A study of multi-stage sludge digestion systems

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Abstract

Various combinations of multi-stage thermophilic and/or mesophilic anaerobic sludge digestion systems were studied to evaluate their solids reduction, odor generation after centrifugal dewatering and indicator organism reduction in comparison to single-stage thermophilic and/or mesophilic anaerobic digestion systems. Pre-aeration of sludge in a thermophilic temperature was also tested followed by single or multi-stage anaerobic digestion systems. It was found that multi stage systems were capable of greater solids removal and placing thermophilic system in multi stage system enhanced indicator organism destruction below EPA Class A biosolids requirement. However, all the digestion systems in the study showed less than 3 log reduction of indicator organism DNA/g solids, which was much smaller than indicator organism reduction measured by standard culturing method. It was also found that the thermophilic anaerobic digestion system could increase organic sulfur-based odors from dewatered biosolids while placing a mesophilic digester reduced odors. It was exclusively observed from sludges containing high sulfate such as ones in this study.

A combined anaerobic and aerobic sludge digestion system was also studied to evaluate their solids and nitrogen reduction efficiencies. The aerobic digester was continuously aerated to maintain dissolved oxygen level below 1 ppm and intermittently aerated. It was found that 90 % or more nitrogen removal was possible at the aerobic SRT greater than 3 days and the optimum aeration ratio could be determined.

- Appreciation remarks -

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- 4ADT / 4Tapered — 4 stage Anaerobic Digestion with Tapered temperature configuration
- 4TAD / 4Ther — 4 stage Thermophilic Anaerobic Digestion
- AER — Aerobic sludge digestion
- ANA — Anaerobic sludge digestion
- Alum — Aluminum sulfate
- BESA — Bromoethanesulfonic Acid
- CER — Cation Exchange Resin
- Ch — Christiansburg wastewater treatment plant
- COD — Chemical Oxygen Demand
- CST — Capillary Suction Time
- DMDS — Dimethyl disulfide
- DMS — Dimethyl sulfide
- DMTS — Dimethyl trisulfide
- DO — Dissolved Oxygen
- GC — Gas Chromatography
- Meso / MAD — Mesophilic anaerobic digestion
- MPN — Most Probable Number
- MS — Mass Spectrometry
- MT — Methanethiol
- OPD — Optimum Polymer Dose
- PCR — Polymerase Chain Reaction
- PF — Peppers Ferry wastewater treatment plant
- SRB — Sulfate-reducing Bacteria
- SRT — Sludge Retention Time
- ThAer — Thermophilic Aerobic digestion
- Thermo / TAD — Thermophilic anaerobic digestion
- TKN — Total Kjeldahl Nitrogen
- TPAD — Temperature Phased Anaerobic Digestion
- tRFLP — terminal Restriction Fragment Length Polymorphism
- TS — Total Solids
- tVOSC — total Volatile Organic Sulfur Compounds
- VS — Volatile Solids

Chapter 1. Executive Summary

There was a report regarding a successful operation of a 4 stage thermophilic anaerobic digestion system that generated much less odorous Class A biosolids (Krugel *et al.*, 1998). Higgins *et al.* (2007) also stated that multi-stage anaerobic digestion system could eliminate sludge odors and reduce indicator organisms significantly. Based on these research results, various combinations of aerobic/anaerobic digestion systems under different temperature conditions were studied to validate advantages of multi-stage sludge digestion systems on stabilization of biosolids (e.g. less solids, easier dewatering, smaller odor generation after dewatering, and more removal of indicator organisms). The following two paragraphs are brief summaries for multi-stage digestion study.

Multi-staged anaerobic sludge digestion processes (Chapter 3) [Revisions submitted to ASCE Journal of Environmental Engineering on June 29, 2010]

Two multi-staged anaerobic digestion systems, a 4 stage-thermophilic anaerobic digestion (4TAD, all at 55 ℃) and a 4 stage-anaerobic digestion with a tapered temperature configuration (4ADT, 55, 49, 43 and 37 ℃, respectively), were studied to evaluate their solids, volatile organic sulfur compounds and indicator organism (E. coli and fecal coliform) reduction potentials. The 4TAD system removed significantly more volatile solids from sludges than the 4ADT system (6%). However, the dewatered sludge cakes from the 4ADT system generated less organic sulfur compounds than those from the 4TAD system. Both multi-stage systems showed better digestion efficiencies than

single-stage mesophilic or single-stage thermophilic anaerobic digesters at the same overall retention time. However, the lowest organic sulfur compounds were observed from the single meso system. Both multi-stage anaerobic digestion systems failed to dramatically remove DNA of the indicator organism, E. coli, quantified by quantitative polymerase chain reaction (qPCR) even though the indicator organism densities measured by standard culturing methods satisfied EPA Class A biosolids requirements.

Digestion performance of various combinations of thermophilic and mesophilic sludge digestion systems (Chapter 4) [Accepted to Water Environment Research]

Various combinations of single and multi-stage anaerobic and aerobic/anaerobic digestion system were studied to evaluate their solids reduction potential along with capabilities to control sulfur based biosolids odor compounds. All the multi-staged digestion systems removed more volatile solids than the single stage anaerobic digestion systems even at the same overall retention time. However, digestion systems with mesophilic digestion as the final stage showed a much lower headspace organic sulfur contents in the dewatered biosolids than the systems with thermophilic digestion as the final stage. This observation leads to the conclusion that placing a mesophilic anaerobic digestion system at the end of multi-stage digestion systems will enable greater sulfur based odor reduction from dewatered biosolids along with greater solid reduction than single stage mesophilic or thermophilic digestion systems.

In the meantime, high biosolids cake odors were measured from thermophilically digested biosolids. In spite of high solids reduction seen from thermophilic systems,

thermophilic biosolids cakes produced much greater cake odors than mesophilic biosolids cakes. In order to learn more about high cake odor generation from thermophilic biosolids cakes, three batch-digestion experiments were carried and some important observation was made. The following paragraph is the brief summary of the biosolids odor study.

How does dewatered biosolids odor increase after thermophilic sludge digestion?: Impact of lignin and sulfate to dewatered biosolids odors (Chapter 5) [Working paper]

Greater amounts of organic sulfur compounds were measured from thermophilically digested and dewatered biosolids when accompanied with excess lignin and sulfate in the feed sludges. High organic sulfur generation was thought to be caused by active methylation of sulfide during anaerobic incubation of thermophilic biosolids cakes and poor aceticlastic methanogenic activities after thermophilic anaerobic digestion. Aceticlastic methanogens are major organic sulfur degraders. Sulfate reducers degraded some organic sulfur compounds from thermophilically digested and dewatered biosolids.

Biological nutrient removal was also studied for the combined anaerobic and aerobic sludge digestion systems. In order to promote aerobic denitrification, the aerobic digestion system was aerated intermittently (i.e. aeration on and off in a cycle). Totally 18 different aeration modes were tested and one optimum mode was determined based on the experiment results. The following two paragraphs are the findings from the

nutrient removal study.

A study of a combined anaerobic/aerobic system: enhanced nitrogen removal by a continuous aeration in the aerobic digestion system (Chapter 6) [Working paper]

A combined anaerobic/aerobic sludge digestion system was studied to test its solids and nitrogen removal efficiencies. After a steady performance of the laboratory anaerobic digester (20 day retention time) was confirmed, effluent from the anaerobic digester was fed to aerobic digesters that were operated at retention times from 2 to 5 days. The anaerobic system was fed with a mixture of primary and secondary sludge from a local municipal wastewater treatment plant. Feeding was done once per a day. The aerobic reactor was continuously aerated with ambient air to maintain a peak dissolved oxygen concentration at 1.1±0.3 ppm. More solids and ammonia reduction was observed from the aerobic system operated for at the longest retention time (5 days) but higher effluent nitrite/nitrate was also measured. Most of total Kjeldahl nitrogen was removed via ammonia oxidation but less than 10 % of removed ammonia was measured as oxidized nitrogen throughout the continuous aeration study. Aerobic denitrification was suspected as the major ammonia removal mechanism in the continuously aerated digestion system operated at low dissolved oxygen concentration.

A study of a combined anaerobic/aerobic system: enhanced N removal by alternating aeration modes in the aerobic digestion system (Chapter 7) [Working paper]

A combined anaerobic/aerobic sludge digestion system was studied to determine the optimum aeration condition in the aerobic system for maximum solids and nitrogen removal with lower effluent oxidized nitrogen. The study was carried for two phases. After a steady performance was observed from the anaerobic digestion system, continuous aeration was applied to the subsequent aerobic system operated for the retention time from 2 to 5 days. Feeding was done once per a day. More solids and ammonia reduction was observed from the aerobic system operated for the longer retention time but higher effluent oxidized nitrogen was also measured. Three days or longer retention times were chosen for the following study since 90% or greater ammonia removal was achieved at the retention times longer than 3 days. As the second half of study, combinations of six aeration modes (50% aeration time for 30 minutes cycle, 66.7% aeration time for 45 minutes cycle, 75% aeration time for 60 minutes cycle, 50% aeration time for 40 minutes cycle, 66.7% aeration time for 60 minutes cycle, and 50% aeration time for 60 minutes cycle) and three retention times (3, 4, and 5 days) were applied to the aerobic system to determine the optimum aeration on/off condition for maximum solids and ammonia removal and lower effluent oxidized nitrogen. Feeding was done continuously. Among 18 alternating aeration modes, 66.7% aeration time (60 minutes cycle) for 4 day retention time was determined as the optimum, which achieved about 7% additional volatile solids reduction, 90% ammonia removal and generated 2.6 mg /L effluent oxidized nitrogen.

A DNA fingerprinting method (e.g. terminal Restriction Fraction Length Polymorphism, tRFLP) was applied to some of aerobic effluents under optimum aeration modes and the result is presented in Appendix 2. Based on the result, greater ammonia removal was

achieved from the aerobic reactor systems, of which tRFs were more evenly abundant but showed more diverse base pairs of tRFs. However, more research should be warranted to validate this claim.

Co-conditioning of field digested biosolids was studied as a separate research topic. In this study, the optimum combination of aluminum sulfate and high molecular-weight cationic polymer was determined to promote better biosolids dewatering properties and lower biosolids cake odor generation. The following paragraph is the brief summary of the co-conditioning study.

Combination of coagulating agents (Aluminum sulfate and Cationic polymer) for biosolids dewatering and its impact to odors (Appendix) [Accepted to KSCE Journal of Civil Engineering on June 16, 2010]

A combination of two conditioning agents, aluminum sulfate and cationic polymer were applied to dewater anaerobically digested biosolids to study their impact to dewatering properties of biosolids and to sulfur-based odor generation from dewatered biosolids. Lower sulfur-based odor compounds were measured from dewatered biosolids conditioned with greater amount of aluminum sulfate (alum) while higher cationic polymer dose resulted in more sulfur-based odors from dewatered biosolids. More alum deteriorated biosolids dewatering properties while more cationic polymer improved dewatering rates for biosolids. Overall data suggest that there exists an optimum combination of alum and cationic polymer dose for better biosolids dewatering characteristics and less sulfur-based odor generation from dewatered biosolids.

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Chapter 2. Literature review

Will the thermophilic anaerobic digestion and staged anaerobic digestion systems be better alternatives for the conventional mesophilic anaerobic digestion?

Sludge generation and its impact to the municipality

Sludge is the remaining slurry from treated wastewater and is usually concentrated for an additional treatment or disposal (Cheremisinoff, 1994). As the treatment options for wastewater sludges, aerobic/anaerobic digestion, dewatering, chemical treatment, composting, etc are widely used while the disposal options include landfilling, incineration, land application, etc. Ever since Clean Water Act, sludge treatment and disposal in the United States has become more regulated and restricted. Due to high cost and possible detrimental impact to environment, landfilling and incineration are adopted by very limited chances (Cheremisinoff, 1994). Land application also appears to be less feasible due to possible soil contamination by toxicity, heavy metal, high nutrient as well as public concerns about vector attraction, odor, pathogens, etc (Cheremisinoff, 1994).

Single-stage anaerobic sludge digestion

Limited sludge treatment and disposal options produced high demands for the better municipal sludge handling strategies so that various advanced sludge digestion methods were studied and exercised in the wastewater treatment industries. Usually these advanced strategies comprise combinations of aerobic/anaerobic and thermophilic (50-60℃) / mesophilic (35-37℃) digestion systems operated at various retention times.

Each of the sludge digestion schemes has its advantages and disadvantages. Aerobic digestion (AER) requires higher energy and nutrient inputs than anaerobic digestion system (ANA), whereas AER can remove a greater amount of sludge solids at shorter retention time than ANA. However, ANA has shown the capabilities to degrade xenobiotic and aromatic compounds which AER may not be able to remove. Likewise, thermophilic anaerobic digestion (TAD) systems have shown enhanced digestion capability owing to the higher kinetic rates as a result of high temperature digestion (Zahler *et al.*, 2007) and greater pathogen removal to meet EPA Class A biosolids requirement (U.S. EPA, 1994), whereas mesophilic anaerobic digestion (MAD) systems, in spite of their less efficient sludge solid reduction and much smaller pathogen removal rate, have been widely used for decades due to less energy requirement and better stability. Many reports have claimed process instability of TAD that was associated with high volatile fatty acid production, odors and high ammonia generation (Kim *et al.*, 2002).

Multi-stage anaerobic sludge digestion

Combinations of thermophilic and mesophilic anaerobic sludge digestion systems have been studied to facilitate advantages of both digestion systems (Effenberger *et al.*, 2006; Han and Dague, 1997; Iranpour *et al.*, 2006; Nielson *et al.*, 2004; Song *et al*., 2004). Most of these investigators studied a combination of thermophilic and mesophilic digestion systems or temperature phased anaerobic digestion systems (TPAD) while Effenberger *et al.* (2006) carried out studies with a three-stage system, a combination of mesophilic, thermophilic and mesophilic system. They all agreed that multi-stage

digestion systems were capable of greater solids reduction than single-stage mesophilic systems. Moreover, placing a thermophilic system in these multi-stage digestion systems improved indicator organism reduction capabilities along with more extensive organic removal from the influent sludges (Han and Dague, 1997). Higgins *et al*. (2007) suggested that the staged thermophilic anaerobic digestion can greatly reduce odors associated with organic sulfur compounds and eliminate indicator organisms. However, poor dewatering properties associated with TPAD systems have been also reported. According to Bivins and Novak (2001), the poor dewatering properties of thermophilically digested sludge were not recovered by the subsequent mesophilic digestion system.

Sludge digestion and its impact on biopolymer

Extracellular polymeric substances (EPS) are an accumulation of biological organics (protein and polysaccharide), cell lysis materials, etc. They are thought to bind sludge flocs due to their glue-like characteristics and to determine the structure of floc materials. Its precise chemical structure and roles in sludge flocs have not been fully determined. One recent EPS model was suggested by Higgins and Novak (1997) that polysaccharides are bound by lectin-like proteins and these biopolymer flocs are interconnected by divalent cations such as calcium and magnesium. This EPS model had a new EPS research tool developed by Park and Novak (2007). They compiled five different extraction methods and showed their uses in EPA studies by extracting biopolymer bound to different cations such as divalent cations, iron and multi-valent cations.

The fate of EPS after various sludge digestion processes is also as important as the composition of EPS in sludge flocs. Novak and Park (2004) observed that greater solids reduction often resulted in more solution biopolymer during anaerobic digestion of sludges. Moreover, greater solution biopolymer in the digestion system was found to cause poorer dewatering properties of digested sludges (Novak *et al*., 2003). However, greater solids reduction from sludges did not necessarily result in less odor generation from anaerobically digested and dewatered sludges (Muller *et al*., 2007). It is reported that more than half of the volatile solids in activated sludge is comprised of protein (Forbes *et al.*, 2004) and more removal of proteins was thought to result in less sludge odors. The major odor-causing compounds from dewatered sludges were generated by the degradation of sulfur containing amino acids, building blocks of proteins (Forbes *et al.*, 2004; Higgins *et al.*, 2006 and 2008).

Odor causing compounds and problems associated with them

Odor causing compounds from an AER were listed by Dincer and Muezzinoglu (2008). Their study revealed that odors from AER were caused by mainly reduced sulfur compounds, especially H_2S. In addition, they observed that aldehydes like propanal and decanal and monoaromatic compounds such as toluene were also produced in significant amount.

