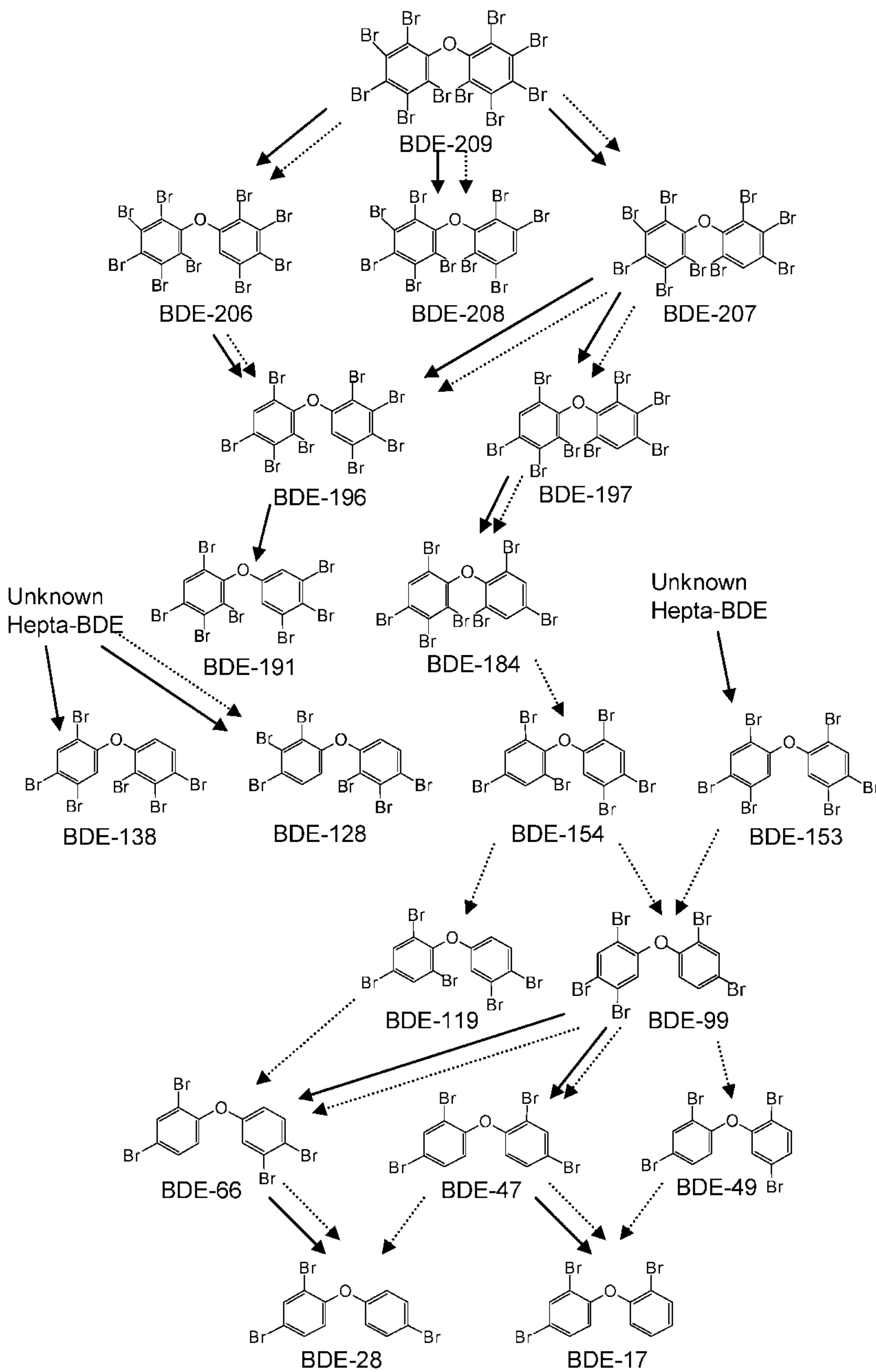
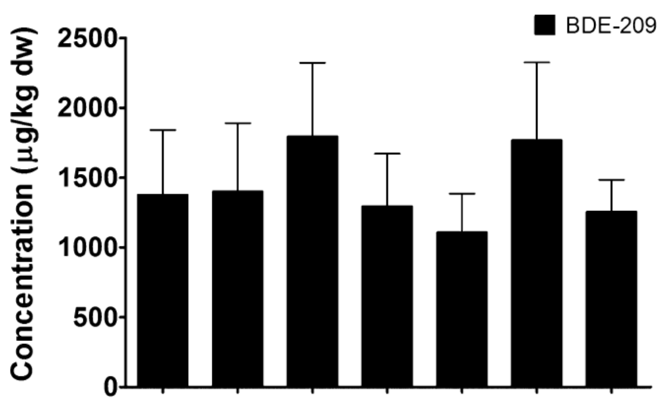


Fig. 7. Major debromination pathways for PBDEs derived in sediment (solid arrows) and in biomimetic debrominations (dash arrows) (Tokarz et al., 2008).

Moreover, a more in-depth investigation and the analysis of the debromination products of BDE-209 were required to better understand this process. Due to the phase out of PBDEs manufacturing and addition since 2004, a decrease trend of PBDEs were observed in biosolids (Kim et al., 2017). As shown in Fig. 8, from 2005 to 20011 in the study by Andrade et al, 2015, a decreasing trend of concentration BDE47+99 was observed. But for BDE-209, the concentration was relative constant. Since the DecaBDE (mainly BDE-209) was phased out in the U.S. after 2013, further studies are needed to determine the impact of phase out in BDE-209 decrease in biosolids. For a better understand for the debromination by THP-AD system, future studies are going to analyze the Class B biosolids samples right before the star of Class A biosolids production at the target NRF. In addition, analysis of PBDEs at different treatment steps among THP-AD system is also helpful to investigate the impacts of THP-AD on PBDEs removal.



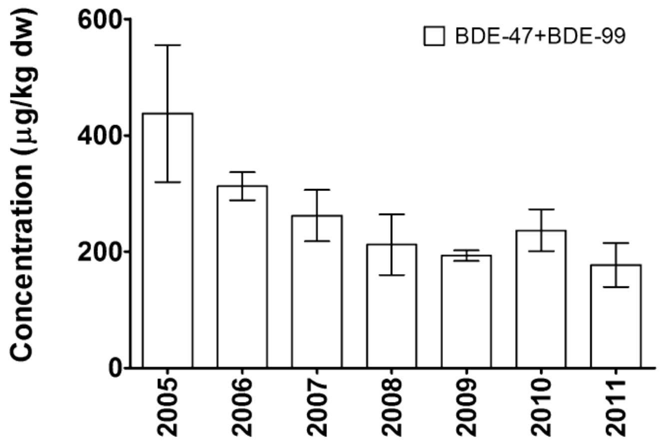


Fig. 8. The trend of PBDEs concentrations in Class B biosolids from 2005 to 2011 at target NRF. (Andrade et al. 2015)

Since the PBDEs are persistent in nature and the trend of concentration decreasing was not such intensive that lead to the extremely decrease of BDE-209 in Class A biosolids, extensive debronmination were expected happened during THP-AD process that degraded BDE-209 into smaller congeners and even to remove. This study provides information on the impact of THP-AD on PBDE concentrations, which can help researchers understand differences between biosolids stabilization processes on microconstituents. In addition, this study shows promise that this technology could be used for microconstituents removal and further research should be conducted to better understand the mechanisms in which these processes rely.

Biosolids samples were collected from the very first day of the Class A biosolids production. It is interesting then, to analyze the data in a temporal scale to observe the impact of the startup of a large-scale stabilization process in PBDEs' biosolids concentrations. The variation of PBDEs concentration from the startup stage to full-operation stage of the THP-AD system was investigated based on the concentrations of the three most common PBDE congeners: BDE-47, -99, and -209

(Fig. 9). Due to the large size of the treatment system and beginning stage, the production of Class A biosolids at the target NRF, during Nov. 26th, 2014 to the end of Feb., 2015, the startup stage, the THP was operated but anaerobic digesters operation parameters were still being optimized and not all digesters were online. From March 2015 until the last sampling event for this study, we characterized as full-operation stage, when the treatment system reached stable conditions and qualified consistently as Class A biosolids. We hypothesized that PBDE concentrations could also be impacted by the differences between the two established stages due to constant changes to operating parameters.

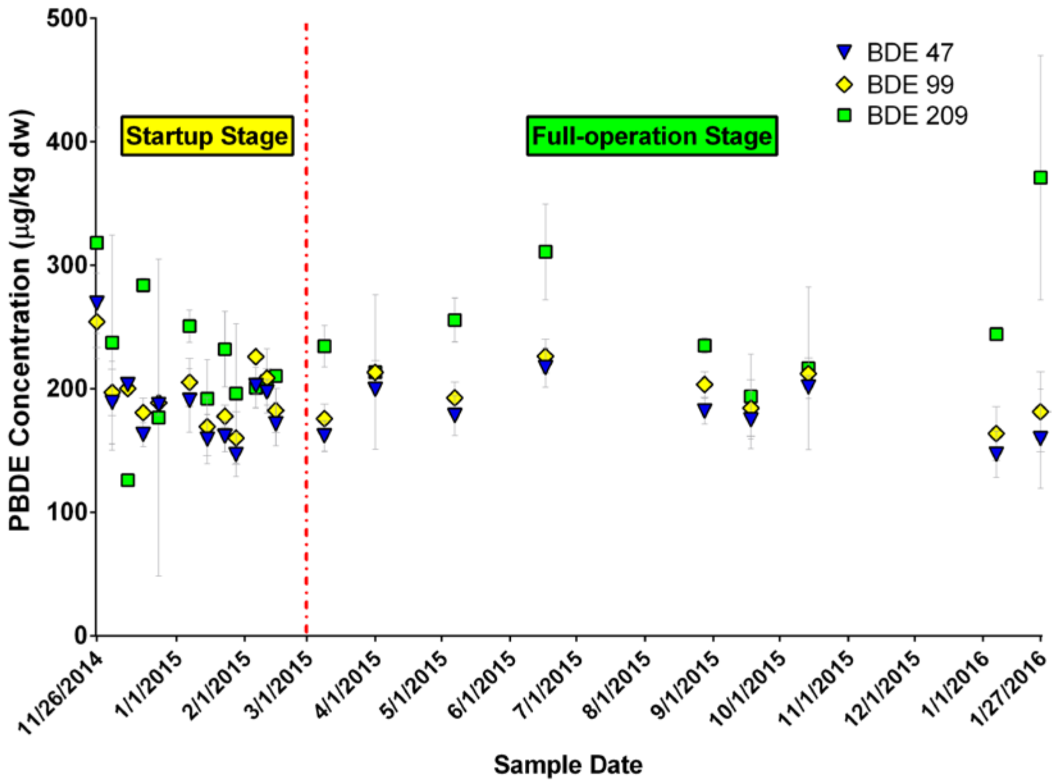


Fig. 9. Concentrations of BDE-47, -99, and -209 in biosolids samples from Nov. 26th, 2014 to Jan. 27th, 2016.

In order to analyze significant differences between the startup stage and full-operation stage, about one year-long data were separated into startup stage (*n*=12) and full-operation stage (*n*=9), as shown in Fig. 9. Concentrations between startup and full-operation stages were not statistically different for BDE-47 (p=0.60), BDE-99 (p=0.92) and BDE-209 (p=0.16) (unpaired t test). The results indicated the variation of THP-AD system in the early operation stage did not affect the debromination of PBDEs in the biosolids products. During the startup stage, the THP tanks were already operated under ideal and stable temperature and temperature. Instead, four anaerobic digesters were gradually filled with the sludge from THP until the end of startup stage to get to stable condition. The population of anaerobic microorganisms in the digesters may have a great variation during the startup stage. But the analysis result of no significant differences between startup and full-operation stages indicated that the AD in this particular facility might be efficient in PBDE removal even when the AD process was not operating at full capacity and optimum conditions.

3.5 Conclusion

Overall, this study determined eight common PBDEs congeners' concentrations in Class A biosolids product that was stabilized with THP-AD processes. The total PBDE concentration in the biosolids in the investigated period Nov. 2014 – Jan. 2016 was 720 ± 110 μg/kg d.w (*n*=21). This reported concentration was lower than the total PBDE concentration found in previously produced Class B biosolids product from the same facility from 2005 to 2011. Among the eight congeners, BDE-47, -99, and -209 were the most common congeners and contributed to about 87% of the total PBDEs concentration. The most prevalent PBDE congener in both Class B and Class A biosolids was BDE-209. However, from Class B to Class A biosolids production, the concentration was decreased from an average 1500 µg/kg d.w. to an average 240 µg/kg d.w. The distribution to the total concentration also decreased from 82% in Class B to 34% in Class A.

It is unknown why the concentration of BDE-209 decreased in the Class A product compared to the Class B product. The study suggests that debromination may be enhanced due to the extensive microbiological activities in the THP-AD process and the phase out of PBDEs production and utilization. The increase concentrations of small PBDEs congeners in Class A biosolids may also due to the debromination of large PBDEs congeners and the great mass production. The increase concentrations of small PBDEs congeners bring the concerns of Class A biosolids land application due to the increasing toxicity for the ambient environment and humans. In addition, the concentration of PBDEs were relatively constant during the startup stage to full-operation stage, which could indicate that the debromination processes may be efficient since the changing operational parameters of the AD in the startup stage seemed sufficient to reduce PBDE concentrations. Further research will investigate the potential impact from the phase out of PBDEs utilization by analyzing Class B biosolids samples close to Class A biosolids production, extend the analyzing PBDEs congeners and extend samples sizes among THP-AD steps to investigate the mechanisms of debromination for a better understanding of THP-AD system impact on microconstituents.

3.6 Acknowledgements

We gratefully thank doctoral candidate Dana Armstrong and research assistant Taylor LaChance from University of Maryland, College Park, for sampling biosolids and assistance in laboratory experiments and instrumentation analysis.

Chapter 4: Conclusions

Since November, 2014, the target NRF at mid-Atlantic area started to operate the newly adopted Cambi^{TM} THP-AD system in order to improve the quality of biosolids from Class B to Class A. Based on EPA standards, biosolids samples were collected at target NRF for over one year and were analyzed for pathogen levels, metals concentrations, and nutrients. The overall results indicated qualified Class A biosolids were produced after one year-long operation. From startup stage (Nov. 2014 – Feb. 2015) to full-operation stage (Mar. 2015 – Dec. 2015), stable Class A quality of biosolids was achieved.

In addition, biosolids samples were analyzed for ubiquitous organic pollutant PBDEs concentrations to study the impacts of THP-AD system on PBDEs residues in Class A biosolids. With the comparison of PBDEs concentrations in Class B biosolids at the same target NRF from the previous study, the total PBDEs were significantly decreased. The weight distribution of different PBDEs congeners shifted from over 80% of large BDE congeners (BDE-209) in Class B biosolids to increased smaller BDE congeners (such as BDE-47 and-99) in Class A biosolids.