The odor compounds from ANA can be sorted into two groups, sulfur-based odor compounds and other protein based compounds, primarily amines (Muller *et al.*, 2004).

Amine based odor chemicals usually originate from protein and amino acid degradation,

which include ammonia, di and tri-methylamine, ethylamine, indole and skatole. Their malodorous characteristics are often described as fishy, fecal or pungent (Bitton, 2005). Other potential odor causing compounds in digested biosolids include toluene and creosol.

Among the malodorous chemicals, sulfur-based odor compounds or volatile organic sulfur compounds (VOSCs) have been reported as major odor constituents of anaerobically digested and dewatered sludges (Erdal *et al.*, 2008, Higgins *et al*., 2006 and 2008, Muller *et al*., 2004).

Reduced sulfur compounds include H_2S and VOSCs such as methanethiol (MT), dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS). These sulfur-based compounds may cause acidification of forests and lakes vastly, while they also attribute to local odor problems from compost sites, wastewater treatment plants and paper and textile industries (Lomans *et al.*, 2002). Some properties of volatile sulfur compounds related to the human health were also found from the literature (Table 1). At much lower concentration, VOSCs are important ingredients of perfume or flavor in beer and cheese. However, they can be a nuisance to local communities or even a threat to the human health at much higher concentration. In this regard, the generation and removal of volatile sulfur compounds from wastewater sludge should be understood to devise countermeasures for enhanced odor removal from dewatered sludge cakes or the accidental emission to the localities, which may cause serious protests or claims.

Table 1. Properties of volatile sulfur compounds.

Compound	Boiling point (℃)	Solubility (g/L) at 20℃	Lethal concentration (LD_{50} in ppm)	Maximum acceptable concentration (ppm)	Odor threshold (ppm)
H_2S	- 60.3	4	444	10	8.5 - 1000
MT	6.0	24	675	0.5	0.9 - 8.5
DMS	37	20	40250	20	0.6 - 40
DMDS	107-110	Insoluble	5	< 20	0.3 - 3.6

* MT: Methanethiol, DMS: Dimethyl sulfide, DMDS: Dimethyl disulfide

** Lomans *et al.* (2002)

Generation of VOSCs

There are two VOSC generation mechanisms available in literatures, which are degradation of sulfur containing amino acids such as cysteine and methionine (Higgins *et al*., 2006; Kiene *et al*., 1990; Kiene and Hines, 1995; Ramsay and Pullammanappallil, 2001; Zinder and Brock, 1978a) and methylation of sulfide or methylated sulfur compound such as MT (Drotar *et al*., 1987; Kiene and Hines, 1995; Lomans *et al*., 1997, 1999a and 2002).

Kiene and Hines (1995) reported that the addition of methionine to anoxic peat samples resulted in rapid accumulation of MT and this led to the production of DMS. Soda *et al.* (1983) also demonstrated the deamination of methionine under anaerobic condition by *Pseudomonas putida, Ps. Taetrolens* and *Ps. Desmolytica.* They all showed high activity of methionine γ-lyase, which enabled methionine degradation to MT, NH_3 and α-ketobutyrate. The generation of MT from sulfur containing amino acids was also described by Ramsay and Pollammanappallil (2001). According to the authors, these sulfur containing amino acids can be degraded by Stickland fermentation using the

following pathways:

$C_3H_6O_2NS$ (Cys) + $2H_2O \rightarrow CH_3COOH + CO_2 + NH_3 + H_2S + 1/2H_2$ + ATP

$C_5H_{11}O_2NS$ (Met) + $2H_2O \rightarrow CH_3CH_2COOH + CO_2 + NH_3 + CH_3SH + H_2$ + ATP

Higgins *et al*. (2006) summarized the VOSC generation mechanism of dewatered sludge cakes. According to the authors, sulfur-containing amino acids such as cysteine and methionine in anaerobically digested sludges are the precursors of H_2S and MT from sludge pellets, respectively. However, they failed to correlate VOSC generation from dewatered mesophilic sludges with protease activities (L-Leucine-Aminopeptidase), amount of solid-bound cysteine, sludge digestion time, etc (Higgins *et al.*, 2008). Only the amount of solid-bound methionine in the mesophilically digested sludges was shown to be proportional to peak VOSCs from the dewatered sludge cakes.

Bak et al. (1992) formulated a DMS production from methoxylated aromatic compounds (e.g. syringate) in accordance with their observation on the anaerobic homoacetogenic isolates from marine mud samples and they formulated a DMS generation sequence as follow,

$R\text{-}O\text{-}CH_3 + H_2S \rightarrow R\text{-}OH + CH_3SH$

$R\text{-}O\text{-}CH_3 + CH_3SH \rightarrow R\text{-}OH + (CH_3)_2S$

Sum: $2R\text{-}O\text{-}CH_3 + H_2S \rightarrow 2R\text{-}OH + (CH_3)_2S$ (R = aromatic residue)

It was suggested that O-demethylation of methoxylated aromatic compounds along with the catalytic effect of methyl transferase excreted by homoacetogens such as *Acetobacterium woodii* and *Clostridium thermoaceticum* may be a possible VOSC formation mechanism in the natural water systems (Kiene and Hines, 1995). Lomans *et al*. (1997) also showed methylation of sulfide and MT with methoxylated aromatic compounds such as syringate or 3,4,5-trimethoxybenzoate under H_2S-rich freshwater sediment samples. It seems that high sulfide condition induced a sulfide-mediated O-demethylation of the methyl groups, which gave rise to the high levels of MT and DMS (Kiene and Hines, 1995; Lomans *et al*., 1997). Dimethyl disulfide (DMDS) can be generated by the oxidation of MT (Lomans *et al.*, 2002). Drotar *et al*. (1987) suggested that pH 7.4 is the optimum pH condition for MT generation from natural water system according to their series of incubation tests with pure cultures. The authors also speculated that MT may be produced by an enzymatic methyl transfer onto sulfide with the catalytic help from S-adenosylmethionine-dependent thiol methyltransferase.

Lomans *et al*. (1997 and 2002) stated that the main source of methoxylated aromatic compounds in the freshwater system may be degradation products of lignin, the most abundant methoxylated biopolymer on earth. In addition, there are a number of reports suggesting the lignin degradation into mono aromatic compounds (Colberg and Young, 1985; Chen *et al.,* 1987) along with VFA (Chen *et al.,* 1987) under mesophilic anaerobic digestion condition at much slower rate (Benner *et al*., 1984). Thermophilic anaerobic biodegradation of lignin and lignocellulose was also studied by Benner and Hodson (1985), who recovered up to 4% of synthetic and natural lignin and about 20% of Kraft lignin in solution form at the elevated temperature.

Removal of VOSCs by methanogens

There are a number of observations that have described the role of methanogens as major degraders of VOSCs in natural water systems (Zinder and Brock, 1978b; Lomans *et al*., 1999a).

Zinder and Brock (1978a) showed the formation of methane, carbon dioxide and hydrogen sulfide from [methyl-^{14}C] methionine with MT as an intermediate. Kien *et al*. (1986) demonstrated methanogenic degradation of DMS with pure cultures isolated from an anoxic sediment sample and formulated the following DMS metabolic sequence.

$$(CH_3)_2S + H_20 \rightarrow 1.5CH_4 + 0.5CO_2 + H_2S \qquad \Delta G^o_f = -73.8 \text{ kJ/mol}$$

An irregular coccoid methanogen was isolated from marine sediments, which was able to grow on MT as a sole energy source (Finster *et al*., 1992). It was also able to degrade DMS with significant intermediate MT production. The following equation was suggested as a sequence of MT degradation by this newly isolated methanogen.

$$4CH_3SH + 3H_20 \rightarrow 3CH_4 + HCO_3^- + 4HS^- + 5H^+ \qquad \Delta G^o_f = -36.9 \text{ kJ/mol}$$

Tallant and Krzycki (1997) isolated a Coenzyme M (CoM) methylase, methylthiol:CoM methyltransferase, which enables acetate-grown *Methanosarcina bakeri* to utilize methylated sulfur compounds such as DMS or methylmercaptopropionate for the

methanogenesis. This CoM enzyme can demethylate corrinoid cofactors, which are final methyl transferers of the methanogenesis mechanism, while methylated sulfur compounds are used to re-methylate these cofactors.

Lomans *et al*. (1999b) found a novel methylotrophic methanogen, *Methanomethylovorans hollandica* gen. nov., sp. nov., from a eutrophic pond sediment. This obligate methylotrophic methanogen could utilize methanol, methylamines, MT and DMS to produce methane. Several freshwater sediments were also tested to quantify VOSC degrading microbes and to learn about their identification by Lomans *et al*. (2001). The authors found that major MT degraders in the samples were obligate methylotrophic methanogens, which appeared to be *M.hollandica* in accordance with selective 16S rRNA amplification tests.

Higgins *et al.* (2006) concluded that methanogens were mainly responsible for VOSC degradation after observing dramatic increase of VOSCs in the headspace of the incubation bottles containing bromoethanesulfonate (BESA) treated sludge cakes. BESA is a methanogenesis inhibitor.

VOSC removal by sulfate-reducing bacteria (SRB)

SRBs can also degrade VOSCs, but they depend on the availability of sulfate or sulfide in the environment.

Lomans *et al*. (1999a) observed MT and DMS degradation from MT- or DMS-amended freshwater sediment slurry with BESA. Furthermore, high sulfide (up to 200 mg/l)

environment was observed to make SRBs outcompete methanogens for VOSC uptake in the freshwater sediments (Lomans *et al.*, 2002). Tanimoto and Bak (1994) also found oxidation of MT and DMS to carbon dioxide and sulfide with sulfate or nitrate as electron acceptors in the presence of sulfate reducers that were isolated from thermophilic fermenter sludge.

The competition between methanogens and SRBs upon DMS in the anaerobic estuarine sediments was reported by Kien *et al*. (1986). At high concentration (in mM), methanogens showed noncompetitive superiority in DMS uptake to SRBs, while SRBs gained an advantage to utilize DMS at less than 10 micromolar concentration. This may be due to greater substrate affinity (low K_m) of SRBs for carbon sources. Lomans *et al*. (1999a) suggested the interspecies H_2 transfer between DMS metabolizing methanogens and hydrogenotrophic SRBs, providing that DMS degradation was retarded from the freshwater sediment slurry incubations with BESA or BESA plus either sulfate or nitrate under H_2 atmosphere.

SRBs may be less involved in VOSC metabolism in the anaerobic sludge digestion systems. In spite of their greater affinity for carbon sources and H_2 (Kien *et al*., 1986; Isa *et al.*, 1986), SRBs are much less competitive in the anaerobic digestion environments than methanogens. Their non competitiveness seems to be originated from SRB's poor floc-forming characteristics together with organic rich environment of anaerobic digesters that offsets SRB's greater substrate affinity (Isa *et al.*, 1986). However, high sulfur environment (e.g. $COD/SO_4^{2-} \leq 4$) may enhance SRBs' VOSC metabolism.

Temperature Vs. Microbial diversity

Microbial communities vary in accordance with different anaerobic digestion conditions. Temperature, especially, is one of the widely used classification criteria for the microbial communities, likely psychrophiles, mesophiles, thermophiles and hyperthermophiles. Thermophilic and mesophilic microbes are of interest in this literature review since most of anaerobic digestion systems are operated at either of these temperature conditions.

It seems that methanogens, which are responsible for the solid removal by converting organic materials into biogas, show little diversity in the TAD compared to the MAD environment. However, dominant thermophilic methanogenic species vary in accordance with different digestion conditions such as sulfate concentration in the influent, temperature, pH, feed composition, etc (Colleran and Pender, 2002; Freeman *et al.*, 2008; Pender *et al.,* 2004; Wilson *et al.,* 2008; Zinder *et al.*, 1984).

It was suggested that *Methanosarcina* sp. like thermophilic aceticlastic methanogens were highly involved in the start-up of the TAD (58℃) with the ground lignocellulose waste as substrate but *Methanothrix* like thermophilic aceticlastic methanogens were mostly observed from the same digester at much later digestion time (Zinder *et al.*, 1984).

Wilson *et al.* (2008) observed retardation of thermophilic aceticlastic methanogenic activities during the anaerobic digestion at 57.5℃. They postulated that acetate

oxidation and hydrogenotrophic methanogenesis were more thermodynamically favorable than aceticlastic methanogenesis under the thermophilic anaerobic digestion condition at the temperature greater than 55℃. Similar thermal preference of methanogenic communities was also observed by Freeman *et al.*, (2008). They found that methanogens utilizing various range of substrate (H_2/CO_2, formate and acetate) such as *Methanobacterium beijingense*, *Methanosarta harundinacea* and *Methanosaeta concilii* were the most archaeal operational taxonomic units (OTU) in the MAD with excess sulfate (1.8 – 5.3 g/L). On the other hand, hydrogenotrophic methanogens such as *Methanothermobacter thermautortophicus* and *Methanobacteriales* were the primary archaeal species in the TAD with the same sulfate concentration in the system.

It is interesting that % total COD flux in the MAD with high sulfate was mostly directed to sulfidogenesis but acetogenesis was the greatest COD flux recipient in the TAD with high sulfate (Freeman *et al.*, 2008). Colleran and Pender (2002) and Pender *et al.* (2004) also observed inhibition of aceticlastic methanogenesis in the TAD with and without high sulfate in the influent. According to Colleran and Pender (2002), aceticlastic methagnogenesis was dominant under MAD with and without sulfate but hydrogenotrophic methanogen like *Methanobacterium thermoautotrophicum* were mainly responsible for methanogenesis during thermophilic anaerobic digestion operated at COD/SO_4^{2-} around 4. However, aceticlastic methanogens showed great susceptibility to high sulfate environment (e.g. COD/SO_4^{2-} ~3), which seemed to happen due to sulfide inhibition with inhibition level at 220-980 mg total sulfide/L (69-150mg free H_2S/L) and pH range at 6.5 - 8.0 (O'Flaherty *et al.*, 1998 and 1999). Pender et al. (2004) also found that *Methanosaeta* spp. was the major OTU in the MAD systems

regardless of sulfate contents in the influent, whereas it was *Methanocorpusculum parvum* and *Methanobacterium thermoautotrophicum* that was the most abundant OTU in no sulfate and sulfate ($COD/SO_4^{2-} \sim 4$) fed TAD systems, respectively.

Thermophilic (55℃) syntrophic metabolism of methanol, which appears to go through a similar degradation pattern of MT (Lomans *et al.*, 2002), was studied by Paulo *et al.* (2004). They carried out the study using two upflow anaerobic sludge bed reactors (UASB), one with sulfate at the COD to sulfate ratio around 10 in the influent and the other without sulfate. They observed that the vancomycin, a homoacetogenesis inhibitor, could significantly inhibit methanol metabolism in the sludge sample incubated in bottles, where H_2 and CO_2 in the initial headspace were flushed out, and speculated that the methanogenic and sulfidogenic metabolisms of methanol could be carried via the conversion of methanol to H_2/CO_2 by homoacetogens as the first step. A strong competition between methanogens and sulfate reducers was also observed from UASB with sulfate in the influent. Interestingly acetate concentration was not critical in both of reactors even though the authors agreed on the detrimental impact of the acetate accumulation to the reactor stability.

Nitrogen removal by single aerobic sludge digestion system

Nitrogen (N) removal from wastewater has been one of major topics among environmental scientists and engineers because this element is a direct cause of eutrophication and water quality deterioration. Anthropogenic activities have been known to be the major source of nitrogen discharge into natural systems. In recent years, the US Environmental Protection Agency forced more strict effluent nitrogen

regulations for treated wastewater and this inevitably generated a strong driving force toward research for advanced wastewater treatment to remove N from wastewater. There are roughly two kinds of N in wastewater, organic N and inorganic N such as ammonia and nitrogen oxides. During anaerobic treatment of wastewater, organic N is converted to ammonia and this inorganic N can be oxidized to nitrogen oxides in the aerobic digestion system. Finally, they can be removed by denitrification in the anoxic condition with organic supply.

There are several studies where aerobic denitrification was seen for low dissolved oxygen environments (Grady *et al.*, 1999; Wang *et al.*, 2007; Zeng *et al.*, 2004). This aerobic N removal is also called simultaneous nitrification and denitrification (SND) and it has drawn great interests since this system does not require an additional anoxic zone for denitrification and organic matter in wastewater can be used as nutrients for denitrifiers. In addition, energy cost can be reduced since aeration can be intermittently turned off.