4.1 Total Solids (TS) and Volatile Solids (VS)

The analysis of TS and VS of Class A biosolids samples were separated into two stages, startup stage and full-operation stage. During startup stage, the average

TS of Class A biosolids was 28.12% ± 2.116% (*n*=199), and the average VS was 59.32% ± 1.972% (*n*=180). Besides, during full-operation stage, the average TS was 31.39% ± 2.180% (*n*=344), and the average VS was 58.40% ± 4.087% (*n*=341). The unpaired t-test comparison between startup and full-operation stages indicated significant difference for both TS and VS. The change of mass loading and microorganism population growth in anaerobic digester seems affect the TS and VS of final biosolids product.

TS and VS of Class B biosolids from Jan. 1st, 2013 to Feb. 13th, 2015 at the target NRF were also analyzed to compare with TS and VS of Class A biosolids during full-operation stage. TS = 33.07% ± 2.899% (*n*=543), and VS = 54.48% ± 6.159% (*n*=733). The TS and VS between Class A and B biosolids were significant different. Although part of organic matters in Class A biosolids was converted into biogas and left, the VS in Class A biosolids was still higher than in Class B biosolids. The overall volatile solids reduction for Class A biosolids was about 65%, which demonstrated a great mass reduction by THP-AD. The decrease of TS and increase of VS from Class B to Class A biosolids indicated efficient dewatering impact of THP-AD system in Class A biosolids production.

4.2 Pathogens

The analysis of pathogen population in Class A biosolids were the measurement of fecal coliforms based on EPA Method 1681. During the startup period, fecal coliform population was 3915 ± 6068 MPN/g d.w. (*n*= 91) that were higher than EPA requirement as below 1000 MPN/g d.w.. However, began from March, 2015, THP-AD system were under full-operation stage and the fecal coliforms population were significantly decreased, with average 35.85± 81.10 MPN/g d.w. (*n*= 301) that qualify to EPA requirement. Significant difference were observed between startup and full-operation stages with $p<0.0001$. The huge number of standard deviation indicated relative unstable condition of fecal coliform population in Class A biosolids. Due to the big size and wide variation of data, fecal coliform population results were divided into each month with mean and standard deviation calculated. A clear decreasing trend was present from the beginning of Class A biosolids production to the last day of analysis. In Dec. 2015, the average fecal coliform population was 6.748± 14.61 MPN/g d.w. (*n*= 56) illustrated the population density hadn't reach to stable condition

Because several factors may affect the fecal coliform population in biosolids, the reasons for the difference between startup and full-operation stages and the huge standard deviations were not clearly suggested. Since the high temperature in THP supposed to eliminate the most fecal coliforms, the later appearance and growth of fecal coliforms in biosolids may come from regrowth of untreated fecal coliforms in the source and the outside feeding seed in anaerobic digesters.

4.3 Metal Pollutants

Totally 11 metal pollutants concentration, including As, Cd, Cr, Cu, Pb, Hg, Mo, Ni, Se, Zn, and K, in biosolids were extracted and measured by ICP. To compare with EPA requirements for the 10 metal pollutants except K, all measurements in biosolids samples contented to Class A EQ biosolids qualification. Among the pollutants, K, Zn, and Cu are the most ubiquitous metal species.

Since metal pollutants concentrations in previous Class B biosolids were also monitored, the comparison was made between Class A and B biosolids. Except for K, the rest 10 metal pollutants had up to five-fold increase from Class B to Class A. After correcting with mass reduction, K, Cr, Mo, and Ni were significantly higher in corrected Class B biosolids; but Cd, Cu, Pb, Hg, and Se were still significantly higher in Class A biosolids. The high temperature of THP can break large organic matters and increase the diffusivity of metal ions and released absorbed metal ions on organic matters that lead to the increase of K, Cr, Mo, and NI. For the rest metal species, which did not significantly change or even significantly increased in Class A biosolids, a further analysis of the metal concentrations in the water phase from THP-AD may need to explain the situation.

4.4 Nutrients

Although EPA doesn't have requirements of nutrients levels for Class A biosolids qualification, understand of nutrients levels in biosolids is important to evaluate the economic benefit and environmental impact in biosolids field application as fertilizer. The measurements of nitrite, TKN, TP, and ammonia indicated no significant difference between startup and full-operation stages, with average 51960 ± 12960 mg.kg d.w., 34895 ± 6185 mg/kg d.w., and 7699 ± 1232 mg/kg d.w.. With the comparison to study by Stehouwer et al., 2000 and commercial organic fertilizer Milorganite™, Class A biosolids from the target NRF contained low total K, but high total P and ammonia, which may help build land application strategy in the future.

4.5 PBDEs

In this study, total eight BDEs congeners (BDE-28, -47, -99, -100, 154, -153, -183, and -209) were extracted and analyzed in Class A biosolids samples. Except concentration of BDE-28 was below the detection limit, the concentrations of rest seven BDEs congeners were determined. The total PBDEs concentration in Class A biosolids in the over one year investigated period was 720 ± 110 µg/kg d.w (n=21). To compare with the data from study Andrade et al. 2015, the Class B biosolids from the same NRF from 2005 to 2011 had total PBDEs concentration was 1790 ± 528 µg/kg d.w. (n=62), which was more than doubled in Class A biosoids. Especially for the most prevalent and the largest PBDEs congeners BDE-209, from Class B to Class A biosolids, the concentration of BDE-209 decreased from 1490 ± 503 µg/kg d.w. (n=62) to 240 ± 72 µg/kg d.w (n=21). And the weight distribution of BDE-209 was also decrease from 82% in Class B to 34% in Class A.

Among the eight congeners in Class A biosolids, BDE-47, -99, and -209 were the most common congeners and contributed to about 87% of the total PBDEs concentration. However, except BDE-209, Class A biosolids contained higher concentrations of more mobile and more toxic small PBDEs than Class B biosolids did that may bring concerns in land application. Due to the great mass reduction and by THP-AD, similar corrections as for metal pollutants concentrations were applied to PBDEs concentrations in Class B biosolids. The new comparison illustrated all BDE congeners decreased from Class B to Class A biosolids. The study suggests debromination may be enhanced by THP-AD that large congener BDE-209 was debrominated to smaller congeners that changed PBDEs distributions significantly. Since the phase out of PBDEs in the U.S. will also decrease the PBDEs concentrations in biosolids, further PBDEs investigations will be on the analysis of the Class B biosolids right from Class A biosolids production, and the analysis of PBDEs debromination mechanisms by extending congeners size. In addition, between startup and full-operation stage no significant differences were observed for BDE-47, -99, and -209 in Class A biosolids. The results suggest the change of mass loading and microorganism growth in anaerobic digesters didn't effectively affect the concentrations of PBDEs significantly. Future study of PBDEs analysis in different THP-AD treatment steps will help explain the reasons for this situation.

Appendices

Appendix A: Fecal coliforms populations, total solids (TS), and volatile solids (VS) of Class A biosolids by THP-AD

Stage	Sample Date	Fecal Coliform (MPN/g d.w.)	TS (%)	VS (%)
Startup Stage	11/29/2014	4750.074286	25.50	59.58
	11/30/2014	3742.968571	26.25	61.37

12/1/2014	5710.324286	26.46	59.58
12/2/2014	6191.56	20.28	58.04
12/3/2014	5746.93	27.84	60.48
12/4/2014	18620.14	26.47	60.26
12/5/2014	18225.27	27.05	59.92
12/6/2014	17967.05	27.52	59.50
12/7/2014	18214.1	27.24	62.09
12/8/2014	8941.92	26.77	60.50
12/9/2014	19390.66	25.45	61.22
12/10/2014	29810.19	26.31	59.10
12/11/2014	12434.47	26.44	59.96
12/12/2014	13285.56	24.69	60.17
12/13/2014	9412.3	25.99	
12/14/2014	5020.89	25.62	59.79
12/15/2014	3099.34	25.81	59.69
12/16/2014	5079.65	25.90	59.64
12/17/2014	1948.08	25.20	58.46
12/18/2014	1261.61	24.82	58.84
12/19/2014	667.83	25.72	59.07
12/20/2014	123.62	26.66	59.10
12/21/2014	125.75	27.53	59.10
12/22/2014	4641.49	27.19	59.59
12/23/2014	284.67	26.72	59.02
12/24/2014	151.52	27.14	60.00
12/25/2014	452.725	26.95	59.34
12/26/2014	527.88	26.64	59.05
12/27/2014	1030.43	27.49	58.59
12/28/2014	1254.085	26.72	59.34
12/29/2014	331.185	26.98	54.84
12/30/2014	687.62	26.82	58.76
12/31/2014	694.59	26.53	59.76
1/1/2015	1355.76	26.94	58.74
1/2/2015	1710.315	27.25	59.32
1/3/2015	707.165	27.77	57.24
1/4/2015	294.85	28.01	59.10
1/5/2015	1698.88	27.94	59.52
1/6/2015	2253.49	27.71	58.82
1/7/2015	4906.205	27.57	57.71
1/8/2015	12527.17	27.83	59.03
1/9/2015	3868.84	28.55	57.27

1/10/2015	955.675	28.33	58.29
1/11/2015	1045.11	29.06	57.38
1/12/2015	2039.27	29.25	57.67
1/13/2015	1726.535	29.79	56.49
1/14/2015		29.97	58.22
1/15/2015	1204.1	29.88	57.77
1/16/2015	1457.72	29.70	57.21
1/17/2015	757.645	28.89	58.51
1/18/2015	1689.27	29.19	58.34
1/19/2015	31191.395	29.99	57.66
1/20/2015	1944.695	28.85	57.92
1/21/2015	1832.45	30.60	57.55
1/22/2015	1106.085	29.75	57.96
1/23/2015	1659.87	29.71	56.54
1/24/2015	2014.74	31.56	57.60
1/25/2015	1520.695	26.81	59.14
1/26/2015	1305.61	30.84	58.23
1/27/2015	1759.79	31.83	57.45
1/28/2015	1674.77	29.54	57.43
1/29/2015	1064.57	31.09	57.32
1/30/2015	1065.7	30.81	61.95
1/31/2015	1924.29	31.46	58.10
2/1/2015	1082.22	29.78	58.87
2/2/2015	2560.8	27.45	58.44
2/3/2015	1106.15	29.78	58.45
2/4/2015	2718.87	29.43	59.26
2/5/2015	2685.345	29.53	58.92
2/6/2015	1757.715	29.20	58.10
2/7/2015	1732.87	28.47	59.20
2/8/2015	1160.58	28.78	60.10
2/9/2015	872.93	28.78	59.81
2/10/2015	880.02	28.29	60.02
2/11/2015	2609.47	28.61	59.75
2/12/2015	1640.215	30.06	60.48
2/13/2015	2263.485	28.35	60.07
2/14/2015	1841.975	30.43	60.56
2/15/2015	1339.83	30.68	60.36
2/16/2015	1951.975	30.12	60.94
2/17/2015	1350.475	30.45	61.00
2/18/2015	1970.97	30.10	60.67

	2/19/2015	639.825	30.10	60.04
	2/20/2015	512.06	29.18	60.43
	2/21/2015	1530.945	30.73	59.85
	2/22/2015	797.485	30.10	61.27
	2/23/2015	366.31	28.56	70.27
	2/24/2015	964.695	30.39	61.59
	2/25/2015	9023.255	30.43	61.47
	2/26/2015	254.26	31.08	61.18
	2/27/2015	272.06	29.60	60.58
Full-operation Stage	2/28/2015	263.22	30.98	61.14
	3/1/2015	162.34	30.43	61.25
	3/2/2015	162.64	30.64	60.72
	3/3/2015	220.745	31.32	60.74
	3/4/2015	99.43	30.55	60.95
	3/5/2015	42.68	32.85	60.13
	3/6/2015	226.655	30.44	
	3/7/2015	150.385	31.30	60.51
	3/8/2015	33.835	32.16	59.96
	3/9/2015	463.68	31.12	60.82
	3/10/2015	6.995	32.36	42.75
	3/11/2015	199.675	29.90	59.58
	3/12/2015	7.235	31.30	59.79
	3/13/2015	7.5	33.85	60.10
	3/14/2015	44.365	42.63	43.81
	3/15/2015	8.74	31.57	60.08
	3/16/2015	8.325	31.61	60.66
	3/17/2015	10.195	32.76	59.45
	3/18/2015	7.35	33.31	59.71
	3/19/2015	4.555	33.21	60.53
	3/20/2015	4.145	31.76	60.42
	3/21/2015	5.26	31.06	60.59
	3/22/2015	8.22	30.66	70.00
	3/23/2015	8.13	29.22	60.87
	3/24/2015	8.39	28.03	61.10
	3/25/2015	14.52	30.41	61.73
	3/26/2015	6.305	30.29	60.07
	3/27/2015	89.505	30.32	61.31
	3/28/2015	43.315	28.65	61.17
	3/29/2015	88.58	30.03	62.10
	3/30/2015	33.215	29.68	58.39

3/31/2015	16.11	30.76	61.15
4/1/2015	305.3	30.85	60.84
4/2/2015	11.105	31.97	61.58
4/3/2015	829.905	31.37	61.02
4/4/2015	91.39	32.08	61.34
4/5/2015	179.85	31.17	60.79
4/6/2015	68.87	31.47	61.55
4/7/2015	265.8	29.80	61.40
4/8/2015	62.72	29.92	61.34
4/9/2015	42.555	31.57	61.19
4/10/2015	248.66	31.69	61.68
4/11/2015	12.17	31.11	60.93
4/12/2015	29.82	31.66	60.17
4/13/2015	63.605	32.42	59.76
4/14/2015	64.84	31.80	59.83
4/15/2015	153.36	32.60	61.50
4/16/2015	13.04	31.53	60.82
4/17/2015	10.435	31.95	61.57
4/18/2015	11.43	30.71	60.12
4/19/2015	57.01	32.24	58.79
4/20/2015	10.265	31.33	60.91
4/21/2015	70.6	32.50	61.95
4/22/2015	11.62	34.03	59.35
4/23/2015	8.805	30.78	60.78
4/24/2015	12.1	32.72	60.12
4/25/2015	6.105	31.29	58.41
4/26/2015	2.45	32.20	59.18
4/27/2015	7.95	32.19	59.18
4/28/2015	9.235	30.86	60.70
4/29/2015			
4/30/2015	4.06	33.60	60.03
5/1/2015	7.03	30.61	59.28
5/2/2015	4.035	32.61	57.97
5/3/2015	17.545	32.12	57.10
5/4/2015	15.955	32.34	59.57
5/5/2015	6.065	31.81	56.95
5/6/2015	6.175	31.24	59.24
5/7/2015	7.78	31.30	59.01
5/8/2015	145.5	31.55	58.08
5/9/2015	2.06	31.29	58.78

5/10/2015	14.81	32.44	58.25
5/11/2015	4.63	33.49	60.61
5/12/2015	1.73	32.68	60.36
5/13/2015	4.22	32.69	56.64
5/14/2015	40.165	32.53	59.52
5/15/2015	238.71	31.67	60.12
5/16/2015	94.515	30.10	59.78
5/17/2015	62.935	32.43	60.13
5/18/2015	27.625	30.99	59.42
5/19/2015	114.21	32.09	59.98
5/20/2015	130.12	31.01	59.74
5/21/2015	9.385	31.96	57.53
5/22/2015	62.19	32.08	59.15
5/23/2015	87.89	32.37	59.40
5/24/2015	372.89	31.98	59.27
5/25/2015	69.025	31.70	59.30
5/26/2015	103.645	32.10	60.39
5/27/2015	149.94	32.88	58.64
5/28/2015	86.735	31.36	59.17
5/29/2015	396.74	31.59	58.84
5/30/2015	17.445	30.26	59.42
5/31/2015	15.845	30.74	58.68
6/1/2015	37.575	31.74	57.32
6/2/2015	33.275	30.94	57.18
6/3/2015	43.38	32.39	57.04
6/4/2015	30.915	31.15	54.83
6/5/2015	44.435	31.91	56.02
6/6/2015	2.905	31.84	56.73
6/7/2015	24.675	32.00	55.97
6/8/2015	23.97	33.04	56.60
6/9/2015	45.105	31.15	57.60
6/10/2015	62.455	31.51	58.15
6/11/2015	28.445	31.50	55.78
6/12/2015			
6/13/2015	33.87	34.69	58.55
6/14/2015	22.89	32.59	58.22
6/15/2015	30.27	31.02	59.08
6/16/2015	40.115	31.36	58.68
6/17/2015	15.46	31.89	57.82
6/18/2015	37.765	32.09	59.20

6/19/2015	25.11	31.54	58.21
6/20/2015	20.445	31.43	59.52
6/21/2015	18.395	31.42	58.82
6/22/2015	7.115	33.73	57.41
6/23/2015	14.63	32.12	57.84
6/24/2015	3.745	32.73	57.86
6/25/2015	4.81	33.58	57.54
6/26/2015	316.915	32.99	57.54
6/27/2015	5.825	32.70	57.21
6/28/2015	7.82	30.68	56.09
6/29/2015	3.72	32.97	56.79
6/30/2015	6.875	32.22	55.77
7/1/2015	6.66	32.44	54.61
7/2/2015	3.31	31.69	55.83
7/3/2015	4.8	33.63	53.87
7/4/2015	4.785	32.40	54.22
7/5/2015	11.66	33.15	54.08
7/6/2015	10.9	32.51	55.27
7/7/2015	10.7	32.80	55.72
7/8/2015	20.405	31.98	55.72
7/9/2015	69.66	32.94	55.96
7/10/2015	7.56	32.14	56.12
7/11/2015	13.41	32.59	54.38
7/12/2015	49.13	33.37	55.98
7/13/2015	15.065	30.01	51.57
7/14/2015	40.73	32.20	56.10
7/15/2015	17.55	31.28	56.58
7/16/2015	6.98	39.46	54.75
7/17/2015	7.06	31.23	56.45
7/18/2015	7.595	31.99	57.49
7/19/2015	77.35	31.99	57.19
7/20/2015	21.46	32.97	56.99
7/21/2015	46.6	32.23	57.21
7/22/2015	13.255	32.78	57.46
7/23/2015	6.725	31.83	57.54
7/24/2015	80.355	32.22	56.15
7/25/2015	3.115	31.90	57.74
7/26/2015	61.425	32.04	58.01
7/27/2015	7.575	30.95	58.25
7/28/2015	8.565	31.82	58.48

7/29/2015	3.925	29.43	58.51
7/30/2015	10.325	32.06	57.26
7/31/2015	3.07	30.30	57.61
8/1/2015	4.645	33.29	58.06
8/2/2015	11.15	33.95	57.77
8/3/2015	5.775	30.04	54.01
8/4/2015	79.485	31.54	57.69
8/5/2015	10.305	33.10	59.66
8/6/2015	8.415	32.21	54.89
8/7/2015	3.875	31.47	53.06
8/8/2015	2.82	32.44	54.57
8/9/2015	5.87	32.80	55.49
8/10/2015	27.595	31.98	52.23
8/11/2015	5.295	32.30	54.03
8/12/2015	6.49	31.51	57.45
8/13/2015	4.59	33.76	55.20
8/14/2015	15.25	32.69	54.45
8/15/2015	2.475	32.50	57.02
8/16/2015	2.21	32.11	57.19
8/17/2015	33.275	31.42	53.97
8/18/2015	2.18	31.65	52.64
8/19/2015	5.29	31.92	55.81
8/20/2015	3.14	31.88	56.62
8/21/2015	2.14	31.81	56.14
8/22/2015	1.05	30.96	56.65
8/23/2015	3.255	31.47	56.31
8/24/2015	2.615	33.44	54.64
8/25/2015	4.44	30.74	54.57
8/26/2015	0.965	31.04	54.36
8/27/2015	3.73	31.09	53.08
8/28/2015	0.62	32.02	55.15
8/29/2015	1.01	32.15	56.21
8/30/2015	3.14	29.60	53.30
8/31/2015	3.905	31.39	57.14
9/1/2015	2.23	30.48	55.53
9/2/2015	2.755	31.59	55.98
9/3/2015	2.95	29.68	55.75
9/4/2015	1.63	31.04	52.87
9/5/2015	0.935	32.07	55.38
9/6/2015	3.405	30.10	55.97

9/7/2015	4.58	30.14	55.28
9/8/2015	3.06	30.37	54.81
9/9/2015	0.98	30.54	58.19
9/10/2015	4.035	31.12	54.61
9/11/2015	3.125	31.53	53.37
9/12/2015	0.945	31.76	53.79
9/13/2015	2.2	34.26	58.22
9/14/2015	0.68	29.49	57.45
9/15/2015	2.81	31.14	58.43
9/16/2015	1.44	31.16	54.50
9/17/2015	4.95	30.50	54.45
9/18/2015	15.38	32.05	56.04
9/19/2015	35.93	29.10	56.38
9/20/2015	3.765	29.22	55.59
9/21/2015	2.445	31.13	57.95
9/22/2015	6.04	31.55	58.40
9/23/2015	4.74	31.52	56.06
9/24/2015	3.92	30.21	55.67
9/25/2015	19.3	29.04	56.34
9/26/2015			
9/27/2015	2.055	30.20	59.53
9/28/2015	9.485	31.63	58.77
9/29/2015	9.505	29.76	58.91
9/30/2015	2.67	29.58	57.88
10/1/2015	1.06	30.65	56.25
10/2/2015	2.895	30.19	56.45
10/3/2015	79.75	30.07	58.88
10/4/2015	0.66	30.46	56.46
10/5/2015	1.005	29.85	56.92
10/6/2015	0.66	30.08	57.81
10/7/2015	0.58	31.01	57.29
10/8/2015	2.105	30.11	58.76
10/9/2015	2.83	30.92	58.92
10/10/2015	1.31	30.47	57.08
10/11/2015	1.085	30.03	56.83
10/12/2015	2.97	29.45	57.95
10/13/2015	2.28	32.20	59.34
10/14/2015	5.68	30.30	57.38
10/15/2015	28.18	30.34	57.70
10/16/2015	59.075	31.29	57.37

10/17/2015	3.07	30.30	57.07
10/18/2015	2.41	30.47	58.23
10/19/2015	2.85	28.25	58.48
10/20/2015	4.18	29.32	59.79
10/21/2015	7.145	30.38	58.12
10/22/2015	15.39	29.89	59.18
10/23/2015	5.855	29.54	57.75
10/24/2015	1.38	30.76	56.25
10/25/2015	4.485	29.90	59.83
10/26/2015	10.535	31.28	58.78
10/27/2015	2.355	31.25	56.15
10/28/2015	3.45	30.98	58.15
10/29/2015	9.655	29.46	59.90
10/30/2015	1.965	31.55	58.82
10/31/2015	4.855	31.74	59.61
11/1/2015	13.445	30.57	59.83
11/2/2015	59.095	31.88	59.92
11/3/2015	8.32	29.27	57.05
11/4/2015	31.855	29.76	58.15
11/5/2015	68.455	31.18	57.78
11/6/2015	4.165	29.40	59.45
11/7/2015	12.2500005	29.92	59.66
11/8/2015	434.275	30.37	59.93
11/9/2015	22.325	31.17	58.54
11/10/2015	5.98	30.36	59.96
11/11/2015	4.715	29.37	59.59
11/12/2015	3.9	30.42	60.09
11/13/2015	1.085	29.93	58.98
11/14/2015	1.54	31.51	54.99
11/15/2015	20.895	30.75	59.52
11/16/2015	76.32	31.42	54.99
11/17/2015	6.105	29.82	59.92
11/18/2015	15	31.09	56.91
11/19/2015	9.275	31.33	57.83
11/20/2015	3.625	30.76	58.63
11/21/2015	1.055	30.87	59.61
11/22/2015	0.995	30.15	59.52
11/23/2015	26.995	30.15	58.40
11/24/2015	112.5	30.71	57.94
11/25/2015	33.225	31.47	60.11

11/26/2015	18.455	30.37	59.12
11/27/2015	6.085	31.48	59.84
11/28/2015	1.02	31.84	59.71
11/29/2015	0.69	28.95	61.10
11/30/2015	41.52	30.30	60.66
12/1/2015	41.08	31.62	60.20
12/2/2015	77.16	31.08	59.73
12/3/2015	5.05	30.59	60.14
12/4/2015	2.605	30.93	59.75
12/5/2015	2.355	31.16	60.23
12/6/2015	1.975	31.41	60.09
12/7/2015	3.035	30.62	62.39
12/8/2015	1.815	31.13	60.64
12/9/2015	2.675	29.90	60.64
12/10/2015	2	30.99	62.75
12/11/2015	1.48	29.73	57.29
12/12/2015	0.97	30.93	57.89
12/13/2015	4.34	30.89	60.10
12/14/2015	1.45	31.03	59.25
12/15/2015	1.785	30.23	61.64
12/16/2015	5.4	31.30	61.38
12/17/2015	0.66	30.22	60.35
12/18/2015	0.96	31.28	61.61
12/19/2015	46.05	30.52	61.09
12/20/2015	6.54	31.27	60.78
12/21/2015	2.785	31.40	61.36
12/22/2015	1.015	32.02	60.49
12/23/2015	0.97	30.99	60.60
12/24/2015	1.47	30.70	61.70
12/25/2015	3.51	30.04	61.04
12/26/2015	1.94	31.94	60.56
12/27/2015	7.115	31.20	60.32
12/28/2015	0.63	31.71	60.61
12/29/2015	1.39	30.52	53.71
Total Average	936.30	30.67	58.44
Total Stand. Dev.	3341.95	2.02	2.51

Appendix B: Total solids (TS) and volatile solids (VS) of Class B biosolids.

Date	(with Lime) Total Solids (%)	(with Lime) Volatile Solids (%)
1/1/2013	31.89	49.15
1/2/2013	31.62	50.32
1/3/2013	35.34	50.94
1/4/2013	27.71	47.32
1/5/2013	30.73	53.79
1/6/2013	29.68	61.22
1/7/2013	30.63	57.33
1/8/2013	30.47	58.4
1/9/2013	30.27	57.53
1/10/2013	31.37	59.29
1/11/2013	34.44	47.39
1/12/2013	32.83	45.31
1/13/2013	32.84	55.06
1/14/2013	32.24	59.04
1/15/2013	33.06	57.48
1/16/2013	37.83	47.82
1/17/2013	31.53	54.08
1/18/2013	32.45	60.95
1/19/2013	30.97	54.49
1/20/2013	31.8	54.26
1/21/2013	31.24	60.11
1/22/2013	30.1	56.49
1/23/2013	33.86	54.26
1/24/2013	34.09	53.08
1/25/2013	29.18	59.79
1/26/2013	29.79	58.23
1/27/2013	31.13	62.6
1/28/2013	30.54	55.22
1/29/2013	30.65	58.71
1/30/2013	30.08	60.28
1/31/2013	33.17	50.57
2/1/2013	33.3	53.32
2/2/2013	37.04	50.21
2/3/2013	31.47	52.49
2/4/2013	31.26	56.65
2/5/2013	36.08	45.66
2/6/2013	34.82	68.4
2/7/2013	29.5	59.02
2/8/2013	28.54	65.25
2/9/2013	28.92	66.21
2/10/2013	29.59	59.73

2/11/2013	29.98	61.29
2/12/2013	31.63	60.51
2/13/2013	32.84	53.75
2/14/2013	30.57	63.52
2/15/2013	31.65	51.58
2/16/2013	32.28	50.48
2/17/2013	27.35	61.21
2/18/2013	29.78	61.43
2/19/2013	29.49	63.57
2/20/2013	28.15	63.85
2/21/2013	30.18	58.87
2/22/2013	32.61	59.26
2/23/2013	32.96	61.68
2/24/2013	31.03	64.77
2/25/2013	30.79	57.04
2/26/2013	32.06	56.13
2/27/2013	33.58	56.71
2/28/2013	32.77	59.39
3/1/2013	33.88	54.28
3/2/2013	30.23	56.81
3/3/2013	32.79	65.46
3/4/2013	29.74	60.78
3/5/2013	29.93	60.95
3/6/2013	30.37	54.08
3/7/2013	31.04	61.26
3/8/2013	34.83	57.98
3/9/2013	28.72	69.51
3/10/2013	30.11	64.98
3/11/2013	28.38	64.69
3/12/2013	33.35	57.65
3/13/2013	33.27	55.33
3/14/2013	30.4	58.91
3/15/2013	30.21	61.14
3/16/2013	29.54	59.48
3/17/2013	31.51	54.26
3/18/2013	31.86	49.04
3/19/2013	27.39	64.69
3/20/2013	29.42	56.1
3/21/2013	31.05	57.35
3/22/2013	28.74	64.69
3/23/2013		
3/24/2013	31.31	65.2
3/25/2013	31.47	53.71

3/26/2013	28.94	57.9
3/27/2013	29.42	59.1
3/28/2013	27.77	59.2
3/29/2013	30	56.81
3/30/2013	31.04	54.57
3/31/2013		
4/1/2013		
4/2/2013	30.84	59.4
4/3/2013		
4/4/2013	33.2	52.08
4/5/2013		
4/6/2013	32.56	57.62
4/7/2013	32.67	54.74
4/8/2013	32.35	54.41
4/9/2013	32.73	57.99
4/10/2013	29.5	57.19
4/11/2013	29.8	64.47
4/12/2013	32.82	60.99
4/13/2013	37.18	51.76
4/14/2013	34.44	54.49
4/15/2013	31.56	60.86
4/16/2013	35.66	58.17
4/17/2013	30.2	55.99
4/18/2013	29.45	57.84
4/19/2013	35.4	51.21
4/20/2013	32.52	53.86
4/21/2013	31.51	57.15
4/22/2013	33.8	49.02
4/23/2013	30.28	56.16
4/24/2013	30.17	57.75
4/25/2013	31.66	49.82
4/26/2013	35.19	53.61
4/27/2013	34.96	55.58
4/28/2013	30.7	61.44
4/29/2013	30.86	61.44
4/30/2013	32.47	60.48
5/1/2013	37.2	46.76
5/2/2013	34.25	59.32
5/3/2013	32.31	56.36
5/4/2013	30.74	58.81
5/5/2013	30.99	63.9
5/6/2013	29.5	61.66
5/7/2013	30.64	57.19