Grady *et al.* (1999) observed greater ammonia-N removal and low effluent nitrate in a sequencing batch reactor (SBR) system by changing the aeration fraction to 60-70% of the total cycle of the SBR. SND was also reported from a lab-scale SBR operated in an alternating anaerobic-aerobic mode under a low dissolved oxygen (DO, ~ 0.5 ppm) condition (Zeng *et al.*, 2004). In this lab-scale SBR study, 2% of removed ammonia-N was measured as nitrite and the rest was converted to nitrogen oxide rather than nitrogen gas. Aerobic denitrification in a single aerobic system was also observed, from which 6-12 % nitrite was converted to nitrogen gas under low DO environment (< 1 ppm) and

low chemical oxygen demand to total Kjeldahl nitrogen ratio (COD/TKN ~ 2.9) in the feed sludge (Wang *et al*., 2007).

Heterotrophic nitrifiers were widely studied for their capability to denitrify under aerobic condition. Among them, *Alcaligenes faecalis* is a heterotrophic nitrifier that is commonly found in soil and activated sludge systems (Van Niel *et al*., 1992). Aerobic denitrification is thought to be a cause for the nitrogen loss that is not measured as oxidized N in the aerobic digestion systems. In addition, high ammonia and low DO environment is found to be required for active SND in an aerobic condition (Van Niel *et al*., 1992).

Combined anaerobic/aerobic (ANA/AER) sludge digestion system can provide benefits from both sludge treatment methods. Park *et al.* (2006) found that the combined reactor system could degrade fractions that were solely anaerobically digestible as well as fractions that were solely aerobically digestible, thus achieving additional solids removal. Along with additional solids reduction, the combined ANA/AER system can achieve greater N reduction that may not be possible by ANA or AER systems alone. In other words, high ammonia-N can be generated in the ANA system and reduced N can be oxidized and converted to a gaseous form via SND in the subsequent AER system.

In order to validate advantages of the combined system, it has been studied by several researchers. A study performed by Kumar (2006) showed that the sequential anaerobic/aerobic (ANA/AER) sludge digestion system removed more than 60% overall volatile solids (VS) with 10 to 15% VS reduction in the subsequent AER system

operated for 3 to 9 day retention times, respectively. Near 50% total N (TKN + oxidized N) removal from ANA effluents was also achieved in the AER system. The author speculated that the most of N removal in the AER system was done via SND since little oxidized N was measured from the AER effluent. A similar result was also reported by Novak et al. (2009) who observed 62% overall VS reduction and 64.5% TKN removal from the combined ANA/AER system with a 15 day anaerobic and a 5 day aerobic retention time. The subsequent AER system of these two studies (Kumar, 2006; Novak et al., 2009) were operated at a DO level greater than 3 ppm and periodic feeding as once per a day was practiced.

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Chapter 3. Multi-staged anaerobic sludge digestion processes

(Revisions submitted to ASCE Journal of Environmental Engineering)

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Abstract

Two multi-staged anaerobic digestion systems, a 4 stage-thermophilic anaerobic digestion (4TAD, all at 55 ℃) and a 4 stage-anaerobic digestion with a tapered temperature configuration (4ADT, 55, 49, 43 and 37 ℃, respectively), were studied to evaluate their solids, volatile organic sulfur compounds and indicator organism (*E. coli* and fecal coliform) reduction potentials. The 4TAD system removed significantly more volatile solids from sludges than the 4ADT system (6%). However, the dewatered sludge cakes from the 4ADT system generated less organic sulfur compounds than those from the 4TAD system. Both multi-stage systems showed better digestion efficiencies than single-stage mesophilic or single-stage thermophilic anaerobic digesters at the same overall retention time. However, the lowest organic sulfur compounds were observed from the single meso system. Both multi-stage anaerobic digestion systems failed to dramatically remove DNA of the indicator organism, *E. coli*,

quantified by quantitative polymerase chain reaction (qPCR) even though the indicator organism densities measured by standard culturing methods satisfied EPA Class A biosolids requirements.

CE Database subject headings: Anaerobic treatment; Wastewater management; Heat treatment; Remediation; Odors; Pathogens.

Introduction

The demand for better municipal sludge handling strategies has resulted in various advanced anaerobic sludge digestion methods, including thermophilic sludge digestion (50-60 ℃), temperature phased anaerobic digestion and autothermal thermophilic aerobic digestion. Higher kinetic rates as a result of higher digestion temperatures have enabled thermophilic sludge digestion systems to improve digestion efficiency (Zahler *et al*. 2007) and to produce Class A biosolids (EPA 1994). However, process instability associated with high volatile fatty acid accumulation, odors and high ammonia generation have also been reported from thermophilic anaerobic digestion systems (Kim *et al*. 2002). On the other hand, temperature phased systems have been shown to provide better odor control than the thermophilic systems, along with greater solid and pathogen reduction than conventional mesophilic systems (Han and Dague 1997). However, poor dewatering properties associated with temperature phased systems have been reported. According to Bivins and Novak (2001), the poor dewatering properties produced by thermophilic digestion were not eliminated by the subsequent mesophilic sludge digestion step.

Extracellular polymeric substances (EPS) are an accumulation of biological organics (protein and polysaccharide) and cell lysis materials. They are thought to bind sludge flocs due to their glue-like characteristics and the composition of these materials will determine the structure of floc materials. Their precise chemical structure and roles in sludge flocs have not been fully determined. One recent EPS model (Higgins and Novak, 1997) suggested that polysaccharides are bound by lectin-like proteins and these lectin proteins are interconnected by the divalent cations, calcium and magnesium. The fate of EPS after various sludge digestion processes is also as important as the composition of EPS in sludge flocs. Novak and Park (2004) observed that greater solids reduction often resulted in more solution biopolymer (protein and polysaccharide) during anaerobic digestion of sludges. Moreover, greater solution biopolymer in the digestion system was found to cause poorer dewatering properties of digested sludges (Novak *et al*., 2003).

Volatile organic sulfur compounds are malodorous sulfur-containing organic compounds such as methanethiol (MT), dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS). It is widely accepted that these sulfur-based volatile organic compounds are mainly responsible for odors from anaerobically digested and dewatered sludges (Erdal *et al*. 2008; Higgins *et al*. 2006 and 2008; Muller *et al*. 2004). The generation of organic sulfur compounds from anaerobically digested biosolids has been detailed by Higgins *et al*. (2006). The main sources of volatile organic sulfurs from dewatered sludge cakes appear to be the sulfur-containing amino acids, cysteine and methionine, which can be degraded to hydrogen sulfide (H_2S) and MT, respectively,

under anaerobic conditions. MT can be also generated by methylation of H_2S. DMS is a methylated form of MT and DMDS can result from the oxidation of MT. Finally, these organic sulfurs can be demethylated and mineralized by methanogens to produce sulfide, methane and carbon dioxide (Higgins *et al*. 2006).

Higgins *et al*. (2007) proposed that the indicator organisms, *E. coli* and fecal coliform, in wastewater sludges can enter a non-culturable state after thermophilic anaerobic digestion so that standard culturing methods tend to underestimate their population in biosolids. Moreover, these non-culturable organisms can be reactivated by centrifugal dewatering of digested sludge and increase further during cake storage.

Staged thermophilic digestion has been suggested as an anaerobic digestion process that can greatly reduce organic sulfur associated odors and eliminate indicator organisms (Higgins *et al*. 2007). In addition, there are several field reports that describe superior solids and pathogen reduction by thermophilic digestion using up to 4 stages.

In this study, two multi-staged anaerobic digestion systems (4TAD and 4ADT) were operated to study their ability to reduce solids, volatile organic sulfur compounds and indicator organisms. The inoculum and feed sludges were shipped from the Western Lake Superior Sanitary District (WLSSD) facility at Duluth, MN for study at Virginia Tech. This facility has the capability to convert their anaerobic digesters to a 4 stage system. The objectives of this study were;

a) To compare the digester performances (e.g. solids reduction, dewatered sludge odors, indicator organism reduction, and sludge dewatering properties) of 4TAD

and 4ADT to each other and to single-stage thermophilic and mesophilic digesters with the same overall retention time; and

b) To relate effluent biopolymer properties (e.g. soluble and extractable) to digester performances and sludge characteristics (e.g. solids reduction, dewatered sludge odors, and sludge dewatering properties).

Methodology

Digestion systems

Four anaerobic digesters were constructed of high density polyethylene (HDPE) brewer tanks (Model No. f6.5, The Hobby Beverage Equipment Company) and the single-stage digester was a 20 L HDPE carboy (Nalgene). Each digester was covered with aluminum foil and temperature adjustable heating tape (Model No. BSAT 101-100, Thermolyne) was placed on top of the foil. The aluminum foil was used to ensure even heat distribution to the reactors and to provide protection from the heating tape so that physical failure of the polyethylene would not occur.

The overall experimental setup is shown in Fig. 1. The temperature of all 4TAD reactors was maintained at 55℃ and the four reactors of the 4ADT system were operated at 55, 49, 46 and 37 ℃. The operational temperature of the single-stage thermophilic anaerobic digestion system was 55 ℃ and the single-stage mesophilic anaerobic digestion system was operated at 37℃. The solids retention time (SRT) of each stage of the multi-stage systems was 6 days and the SRT of each single-stage reactors was 24 days so the overall retention times of multi and single-stage systems were the same. The

operational volumes of the 4 stage-anaerobic reactors were 21 L, 15 L, 9 L and 9 L, respectively and the sludge volume of the single-stage reactors was 12 L. The waste sludges from each reactor were fed to the subsequent reactors. Each successive reactor was operated at a smaller volume to save about 1 L of waste sludges per day for analysis. Gas mixing was applied to each of the multi-stage anaerobic reactors by circulating head space gas to the bottom of reactors using a peristaltic pump (Model No. 7553-70, Cole Parmer). Mechanical mixing by a stirring plate (Cimarec 2, Thermolyne) and a magnetic stir bar was used for the single-stage reactors. Each reactor was equipped with a gas collection bag to alleviate excessive gas pressure and to measure gas volume production. The 4TAD and single-stage thermo systems were operated side by side with the same feed sludge. These were followed by operation of the 4ADT and single-stage meso system, also in a side by side mode with the same feed sludge.

The inocula for the 4TAD and the single-stage thermo system were thermophilically digested anaerobic sludge from the Western Lake Superior Sanitary District (WLSSD) at Duluth, MN, USA. Seeding was not needed for the 4ADT and the single-stage meso system since they were converted from the thermophilic systems and responded well without a seed sludge. The feed sludge was thickened waste activated sludge (5% TS_{avg}) shipped weekly from the WLSSD and diluted with tap water to make a feed of 3% total solid (TS). WLSSD does not practice primary treatment. High sulfate was found in the feed sludge because WLSSD accepts wastewater from pulp and paper companies. The average sulfate concentration in the feed sludge was 171.3 ± 3.7 mg/L as sulfate and dissolved sulfide in the feed was 0.6 mg/L.

Chemical / physical analysis

In this study, stable digester performance was assumed to have occurred when the solids reduction varied with a standard deviation less than 10%. In this regard, all data collection was performed during the circled days (Fig. 2), when the standard deviation for the volatile solids reduction data was 8.0 ± 1.4%.

Total and volatile solids (TS/VS) were determined according to standard methods (APHA 1998). Total cations in the sludge samples were measured in accordance with EPA method 3050B (1996). Initially 15 mL of each raw sludge sample was acidified with the same amount of 1:1 HNO_3 (v/v) and the mixture was kept at room temperature until acid-digestion. After acid-digestion, each cation (Ca, Mg, Al and Fe) was quantified with atomic absorption spectrophotometry (Model No. 5100PC, Perkin-Elmer). Solution sulfate (SO_4^{2-}) was analyzed using an Ion Chromatograph (Model No. DX120, Dionex) equipped with AS9-HC column (Model No. 051786, IonPac). The eluent was 9.0 mM Na_2CO_3 and the flow rate was 1.0 ml/min. Dissolved sulfide was measured by method 4500-S^{2-} in standard methods (APHA 1998).

The concentration of soluble polysaccharides was measured as described by Dubois *et al*. (1956). Dextrose was used as a standard. The concentration of soluble proteins was measured by the method of Frølund *et al*. (1996). Bovine serum albumin (Prod No. 23209, Thermo scientific) was used as a standard. Biopolymer herein refers to the sum of polysaccharides and proteins in mg/L for solution biopolymer and in mg/g VS for extractable biopolymer.

Four extraction methods were used to quantify solid-bound exocellular polymeric substances. They are cation exchange resin (CER), base, shearing and sonication extraction. These are described in detail below.

a) **CER extraction** was adopted from the method of Park and Novak (2007). One hundred mL of sludge sample was centrifuged at 10000 rpm (17700 G) for 15 min at 4 ℃. The sludge pellet was resuspended in 200 mL of phosphate buffer solution (PBS, 10 mM NaCl + 6 mM Na_2HPO_4 + 1.2 mM KH_2PO_4) and the mixture was put into a plastic mixing beaker with 4 baffles where previously PBS-washed CER (Dowex 50_8, Sigma-Aldrich) was placed as 60 g resin/g TS. A shearing by a mixing paddle at 600 rpm (8 G) was applied for an hour and extracted material was separated from the resin beads by filtering the extraction through a 1.5 μm glass fiber filter. The filtrate was centrifuged at 10000 rpm (17700 G) for 15 min at 4 ℃ and the centrate was filtered through 0.45 μm membrane filter. The filtrate was stored in a freezer until biopolymer analysis.

b) **Base extraction** was also carried out in accordance with the method of Park and Novak (2007). The sample preparation was mostly same as that of CER extraction but the centrifuged sludge pellet was resuspended in 200 mL 10 mM NaCl solution. The pH of the solution was adjusted to 10.5 by 1 N NaOH. During mixing, N_2 atmosphere was maintained to prevent pH change by CO_2 in atmosphere. The rest of extraction procedure was identical to that of CER extraction.

c) **Shearing extraction** was applied to sludge pellets that were resuspended in PBS

buffer as described in CER extraction procedure. After resuspension of the sludge pellet in the PBS, 1 minute of mechanical shear was applied to the resuspended sample followed by 1 minute rest and re-shearing for 1 minute. A Waring blender was used to apply mechanical shear (Muller *et al.,* 2004). The rest of the extraction procedure was same as that of the CER extraction process.

d) **Sonication extraction** was carried out using a lab sonication unit (Model No. Dukane 2120, Dukane Corp.) for 30 seconds followed by 30 seconds rest and re-sonication for 30 seconds. The sonication unit imparted power at 20 KHz, 166 W. The sample preparation and the extraction procedure after sonication were same as the CER extraction.

The method described by Muller *et al*. (2004) was used for the sludge dewatering testing and for the preparation of dewatered sludge cakes for organic sulfur analysis. One percent high molecular weight cationic polymer (Clarifloc 3275, Polydyne) was used as the sludge conditioner. The polymer dose that resulted in the lowest capillary suction time or the best dewaterability was selected as the optimum polymer dose. A mixture of optimum polymer and sludge was then sheared in a Waring blender for 30 sec and centrifuged in a lab centrifuge at 10000 rpm (17700 G) for 15 min under the room temperature. The sludge pellet was collected and pressed at 207 kPa for 15 min by a lab press. This provided a dewatered sludge cake similar to that generated by a high-solids centrifuge (Muller *et al*., 2004). These pressed sludge cakes were used for volatile organic sulfur compounds measurement.

Volatile organic sulfur compounds were measured by the method of Glindemann *et al*. (2006). Twenty five grams of dewatered sludge cake were incubated in a glass bottle (250 mL, I-Chem) and each bottle was sealed by a cap with a Teflon lined septa. One hundred μL headspace gas from each incubation bottle was periodically collected and injected into gas-chromatography/mass spectrometry (Model No. GC 6890, MSD 5970, Hewlett-Packard) with a cryo-trapping system. The cryo-trap was employed to accumulate gas samples and to generate narrow chromatographic peaks. A 30 m long and 0.25 mm I.D. column (Model No. 20751-01A, Supelco) was connected to the gas injection inlet (200 ℃) and helium was used as a carrier gas (2 ml/min). The oven temperature was increased from 50 to 265 ℃ at a rate of 35 ℃/min. Total analysis time was 7.64 min. Odorous compounds that were measured in the study were H_2S, MT, DMS and DMDS. Peak areas of each organic sulfur compound were integrated by the data analysis program, G1034C version C.03.00 (Hewlett-Packard). The amount of organic sulfur in each sample was quantified by comparing the sample peak area with the area of a standard gas mixture of known amounts of H_2S, MT and DMS (Scott Specialty Gases Inc.). DMDS was quantified using DMS as a reference.