5/8/2013	32.42	60.67
5/9/2013		
5/10/2013		
5/11/2013	33.39	67.07
5/12/2013		
5/13/2013	32.86	56.51
5/14/2013	29.6	55.85
5/15/2013	31.02	55.58
5/16/2013	30.19	63.45
5/17/2013	33.72	52.2
5/18/2013	32.43	56.71
5/19/2013	31.28	57.59
5/20/2013	31.84	57.82
5/21/2013	29.85	57.01
5/22/2013	30.49	58.18
5/23/2013	32.65	53.84
5/24/2013	32.37	57.81
5/25/2013	33.18	57.83
5/26/2013	31.46	59.19
5/27/2013	32.3	58.75
5/28/2013	31.37	59.05
5/29/2013	28.64	61.47
5/30/2013	30.38	61.57
5/31/2013	29	61.04
6/1/2013	31.81	56.74
6/2/2013	33.64	52.72
6/3/2013	30.58	57.43
6/4/2013	31.2	55.45
6/5/2013	32.86	58.12
6/6/2013	28.7	65.28
6/7/2013	30	59.55
6/8/2013	32.47	56.16
6/9/2013	34.68	53.75
6/10/2013	30.58	58.89
6/11/2013	31.97	54.04
6/12/2013	34.8	47.08
6/13/2013	33.42	53.76
6/14/2013	32.17	58.04
6/15/2013	31.63	47.76
6/16/2013	34.5	51.32
6/17/2013	32.26	44.76
6/18/2013		
6/19/2013	35.71	55.91

6/20/2013	33.81	54.06
6/21/2013	31.43	61.03
6/22/2013	30.06	66.15
6/23/2013	31.12	59.12
6/24/2013	35.59	
6/25/2013	31.28	
6/26/2013		
6/27/2013	31.42	62.38
6/28/2013	32.56	57.14
6/29/2013		
6/30/2013	36.54	63
7/1/2013	37.8	63.81
7/2/2013	30.31	53.19
7/3/2013	33.22	54.1
7/4/2013	29.87	56.27
7/5/2013	31.24	62.41
7/6/2013	27.75	60.55
7/7/2013	34.96	42.23
7/8/2013	34.85	50.9
7/9/2013	34.2	56.68
7/10/2013	33.9	57.76
7/11/2013	33.07	58.41
7/12/2013	35.29	65.54
7/13/2013	38.67	46.92
7/14/2013	34.15	50.72
7/15/2013	32.89	58.71
7/16/2013	33.63	55.14
7/17/2013	33.17	60.99
7/18/2013	55.63	34.52
7/19/2013	32.1	58.8
7/20/2013	35.48	54.2
7/21/2013	31.57	57.62
7/22/2013	33.06	53.65
7/23/2013	32.1	58.02
7/24/2013	30.48	64.83
7/25/2013	36.36	52.41
7/26/2013	33.12	59.44
7/27/2013	30.3	50.46
7/28/2013	29.07	48.71
7/29/2013	31.26	54.98
7/30/2013	30.3	57.54
7/31/2013	31.26	58.17
8/1/2013	31.84	50.45

8/2/2013	31.74	54.93
8/3/2013	33.57	56.48
8/4/2013	32.95	50.68
8/5/2013	35.72	52.63
8/6/2013	32.12	54.11
8/7/2013	36.27	56.73
8/8/2013	36.47	59.68
8/9/2013	29.64	66.04
8/10/2013	36.89	48.09
8/11/2013	30.94	58.7
8/12/2013	29.54	57.51
8/13/2013	33.64	54.62
8/14/2013	33.03	59.8
8/15/2013	31.97	53.98
8/16/2013	31.28	60.65
8/17/2013	31.18	60.66
8/18/2013	32.86	52.3
8/19/2013	34	42.17
8/20/2013	33.05	51.13
8/21/2013	34.99	54.04
8/22/2013	29.37	57.84
8/23/2013	31.7	54.44
8/24/2013	34.48	52.54
8/25/2013	34.45	52.35
8/26/2013	30.83	54.35
8/27/2013	30.04	50.28
8/28/2013	34.97	56.78
8/29/2013	31.9	58.06
8/30/2013	30.46	57.69
8/31/2013	32.73	57.87
9/1/2013	36.95	65.3
9/2/2013	34.61	57.96
9/3/2013	38.52	68.77
9/4/2013		
9/5/2013	32.53	54.09
9/6/2013	32.06	56.31
9/7/2013	30.99	58.46
9/8/2013	32.39	54.75
9/9/2013	33.78	54.17
9/10/2013	41.33	56.79
9/11/2013	32.06	51.07
9/12/2013	32.16	55.56
9/13/2013	35	57.44

9/14/2013	30.56	51.97
9/15/2013	33.54	50.59
9/16/2013	29.81	53.96
9/17/2013	32.06	51.03
9/18/2013	36.53	52.41
9/19/2013	33.91	48.44
9/20/2013	29.71	51.14
9/21/2013	29.87	59.31
9/22/2013	29.51	56.12
9/23/2013	34.61	49.77
9/24/2013	33.39	47.7
9/25/2013	36.7	49.12
9/26/2013	33.76	46.92
9/27/2013	30.4	51.1
9/28/2013	28.67	54.89
9/29/2013	27.52	49.35
9/30/2013	29.21	53.53
10/1/2013	29.36	55.66
10/2/2013	30.28	60.87
10/3/2013	32.48	57.78
10/4/2013	33.29	49.99
10/5/2013	37.34	53.12
10/6/2013	33.75	58.94
10/7/2013	37.23	55.97
10/8/2013	39.34	54.01
10/9/2013	34.85	54.27
10/10/2013	36.1	64.55
10/11/2013	40.13	53.82
10/12/2013	38.55	50.86
10/13/2013	33.44	44.16
10/14/2013	31.03	48.4
10/15/2013	38.46	52.95
10/16/2013	32.3	59.88
10/17/2013	31.98	52.63
10/18/2013	31.11	56.01
10/19/2013	27.18	51.95
10/20/2013	26.31	51.09
10/21/2013	28.68	52.78
10/22/2013	28.55	52.45
10/23/2013	30.89	51.42
10/24/2013	28.35	63.91
10/25/2013	34.98	58.66
10/26/2013	32.09	56.27

10/27/2013	32.45	57.92
10/28/2013	31.16	55.11
10/29/2013	30.5	57.21
10/30/2013	31.92	53.06
10/31/2013	33.2	53.95
11/1/2013	34.04	51.73
11/2/2013	31.85	58.42
11/3/2013	32.53	48.81
11/4/2013	34.4	48.05
11/5/2013	34.9	48.55
11/6/2013	32.38	49.78
11/7/2013	32.86	59.59
11/8/2013	33.76	55.82
11/9/2013	32.88	54.45
11/10/2013	33.87	47
11/11/2013	35.22	42.26
11/12/2013	35.72	44.88
11/13/2013		
11/14/2013	33.99	67.76
11/15/2013	35.62	67.53
11/16/2013	33.57	58.47
11/17/2013	33.54	60.24
11/18/2013	35.42	48.37
11/19/2013	37.16	51.79
11/20/2013	34.58	53.15
11/21/2013	35.94	71.9
11/22/2013	35.6	66.21
11/23/2013	36.7	53.25
11/24/2013	33.82	56.44
11/25/2013	30.36	51.68
11/26/2013	31.37	54.33
11/27/2013	31.37	54.33
11/28/2013	33.49	52.36
11/29/2013	33.04	56.99
11/30/2013	30.41	62.11
12/1/2013	31.8	62.82
12/2/2013	31.52	45.08
12/3/2013	29.69	62.16
12/4/2013	32.37	52.52
12/5/2013	31.88	53.3
12/6/2013	29.77	58.03
12/7/2013	31.86	51.3
12/8/2013	48.31	60.84

12/9/2013	32.92	47.2
12/10/2013	35.74	38.82
12/11/2013	31.38	56.93
12/12/2013	27.33	59.24
12/13/2013	31.14	53.66
12/14/2013	30.56	59.95
12/15/2013	35.59	38.59
12/16/2013	31.06	60.51
12/17/2013	30.3	60.03
12/18/2013	31.22	59.6
12/19/2013	29.24	54.26
12/20/2013	36.53	44.88
12/21/2013	38.26	43.38
12/22/2013	33.4	57.03
12/23/2013	35.02	55.69
12/24/2013	38.13	51.87
12/25/2013	38.58	47.9
12/26/2013	32.4	55.03
12/27/2013	30.13	59.48
12/28/2013	30.66	58.48
12/29/2013	31.4	53.29
12/30/2013		
12/31/2013	33.24	49.71
1/1/2014	33.41	50.94
1/2/2014	30.52	54
1/3/2014	35.47	45.41
1/4/2014	31.32	60.01
1/5/2014	30.25	58.16
1/6/2014	30.3	60.16
1/7/2014	31.54	53.51
1/8/2014	30.79	60.77
1/9/2014	28.88	51.21
1/10/2014	30.83	58.7
1/11/2014	29.06	58.4
1/12/2014	30.64	55.93
1/13/2014	31.64	56
1/14/2014	29.65	58.61
1/15/2014	31.62	55.18
1/16/2014	36.19	57.37
1/17/2014	32.65	53.98
1/18/2014	30.23	55.79
1/19/2014	34.86	48.79
1/20/2014	31.48	53.19

1/21/2014	31.43	55.98
1/22/2014	29.4	57.82
1/23/2014	29.35	55.77
1/24/2014	31.22	53.83
1/25/2014	29.25	60.89
1/26/2014	28.9	59.79
1/27/2014	31.87	52.43
1/28/2014	29.89	60.03
1/29/2014	30.12	56.35
1/30/2014	34.44	56.6
1/31/2014	31.77	55.06
2/1/2014	30.83	58.38
2/2/2014	32.34	32.49
2/3/2014	33.45	48.87
2/4/2014	34.3	52.2
2/5/2014	32.87	53.61
2/6/2014	33.06	52.33
2/7/2014	30.17	62.1
2/8/2014	32.77	50.11
2/9/2014	31.37	56.92
2/10/2014		
2/11/2014	32.66	55.15
2/12/2014	34.92	55.76
2/13/2014		
2/14/2014	33.93	53.09
2/15/2014	33.56	60.1
2/16/2014	31.13	61.4
2/17/2014	34.68	51.82
2/18/2014	31.89	57.85
2/19/2014	34.26	51.75
2/20/2014	32.05	58.42
2/21/2014	31.05	54.38
2/22/2014	34.03	54.18
2/23/2014	32.8	56.02
2/24/2014	31.22	57.5
2/25/2014	31.48	57.08
2/26/2014	31.19	59.18
2/27/2014	32.53	54.87
2/28/2014	34.45	58.04
3/1/2014	31.07	52.43
3/2/2014	33.27	61.01
3/3/2014	31.05	52.43
3/4/2014	33.01	61.89

3/5/2014	29.03	64.43
3/6/2014	42.69	54.64
3/7/2014	30.71	70.2
3/8/2014	28.75	63.37
3/9/2014	25.77	51.72
3/10/2014	35.1	45.18
3/11/2014	30.17	64.72
3/12/2014	34.64	54.43
3/13/2014	34.81	47.41
3/14/2014	30.49	59.03
3/15/2014	36.07	55.24
3/16/2014	31.38	63.84
3/17/2014	31.55	64.94
3/18/2014	32.22	70.31
3/19/2014	33.78	56.16
3/20/2014	30.42	71.46
3/21/2014	32.12	58.41
3/22/2014	30.76	62.36
3/23/2014	30.87	60.89
3/24/2014	30.29	64.08
3/25/2014	36.92	49.28
3/26/2014	37.7	44.74
3/27/2014	31.01	59.35
3/28/2014	34.1	66.52
3/29/2014	31.31	59.24
3/30/2014	32.32	58.24
3/31/2014	37.8	52.29
4/1/2014	35.18	61.53
4/2/2014	33.58	66.56
4/3/2014	32.77	55.88
4/4/2014	31.03	65.26
4/5/2014		
4/6/2014	33.33	52.64
4/7/2014	33.39	48.74
4/8/2014	32.22	55.55
4/9/2014	32.42	54.37
4/10/2014	28.8	59.95
4/11/2014		
4/12/2014	29.78	59.43
4/13/2014	33.69	51.59
4/14/2014	31.85	62.11
4/15/2014	32.96	51.1
4/16/2014	33.99	55.13

4/17/2014	35.23	57.16
4/18/2014	39.46	51.53
4/19/2014	31.42	57.5
4/20/2014		
4/21/2014	31.78	61.94
4/22/2014	37.45	36.59
4/23/2014	36.26	62.26
4/24/2014	31.21	58.99
4/25/2014	31.92	57.45
4/26/2014	30.38	58.12
4/27/2014	33.57	57.51
4/28/2014	42.49	66.81
4/29/2014	32.04	49.56
4/30/2014	31.34	52.56
5/1/2014	35.29	51.55
5/2/2014	37.86	40.17
5/3/2014	37.62	45.86
5/4/2014	35.01	50.03
5/5/2014	30.73	58.44
5/6/2014	33.4	52.78
5/7/2014	33.32	59.88
5/8/2014	33.06	59.84
5/9/2014	33.03	50.87
5/10/2014	32.21	55.88
5/11/2014	32.92	52.06
5/12/2014	34.8	57.32
5/13/2014	33.54	51.79
5/14/2014	36.91	55.38
5/15/2014	33.88	48.76
5/16/2014	35.19	43.11
5/17/2014	35.15	52.86
5/18/2014	37.23	49.46
5/19/2014	38.4	57.17
5/20/2014	32.72	53.06
5/21/2014	33.24	54.23
5/22/2014	31.3	56.25
5/23/2014	35.46	59.88
5/24/2014	32.86	53.16
5/25/2014	35.19	53.94
5/26/2014	30.75	58.46
5/27/2014		
5/28/2014	35.1	50.34
5/29/2014	38.61	49.82

5/30/2014	35.21	52.97
5/31/2014	30.04	59.44
6/1/2014	32.78	53.69
6/2/2014	31.18	54.92
6/3/2014	32.55	53.12
6/4/2014	31.29	56.67
6/5/2014	34.25	52.79
6/6/2014	32.49	56.46
6/7/2014	32.61	56.38
6/8/2014	31.06	58.57
6/9/2014	34.11	53.9
6/10/2014	32.79	58.59
6/11/2014	32.79	54.65
6/12/2014	35.63	51.89
6/13/2014	30.36	48.15
6/14/2014	32.21	56.69
6/15/2014	34.59	51.71
6/16/2014	32.88	54.17
6/17/2014	32.78	50.81
6/18/2014	34.69	55.84
6/19/2014	32.99	51.31
6/20/2014	33.72	57.21
6/21/2014	33.97	46.37
6/22/2014	34.58	48.5
6/23/2014	33.95	45.75
6/24/2014	33.32	47.95
6/25/2014	36.2	53.37
6/26/2014		
6/27/2014	34.15	54.43
6/28/2014	33.11	59.66
6/29/2014	33.81	55.33
6/30/2014	32.13	54.31
7/1/2014	32.6	52.79
7/2/2014	33.36	65.18
7/3/2014	34.61	52.4
7/4/2014	41.27	36.69
7/5/2014		
7/6/2014	35.56	43.4
7/7/2014		
7/8/2014	35.93	51.14
7/9/2014	38.51	42.68
7/10/2014	32.63	65.33
7/11/2014	35.73	46.99

7/12/2014	36.75	51.84
7/13/2014	37.89	34.72
7/14/2014	38.77	46.83
7/15/2014	34.78	52.63
7/16/2014	40.55	46.82
7/17/2014	35.4	50.41
7/18/2014	34.1	49.69
7/19/2014	36.07	50.45
7/20/2014	32.29	53.81
7/21/2014	31.3	55.53
7/22/2014	34.97	52.51
7/23/2014	41.5	50.72
7/24/2014	32.93	57.69
7/25/2014	32.37	61.77
7/26/2014	34.4	54.22
7/27/2014	31.22	52.37
7/28/2014	33.44	50.62
7/29/2014	36.26	53.21
7/30/2014	40.04	63.87
7/31/2014	23.81	49.07
8/1/2014	31.7	56.78
8/2/2014	31.02	60.28
8/3/2014	32.84	55.69
8/4/2014	30.18	58.56
8/5/2014	36.3	50.47
8/6/2014	35.84	48.04
8/7/2014	34.21	50.34
8/8/2014	32.96	57.16
8/9/2014	34.56	51.04
8/10/2014	34.53	50.06
8/11/2014	34.39	43.86
8/12/2014	32.57	49.11
8/13/2014	34.48	50.49
8/14/2014	36.97	42.7
8/15/2014	37.1	43.11
8/16/2014	33.66	43.69
8/17/2014	31.47	49.36
8/18/2014	33.02	43.2
8/19/2014	33.15	48.35
8/20/2014	32.5	51.89
8/21/2014	35.1	53.6
8/22/2014	31.11	58.85
8/23/2014	32.19	53.92

8/24/2014	31.44	56.78
8/25/2014	30.01	56.3
8/26/2014	31.18	51.78
8/27/2014	33.31	50.82
8/28/2014	34.75	47.13
8/29/2014	37.87	51.13
8/30/2014	32.42	56.08
8/31/2014	38.13	46.82
9/1/2014	38.65	44.95
9/2/2014	38.71	36.52
9/3/2014	36.93	45.67
9/4/2014	42.81	35.59
9/5/2014	38.57	39.35
9/6/2014	36.23	39.69
9/7/2014	35.99	45.78
9/8/2014	36.27	48.93
9/9/2014	34.54	48.41
9/10/2014	36.99	53.58
9/11/2014	37.03	61.95
9/12/2014	32.42	56.61
9/13/2014	32.13	53.53
9/14/2014	38.61	48.57
9/15/2014	34.38	50.16
9/16/2014	33.34	53.1
9/17/2014	31.49	55
9/18/2014	31.7	57.56
9/19/2014	33.45	59.32
9/20/2014	34	53.86
9/21/2014	31.35	57.01
9/22/2014	33.53	54.17
9/23/2014	36.53	45.35
9/24/2014	31.97	58.61
9/25/2014	30.23	57.53
9/26/2014	31.47	58.09
9/27/2014	31.7	56.73
9/28/2014	36.55	44.05
9/29/2014	31.52	58.52
9/30/2014	32.25	57.5
10/1/2014	32.84	50.3
10/2/2014	35.86	55
10/3/2014	38.94	46.78
10/4/2014	34.39	52.19
10/5/2014	36.77	47.86

10/6/2014	32.79	60.04
10/7/2014	32.19	48.9
10/8/2014	35.38	49.47
10/9/2014	36.4	55.2
10/10/2014	32.46	54.78
10/11/2014	35.18	50.31
10/12/2014	33.79	55.98
10/13/2014	32.53	59.39
10/14/2014	32.47	52.06
10/15/2014	33.26	51.25
10/16/2014	33.8	52.45
10/17/2014	39.06	39.94
10/18/2014	31.53	56.72
10/19/2014	33.05	51.25
10/20/2014		
10/21/2014	31.29	46.91
10/22/2014	37.6	39.51
10/23/2014	32.92	57.06
10/24/2014	33.29	51.41
10/25/2014	33.23	50.33
10/26/2014	30.79	46.75
10/27/2014	37.06	42.41
10/28/2014	31.1	54.48
10/29/2014	33.97	48.25
10/30/2014	33.3	48.85
10/31/2014	34.09	50.2
11/1/2014	32.28	54
11/2/2014	31.61	60.36
11/3/2014	32.98	54.49
11/4/2014	33.02	53.97
11/5/2014	33.47	55.06
11/6/2014	33.08	60.55
11/7/2014	36.14	52.93
11/8/2014	38.85	48.6
11/9/2014	36.67	47.84
11/10/2014	32.66	55.34
11/11/2014	34.99	51.75
11/12/2014		
11/13/2014	35.89	48.05
11/14/2014	31.63	59.69
11/15/2014		
11/16/2014		
11/17/2014		

11/18/2014	37.25	52.62
11/19/2014	41.31	38.63
11/20/2014		
11/21/2014		
11/22/2014	33.98	56.59
11/23/2014	33.75	49.56
11/24/2014	29.55	62.88
11/25/2014	33.23	54.66
11/26/2014	32.95	51.43
11/27/2014	36.38	48.43
11/28/2014	36.3	49.11
11/29/2014	34.76	42.67
11/30/2014		
12/1/2014	35.61	48.93
12/2/2014	36.63	46.29
12/3/2014	34.13	50.75
12/4/2014	34.01	45.11
12/5/2014	34.15	47.94
12/6/2014	32.03	51.09
12/7/2014	33.59	55.07
12/8/2014	32.81	49.06
12/9/2014	37.49	40.93
12/10/2014	30.76	61.87
12/11/2014	37.99	51.92
12/12/2014	34.6	50.5
12/13/2014	35.36	48.76
12/14/2014	26.35	66.79
12/15/2014	32.03	64.44
12/16/2014	29.87	51.36
12/17/2014	31.13	62.82
12/18/2014	31.65	63.91
12/19/2014		
12/20/2014	33.73	65.3
12/21/2014	33.83	58.14
12/22/2014	37.22	54.9
12/23/2014	29.9	62.33
12/24/2014	35.52	40.5
12/25/2014	34.5	49.2
12/26/2014		
12/27/2014	32.54	45.94
12/28/2014	32.2	46.92
12/29/2014	32.77	46.9
12/30/2014	32.75	39.37

12/31/2014	31.64	50.47
1/1/2015	31.1	55.35
1/2/2015	31.69	53.24
1/3/2015	30.5	49.96
1/4/2015	31.56	46.46
1/5/2015	40.17	40.39
1/6/2015		
1/7/2015		
1/8/2015	29.57	57.39
1/9/2015	35.87	45.6
1/10/2015	31.55	55.6
1/11/2015	32.36	56.77
1/12/2015		
1/13/2015	34.25	55.24
1/14/2015	34.42	51.92
1/15/2015	30.77	56.69
1/16/2015	34.79	52.12
1/17/2015	36.55	46.31
1/18/2015	37.49	40.94
1/19/2015	36.29	47.2
1/20/2015	35.57	46.93
1/21/2015	37.58	45.29
1/22/2015	37.87	44.49
1/23/2015	36.8	44.46
1/24/2015	37.96	45.1
1/25/2015	35.72	55.34
1/26/2015	35.02	56.22
1/27/2015	42.16	39.61
1/28/2015		
1/29/2015	32.85	54.46
1/30/2015	36.03	43.86
1/31/2015	34.91	48.18
2/1/2015	37.14	38.02
2/2/2015	35.36	43.74
2/3/2015	32.01	53.15
2/4/2015		
2/5/2015	38.07	44.67
2/6/2015	34.61	58.75
2/7/2015	31.4	40.94
2/8/2015	31.56	61.45
2/9/2015	31.76	56.2
2/10/2015	35.16	44.33
2/11/2015	39.81	41.34

2/12/2015		
2/13/2015	31.55	59.63

Appendix C: Metal pollutants concentrations of Class B biosolids from 2013 to 2015

	Date	As mg/Kg	Cd mg/Kg	Cr mg/Kg	Cu mg/Kg	Pb mg/Kg	Hg mg/Kg	Mo mg/Kg	Ni mg/Kg	K mg/Kg	Se mg/Kg	Zn mg/Kg
2013	January 1 -15	1.5	1.80	33	99	19	0.15	5.5	14	1750.00	4.1	209
	January 16 -31	1.7	0.61	35	107	16	0.23	5.0	11	1770.00	2.4	257
	February 1-15	2.10	0.77	30	125	21	0.32	4.9	10.8	2150.00	2.50	284
	February 16-29	1.70	0.77	23	128	14	0.14	4.5	8.3	1730.00	2.80	314
	March 1-15	1.9	0.71	27	117	17	0.31	4.9	11	1490.000	2.5	246
	March 16 -31	1.7	0.66	25	112	12	0.30	4.4	10	2240.000	2.2	223
	April 4 -15	1.8	0.77	26	120	13	0.40	4.4	8.7	1920	2.6	253
	April 16-30	1.5	0.68	29	107	14	0.23	4.9	9.3	1710	2.5	242
	May 1-15	1.8	0.80	40	146	19	0.39	4.8	11	1770	2.60	340
	May 16 -31	1.6	0.75	47	125	15	0.38	5.5	9	1790	2.50	294
	June 1-15	2.6	0.67	48	130	25	0.36	6.4	15	1750.000	2.5	286
	June 16-30	2.5	0.82	38	143	28	0.25	6.6	9.3	1640.000	2.3	288
	July 1-15	2.2	0.72	36	135	21	0.29	9.1	10.9	1610	2.6	306
	July 16 -31	1.6	0.63	35	119	15	0.18	6.7	8.9	1670	2.4	276
	August 1-15	1.90	0.80	39	168	19	0.30	11.7	13.9	1620.00	2.4	377
	August 16 -31	1.70	0.69	48	145	16	0.60	11	16	1900.00	2.4	315
	September 1-15	1.7	0.73	45	156	17	0.24	12.2	11.6	1390.00	2.4	322
	September 16-30	2.2	0.52	60	152	19	0.28	13.4	15.0	1330.00	2.5	354
	October 1-15	2.3	0.74	47	125	19	0.33	10.5	15.7	1800.000	2.3	289
	October 16 -31	2.1	0.75	35	127	15	0.36	10.0	9.6	503.000	2.4	285

	November 1-15	1.6	0.73	33	131	17	0.61	7.6	9.5	1370.00	2.4	288
	November 16-30	1.8	0.76	41	149	18	0.24	7.0	13	1340.00	2.4	329
	December 1-15	2.4	0.72	38	114	17	0.20	8.40	12	1590.000	2.4	224
	December 16 -31	1.9	0.67	42	111	18	0.23	6.9	12	1260.000	1.9	250
	January 1 -15	2.2	1.0	51	125	22	0.17	8.3	16	1460.00	2.2	328
	January 16 -31	1.8	0.77	44	125	19	0.25	5.5	12	1320.00	1.90	277
	February 1-15	2.0	0.77	49	130	17	0.64	7.2	15.4	1730.00	2.10	247
	February 16-28	2.30	0.82	42	127	18	0.29	5.7	12.5	1180.00	1.80	278
	March 1-15	1.7	0.76	36	112	14	0.24	5.2	11	1210.000	2.4	264
	March 16 -31	2.3	0.86	46	140	17	0.29	7.1	16	1260.000	2.5	305
	April 4 -15	2.8	0.96	63	163	20	0.32	7.2	22.6	1060.000	2.6	357
	April 16-30	2.4	0.84	47	138	25	0.27	6.1	17.1	1410.000	2.5	304
	May 1-15	2.9	0.85	55	153	25	0.34	8.5	25	1440.000	2.5	306
	May 16 -31	2.3	0.75	31	126	22	0.32	6.2	14	1220.000	2.6	291
2014	June 1-15	2.4	0.91	41	141	24	0.36	7.1	17	1280.000	2.5	332
	June 16-30	2.1	0.70	41	132	16	0.25	8.7	14.7	1230.000	2.4	300
	July 1-15	2.3	0.76	38	133	22	0.27	8.4	13	1460.00	2.4	296
	July 16 -31	1.7	0.69	28	121	14	0.29	8.7	10	1050.00	2.4	297
	August 1-15	2.1	0.81	46	136	18	0.26	11.3	18	1490	2.5	326
	August 16 -31	1.9	0.68	37	135	15	0.26	10.1	14	1430	2.5	308
	September 1-15	1.6	0.65	36	136	19	0.28	10.2	12	1150	2.6	309
	September 16-30	3.2	0.61	39	135	13	0.32	11.1	14	1340	5.3	293
	October 1-15	1.5	0.69	30	131	15	0.29	10.5	10	928	2.5	273
	October 16 -31	2.6	0.86	43	139	20	0.32	10.6	12	1190	4.3	290
	November 1-15	2.6	0.61	42	105	13	0.20	13	9	1330	4.3	239
	November 16-30	2.3	0.76	48	133	21	0.24	8.6	13	1310	2.5	273

	December 1-15	2.9	0.98	38	111	16	0.18	5.6	8.8	1260	4.9	238
	December 16 -31	2.2	0.67	42	113	16	0.22	5.7	11	1460	2.3	237
	January 1 -15	2.3	0.61	41	108	14	0.27	5.0	11	1590	2.2	254
2015	January 16 -31	1.8	0.53	28	92	12	0.18	4.6	8.0	1160	2.5	219
	February 1-15	1.6	0.60	21	99	9.4	0.16	4.7	8.2	1220	2.2	207
	Average	2.1	0.8	39.2	128.0	17.6	0.3	7.6	12.5	1456.1	2.6	284.3
	Std. Dev.	0.4	0.2	8.9	16.6	3.8	0.1	2.5	3.5	310.2	0.7	38.9