Microbial test for pathogen

Feed sludge and effluents from each digestion system (500 mL per each) were collected and shipped overnight in ice to the Bucknell University Environmental Engineering and Science Laboratory, PA, USA. The indicator organisms, *E. coli* and fecal coliform, in these sludge samples were quantified by Standard Method 9221F and 9221E (APHA 1998). In addition to the standard culturing method, total solids concentration was also measured for each sludge sample. Feed sludge and effluents from each digestion system

(50 mL per each) were centrifuged at 10000 rpm (17700 G) for 15 minutes at 4℃ and pellets were shipped overnight in dry ice to the Bucknell University. All molecular work was conducted in accordance with the protocol described by Chen *et al*. (2006).

DNA in each sludge sample was extracted in accordance with the DNA extraction protocol described by Chen *et al*. (2006). Real time PCR was used to enumerate the *E. coli* specific gene, glutamate decarboxylase (gadA/B) in the extracted DNA samples. In brief, the extracted DNA was amplified with a forward (50–GCG TTG CGT AAA TAT GGT TGC CGA–30) and a reverse primer (50–CGT CAC AGG CTT CAA TCA TGC GTT–30) by the Brilliants SYBRs Green QPCR Master Mix (Stratagene, La Jolla, CA). The *E. Coli* – specific DNA was quantified by the Stratagene MX3005P rt-PCR system (La Jolla, CA). Serially diluted *E. coli* DNA (2 to 7620 copies) was used as an external DNA standard for each real time PCR analysis. DNA quantification was preformed three times per sample.

Error bars in the figures represent the standard deviation using three or more sets of data.

Results

Solids reduction

VS reduction data of multi-staged systems were compared to each other as well as to those of single-stage systems in order to quantify and compare each reactor system's solids removal efficiencies in accordance with their unique digestion conditions. All the multi-staged reactors removed more solids than single-stage reactors. The greater solids

reduction associated with the higher temperature was also observed from the single-stage reactors. For the single-stage systems, the VS reduction of the single thermo system (39.92 ± 2.60 %) was about 6% greater than that of the single meso system (34.14 ± 3.12 %).

The 4TAD system removed about 6% more VS than the 4ADT (Fig. 3). Statistical analysis showed that first two reactors of each multi-stage systems removed similar % VS while the last reactors removed a statistically different % VS (Table 1). A comparison of the solids reduction in the second stage digesters for the 4TAD at 55 ℃ and the 4ADT at 49 ℃ shows that a similar solids reduction occurs for both systems, even though the temperatures differ.

Overall, the 4 stage systems degraded more solids than the single-stage systems and the thermophilic systems degraded more solids than the mesophilic or partially mesophilic systems.

Gas generation

Most of thermophilic digestion systems operated at the temperature greater than 49 ℃ generated more gas volume per g VS removed (Table 2). However, high VS reduction in the first reactors of both multi-stage systems did not result in greater gas volume generation. All the gas volume data fell in the range, 0.62 to 1.56 L/g VS removal, which was suggested by Girardi (2002).

Indicator organism reduction

Pathogen reduction in sludges to be land applied is an important consideration in the selection of an advanced digestion method. If the Class A biosolids requirements (EPA 1994) are met, digested sludges can be dewatered and land-applied as a disposal method without restrictions. The single-stage thermo system is listed as a sludge treatment method to attain Class A biosolids (EPA 1994). Standard culturing data for both of the multi-stage anaerobic digestion systems and the single thermo system showed near complete removal of *E.coli* and fecal coliform, which meets the Class A biosolids requirement of less than 1000 MPN/g DS (Fig. 4). It was observed that most of indicator organism removal was achieved in the first thermophilic anaerobic digester of multi-stage digestion systems (Fig. 5). Regardless of their digestion temperature conditions, subsequent reactors of multi-stage systems provided little additional indicator organism destruction. The single meso system removed a smaller percentage of indicator organisms than the multi-stage systems and the single thermo system, even though all of them were operated for the same overall retention time.

However, DNA data generated by qPCR failed to confirm the dramatic reduction of *E.coli* DNA. In general, the thermophilic digestions systems, either single-stage or 4 stage systems showed about one log *E.coli* reduction from the feed sludge, from which 6.86 ± 0.13 *E.coli* DNA copies / g DS in log scale were measured. This was comparable to the single-stage mesophilic digester. The ADT system showed a 2.6 log reduction but this was still considerably lower than the 5 to 6 log reduction indicated by the standard culturing method data. In addition, all the DNA removal occurred in reactor 1 of the 4TAD system. Additional thermophilic digestion of sludge by subsequent reactors (e.g. reactor 2, 3 and 4) of the 4TAD system did not provide any additional *E.coli* DNA

reduction.

Reduction of volatile organic sulfur compound from dewatered sludge cakes

Odors from sludge biosolids were voted as the top odor problem associated with wastewater treatment at a Water Environment Federation workshop, held at Anaheim, CA (Forbes *et al*. 2004). Since proteins usually comprise from 50 to 70% of volatile solids of wastewater sludges (Forbes *et al*. 2004) and sulfur-based odors are mainly generated from degradation of these proteins, greater VS removal is expected to result in low odor generation from dewatered sludges.

In spite of the greater VS reduction by the 4TAD, the dewatered sludge from the 4^{th} reactor of 4TAD system (376 ± 261 ppmv as S/g VS) produced a much greater peak volatile organic sulfur concentration than the dewatered sludge from the 4^{th} reactor of 4ADT system (29 ± 13 ppmv as S/g VS) and the single meso system (5 ± 3 ppmv as S/g VS). The single-stage thermo system also produced as much peak organic sulfur (335 ± 74 ppmv as S/g VS) as the 4^{th} reactor of the 4TAD system. The lowest peak total organic sulfur compounds were measured from the dewatered sludge cakes of the single meso system, which removed the least VS. Moreover, neither the solids (R^2 = 0.02 from the linear regression) nor solution biopolymer profiles (R^2 = 0.18 from the linear regression) showed a reasonable relationship with volatile organic sulfur compound data.

Solution biopolymer and sludge dewaterability

Solution biopolymer tends to accumulate in digestion systems as more solids are destroyed. More solution biopolymer was measured in the final effluent of multi-staged

digestion systems (649.63 ± 147.05 mg/L for 4TAD and 413.38 ± 56.55 mg/L for 4ADT) than in the single-stage systems (404.95 ± 99.64 mg/L for single thermo and 139.37 ± 33.81 mg/L for single meso) where less VS was removed than in the multi-stage systems. Likewise, thermophilic systems accumulated more solution biopolymer than mesophilic systems.

The relationship between VS reduction of the digestion system and solution biopolymer content can be clearly seen in Fig. 6. More biopolymer was accumulated in solution as greater VS was removed. A similar result was observed by Novak and Park (2004). They found that during anaerobic digestion of wastewater sludge, greater solids reduction resulted in more solution biopolymer in the digestion system.

Greater solution biopolymer can lead to poorer dewatering properties of anaerobically digested sludges (Novak and Park 2004). As seen in Fig. 7, more solution biopolymer resulted in a greater optimum polymer dose requirement and poorer dewaterability of the unconditioned sludges.

Extractable biopolymer

A major component of the organic solids in sludges is biopolymer. During anaerobic digestion, some biopolymer is solubilized while some remains bound to solids. In this respect, quantifying solid-bound biopolymer may provide information regarding the destruction of these materials by anaerobic digestion as well as potential amounts of degradable biopolymer under advanced or multi-stage digestion. Four biopolymer extraction methods were applied to sludge samples from each anaerobic digestion

system. Details of the extractions are provided below.

The amount of extractable biopolymer in the feed sludges changed while shifting from the 4TAD and single thermo system to the 4ADT and single meso system. However, the trends in the data are the same. The lowest extractable fraction is for mechanical shearing and the highest is for base and CER extraction.

In order to evaluate the changes in the feed sludge following anaerobic digestion, the ratios of the extractable biopolymer in the effluent sample to the initial extractable biopolymer in the feed were used. The ratios are shown in Fig. 8.

The data suggest that single or multi-stage anaerobic digestion of sludges at different temperature conditions (e.g. thermophilic and mesophilic) for the same total retention time could cause changes to different pools of extractable biopolymer. All the digestion systems in this study reduced biopolymer that was extractable by sonication, CER and base extraction, respectively. However, more release of biopolymer also observed from mechanically sheared sludges after single or multi-stage sludge digestions in the study.

Discussions

Solids reduction

Overall, the 4 stage systems degraded more solids than the single-stage systems and reactors operated at a temperature greater than 49℃ removed more solids than the reactors operated at a lower temperature. The different mixing configurations could

have had a small effect on the solids reduction. However, both the gas mixed and mechanically mixed reactors were well mixed. More solids removal in the thermo systems resulted in greater gas volume generation than reactors operated at lower temperatures. Interestingly, greater VS removal in reactor 1 of both multi-stage systems did not result in more gas volume generation. It seems that most of soluble organics, generated from reactor 1 of both multi-stage systems were consumed in the subsequent reactors resulting in more gas production. Overall gas generation per g VS consumed for 4 stage systems were smaller than single stage systems and this seems to be caused by much lower gas yield in the first reactors of each multi-stage systems. More gas generation per g VS consumed was observed from thermophilic systems throughout the study. The combination of 4 different digestion temperatures was expected to offer advantages over the 4 stage digestion system at the same digestion temperature, but that was not seen in the data.

E.coli DNA reduction

It has been suggested, based on a successful full-scale operation, that a 4 stage thermophilic digestion system might result in multiple log reduction in *E.coli* based on DNA analysis and lower organic sulfur gas generation (Higgins *et al*. 2007). However, DNA data did not show dramatic reduction of *E.coli* DNA in the multi-staged systems even though their effluents fulfilled EPA Class A biosolids requirements (EPA 1994). This implies that there is potentially a large population of non-culturable *E.coli* in both of the multi and single-stage systems, which could result in reactivation after centrifuge dewatering (Higgins *et al*. 2007).

Sulfur-based odor reduction

Greater sulfur-based odor reduction potential of multi-staged thermophilic anaerobic digestion system was also not observed in this study. It was thought that the greater sulfur-based compound generation from the thermo systems could be explained by microbial aspects of sludge digestion environment. Aceticlastic or methylotrophic methanogens, the major organic sulfur degraders (Higgins *et al.* 2006), may be less active in a single thermo system with excess sulfate in the influent than in a single meso system with same excess sulfate in the influent. This alteration of microbial activity may have resulted in the accumulation of organic sulfurs in the headspace of the incubation bottles with thermophilically digested sludge cakes. Similar observations were found from the literature. Wilson *et al.* (2008) proposed that acetate oxidation and hydrogenotrophic methanogenesis were more thermodynamically favorable than aceticlastic methanogenesis under thermophilic anaerobic digestion conditions at a temperature greater than 55°C. A similar thermal preference of methanogenic communities was observed by Freeman *et al.* (2008), who found that mixture of methanogenic communities utilizing various range of substrate (H_2/CO_2, formate and acetate) were mostly observed in the single meso system with excess sulfate (1.8 – 5.3 g/L) while hydrogenotrophic methanogens were the primary archaeal species in the thermophilic anaerobic digestion system with the same sulfate concentration in the system. From these data, it appears that the reduction in organic sulfur gas and *E.coli* in the 4 stage full-scale system are due to something other than the thermophilic temperatures and multiple stages at that facility.

Soluble and extractable biopolymer

Overall, the data showed that more VS reduction gave rise to greater solution biopolymer, which caused poor dewatering properties of digested sludges and higher polymer conditioning requirements. Although more VS reduction is usually considered desirable for anaerobic digestion systems, poorer dewatering properties are likely to be found due to the accumulation of additional biopolymer in solution

The CER extraction method is specific for divalent cation-associated biopolymer (Park and Novak 2007). Since the divalent cation content of the sludge was much higher than the monovalent and trivalent cations, it was expected that the CER-extractable biopolymer would decrease more than the other fractions. However, both the CER and base-extractable fractions decreased substantially during digestion. The base-extractable fraction is the least specific extraction method but includes the aluminum-associated biopolymer fraction (Park and Novak 2007). The largest decrease in base-extractables was for the single-stage and four-stage thermophilic digestion systems, suggesting that the aluminum-associated materials are better degraded by thermophilic processes. This might account for some of the increased solids destruction often seen for thermophilic digestion compared to mesophilic.

The amount of biopolymer extracted by shearing increased following digestion. It was thought that the reason for the increase was that anaerobic digestion appears to weaken the floc structure, making it more susceptible to shear. The generation of odor-causing organic sulfur compounds has been associated with high intensity shear in centrifuges (Muller *et al*. 2004) and these data are consistent with that observation. The increase in biopolymer, especially protein, as a result of mechanical shear would be expected to

lead to organic sulfur odors.

Only the sonication extractable ratios of final effluents of digestion systems correlated with the VS reduction from the digestion systems as shown in Fig 9. The greatest VS reduction of 4TAD system corresponded to the lowest sonication extractable biopolymer ratio while the lowest VS reduction of the single meso system brought about the greatest sonication extractable biopolymer ratio.

Among the four solid-bound biopolymer extraction methods, the extractable protein profile from shearing extraction showed a correlation with peak total volatile organic sulfurs from sludge cakes (Fig. 10). The single meso system was excluded from the correlation in Fig. 10 since it produced a much less odorous sludge and was the only system that did not include at least one thermophilic stage. It appears that more shear extractable protein in digested sludge may lead to greater peak total volatile organic sulfur generation from dewatered biosolids from digestion systems that contained one or more thermophilic stage. This agrees with research that suggests that shearing within a centrifuge during dewatering may release materials that become bioavailable which are then degraded to produce odorous compounds (Higgins *et al*. 2006).

Conclusion

- The 4TAD removed more solids than 4ADT and both removed more volatile solids than single-stage digesters at the same overall retention time.
- The multi-stage anaerobic digestion systems did not show dramatic reduction of

indicator organism DNA even though they removed most of indicator organisms in accordance with standard culturing method data. This suggests that the systems may have reactivation potential.

- Systems with a mesophilic digestion as the final stage had much lower total volatile organic sulfur compounds compared to systems with a thermophilic system as the final stage.
- The pattern of volatile organic sulfur compound generation could not be predicted by either of VS reduction or solution biopolymer data. Among 4 solid-bound biopolymer extraction methods, shearing extractable protein ratios showed a correlation with peak total volatile organic sulfur compounds from dewatered sludge cakes.

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Table 1. Statistical analysis of % VS reduction of multi-stage systems (α = 0.05)

	Reactor 1	Result	Reactor 2	Result	Reactor 4	Result
Variance test	F = 1.65, p = 0.30	Equal variance	F = 0.66, p = 0.29	Equal variance	F = 1.64, p = 0.30	Equal variance
Sample mean test	t(13) = 0.68, p = 0.51	Similar	t(13) = 1.32, p = 0.21	Similar	t(13) = 3.42, p = 0.0046	Different

* Equal variance was assumed for all t-tests.

* When p-value is greater than α, either equal variances (F-test) or equal sample means (t-test) can be assumed

Table 2. Gas generation data

	Reactor	L-gas / day	g VSR / day	L-gas/ g VSR	Total L-gas/ g VSR
4TAD	1	7.87 (1.67)	20.48	0.38	
	2	8.56 (1.29)	6.39	1.34	
	3	2.31 (0.44)	1.38	1.67	
	4	3.14 (2.42)	2.84	1.11	**0.70**
Single thermophilic		4.08 (0.48)	4.38	0.93	**0.93**
4ADT	1	7.89 (1.97)	20.33	0.39	
	2	6.86 (2.09)	4.39	1.56	
	3	1.37 (0.17)	2.22	1.30	
	4	1.52 (0.48)			**0.65**
Single mesophilic		2.45 (0.68)	3.25	0.75	**0.75**

* Numbers in parentheses are standard deviations.