Appendix D: Metal pollutants concentrations in Class A biosolids by THP-AD

Date	Cu (mg/kg)		K (mg/kg)		Zn (mg/kg)		As (mg/kg)		Cd (mg/kg)		Cr (mg/kg)		Hg (mg/kg)		Mo (mg/kg)		Ni (mg/kg)		Pb (mg/kg)		Se (mg/kg)	
11/27/2014	445.58	423.98	1000.58	922.34	1050.97	989.29	9.21	8.63	3.28	3.06	87.82	84.80	0.05	0.50	16.20	15.55	16.27	15.47	80.62	75.13	3.66	3.65
11/28/2014	316.16	279.48	666.49	578.48	710.82	607.03	3.70	2.36	2.73	2.51	62.51	55.44	0.12	-0.15	11.63	10.37	21.00	18.86	64.25	56.80	2.99	2.54
11/29/2014	477.92	464.21	1007.71	973.57	963.25	938.29	4.78	6.05	4.02	3.80	90.40	87.48	-0.05	0.37	16.67	15.80	29.34	28.29	91.14	89.60	3.12	2.71
11/30/2014	509.64	497.76	1057.48	1031.32	1020.76	981.19	6.61	5.60	4.18	3.97	96.20	93.82	0.17	0.26	16.96	16.62	30.84	30.08	100.61	95.97	3.10	3.30
12/1/2014	502.18	495.80	973.84	937.59	922.97	975.10	11.34	7.50	3.68	4.04	93.75	92.26	3.62	3.46	16.35	16.50	23.47	23.18	52.62	53.26	8.65	7.07
12/2/2014	393.72	402.12	706.15	708.40	753.13	770.90	6.88	5.08	3.18	3.19	76.80	77.78	2.24	3.00	13.42	13.75	19.16	19.45	43.70	43.68	5.30	6.35
12/3/2014	496.27	482.17	904.32	843.26	904.32	879.30	3.63	3.43	3.72	3.75	96.31	93.70	1.84	4.40	17.20	17.01	26.69	25.59	88.96	82.16	23.53	23.86
12/4/2014	485.10	484.45	882.00	872.01	911.90	909.27	6.44	4.75	3.89	3.78	99.41	99.13	2.98	2.00	14.87	14.68	24.67	24.30	49.03	47.25	0.96	2.57
12/5/2014	487.54	465.57	879.05	836.99	908.60	881.83	1.99	4.60	4.07	3.98	97.51	96.40	1.69	1.53	16.33	16.37	28.81	29.22	73.28	69.95	11.38	10.09
12/6/2014	486.77	459.02	911.36	845.57	911.36	859.78	8.27	3.62	4.03	3.69	99.61	94.50	3.29	1.01	15.26	15.06	28.61	27.71	81.25	80.29	13.92	8.53
12/7/2014	420.52	260.41	790.34	494.86	820.16	536.40	0.60	5.14	3.65	2.27	88.73	58.31	1.45	1.33	15.81	9.50	26.47	17.88	63.23	39.91	3.69	5.78
12/8/2014	498.77	501.78	913.56	925.10	920.92	925.10	4.07	2.78	4.19	4.24	104.62	101.39	2.84	2.93	16.50	16.50	30.43	30.57	79.57	76.97	11.86	9.40
12/9/2014	466.49	485.86	854.87	885.42	869.61	907.74	5.31	8.26	3.95	3.83	98.75	114.58	2.56	2.18	16.21	17.71	28.67	42.41	81.07	82.59	7.02	11.24

12/10/2014	437.42	460.21	792.13	796.24	792.13	840.07	6.15	4.68	3.97	4.20	87.63	91.31	1.96	1.53	12.55	13.51	20.68	21.40	87.63	92.77	11.15	12.05
12/11/2014	476.79	469.34	851.06	829.42	836.56	819.48	6.72	5.02	4.26	4.17	92.36	91.38	1.61	2.41	13.44	13.31	20.89	21.01	84.14	80.46	10.49	10.78
12/12/2014	471.83	461.98	875.04	853.12	810.41	803.23	5.72	7.28	4.14	4.05	91.48	90.30	1.81	1.29	13.27	13.02	20.68	20.26	82.04	81.32	11.98	12.32
12/13/2014	341.09	492.49	660.07	899.09	619.19	838.70	6.51	9.53	3.11	4.23	70.36	95.28	2.10	1.55	9.92	13.29	17.16	22.07	66.68	89.91	11.26	13.49
12/14/2014	464.25	456.31	1166.83	1007.44	774.58	765.46	4.13	1.30	5.06	4.93	95.83	93.34	1.58	8.59	13.51	13.19	17.08	16.59	81.43	80.99	8.79	7.85
12/15/2014	478.25	428.66	1019.87	870.24	787.18	721.06	4.30	4.39	5.10	4.64	97.04	92.49	1.63	0.67	13.07	12.18	17.48	18.60	97.04	86.53	5.74	5.92
12/16/2014	476.68	493.87	1014.11	1008.72	783.85	798.99	4.95	3.97	5.19	5.19	98.47	101.87	0.55	1.75	13.57	14.03	17.05	17.93	91.61	90.39	4.70	6.64
12/17/2014	503.72	505.25	1057.32	1040.22	807.95	807.41	3.75	3.64	5.39	5.45	115.21	102.54	1.13	1.59	15.61	14.12	33.17	17.93	89.27	89.66	8.58	9.51
12/18/2014	501.26	466.29	1047.18	940.55	808.96	776.33	4.09	3.89	5.26	4.96	102.24	96.54	1.06	1.58	13.85	13.39	19.50	18.66	99.26	94.55	4.76	7.86
12/19/2014	489.87	494.92	1305.82	1508.20	826.28	819.99	-2.29	3.51	5.16	5.17	106.97	107.62	2.27	1.36	15.20	15.23	29.07	29.43	90.01	87.86	14.68	8.42
12/20/2014	447.68	566.29	1150.56	1395.69	759.66	941.59	4.57	2.41	4.79	6.00	95.14	115.53	2.12	1.82	14.38	16.69	24.26	28.98	95.88	117.53	9.07	8.35
12/21/2014	568.14	523.95	1215.34	968.06	913.97	878.24	5.83	2.38	5.98	5.59	115.61	108.28	1.06	0.45	16.11	15.22	27.22	26.45	124.50	115.27	8.30	7.29
12/22/2014	254.86	314.79	557.51	628.59	478.36	579.09	4.60	4.92	2.59	3.12	55.75	67.81	1.00	0.95	8.41	10.05	12.59	14.95	56.75	70.28	9.71	11.73
12/23/2014	546.70	397.79	1035.09	755.94	940.32	725.12	10.35	8.29	5.26	4.02	115.17	87.34	2.22	0.91	17.13	13.06	25.29	19.38	118.09	88.07	20.85	11.82
12/24/2014	530.62	474.29	971.76	809.08	908.84	850.93	6.86	7.81	5.15	4.81	112.56	104.62	0.85	0.53	16.92	15.83	24.54	23.44	114.65	109.50	18.46	15.07
12/25/2014	330.31	334.79	579.49	550.93	618.12	640.98	7.15	6.09	3.29	2.86	72.92	61.45	1.32	0.38	10.87	9.11	16.18	13.77	75.33	63.57	12.12	9.06
12/26/2014	706.80	726.17	1438.48	1403.60	1000.47	1023.46	7.52	7.85	4.03	4.08	129.41	132.56	1.59	1.36	18.91	19.49	28.57	28.66	137.38	140.85	8.11	7.94
12/27/2014	663.20	674.74	1350.78	1319.94	916.78	930.85	7.07	7.93	3.68	3.73	119.96	120.17	2.15	2.89	17.51	17.43	26.28	26.40	115.08	114.76	9.22	8.22
12/28/2014	444.63	436.67	1071.74	1037.18	847.25	826.74	6.79	5.91	4.74	4.50	97.04	94.70	0.80	1.06	14.27	13.83	25.13	24.88	88.35	87.18	21.07	19.32
12/29/2014	445.42	406.73	1078.93	964.87	816.49	749.63	7.95	6.75	4.74	4.39	93.31	85.35	0.77	1.14	13.56	12.77	25.22	23.53	88.21	78.67	20.56	17.96
12/30/2014	528.55	521.95	1226.58	1204.50	958.97	927.10	8.62	8.98	5.35	5.39	109.28	111.69	1.36	1.00	15.91	16.21	29.59	31.10	99.61	97.09	22.60	22.48
12/31/2014	559.12	569.86	1351.88	1366.79	977.17	983.51	9.04	8.82	5.79	5.86	113.15	114.26	0.90	1.16	16.46	16.27	29.83	29.58	98.45	100.52	24.25	23.94
1/1/2015	423.26	430.79	1059.99	1064.04	800.55	812.81	7.71	7.54	4.66	4.63	92.66	93.10	0.10	1.03	13.56	13.00	24.76	24.38	85.99	90.89	19.79	20.10
1/2/2015	439.07	480.10	1032.69	967.69	850.04	840.17	12.15	12.68	5.02	5.01	96.95	96.02	0.27	0.30	14.19	14.10	29.08	27.61	96.24	89.27	17.00	13.65
1/3/2015	558.37	567.83	1202.30	1269.95	981.02	985.31	14.09	14.82	6.20	6.23	137.20	152.54	0.60	0.29	19.47	21.24	50.89	53.72	112.85	113.86	13.57	18.68

1/4/2015	489.70	378.46	992.83	678.10	843.54	743.53	11.94	11.00	5.02	4.42	100.03	86.25	0.78	0.42	14.48	12.19	27.92	24.76	97.79	84.02	14.71	13.23
1/6/2015	435.63	179.73	1090.94	467.58	816.35	657.01	10.54	8.35	4.99	4.55	92.02	40.42	0.77	1.08	13.73	10.44	24.94	15.36	94.25	50.86	8.02	10.74
1/7/2015	96.65	99.43	236.42	243.54	388.08	399.89	5.06	5.01	2.71	2.71	22.16	22.70	0.22	0.17	6.10	6.23	9.74	9.87	29.59	30.91	6.90	7.18
1/8/2015	109.43	170.51	269.70	415.88	438.44	637.54	5.26	8.82	2.89	4.34	26.12	39.66	0.29	0.07	7.00	10.38	10.45	15.05	34.03	50.93	6.16	10.45
1/9/2015	539.16	533.44	1277.35	1281.74	1061.99	1059.47	13.44	13.41	6.40	6.33	119.57	120.76	-0.18	0.16	17.30	17.71	37.95	38.38	103.97	104.47	10.99	11.48
1/11/2015	321.53	306.68	724.88	684.65	636.60	610.40	7.54	9.13	3.85	3.64	80.38	69.28	0.25	0.92	10.41	10.10	27.92	23.99	60.79	58.14	9.47	8.76
1/13/2015	199.50	174.75	787.50	802.50	273.75	228.75	-12.75	20.70	5.35	3.87	120.75	84.75	-14.70	-19.05	14.10	9.38	131.25	35.48	67.95	62.85	-31.65	12.00
1/15/2015	151.50	154.50	600.75	638.25	152.25	125.25	35.10	-51.08	1.70	2.00	54.23	43.88	-0.58	6.32	8.70	8.33			43.88	44.48	59.63	-35.55
1/16/2015	154.50	125.25	564.00	810.00	100.50	106.50	26.55	32.93	1.38	2.75	33.00	39.38	-4.03	11.78	7.28	10.20	22.88	23.55	40.05	53.70	-41.78	29.93
1/19/2015	290.00				677.00		3.30		1.60		79.20		0.41		11.00		17.20		35.00		4.20	
1/23/2015	320.00				781.00		3.70		1.90		85.70		0.50		11.00		19.00		39.00		4.20	
1/26/2015	303.00				647.00		3.80		1.80		71.00		0.67		12.00		17.60		37.00		4.70	
1/30/2015	313.00				736.00		3.70		1.80		85.20		0.64		14.00		20.10		40.00		4.70	
2/2/2015	287.00				696.00						76.00		0.53		12.00		19.00		34.00			
2/5/2015	375.00				893.00		4.90		2.10		96.00		0.53		16.00		24.00		44.00			
2/9/2015	339.00				825.00				2.00		79.00		0.75		13.00		20.00		36.00			
2/12/2015	275.00				589.00				1.70		63.00		0.66		12.00		17.00		36.00			
2/15/2015	325.00				726.00		4.20		2.10		80.00		0.64		15.00		24.00		39.00		5.70	
2/17/2015	285.00				611.00				1.90		59.00		0.80		13.00		16.00		39.00			
2/19/2015	331.00				674.00				2.00		60.00		0.58		12.00		18.00		31.00			
2/23/2015	345.00				759.00				2.20		66.00		0.71		15.00		19.00		36.00			
2/26/2015	295.00				674.00				1.80		52.00		1.00		11.00		16.00		35.00			
2/28/2015	311.00				727.00		3.60		1.90		71.00		0.63		12.00		21.00		38.00		4.40	
3/2/2015	302.00		685.00		660.00		4.90		1.70		48.00		0.64		10.00		19.00		31.00		8.10	
3/9/2015	317.00		864.00		765.00		4.40		2.00		49.00		0.80		12.00		19.00		33.00		7.40	

3/16/2015	282.00		701.00		593.00		4.80		1.60		45.00		0.63		10.00		17.00		36.00		8.00	
3/24/2015	319.00		838.00		588.00		4.50		1.80		45.00		0.67		11.00		18.00		32.00		7.60	
3/31/2015	300.00		812.00		704.00		4.60		1.80		51.00		0.61		13.00		19.00		36.00		7.70	
4/7/2015	302.00		685.00		660.00		4.90		1.70		48.00		0.60		10.00		19.00		31.00		8.10	
4/14/2015	331.00		754.00		811.00		5.00		1.70		61.00		0.50		11.00		20.00		38.00		8.30	
4/21/2015	32.00		717.00		705.00		4.80		1.80		63.00		0.70		13.00		20.00		39.00		8.00	
4/28/2015	443.00		728.00		1090.00		5.50		2.30		89.00		0.70		15.00		27.00		57.00		7.40	
5/5/2015	316.00		620.00		670.00		4.40		2.00		71.00		0.61		15.00		21.00		49.00		7.20	
5/12/2015	334.00		562.00		770.00		4.00		1.80		71.00		0.76		13.00		19.00		40.00		6.70	
5/19/2015	323.00		551.00		757.00		4.00		1.80		64.00		0.36		12.00		20.00		39.00		6.70	
5/26/2015	360.00		719.00		849.00		4.60		2.10		73.00		0.76		16.00		22.00		48.00		7.60	
6/2/2015	390.00		722.00		804.00		4.90		2.30		79.00		0.69		17.00		23.00		54.00		6.90	
6/9/2015	337.00		804.00		783.00		4.70		1.90		71.00		0.69		14.00		21.00		49.00		7.80	
6/16/2015	371.00		677.00		799.00		4.70		2.00		75.00		0.62		16.00		21.00		56.00		7.90	
6/23/2015	403.00		669.00		875.00		5.30		2.40		85.00		0.75		18.00		24.00		68.00		8.30	
6/30/2015	347.00		750.00		716.00		5.30		2.10		83.00		0.66		19.00		23.00		59.00		7.70	
7/7/2015	353.00		774.00		772.00		5.80		2.00		92.00		0.99		16.00		24.00		62.00		7.40	
7/14/2015	357.00		788.00		792.00		6.20		2.20		99.00		0.73		17.00		23.00		91.00		7.40	
7/21/2015	337.00		627.00		731.00		5.00		1.80		97.00		0.76		16.00		24.00		51.00		7.60	
7/28/2015	467.00		679.00		955.00		6.00		2.40		125.00		0.67		20.00		24.00		56.00		7.70	
8/4/2015	373.00		685.00		921.00		4.90		1.80		114.00		0.55		18.00		21.00		56.00		7.00	
8/11/2015	398.00		639.00		831.00		4.50		1.90		112.00		1.00		17.00		20.00		61.00		7.50	
8/18/2015	384.00		652.00		812.00		4.60		1.80		108.00		0.73		19.00		21.00		54.00		7.60	
8/25/2015	394.00		650.00		839.00		4.40		1.90		110.00		0.43		20.00		20.00		49.00		7.10	
9/1/2015	446.00		698.00		923.00		5.30		2.30		131.00		0.64		28.00		26.00		63.00		7.90	