Figure captions

- Figure 1. Overall experimental settings
- Figure 2. VS reduction by date (Circled data used for steady state analysis).
- Figure 3. VS reduction of each multi-stage reactor
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- Figure 7. Relationship between solution biopolymer, optimum polymer dose and dewaterability of unconditioned sludge
- Figure 8. Extractable biopolymer profiles of single and multi-stage systems.
- Figure 9. Sonication extractable biopolymer ratio and VS reduction
- Figure 10. Shearing extractable protein ratios and peak sulfur-based odor compounds

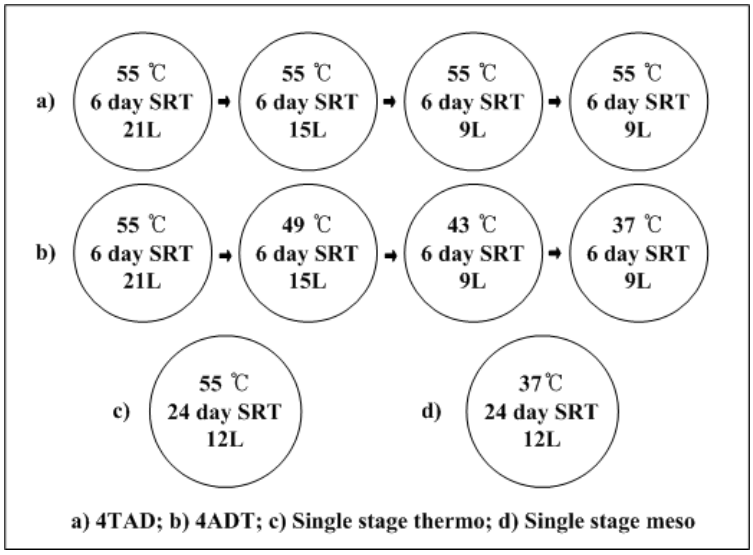

Figure 1. Overall experimental settings

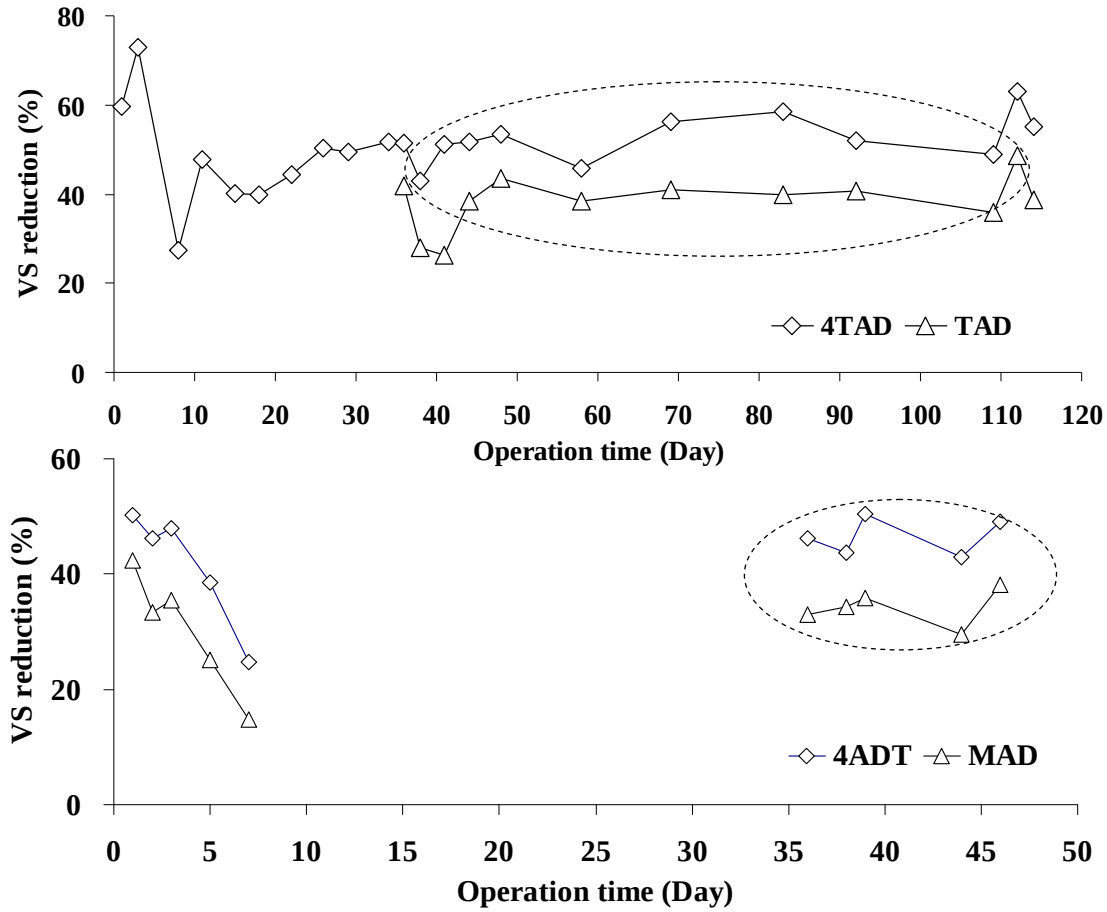

Figure 2. VS reduction by date (Circled data used for steady state analysis).

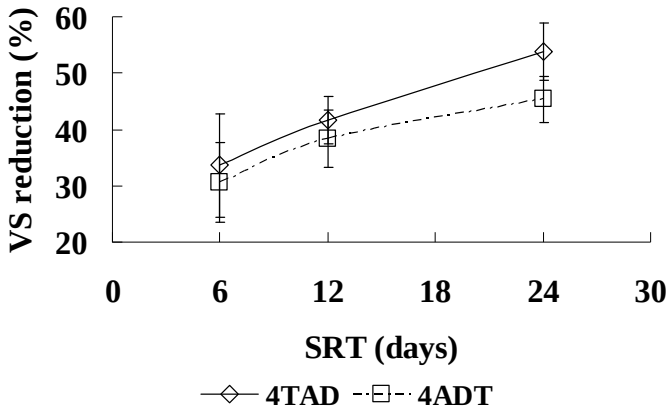

Figure 3. VS reduction of each multi-stage reactor

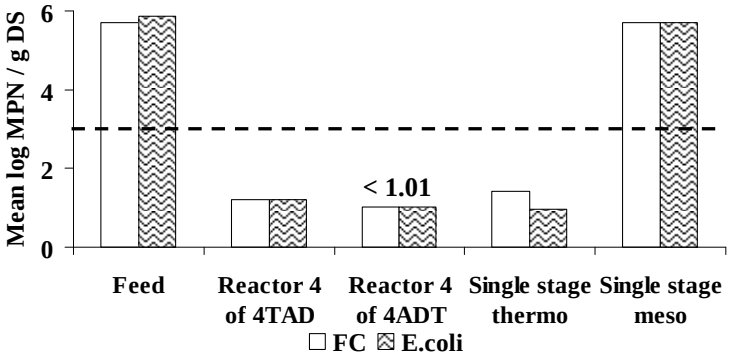

Figure 4. Mean log MPN of indicator organisms in single and multi-stage digestion systems (Line indicates EPA Class A biosolids requirement)

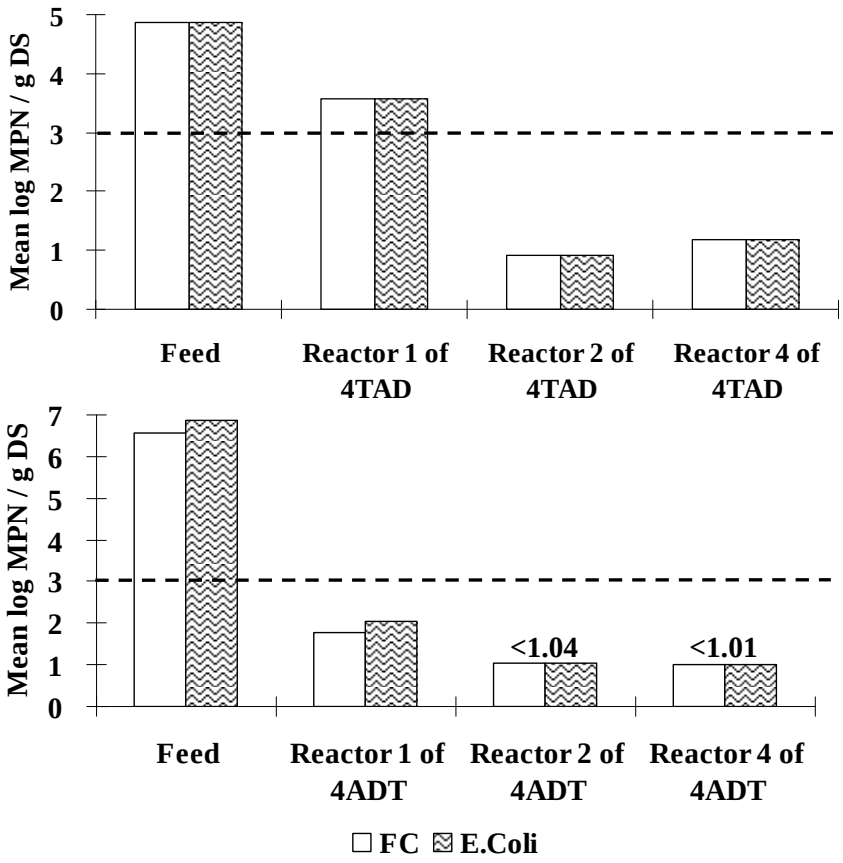

Figure 5. Effect of temperature to mean log MPN of indicator organisms in multi-stage digestion systems (Lines indicate EPA Class A biosolids requirement.)

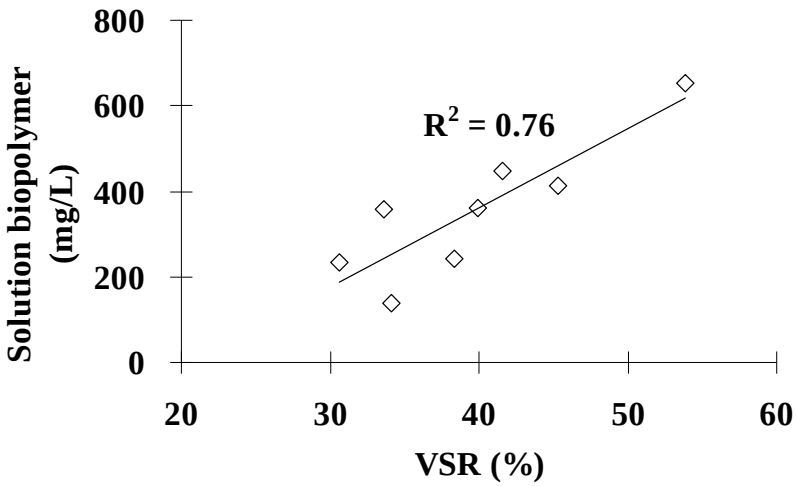

Figure 6. VS reduction and solution biopolymer.

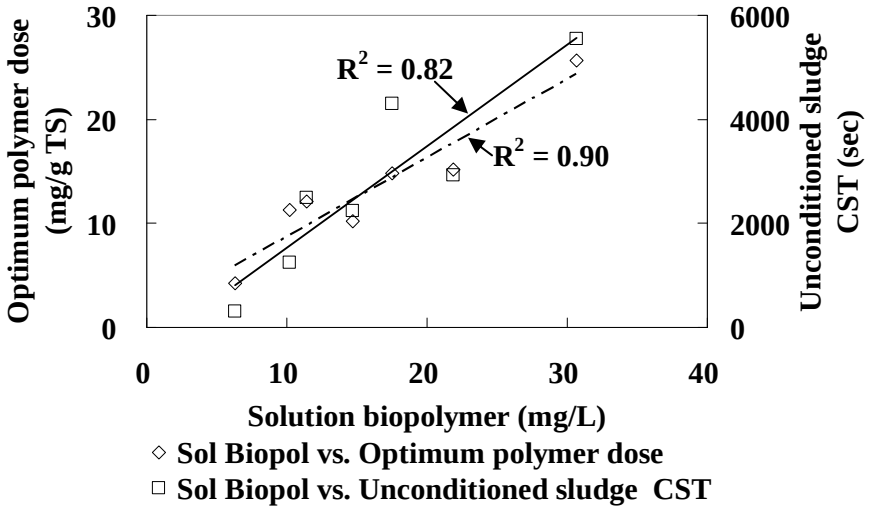

Figure 7. Relationship between solution biopolymer, optimum polymer dose and dewaterability of unconditioned sludge.

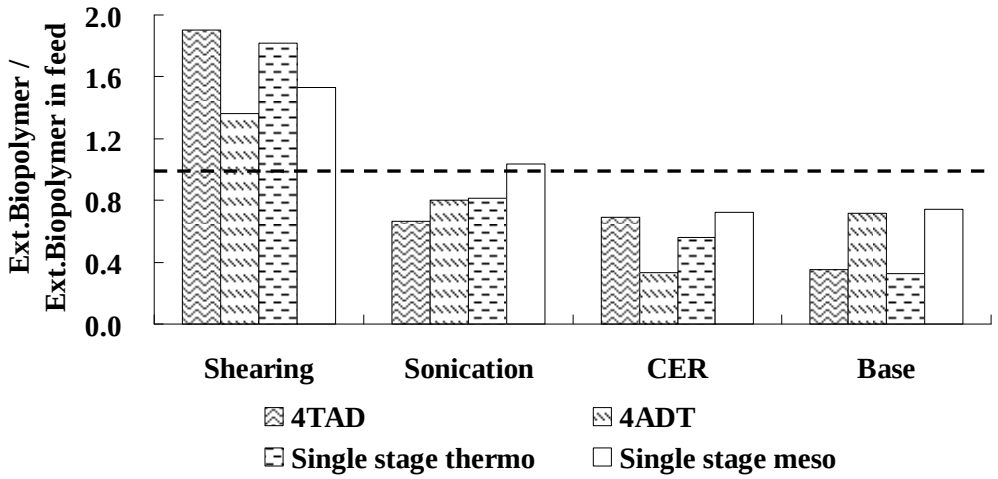

Figure 8. Extractable biopolymer profiles of single and multi-stage systems.

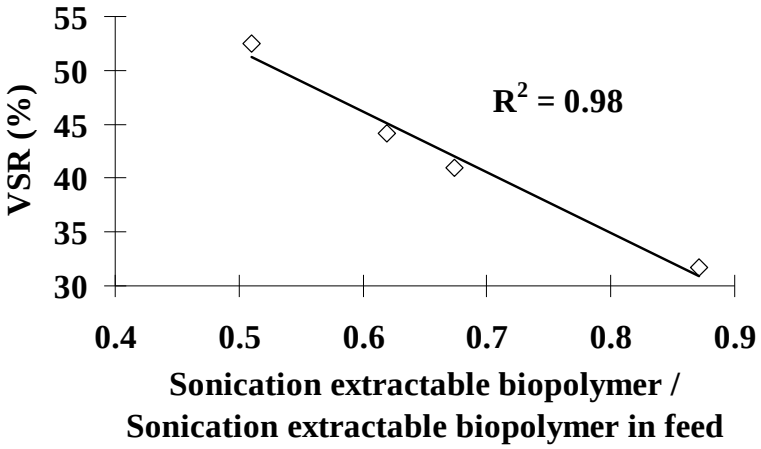

Figure 9. Sonication extractable biopolymer ratio and VS reduction

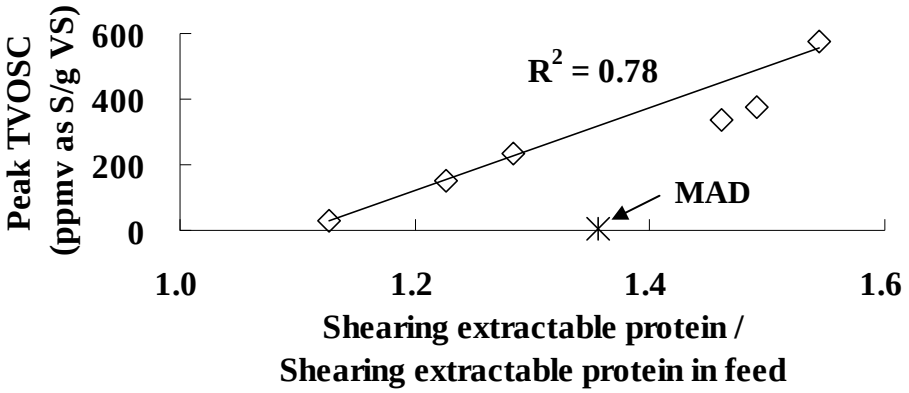

Figure 10. Shearing extractable protein ratios and peak sulfur-based odor compounds

Chapter 4. Digestion performance of various combinations of thermophilic and mesophilic sludge digestion systems

(Accepted to Water Environment Research)

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Abstract

Various combinations of single and multi-stage anaerobic and aerobic/anaerobic digestion system were studied to evaluate their solids reduction potential along with capabilities to control sulfur based biosolids odor compounds. All the multi-staged digestion systems removed more volatile solids than the single stage anaerobic digestion systems even at the same overall retention time. However, digestion systems with mesophilic digestion as the final stage showed a much lower headspace organic sulfur contents in the dewatered biosolids than the systems with thermophilic digestion as the final stage. This observation leads to the conclusion that placing a mesophilic anaerobic digestion system at the end of multi-stage digestion systems will enable greater sulfur based odor reduction from dewatered biosolids along with greater solid reduction than single stage mesophilic or thermophilic digestion systems.