9/8/2015	368.00		600.00		763.00		5.00		1.80		107.00		0.77		22.00		20.00		53.00		8.30	
9/15/2015	425.00		638.00		906.00		4.40		2.10		124.00		0.78		26.00		22.00		60.00		6.70	
9/22/2015	275.00		363.00		601.00		5.10		1.70		76.00		0.84		17.00		13.00		52.00		8.40	
9/29/2015	357.00		628.00		855.00		4.20		2.10		108.00		1.00		23.00		18.00		50.00		7.00	
10/6/2015	413.00		711.00		939.00		4.80		2.30		119.00		0.58		27.00		23.00		62.00		8.00	
10/13/2015	410.00		660.00		858.00		5.00		2.10		113.00		0.78		25.00		23.00		53.00		7.10	
10/20/2015	388.00		584.00		828.00		4.30		1.90		107.00		0.72		23.00		21.00		53.00		7.10	
10/27/2015	416.00		720.00		923.00		4.30		2.10		112.00		0.62		23.00		23.00		51.00		7.90	
11/3/2015	401.00		689.00		850.00		4.90		2.00		103.00		0.80		22.00		21.00		49.00		8.00	
11/10/2015	361.00		605.00		748.00		4.70		1.80		93.00		0.87		19.00		19.00		47.00		7.80	
11/17/2015	375.00		711.00		837.00		5.00		2.10		96.00		0.51		20.00		18.00		47.00		8.40	
11/24/2015	436.00		636.00		926.00		4.50		2.10		104.00		0.67		21.00		21.00		56.00		7.50	
12/1/2015	348.00		614.00		798.00		4.50		1.80		89.00		0.68		18.00		20.00		46.00		7.50	
12/8/2015	333.00		811.00		623.00		4.60		1.50		69.00		0.78		15.00		18.00		38.00		7.70	
12/15/2015	372.00		887.00		633.00		4.80		2.00		69.00		0.63		20.00		20.00		36.00		8.00	
12/22/2015	407.00		975.00		972.00		4.70		2.10		79.00		0.67		17.00		22.00		34.00		7.90	
12/29/2015	398.00		665.00		898.00		4.00		1.70		67.00		0.63		16.00		23.00		34.00		6.70	

Appendix E: TKN, TP, and Ammonia in Class A biosolids by THP-AD

Sample Date	TKN (mg/kg)		TP(mg/kg)		Ammonia (mg/kg)
11/29/2014	49564.07	49599.7	31264.6	30199.3	2910.8
11/30/2014	71059.48	60988.5	46405.5	36381.7	5670.4
12/1/2014	39541.39	50607.2	29117.7	34050.3	6311.0
12/2/2014	54858.77	41007	38614.1	30701.5	6429.4
12/3/2014	64826.85	68465.7	40781	40142	5827.9
12/4/2014	54428.55	66802.1	32868.5	34214.7	3661.5
12/5/2014	77385.81	62351	35851.1	34020.9	6485.9
12/6/2014	60809.15	65064.6	37931.6	34531.7	5655.0
12/7/2014	67676.88	96762.2	28380.5	39497	6268.1
12/8/2014	58647.91	55750.8	34967.4	33231.8	5208.7
12/9/2014	62513.23	67092.6	39114.3	36038.1	5181.8
12/10/2014	61098.18	61125.2	36000.7	34233.1	6305.7
12/11/2014	59353.6	71189.5	38190.1	39262.5	5016.5
12/12/2014	60870.4	78995.8	35397.6	41406.4	5866.6
12/13/2014	49487.29	60895.3	29861.8	33246.3	6073.2
12/14/2014	64039	61486.4	31181.4	35127.2	6489.9
12/15/2014	43224.88	35458.6	32588.2	22364	5213.4
12/16/2014	36832.11	36572.4	29170.8	26324.7	6702.1
12/17/2014	59690.02	66358.3	34611.4	37961.7	8631.1
12/18/2014	71239.28	80126.9	37316.1	42321.4	7057.9
12/19/2014	26605.75	32396.5	19782.4	22967.5	7625.6
12/20/2014	25764.6	28686	27494.9	22811.9	5829.8
12/21/2014	61001.7	70095	36156.9	38955	4444.9
12/22/2014	40870.52	39961.6	20511.5	20964.8	3982.2
12/23/2014	73777.7	64827.3	31817.1	34545.9	7131.7
12/24/2014	71733.32	64353.4	36821.7		5545.6
12/25/2014	59652.04	64101.3	34811.6	32728.4	6446.2
12/26/2014	53263.29	51659.2	24849.6	31917.2	3767.1
12/27/2014	45973.69	30040	30439.2	24704.6	6739.9
12/28/2014	57640.13	53286	32736.8	33849.2	6790.6
12/29/2014	66805.24	65577	35357.8	36236.7	6413.7
12/30/2014	61286.52	59645.8	40297.2	35652	7344.3
12/31/2014	59687.6	60379.8	30807.8	36034.8	6970.3
1/1/2015	40197.46	42786.4	22670.6	23798.5	7393.6
1/2/2015	29859.32	7542.98	35016.8	7604.93	6154.1
1/3/2015	58706.57	49142.9	39362.3	33758.9	6957.7
1/4/2015	57340.84	54019.9	31434.3	30099.2	5451.6

1/5/2015					
1/6/2015	43287.66	49266.9	28242.5	29214.9	7087.5
1/7/2015	56357.34	66262.7	31918.2	17338	5886.0
1/8/2015	51659.24	26452	33327.6	34700.5	5009.3
1/9/2015	45092.42	47491.5	24988.1	18995.3	6335.3
1/10/2015					8151.4
1/11/2015	53274.63	58154.5	31450.5	36278	
1/12/2015					
1/13/2015			22481.3	43519.5	7151.1
1/14/2015	47057.37	81958.9			
1/15/2015					6876.8
1/16/2015					7903.6
1/17/2015					
1/18/2015					
1/19/2015					
1/20/2015					
1/21/2015					
1/22/2015					
1/23/2015	35489.15	33082.3	18982.3	16647.5	
3/2/2015	39000				7690.0
3/9/2015	67300		34500		12000.0
3/16/2015	55600				8340.0
3/24/2015	26500		39200		8070.0
3/31/2015	58000		29600		9730.0
4/7/2015	68000		31000		7430.0
4/14/2015	30900		32800		
4/21/2015	29500		32200		8610.0
4/28/2015	26900		44900		8530.0
5/5/2015	36000		34300		8030.0
5/12/2015			24500		7980.0
5/19/2015	51000		37400		8090.0
5/26/2015	53700		50600		7450.0
6/2/2015	42900		31300		8170.0
6/9/2015	59100		33100		7130.0
6/16/2015	61800		36700		8150.0
6/23/2015	48700		31800		8530.0
6/30/2015	43700		29600		8430.0
7/7/2015	43200		27600		7720.0
7/14/2015	51700		27300		8050.0
7/21/2015	49200		34500		6930.0
7/28/2015	47700		33000		7730.0

8/4/2015	77400	36200	7540.0
8/11/2015	46700	36400	7550.0
8/18/2015	36100	30000	5940.0
8/25/2015	58700	30800	4530.0
9/1/2015	49400	30900	5150.0
9/8/2015	47500	33400	10400.0
9/15/2015	78100	41600	7790.0
9/22/2015	54200	40000	6740.0
9/29/2015	50800	38400	7870.0
10/6/2015	50800	28400	6630.0
10/13/2015	55300	27100	6300.0
10/20/2015	52800	36300	7960.0
10/27/2015	45800	33600	7690.0
11/3/2015	66900	42100	8490.0
11/10/2015	53600	31600	7510.0
11/17/2015	64600	57400	8160.0
11/24/2015	90200	32700	6840.0
12/1/2015	48700	32500	8920.0
12/8/2015	51700	34600	8550.0
12/15/2015	49000	33200	5280.0
12/22/2015	66000	33800	10400.0
12/29/2015	52200	33000	9100.0

Appendix F: PBDEs in Class A biosolids

unit: ug/kg d.w.

Sample date	Recovery	PCB209 (surrogate)	BDE28	BDE47	BDE100	BDE99	BDE154	BDE153	BDE183	BDE209	Weight (g)	TS %
11/26/2014	90.40%	102.53	0.00	249.57	0.00	248.83	23.39	38.96	8.05	180.30	1.5085	23.38%
	100.73%	114.37	0.00	302.90	59.84	285.04	25.55	39.80	8.18	339.52	1.5067	23.38%
	100.13%	110.66	7.90	271.83	56.56	244.94	24.23	30.70	6.60	373.84	1.5480	23.38%
	97.33%	109.49	6.47	252.84	52.26	238.78	24.92	25.59	6.07	379.34	1.5208	23.38%
12/3/2014	107.15%	102.05	0.00	216.66	41.07	204.69	13.64	23.74	4.57	136.12	1.5125	27.83%
	103.15%	99.19	0.00	148.98	47.24	173.50	17.21	20.00	7.19	277.85	1.4947	27.83%
	109.13%	104.49	0.00	198.74	56.02	207.00	21.14	24.25	7.56	272.26	1.5010	27.83%
	102.78%	98.19	0.00	179.45	46.72	191.99	18.53	22.74	9.53	292.28	1.5044	27.83%
12/10/2014	108.58%	109.96	0.00	202.27	36.41	196.07	15.44	19.52	4.35	124.49	1.5012	26.31%
	89.40%	90.31	0.00	204.56	36.64	204.39	15.91	20.15	5.10	127.66	1.5050	26.31%
12/17/2014	104.88%	111.25	0.00	174.33	51.02	194.17	18.40	22.04	7.66	279.08	1.4964	25.20%
	107.75%	113.95	0.00	158.57	42.19	172.79	17.24	20.62	7.72	282.46	1.5010	25.20%
	105.58%	111.53	0.00	156.32	43.76	175.52	18.46	20.41	7.90	290.13	1.5026	25.20%
12/24/2014	99.75%	98.02	0.00	193.04	33.14	185.94	14.30	18.99	4.37	86.08	1.4999	27.14%
	104.60%	102.35	0.00	181.21	42.73	191.31	17.20	21.21	6.92	267.48	1.5063	27.14%
1/7/2015	98.30%	95.11	0.00	172.93	51.38	192.74	18.58	21.67	6.36	237.35	1.5000	27.56%
	100.30%	96.54	0.00	178.96	46.59	195.46	18.24	22.23	6.74	263.44	1.5079	27.56%
	107.23%	104.03	0.00	220.37	51.83	227.91	20.91	23.41	6.38	251.58	1.4959	27.56%
1/15/2015	105.50%	93.69	0.00	173.41	38.72	186.20	16.69	21.69	5.33	214.41	1.5075	29.88%
	108.53%	97.13	0.00	145.19	36.38	152.82	16.11	18.64	5.06	169.66	1.4958	29.88%
1/23/2015	106.93%	95.71	0.00	166.72	37.44	180.70	16.36	20.81	5.06	190.48	1.5046	29.70%
	101.15%	90.33	0.00	168.61	41.35	184.39	16.83	19.87	6.63	238.53	1.5081	29.70%
	106.20%	94.81	0.00	168.13	41.25	181.63	16.96	20.38	6.85	235.89	1.5086	29.70%

	104.38%	93.63	0.00	143.14	36.44	164.72	15.94	19.11	6.57	263.73	1.5014	29.70%
1/28/2015	103.65%	93.15	0.00	134.31	30.15	145.22	13.35	17.41	4.99	156.32	1.5073	29.53%
	105.45%	94.76	0.00	159.34	39.76	175.32	16.24	20.02	7.03	236.18	1.5074	29.53%
2/6/2015	99.55%	90.56	0.00	198.18	55.17	223.27	19.92	23.27	8.28	212.69	1.5058	29.20%
	99.95%	91.27	0.00	207.21	55.86	228.78	21.18	23.56	8.36	189.47	1.5002	29.20%
2/11/2015	102.75%	95.24	0.00	186.49	37.89	202.64	18.28	23.08	9.45	229.89	1.5084	28.61%
	98.85%	92.04	0.00	198.27	53.79	206.51	20.02	22.49	8.59	178.33	1.5016	28.61%
	101.98%	94.84	0.00	207.28	53.83	217.37	20.44	23.16	8.70	211.23	1.5033	28.61%
2/15/2015	107.33%	92.78	0.00	184.22	39.20	195.28	18.22	22.91	8.56	211.43	1.5082	30.68%
	103.53%	89.86	0.00	159.34	35.85	169.95	15.21	20.18	8.55	209.22	1.5021	30.68%
3/9/2015	106.70%	90.93	6.54	149.63	34.13	163.97	0.00	18.92	8.76	221.88	1.5082	31.12%
	101.48%	87.03	8.75	174.41	44.36	187.45	16.55	20.43	9.03	228.00	1.4986	31.12%
	104.70%	89.32	7.06	161.73	39.99	176.54	16.38	19.84	8.89	253.55	1.5066	31.12%
4/1/2015	97.85%	84.24	8.93	195.05	48.60	202.60	18.73	21.39	8.31	173.65	1.506	30.85%
	106.53%	91.92	8.54	199.46	49.21	220.45	19.83	22.91	8.15	181.43	1.5026	30.85%
	107.05%	92.07	7.80	203.96	49.17	217.20	19.16	22.92	8.19	285.77	1.5076	30.85%
5/7/2015	108.35%	91.80	8.43	189.81	45.37	201.76	17.81	21.97	10.36	243.23	1.5083	31.30%
	107.15%	91.37	8.06	166.94	43.42	183.63	18.08	21.02	9.15	268.26	1.4987	31.30%
6/17/2015	107.88%	90.09	8.08	213.88	51.19	232.94	21.57	24.57	9.31	326.75	1.5019	31.89%
	105.75%	88.42	8.01	220.44	51.17	237.05	20.53	24.20	9.72	356.26	1.5002	31.89%
	107.68%	87.37	7.22	235.99	44.18	229.80	21.46	27.26	5.58	293.88	1.5459	31.89%
	97.53%	79.34	5.47	198.16	38.48	206.44	19.40	23.51	4.31	267.27	1.5418	31.89%
8/28/2015	99.78%	82.87	0.00	172.90	47.26	194.16	18.58	22.34	8.35	241.56	1.5041	32.02%
	105.83%	87.76	0.00	179.77	40.74	201.64	18.99	25.40	8.54	228.94	1.5064	32.02%
	108.23%	89.77	0.00	193.32	43.86	214.82	19.72	23.06	8.81	234.69	1.5061	32.02%
9/18/2015	102.05%	84.68	0.00	179.96	41.34	191.61	17.96	21.20	8.40	232.94	1.5041	32.05%
	92.08%	76.19	0.00	149.30	36.58	158.92	14.83	17.36	7.12	168.83	1.5082	32.05%