KEY WORDS: *anaerobic digestion, volatile solid reduction, volatile organic sulfur compound, combined aerobic-anaerobic digestion*

1. Introduction

A high temperature environment enhances the solids reduction capability of anaerobic digestion systems by increasing microbial kinetics (Zahler *et al*., 2007) and enables much greater indicator organism removal (EPA, 1994). However, enhanced solids removal by thermophilic systems tends to produce high volatile fatty acids, high ammonia concentrations and odors (Kim *et al*., 2002). On the other hand, mesophilic anaerobic digestion systems have been found to be relatively more stable and remove less solids and pathogens than thermophilic anaerobic digestion systems. In this regard, combinations of these anaerobic biosolids digestion systems have been studied to facilitate advantages of both mesophilic and thermophilic digestion systems (Effenberger *et al.*, 2006; Han and Dague, 1997; Iranpour *et al.*, 2006; Nielsen *et al.*, 2004; Song *et al*., 2004).

Most of these investigators studied a combination of thermophilic and mesophilic digestion systems or temperature phased anaerobic digestion systems while Effenberger *et al.* (2006) carried out studies with a 3-stage system, a combination of mesophilic, thermophilic and mesophilic systems. These studies all agreed that multi-stage digestion systems were capable of greater solids reduction than single stage mesophilic systems. Moreover, placing a thermophilic system in these multi-stage digestion systems improved indicator organism reduction capabilities along with providing more extensive organic removal from the influent biosolids (Han and Dague, 1997).

Higgins *et al*. (2007) conducted studies of four completely mixed thermophilic anaerobic reactors in series and suggested that multi-stage thermophilic anaerobic digestion can greatly reduce odors associated with organic sulfur compounds and eliminate indicator organisms. However, poor dewatering properties associated with the tapered temperature system have been also reported. According to Bivins and Novak (2001), the poor dewatering properties of thermophilically biosolids were not recovered by a subsequent mesophilic digestion stage.

It is widely agreed that odors from anaerobically digested and dewatered biosolids consist mainly of volatile organic sulfur compounds (VOSCs) (Erdal et al., 2008, Higgins *et al*., 2006, and Muller *et al*., 2004). These organic sulfur compounds include methanethiol (MT), dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS). Higgins *et al*. (2006) described the generation mechanisms of VOSCs from anaerobically biosolids. According to these authors, the degradation of sulfur-containing amino acids such as cysteine and methionine along with methylation of hydrogen sulfide (H_2S) and MT are the major VOSC generation pathways in anaerobically biosolids. Among VOSCs, DMDS can be generated by chemical oxidation of MT. Finally, these VOSCs can be demethylated and mineralized by methanogens to produce sulfide, methane and carbon dioxide (Higgins *et al*., 2006).

2. Study background

The Western Lake Superior Sanitary District (WLSSD) facility at Duluth, MN was trying to convert its anaerobic digestion system to multi stage systems to produce more

stabilized Class A biosolids (EPA, 1994). This project was initiated based on a report regarding a successful operation of 4 stage thermophilic anaerobic digestion system at Annacis island wastewater treatment plant, Vancouver, Canada (Krugel *et al.*, 1998). In order to build performance data of various multi stage sludge digestion systems, WLSSD sludges were digested in 10 combinations of aerobic and anaerobic digestion systems under different digestion temperatures and their sludge stabilizing capabilities were assessed based on the characteristics of biosolids. The specific objectives of this study are as follows:

a) To compare the digester performances (For example, solids reduction, dewatered biosolids odors, and dewatering) of multi stage digestion systems to each other and to single stage thermophilic and mesophilic digesters with the same overall retention time; and

b) To relate effluent biopolymer properties (For examples, soluble and extractable) to digester performances and sludge characteristics (For example, solids reduction, dewatered sludge odors, and dewatering).

3. Methodology

3.1. Digester setup

The multi-stage anaerobic digesters were prepared using high density polyethylene (HDPE) brewer tanks (Model No. f6.5, The Hobby Beverage Equipment Company, CA) and the single stage digester was built with a 20 L HDPE carboy (Nalgene, USA). A 26.5 L stainless conical fermentator (Model No. CF01, Blichmann Engineerng, PA) was used for the thermophilic aerobic digestion system. All the reactors were kept in a 37 ℃

constant temperature room and the reactors that were operated at the temperature greater than 37 ℃ were heated by a heating tape with a temperature controller (Model No. BSAT 101-100, Thermolyne, IA). To ensure an even heat distribution and to protect the reactors, aluminum foil tape was applied between the heating tapes and reactors. Details of each multi- and single stage system are given in Table 1. A peristaltic pump (Model No. 7553-70, Cole Parmer, IL) was used to circulate head space gas to the bottom of reactors for mixing and mechanical mixing by a stirring magnetic bar and a stirring pad (Model No. Cimarec 2, Thermolyne, IA) was used for the single stage systems. Ambient air (250 ml/min) was applied to the bottom of the thermophilic aerobic system for aeration and mixing. Each anaerobic reactor was equipped with gas collection bags to alleviate excessive gas pressure and to measure the volume of gas produced. Ferric chloride (500 mg/L) was added to feed sludges to some single or multi digestion systems to simulate field condition. WLSSD applies iron to suppress H_2S generation during anaerobic digestion.

Each thermophilic digester of the 4Thermo system was seeded with thermophilically digested anaerobic biosolids from WLSSD. Seeding was not practiced for the other systems operated at the lower temperatures since they responded well without a seed. It seems that some mesophilic methanogens can survive a thermophilic temperature environment and they can proliferate when the appropriate condition (For example, temperature) is provided. The temperatures of digesters that were operated at the temperature lower than 55 ℃ were changed by adjusting the temperature controller of heating tape. Heating was turned off for the mesophilic systems at 37 ℃. All temperature changes were done within a day. Steady performance of each digestion

system was confirmed by stable percent solids reduction with about 10 % or smaller standard deviation (Fig. 1).

The feed sludge was a mixture of thickened waste activated biosolids shipped from the WLSSD wastewater treatment plant at Duluth, MN and tap water to make a feed of 3% total solid (TS). WLSSD does not practice primary treatment. High sulfate was found in the feed sludges since WLSSD accepts wastes from pulp and paper industries. The average sulfate concentration in the feed sludges was 171.3 ± 3.7 mg/L as sulfate and dissolved sulfide in the feed was 0.6 mg/L.

3.2. Analyses

pH of biosolids sample was measured by a pH probe (Model No. 13-620-287, Accumet, Malaysia) and a pH meter (Model No. 910, Accumet, USA). Total solids (TS), volatile solids (VS), ammonia and total nitrogen were determined according to standard methods (APHA,1998).

Total cations in the biosolids samples were measured in accordance with EPA method 3050B (1996). Each cation (Ca, Mg, Al and Fe) was quantified by an atomic absorption spectrophotometry (Model No. 5100PC, Perkin-Elmer, MA).

Solution sulfate (SO_4^{2-}) was measured by an Ion Chromatograph (Model No. DX120, Dionex, CA). Samples were filtered through 0.45 μm membrane filter (Cat No. 09 719 2D, Fisher, PA) before testing. Solution sulfide was measured by the iodometric method (APHA, 1998).

The concentration of soluble polysaccharides was measured as described by Dubois *et al*. (1956). Dextrose was used as a standard. Soluble proteins were quantified by the method developed by Frølund *et al*. (1996). Bovine serum albumin (Prod No. 23209, Thermo scientific, IL) was used as a standard. Biopolymer herein refers to the sum of protein and polysaccharide in mg/L for solution biopolymer and in mg/g VS for extractable biopolymer.

Three extraction methods were used to quantify solid-bound biopolymer. They are cation exchange resin (CER), base and sulfide extraction. Details of these extraction methods are described in the following paragraphs.

a) **CER extraction** was adopted from the method of Park and Novak (2007). One hundred mL of biosolids sample was centrifuged at 10000 rpm (17700 G) for 15 min under 4 ℃. The biosolids pellet was resuspended in 200 ml of phosphate buffer solution (PBS, 10 mM NaCl + 6 mM Na_2HPO_4 + 1.2 mM KH_2PO_4) and the mixture was put into a plastic mixing beaker with 4 baffles where previously PBS-washed CER (Dowex 50_8, Sigma-Aldrich, MO) was placed as 60 g resin/g TS. Shearing by a mixing paddle at 600 rpm (8 G) was applied for an hour and extracted material was collected by filtering the extraction through a 1.5 μm glass fiber filter (Cat. No. 1827 055, Whatman, NJ). The filtrate was centrifuged at 10000 rpm (17700 G) for 15 min under 4 ℃ and the centrate was filtered through 0.45 μm membrane filter (Cat No. 09 719 2D, Fisher, PA). The filtrate was stored in -20 ℃ until biopolymer analysis.

b) **Base extraction** was also carried in accordance with the method of Park and Novak (2007)**.** The sample preparation was mostly same as that of CER extraction but the centrifuged biosolids pellet was resuspended in 200ml 10mM NaCl solution. The pH of the solution was adjusted to 10.5 by 1 N NaOH. During mixing, N_2 atmosphere was maintained to prevent pH change by CO_2 in atmosphere. The rest of extraction was identical to that of CER extraction except for the CER bead separation.

c) **Sulfide extraction** was applied to biosolids samples to extract iron-bound biopolymer. Sulfide tends to strongly bind to iron to produce ferrous sulfide and release the iron-bound biopolymer. Initial sample preparation was same as that of base extraction. Six hundred mM anhydrous sodium sulfide solution was added to the resuspended biosolids sample in a manner to add 300 mmole of sulfide to 1 mmole of total iron in the biosolids sample. The mixture was transferred to a 250 ml flask with air-tight sealing with Parafilm and shaken by a wrist action shaker (Burrell Corp, PA) until solution turns black. The rest of extraction procedure was same as other extraction methods.

Dewatering testing was performed by the method described by Muller *et al*. (2004). One % (w/w) high molecular weight cationic polymer (Clarifloc 3275, Polydyne, GA) was used as a coagulant and the dewatering time was measured by a capillary suction time (CST) apparatus (Model #: W.R.C type 165, Triton Electronics Ltd., England). Initially, a mixture of cationic polymer and 100 ml biosolids sample was sheared in a Waring laboratory blender (Model #: 55A60VL22, General Electronics, IN) at 2300 rpm (120 G) for 30 sec. This sheared mixture was tested for CST and the amount of

cationic polymer that promoted the lowest CST was chosen as an optimum polymer dose.

The mixture of optimum polymer and biosolids sample was sheared in a Waring blender at 2300 rpm (120 G) for 30 sec and centrifuged at 10000 rpm (17700 G) for 15 min under the room temperature. This centrifuged biosolids pellet was pressed under 30 psi for 15 min by a lab press. This provided a dewatered biosolids cake similar to that generated by a high-solids centrifuge (Muller *et al*., 2004). This pressed biosolids cake was used for VOSC measurement. Twenty five gram of pressed biosolids pellet was collected and incubated in a glass bottle (250 mL, I-Chem, NY) with Teflon lined septa for VOSC measurement. Bromoethane sulfonic acid (BESA, 1 ml of 0.127 M to 10 g of dewatered biosolids), a chemical inhibitor for methanogenesis (Higgins *et al*., 2006) was applied to some dewatered biosolids to observe the role of methanogens in VOSC generation from dewatered biosolids.

Organic sulfur compounds were measured by the method of Glindemann *et al*. (2006). One hundred μL headspace gas from each incubation bottle was periodically collected and injected into gas-chromatography/mass spectrometry (Model No. GC 6890, MSD 5970, Hewlett-Packard, PA) with cryo-trapping system. Cryo-trap was employed to accumulate gas samples and to generate narrow chromatographic peaks. A 30m long and 0.25 mm I.D. column (Model No. 20751-01A, Supelco, PA) was connected to the gas injection inlet (temperature at 200 ℃) and helium was used as a carrier gas (2 ml/min). The oven temperature was programmed to rise from 50 to 265 ℃ at a rate of 35 ℃/min. Total analysis time was 7.64 min. Odorous compounds that were measured

in the study were H_2S, MT, DMS and DMDS. Peak areas of each organic sulfur compound were integrated by the data analysis program, G1034C version C.03.00 (Hewlett-Packard, PA). The amount of organic sulfurs in each sample was quantified by comparing the sample peak area with the area of a standard gas mixture of known amount of H_2S, MT and DMS (Scott Specialty Gases Inc., PA). DMDS was quantified using DMS as a reference. All the biosolids odor data are presented as total VOSC (tVOSC), which is the sum of MT, DMS and DMDS.

3.3 Statistical study

All statistical studies were carried using Excel 2003 software (Microsoft, USA). Linear regression was used to show data correlation along with the coefficient of determination, R^2. This statistical value implies a better fit of data to the regression line if it approaches 1. F-test was used to test variance equality and the significance of similarity between two sample means was shown by two tailed t-test. The assumption for t-test (i.e. equal or unequal variances) was determined based on the result of F-test. Both statistical analysis implies positive if p-value is greater than statistical significance, α.

pH, VS removal, solution biopolymer, dewaterability (CST), peak tVOSC and total cation data of each digestion systems are tabulated in Table 2. Unless otherwise indicated, results from the final effluent of each digestion system are presented here.

4. Results and Discussions

4.1 Solids reduction and VOSCs

It is reported that more than half of VS in activated sludge is comprised of protein (Forbes *et al.*, 2004) and VOSCs are mainly generated by the degradation of sulfur containing amino acids, building blocks of proteins (Forbes *et al.*, 2004; Higgins *et al.*, 2006 and 2008). Therefore VS reduction efficiencies of different sludge digestion systems were evaluated to assess their impact on VOSC generation from dewatered biosolids in this section.

Most of the multi-digestion systems removed more VS than single stage anaerobic digestion systems (Fig.1). More than 50% VS reduction was observed for the two digestion systems with a thermophilic aeration step at the front (For example, ThAer+TPAD+Iron and ThAer+Thermo+Iron) and the 4Thermo system. The least solids removal was for the Meso+Iron system. However, the greatest peak total volatile organic sulfur compounds (tVOSC) were generated from the 4Thermo system while the least was measured from ThAer+Meso+Iron system. This result was also observed by Muller *et al*. (2007), who found that more VS reduction from biosolids did not necessarily result in less tVOSC generation from dewatered biosolids.

Overall, the data show that solids removal efficiencies of the pre-aerated systems in association with anaerobic digestion systems are comparable to multi stage thermophilic systems even at a smaller overall retention time. In addition, digestion systems with a thermophilic digestion system as the final stage (For example, Thermo, 4Thermo and ThAer+Thermo+Iron) produced greater total organic sulfur based odor compounds than digestion systems with a mesophilic digestion system as a final stage (For example, Meso, Meso+Iron, 4Tapered, 4Tapered+Iron, ThAer+Meso+Iron and 3stage+Iron).

Methanogens are known to convert organic sulfur to sulfide in dewatered biosolids and anaerobic sediments (Higgins *et al*., 2006; Lomans *et al*., 2002). If methanogenesis in dewatered biosolids is inhibited by BESA, it is possible to measure the amount of peak tVOSC that can be otherwise consumed by methanogens (Higgins *et al*., 2006). Much greater peak tVOSC was measured from ThAer+Meso+Iron biosolids with BESA (586.4 ± 373.7 ppmv as S/g VS) than the one without BESA (2.7 ± 3.2 ppmv as S/g VS) while a similar amount of peak tVOSC was measure from ThAer+Thermo+Iron biosolids with and without BESA (152.1 ± 38.7 ppmv as S/g VS and 132.6 ± 15.9 ppmv as S/g VS, respectively). This indicates that methanogenic activity of thermophilic biosolids was greatly suppressed, which caused much greater peak tVOSC generation from dewatered thermophilic biosolids than from dewatered mesophilic biosolids.

The 4Tapered and mesophilic systems were chosen to compare the effects of iron on sulfur based odor generation from dewatered biosolids since these two systems were operated with and without iron in the study. As expected, a significant decrease of H_2S was observed from dewatered biosolids that were generated from digestion systems with additional biosolids iron. Peak H_2S from dewatered biosolids of the reactor 4 of 4Tapered+Iron and the Meso+Iron system (72.2 ± 54.6 ppmv as S and 199.0 ± 205.6 ppmv as S, respectively) were much less than those from the 4Tapered and Mesophilic systems (544.7 ± 711.6 ppmv as S and 10119.1 ± 2975.7 ppmv as S, respectively). However, additional iron in the feed sludge did not ensure less VOSC generation from biosolids. Dewatered biosolids from iron added digestion systems produced greater VOSCs than the digestion systems without iron addition (Table 2). Iron seems to be an

excellent measure to eliminate H_2S odors from biosolids cakes, but other strategies will be needed to control sulfur based organic odors from dewatered anaerobic biosolids.

Incubation times when the peak tVOSC was measured were also of interest (Fig. 3). It was observed that adding iron to the feed sludge could shorten the incubation time to peak tVOSC generation from dewatered biosolids. If no iron was added to the feed sludges, thermophilically digested and dewatered biosolids generated peak tVOSC in 2 weeks or later while peak tVOSC was observed in less than 10 days from mesophilically digested and dewatered biosolids. A similar result was observed by Wilson (2006), who found that dewatered thermophilic biosolids generated peak tVOSC at much later incubation time than dewatered mesophilic biosolids. However, iron addition shortened the incubation time for thermophilically digested and dewatered biosolids to less than 10 days (For example, 15 days for reactor 1 of 4Tapered vs. 3 days for reactor 1 of 4Tapered+Iron). Additional iron did not shorten the time to peak tVOSC from most of dewatered biosolids from ThAer systems. They generated peak tVOSC at the incubation time of 10 days or longer even though iron was added to the feed sludges. One exception was the ThAer+Meso single system, where peak tVOSC was generated in about 5 days. Adding iron may not reduce the peak tVOSCs from dewatered biosolids, but iron may shorten the necessary storage time of dewatered biosolids if storing is used as a biosolids stabilization method.

4.2 Solution biopolymer and dewatering properties

Greater solids reduction often results in more solution biopolymer during anaerobic digestion of biosolids (Novak and Park, 2004). Moreover, greater solution biopolymer

in the digestion system can cause poorer dewatering properties of biosolids (Novak *et al*., 2003). A similar trend was also observed in this study.

More solution biopolymer was measured from biosolids samples as more VS was removed. However, the ThAer systems with the mesophilic digestion system as the final stage (For example, ThAer+TPAD+Iron and ThAer+Meso+Iron) did not follow this trend. Effluent solution biopolymer from these ThAer systems was 40 to 60 % smaller than solution biopolymer in the effluents of multi stage anaerobic digestion systems that removed similar or greater % VS from the feed sludge (For example, 4Thermo, 4Tapered. 4Tapered+Iron). So the data for systems that included an aerobic digestion step were not considered to be comparable. Some studies (Park *et al.*, 2006) found that aerobic digestion of sludge could promote greater solution polysaccharide release while mesophilic anaerobic digestion of sludge resulted in greater solution protein in the system. In addition, high solids destruction by thermophilic anaerobic digestion usually results in much greater solution protein. It was observed that greater soluble polysaccharides were generated from pre-aerobic digestion of sludges (Table 2), which were consumed during the following anaerobic digestion. Effluent polysaccharides from the all three ThAer systems contained less than 30% of solution polysaccharides in the effluents of multi stage anaerobic digestion systems that removed similar % VS from the feed sludge (For example, 4Thermo, 4Tapered). Lower concentration of polysaccharides resulted in lower biopolymer contents in the effluents of the ThAer systems. However, the subsequent thermophilic anaerobic digestion (For example, ThAer+Thermo+Iron) resulted in higher protein release thus resulting in greater biopolymer contents in effluents (Table 2). Since greater solution biopolymer worsened

the dewatering properties of biosolids regardless of their digester configurations, the ThAer systems with a mesophilic reactor as the final stage produced biosolids easier to dewater than those from other multi stage systems while maintaining comparable VS reduction efficiencies.

As shown in Fig. 3, greater solution biopolymer gave rise to greater polymer dosage requirements along with higher unconditioned biosolids CST values. In other words, enhanced solids removal efficiencies lead to the release of greater amounts of solution biopolymer and poorer dewatering properties of biosolids. Much of this solution biopolymer is colloidal material and that accounts for the effect on biosolids filtration (Novak and Park, 2004).

4.3 Extractable biopolymer

Various extraction methods were applied to each single and multi-staged digestion system to evaluate their solid-bound biopolymer. The extractable biopolymer is important since more solid-bound biopolymer in biosolids may lead to greater odor generation from dewatered biosolids pellets as well as more expensive biosolids hauling cost.

a. CER extraction

CER tends to extract biopolymer bound to divalent cations, primarily calcium and magnesium (Park and Novak, 2007). Since calcium was the major cation in the biosolids samples (Table 2), CER extraction profiles of each single and multi-staged anaerobic digestion system should provide useful information about the fate of a major

portion of the bound biopolymer. Two thermophilic systems (For example, 4Thermo and single thermophilic) removed as much CER extractable biopolymer from feed sludge as multi stage systems with a mesophilic digestion system at the last stage except for 4ADT system (Fig. 4). This tapered temperature system removed the most CER extractables. Statistical analysis was applied to determine the significance of differences between % removals of CER extractable from both groups. Initially equal variance could be assumed in accordance with F-test result (F-value = 0.5, p-value = 0.36 at α = 0.05). The statistical similarity of sample means were tested using two-sided t-test assuming equal variances and % removals of CER extractables from both groups were found to be statistically equal (t-value = -0.59, degree of freedom = 6, p-value = 0.58 at α = 0.05). Since two thermophilic systems (For example, 4Thermo and single thermophilic) and 5 multi stage systems with a mesophilic system at the last stage (For example, 4Tapered+Iron, Meso+Iron, 3 stage+Iron, ThAer+TPAD+Iron, and ThAer+Meso+Iron) removed statistically same % CER extractable biopolymer from feed sludge and 4Tapered system removed 20% more CER extractable biopolymer than these two groups, it was concluded that single stage thermophilic or multi stage systems with thermophilic digestion as the last stage removed equal or less CER extractable biopolymer from the feed sludges than multi stage systems with mesophilic digestion system as the last stage. Single stage mesophilic digestion system was the only system that removed much less CER extractable material from the feed sludges than thermophilic systems. Divalent cation bound biopolymer may be more susceptible to mesophilic anaerobic digestion than thermophilic digestion. When designing a multi stage biosolids digestion system, placing a mesophilic system as the final stage may enhance solids reduction from biosolids that are high in the divalent cations, calcium

and magnesium.

However, the CER extractable biopolymer removal data did not show a meaningful relationship with VS reduction ($R^2 = 0.10$). This suggests that there may be different pools of biopolymer involved in destruction of volatile solids. VOSC generation data was also poorly correlated to CER extractable protein removal data ($R^2 = 0.036$).

b. Base extraction

At high pH (~ 10.5), multi-valent cations such as aluminum become ionized and release attached biopolymer (Park and Novak, 2007). If ThAer systems are excluded, the % base-extractable removal data shows a modest correlation with VS reduction of each digestion system ($R^2 = 0.47$). The VS removal efficiencies of ThAer systems were comparable to other multi stage systems but less base extractable biopolymer was removed by ThAer systems with mesophilic digestion system as the last stage. Higher peak tVOSC was measured from the dewatered biosolids from the digestion systems that removed more base extractable protein. Moreover, more base-extractables were removed from the single stage thermophilic system and the multi stage systems with thermophilic digestion as the last stage than the single stage mesophilic systems and the multi stage systems with mesophilic digestion as the last stage (Fig. 5). This suggests that thermophilic systems can degrade more base extractable biopolymer than mesophilic systems. In addition, base extractable materials may not contribute to sulfur based odor generation from dewatered biosolids since high VOSC was generated from thermophilic biosolids cakes, from which much greater base extractables were removed during thermophilic digestion. Additional study should be warranted to further

understand the roles of aluminum in biosolids digestion systems under different temperature conditions.

c. Sulfide extraction

Sulfides preferentially bind to ferrous iron resulting in the formation of ferrous sulfide, which forms a precipitate that gives anaerobic sludge its distinct black color. Park and Novak (2007) extracted iron bound biopolymer from the biosolids samples by adding sulfide and incubating the mixture until the color of mixed biosolids turned black. The staged-digestion system with a tapered temperature configuration (For example, 4Tapered and 4Tapered+Iron) and single stage mesophilic systems with and without iron addition were chosen for comparison purpose since they were operated under identical condition except that one reactor system was operated with iron addition.

Biosolids from single or multi stage systems with iron addition generated much greater sulfide extractable biopolymer (overall increase from feed = 147.2 ± 49.8 %) while systems without iron addition removed sulfide extractable biopolymer (64.6 % removal for the 4Tapered and 16.8% removal for single mesophilic system). The dewatered biosolids from some digestion systems that received additional iron also generated higher headspace organic sulfur than those from the digestion systems without iron addition (For example, 4Tapered and Meso systems vs. 4Tapered+iron and Meso+iron systems). This indicates that greater iron in the sludge digestion system, while lowering hydrogen sulfide gases, may increase the organic sulfur odors from centrifugally dewatered biosolids. A similar result was also found by Verma (2005), who observed that the dewatered biosolids from the digester with greater iron content in feed sludge

generated more headspace organic sulfur compounds. It also appears that the sulfide extractable biopolymer content may be useful as a predictor of the potential for organic sulfur-based odor generation from dewatered biosolids. This observation was done for two identical digestion systems (4Tapered and single meso) with or without iron addition. However, if all single and multi stage systems are considered, the change of sulfide extractable biopolymer poorly correlated to VS reduction (R^2=0.071). Poor correlation was also observed between % change of sulfide extractable protein and peak tVOSCs from dewatered biosolids (R^2 = 0.00024).

5. Conclusion

Several multi and single staged sludge digestion systems were studied and their biosolids stabilizing capabilities were studied. A number of parameters were collected from each digestion systems and the findings are as follow:

• Multi staged anaerobic digestion systems remove more solids than single staged mesophilic or thermophilic anaerobic digestion systems.

• Placing the thermophilic aerobic digestion system as the first stage may provide better solids reduction and odor control at a shorter retention time than digestion systems comprised of anaerobic digesters only.

• Greater VS reduction does not always result in less sulfur based odor generation from dewatered biosolids. More study is required to understand this decoupling between VS reduction and sulfur based odor generation from dewatered biosolids.

• Placing a mesophilic anaerobic digestion system as the last digester in the multi staged digestion system may provide better sulfur based volatile odor control from dewatered

biosolids, especially if the influent to the digester contains a high sulfate concentration.

• The extractable biopolymer profiles did not show an appreciable relationship with either VS reduction or sulfur based odor generation from dewatered biosolids. It appears that the contributions of pools of biopolymer to VS reduction and to VOSC generation from dewatered biosolids are very different.

• More CER extractables were removed from multi-staged digestion systems than single staged digestion systems. In addition, more CER extractable (divalent cation bound) biopolymer was removed from multi stage systems with a mesophilic system as the final stage than from multi stage systems with a thermophilic system as the final stage.

• More base extractables were removed from single thermophilic system and multi stage systems with a thermophilic system as the final stage than single mesophilic system and multi stage systems with a mesophilic system as the final stage.

• Iron addition provided a good control over H_2S generation from dewatered biosolids but did not remove sulfur based odors. However, iron addition shortened the time to reach peak organic sulfur concentration from dewatered thermophilic anaerobic biosolids.

6. Acknowledgements

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Table 1. Description of digestion systems

	No. of stages	Temperature	Solid Retention Time	Biosolids volume	Iron addition
4Thermo	4	55 ℃	6 day/each (total 24 day)	21, 15, 9, 9 L	
4Tapered	4	55, 49, 43, 37 ℃	6 day/each (total 24 days)	21, 15, 9, 9 L	
4Tapered + Iron	4	55, 49, 43, 37 ℃	6 day/each (total 24 day)	21, 15, 9, 9 L	500 mg/L to influent
3 stage + Iron	3	55, 49, 30 ℃	6 day/each (total 18 days)	21, 15, 9 L	500 mg/L to influent
ThAer + TPAD + Iron	3	55, 55, 37 ℃	2.5 day (THAER), 6 day/each (TPAD, total 12 days)	6.25, 9, 6 L	500 mg/L to influent
ThAer + Meso + Iron	2	55, 37 ℃	2.5 day (THAER), 12 day (Meso)	6.25, 6 L	500 mg/L to influent
ThAer + Thermo + Iron	2	55, 55 ℃	2.5 day (THAER), 12 day (Thermo)	6.25, 6 L	500 mg/L to influent
Thermo	1	55 ℃	24 day	12 L	
Meso	1	37 ℃	24 day	12 L	
Meso + Iron	1	37 ℃	24 day	12 L	500 mg/L to influent

- 4Thermo: 4 stage thermophilic anaerobic digestion system
- 4Tapered: 4 stage anaerobic digestion system with tapered temperature configuration
- 3 stage: 3 stage anaerobic digestion system with tapered temperature configuration
- ThAer: Single stage thermophilic aerobic digestion system
- TPAD: Temperature phased anaerobic digestion system (Thermo and Mesophilic)
- Meso: Single stage mesophilic anaerobic digestion system
- Thermo: Single stage thermophilic anaerobic digestion system