	107.10%	88.69	0.00	195.15	44.88	202.90	19.11	22.32	7.76	179.56	1.5072	32.05%
10/14/2015	103.95%	91.10	0.00	195.03	46.10	203.38	19.52	22.74	8.13	263.32	1.5064	30.30%
	109.88%	96.17	0.00	208.07	47.64	221.37	20.94	24.40	8.16	170.06	1.5083	30.30%
1/7/2016	107.05%	90.32	0.00	160.27	37.67	179.17	18.16	20.38	8.46	245.00	1.5074	31.45%
	87.65%	73.84	0.00	133.85	31.95	148.57	14.26	17.25	8.19	243.39	1.5097	31.45%
	107.05%	95.67	8.62	183.70	47.19	207.19	19.82	21.92	9.76	349.94	1.5095	29.65%
1/27/2016	99.93%	89.62	7.44	119.33	40.81	147.18	15.65	17.44	8.72	350.72	1.5042	29.65%
	98.18%	87.93	7.21	132.57	42.32	160.47	16.10	19.43	9.29	509.12	1.5063	29.65%
	97.33%	84.92	5.69	203.31	41.73	211.07	19.72	22.12	4.93	274.47	1.5461	29.65%

Appendix G: Spike PBDEs concentrations

Batch		**PCB209**	**BDE28**	**BDE47**	**BDE100**	**BDE99**	**BDE154**	**BDE153**	**BDE183**	**BDE209**
1		118.05%	65.04%	77.44%	85.62%	95.40%	94.36%	91.68%	79.00%	61.90%
2		117.50%	63.92%	68.36%	81.04%	82.70%	80.32%	78.40%	70.82%	59.37%
3		102.40%	80.68%	77.76%	87.90%	94.24%	92.22%	93.38%	90.92%	81.36%
4		107.60%	50.08%	56.06%	72.64%	74.80%	79.58%	77.46%	72.48%	82.95%
5		15.58%	17.04%	20.04%	38.30%	33.88%	43.28%	41.54%	40.54%	58.90%
6		96.90%	60.88%	80.66%	94.68%	98.96%	100.58%	97.48%	93.80%	97.79%
	Average	108.49%	64.12%	72.06%	84.38%	89.22%	89.41%	87.68%	81.40%	76.67%
	Std dev.	9.28%	10.99%	10.06%	8.20%	10.11%	9.17%	9.15%	10.51%	16.01%

Appendix H: Standard Operation Procedure (SOP) of Preparation, Extraction, Cleanup, and Analysis of PBDEs in Biosolids

Preparation, Extraction, Cleanup and Analysis of Polybromminated Diphenyl Ethers (PBDEs) in Biosolids

Standard Operating Procedure

University of Maryland, College Park
Civil and Environmental Engineering
Prepared by: Victor (Xuanzhao Wang)
Date Prepared: 4/4/2016

The intention of this document is to summarize, in detail, the experiment operating procedure for the determination of polybrominated diphenyl ethers (PBDEs) in DC Water biosolids.

SOP Method Overview

This SOP describes how to determine PBDEs in DC Water biosolids. The method is developed based on EPA Method 1614 (U.S. EPA, 2010b), Deng et al., 2015, Krol et al., 2012, and Giergielewicz-Mozajska et al., 2001.Total solids (TS) of biosolids are measured according to EPA Method 1684 before this procedure. Biosolids are homogenized by grinding before the PBDEs extraction by Accelerated Solvent Extraction (ASE). The extract is cleaned up by multi-layers silica gel chromatographic column. The final solution is analyzed by gas chromatography and mass spectrometry (GC-MS).

1. Biosolids Sample Preparation

1.1 Laboratory Work Area and Apparatus Clean Procedure

1.1.1 Make sure all the laboratory work area and apparatus are clean and PBDEs – free for use.

1.1.2 Wipe and clean the laboratory work surface area, such as the balance area, the experiment bench area, and the fume hood.

1.1.3 All the apparatus must be clean and dry before use.

1.2 Biosolids Samples Storage

1.2.1 All biosolids samples were collected from DC Water and stored in 250ml amber glass jars with properly labeled, including sample name, sampling date and location, project name, and the person who collected, as shown in Fig. 1.

Fig. 1. Biosolids samples stored in amber jars with labels

1.2.2 Biosolids samples were stored in freezer at -20^{o}C at USDA BARC before processing.

1.2.3 Before the experiment, the needed samples should be thawed in refrigerator at -4 ^{o}C overnight.

1.3 Biosolids Samples Preparation

1.3.1 Before the sample preparation, all samples need to reach room temperature for processing.

1.3.2 For each batch of extraction run, prepare:

- 1 sand blank
- triplicate analysis for each samples

- 1 sand spike
- 1 matrix spike

1.3.3 Prepare enough 22-mL ASE 200 extraction cells for samples to be extracted by hand-tightening a bottom cell cap onto each cell body. The symbol should be at the top of the cell. Then insert a disposable cellulose filter into the bottom of each extraction cell using the insertion tool. The cellulose filter prevents blockage of the stainless steel frit in the bottom cap. Check the end of each cap to verify that the white O-rings are in place and in good condition.

1.3.4 Weigh out a sample approximately 1.5g of wet weight into an aluminum weighing dish and record the weight to nearest 0.001 g. Transfer this to a mortar.

1.3.5 Add about 3g of hydromatrix to the sample to absorb the moisture in the sample. With the mortar and pestle, mix until a free flowing sample is observed.

1.3.6 Add enough clean/baked sand to cover the filter at the bottom of the extraction cell. Transfer the sample with hydromatrix into the extraction cell labeled with the laboratory sample ID, being careful to keep the threads clean on the cell body and cap.

1.3.7 Using an electronic syringe, add 10uL of surrogate solutions (4ug/ml of PCB-209) to each cell.

1.3.8 The sand blank is the cell with baked sand and surrogate only.

The sand spike is the cell with baked sand, surrogate, and the addition of 50uL of BDE-mix by the electronic syringe.

The matrix spike is the cell with biosolids sample, surrogate, and the addition of 50uL of BDE-mix by the electronic syringe.

Note 1: Allow surrogate and BDE-mix solutions to come to room temperature before using. Re-mix the solutions by shaking or sonicating.

Note 2: The BDE-mix contains 1ug/ml of BDE-28, -47, -99, -100, -153, -154, -183, and 10ug/ml of BDE-209.

1.3.9 Fill any void volume in the cell with clean/baked sand. Level off the sand, and use a brush to clean any remaining sand from the threads. Screw the top cap on to the cell body and hand-tighten.

<u>2. Sample Extraction</u>

2.1 Manually run the rinse procedure on the Accelerated Solvent Extraction (ASE) system 200 several times before beginning an extraction run.

2.2 Load the tray slots in numerical order with all of the full sample cells. Hang the cells vertically in the tray slots from their top caps.

2.3 Load 60-mL vials as rinse tubes into the four open slots, labeled R1 through R4.

2.4 Load a 60-mL collection vials labeled with the laboratory sample ID onto the corresponding vial tray positions, shown in Fig. 2.

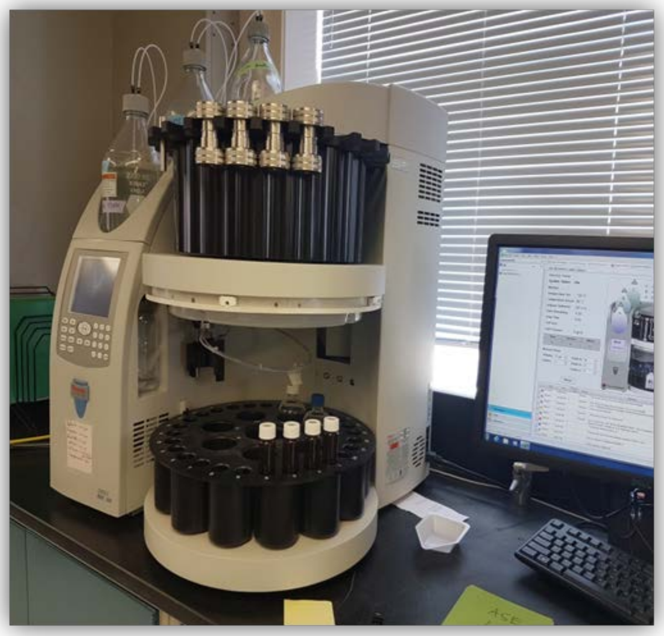


Fig. 2. ASE 200 system with loaded cells and amber vials

Note: During the extraction process, sensors determine if a vial is present, contains 1 mL of solvent, or is full. Because of this, vial labels must be placed where they do not block areas of the vial read by the sensors.

IMPORTANT: Make sure that the gas (N2) supply pressure is ≥150 psig. The ASE unit may not extract samples reliably with the N2 supply pressure below 150 psig.

2.5 Load the method and begin the extraction run.

Dionex ASE Parameters:

Preheat time: 5 minutes

Temperature: 120ºC

Pressure: 2000 psi

Static time: 10 minutes

Flush %: 60%

Purge time: 200 seconds

Cycles: 2

Solvent A, % 20 Acetone

Solvent B, % 80 Hexane

Note: Ensure the waste container is properly connected and labeled.

2.6 When complete, allow the extracts to cool to room temperature before proceeding with filtering. Samples may be stored in the freezer once caps are replaced with new ones.

2.7 Discard the samples from the cells into the waste container. And keep the extract in amber vails in -4 ^{o}C refrigerator for further cleanup.

3. Extract Cleanup

3.1 Before the process, the extract in amber vials need to reach room temperature. Prepare clean corresponding amber vials with the proper labels to take the cleaned extract after cleanup process.

3.2 For each extract, prepare one clean chromatographic column (300-mm long x 22-mm ID, with coarse-glass frit, 300-mL reservoir, and fluoropolymer stopcock) and fix it on the stand in the fume hood.

3.3 Place about 2cm long of glass wool on the bottom of the column Then weight and put the materials in the order as shown in Fig. 3.

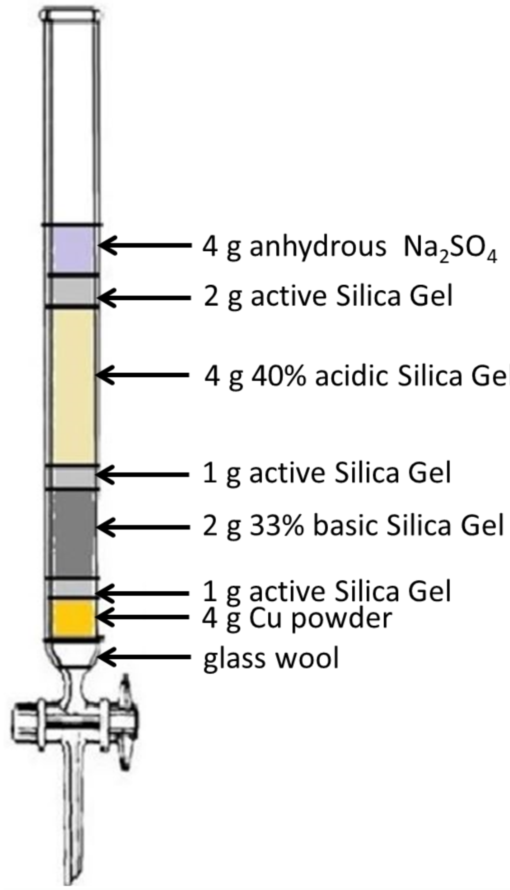


Fig. 3. Weight and the order of cleanup materials in chromatographic column.

Note 1: The activated silica gel is 100-200 mesh, baked at 180 °C for a minimum of 1 hour, cooled in a desiccator, and stored in a precleaned glass bottle with screw-cap that prevents moisture from entering.

Note 2: Acid silica gel (40% w/w) is 100g of activated silica gel well-mixed with 67g of concentrated sulfuric acid.

Note 3: Basic silica gel (33%) is 100g of activated silica gel well-mixed with 50ml of 1N sodium hydroxide solution.

3.4 After pack all materials in chromatographic column, rinse through the column with 20ml of n-hexane. Use gentle air flow drain out the n-hexane.

3.5 Transfer the uncleaned extract to the column with Pasteur pipette. Apply gentle air flow to grain the extract into corresponding clean labeled amber vial.

3.6 Use 5ml of n-hexane to rinse the amber vial for uncleaned extract and Pasture pipette. Rinse three times and put into chromatographic column.

3.7 Put 10ml of n-hexane into the chromatographic column and rinse the left PBDEs inside the column into amber vial with gentle air flow. Cap the amber vial.

3.8 After clean all extract, use Zymark TurboVap evaporator to completely dry the samples. Adjust the bath temperature to about 40°C, and set the vials with the sample extracts into the evaporator.

3.9 Set the pressure to 0.6-0.8 psi, and run for 45 minutes or until completely dry.

3.10 Add 1.0mL of n-hexane to each vial and vortex until dissolved completely.

3.11 Prepare 1.0mL clean GC vials with properly labeled for GC-MS analysis:

3.11.1 Transfer 1.0ml of dissolved extract into clean amber GC vials with Pasteur pipette.

3.11.2 Use electronic pipette add 10uL of Internal Standard (4ug/ml PCB-138) in each amber GC vials. Cap the GC vials and send to GC-MS for analysis.

Note: Internal Standard (4ug/ml PCB-138) need to reach room temperature before use.

4. Gas Chromatography and Mass Spectrometry (GC-MS) Analysis

4.1 Before put GC vials on the analysis tray, vortex the vials to mix well.

4.2 Before the sample batch, put PBDEs standards vials for GC-MS to make calibration curves.

4.3 The GGC-MS is 6890N/5975 with negative chemical ionization in selected monitoring mode and DB-5MS capillary column. The instrument is shown in Fig. 4.

4.4 The injection volume is 1.0uL and the running time is 22 minutes. Pressure is 4.80 psi. And the temperature is about 300 ˚C.

4.5 After the analysis finish, put the PBDEs standards vials and sample vials in designated refrigerators.

Fig. 4. GC-MS 6890N/5975 for PBDEs analysis.

5. Cleaning Procedure

5.1 To clean the apparatus after experiment, all apparatus need to be washed with brush and tap water three times, then be rinsed with DI Water three times.

5.2 For ASE Extraction cells:

5.2.1 Unscrew an end cap from the extraction cell body and remove the extracted sand/soil/hydromatrix. Discard the sand/soil/hydromatrix into a waste container.

5.2.2 Unscrew the other end cap. Remove and discard the cellulose filters from the end cap.

5.2.3 Let the cell bodies and end caps soak in soapy water (Contrad or equilavent).

5.2.4 Scrub cell bodies and end caps, and rinse well with DI water.

5.2.5 Soak end caps in DI water for at least 30 minutes, then rinse well with DI water again.

5.2.6 Rinse cell bodies and end caps with acetone and let air dry.

5.3 For collection vials, caps, funnels and other glassware:

5.3.1 Soak in soapy water (Contrad or equivalent) for at least 4 hours.

5.3.2 Scrub with a brush and rinse thoroughly with tap water.

5.3.3 Rinse 3 times with DI water, and let air dry.

5.3.4 Rinse vials with acetone and let air dry.

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