Table 2. Overall characteristics of digested biosolids

	Reactor No.	pH	VS reduction (%)	Solution protein (mg/L)	Solution polysaccharide (mg/L)	Unconditioned CST (sec)	Peak tVOSC (ppmv as S/g VS)	Total cation (mg/g TS)			
								Ca	Mg	Fe	Al
4Thermo	1	7.6 (0.4)	29.1 (10.6)	195.2 (60.1)	163.7 (51.8)	2233 (1063)	662.8 (86.1)	25.1 (10.7)	4.0 (0.7)	8.0 (1.1)	5.2 (0.9)
	2	8.0 (0.2)	40.1 (5.6)	255.0 (115.2)	193.9 (58.4)	4298 (1495)	577.7 (278.9)	36.6 (13.0)	5.1 (1.9)	11.3 (3.2)	6.8 (1.7)
	3	-	44.3 (4.5)	-	-	-	-	32.7	3.8	10.5	5.8
	4	8.4 (0.2)	53.3 (5.2)	360.6 (132.4)	292.8 (75.5)	5540 (1800)	344.5 (275.0)	37.1 (18.6)	4.4 (0.9)	10.5 (3.5)	6.5 (1.9)
4Tapered	1	7.3 (0.1)	30.8 (2.1)	126.3 (59.6)	106.7 (2.4)	1243 (414)	235.1 (75.8)	44.3 (0.9)	3.4 (0.8)	3.3 (0.3)	5.0 (0.4)
	2	8.0 (0.0)	39.1 (2.2)	122.4 (59.8)	120.7 (14.9)	2501 (378)	151.4 (165.3)	56.1 (11.3)	3.3 (0.8)	3.8 (0.5)	5.8 (1.7)
	4	8.2 (0.1)	46.1 (3.2)	206.5 (28.9)	206.9 (27.7)	2931 (784)	29.4 (13.2)	55.6 (4.2)	4.3 (1.4)	4.7	7.4 (1.7)
4Tapered + Iron	1	7.5 (0.1)	35.3 (8.1)	75.2 (79.6)	40.5 (15.4)	1960	1.4 (1.0)	62.4 (4.0)	63.9 (19.3)	15.6 (0.1)	15.1 (2.4)
	2	7.7 (0.1)	42.4 (6.6)	125.6 (73.9)	51.9 (15.1)	1658	15.5 (14.1)	60.0 (3.6)	62.8 (4.6)	16.4 (1.6)	15.4 (4.1)
	3	8.5 (0.3)	42.7 (3.0)	193.5 (39.1)	77.3 (32.9)	-	-	50.6 (3.6)	40.1 (1.1)	12.4 (0.5)	8.1 (0.6)
	4	8.1 (0.1)	49.6 (3.6)	195.6 (107.3)	73.8 (41.9)	1791	48.8 (56.5)	55.5 (15.1)	19.3 (10.7)	11.7 (2.4)	9.8 (4.4)
3 stage + Iron	1	7.5 (0.1)	-	56.8 (12.6)	33.9 (9.7)	1960	1.5 (0.7)	62.4 (4.0)	63.9 (19.3)	15.6 (0.1)	15.1 (2.4)
	2	7.7 (0.1)	-	123.5 (21.8)	56.0 (15.5)	1658	15.4 (14.2)	60.0 (3.6)	62.8 (4.6)	16.4 (1.6)	15.4 (4.1)
	3	7.6 (0.1)	39.9 (1.8)	145.9 (34.3)	37.6 (16.5)	2126	50.8	61.6 (4.2)	1.7 (0.4)	16.2 (2.2)	11.3 (2.3)
ThAer + TPAD + Iron	1	6.6 (0.4)	33.7 (1.7)	129.6 (2.9)	208.1 (31.6)	2582 (383)	620.7 (158.0)	30.6 (9.8)	4.0 (0.8)	10.7 (2.5)	5.5 (2.1)
	2	8.0 (0.1)	46.9 (4.8)	147.3 (4.6)	74.2 (16.3)	3601 (1382)	92.0 (48.3)	32.7 (4.8)	4.5 (0.1)	10.9 (0.8)	5.9 (0.4)
	3	7.8 (0.1)	50.3 (3.8)	140.5 (23.9)	68.8 (26.8)	2556 (814)	67.3 (62.1)	46.5 (9.4)	4.8 (0.4)	12.2 (2.0)	6.6 (0.6)
ThAer + Meso + Iron	1	6.6 (0.4)	33.7 (1.7)	129.6 (2.9)	208.1 (31.6)	2582 (383)	620.7 (158.0)	30.6 (9.8)	4.0 (0.8)	10.7 (2.5)	5.5 (2.1)
	2	7.6 (0.1)	46.2 (4.3)	98.9 (5.7)	43.7 (24.9)	1494 (473)	2.7 (3.2)	46.2 (3.4)	5.2 (0.1)	12.8 (1.1)	6.8 (0.6)
ThAer + Thermo + Iron	1	6.6 (0.4)	33.7 (1.7)	129.6 (2.9)	208.1 (31.6)	2582 (383)	620.7 (158.0)	30.6 (9.8)	4.0 (0.8)	10.7 (2.5)	5.5 (2.1)
	2	7.9 (0,1)	50.4 (6.6)	402.5 (14.9)	88.8 (21.3)	4396	132.6 (15.9)	17.5 (3.1)	5.8 (0.8)	15.6 (3.7)	9.7 (2.3)
Thermo	-	8.0 (0.1)	41.6 (2.5)	181.6 (103.5)	180.3 (33.6)	4170 (351)	295.5 (79.3)	33.6 (8.9)	4.0 (0.3)	11.4 (3.4)	7.0 (1.4)
Meso	-	7.4 (0.2)	34.1 (2.1)	74.7 (15.0)	64.7 (18.8)	314 (75)	4.9 (2.5)	45.2 (14.8)	3.6 (0.2)	4.5	7.2 (1.0)
Meso + Iron	-	7.4 (0.1)	28.1 (2.3)	72.1 (49.5)	17.6 (16.3)	257	30.8 (1.2)	50.7 (0.5)	63.7 (9.1)	12.6 (0.7)	13.0 (1.0)

* Numbers in parentheses are standard deviations.

Figure captions

- Figure 1. VS reduction of biosolids and peak tVOSC generation from dewatered biosolids.
- Figure 2. Time to peak tVOSC from dewatered biosolids.
  *( ) is the digestion temperature in ℃.
- Figure 3. Dewatering properties of biosolids and solution biopolymer
- Figure 4. % removal of CER extractable biopolymer
  Each multi stage system represents the last digestion stage.
- Figure 5. % removal of base extractable biopolymer
  Each multi stage system represents the last digestion stage.

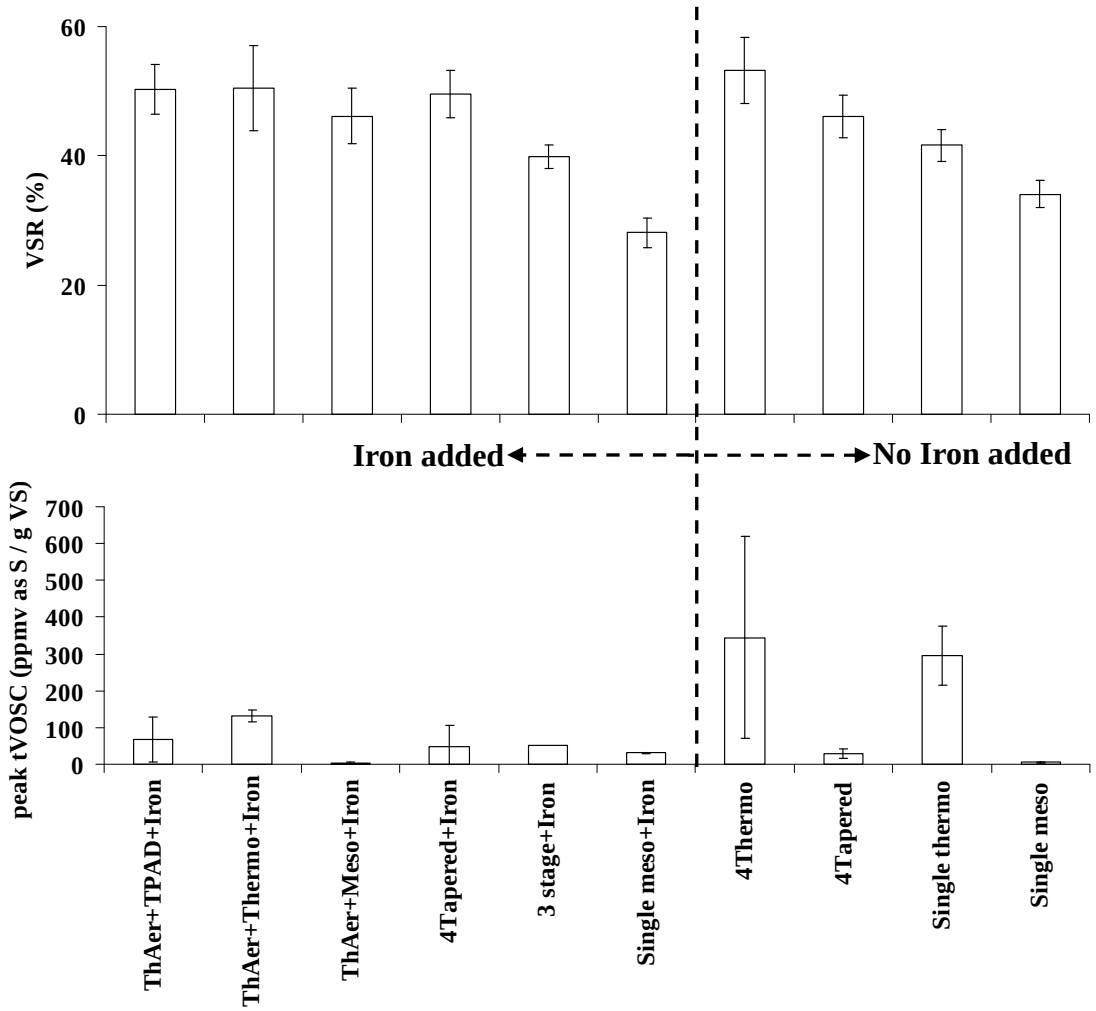

Figure 1. VS reduction of biosolids and peak tVOSC generation from dewatered biosolids.

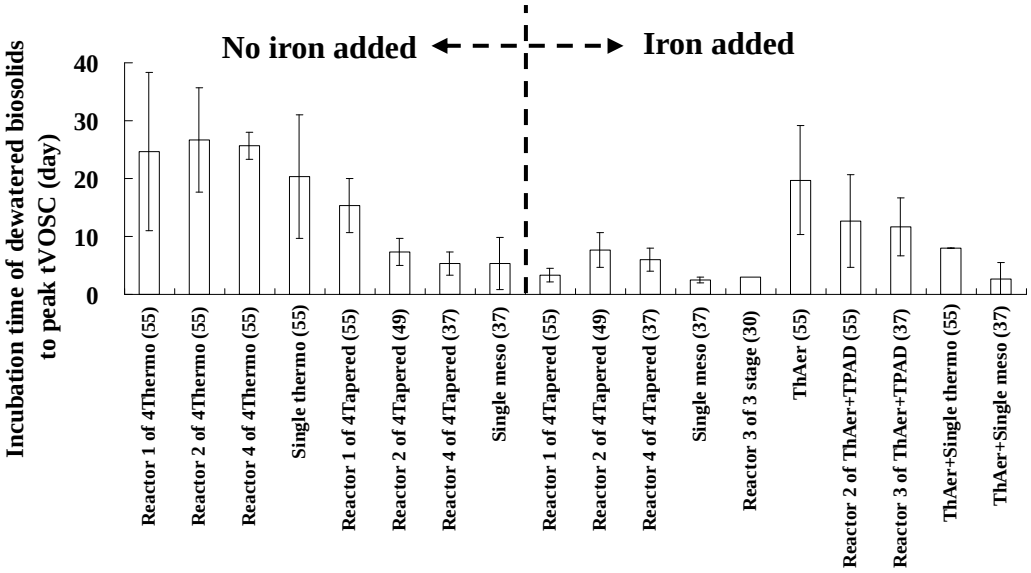

Figure 2. Time to peak tVOSC from dewatered biosolids.

*( ) is the digestion temperature in ℃.

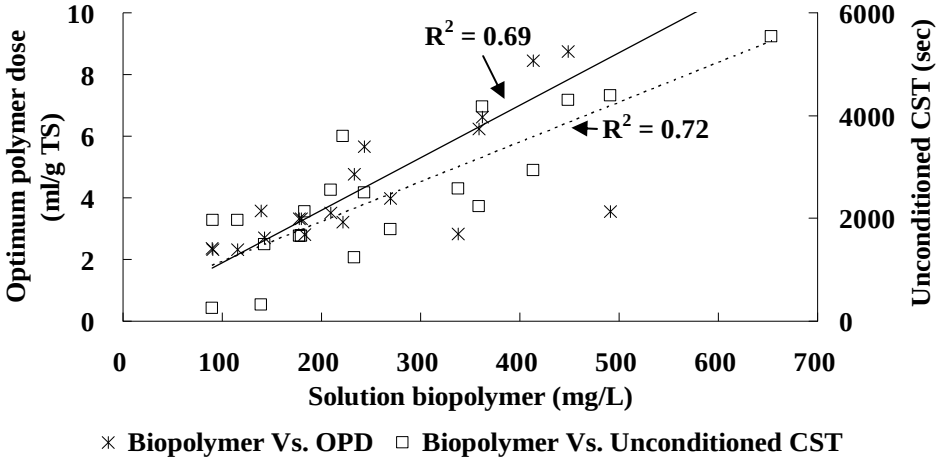

Figure 3. Dewatering properties of biosolids and solution biopolymer

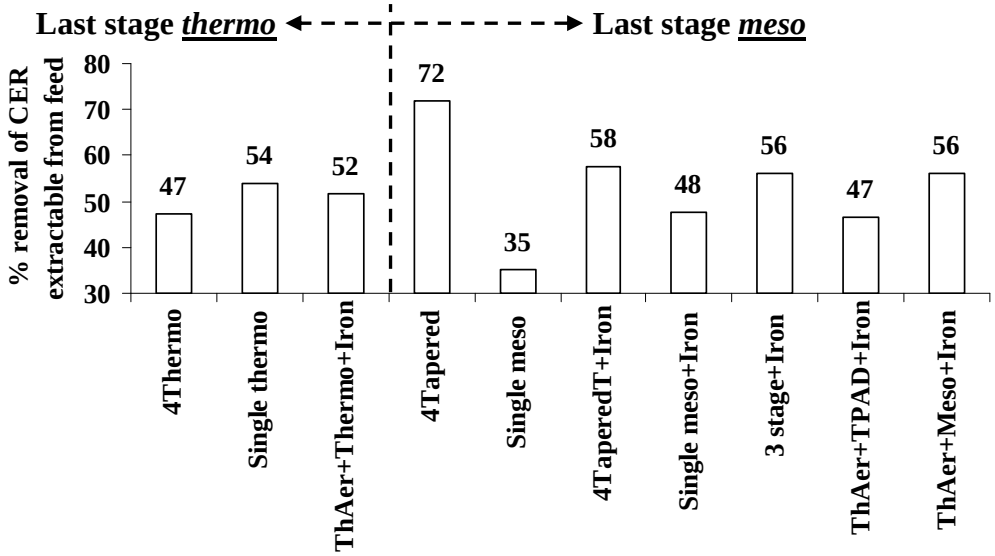

Figure 4. % removal of CER extractable biopolymer

* Each multi stage system represents the last digestion stage.

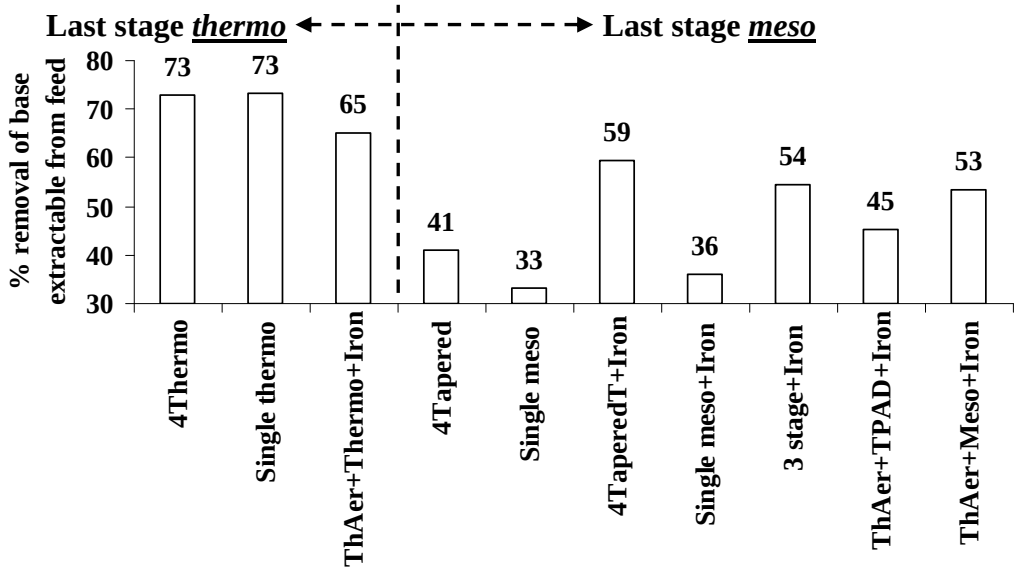

Figure 5. % removal of base extractable biopolymer

* Each multi stage system represents the last digestion stage.

How does dewatered biosolids odor increase after thermophilic sludge digestion?: Impact of lignin and sulfate to dewatered biosolids odors

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Abstract

A greater concentration of organic sulfur compounds were measured from thermophilically digested and dewatered biosolids when accompanied with excess lignin and sulfate in the feed sludges. High organic sulfur generation was thought to be caused by active methylation of sulfide during anaerobic incubation of thermophilic biosolids cakes and poor aceticlastic methanogenic activity under thermophilic conditions. Aceticlastic methanogens are major organic sulfur degraders. So their reduced activity resulted in accumulation of the intermediate degradation products, organic sulfides.

Key words

Thermophilic anaerobic digestion, sulfate, lignin, volatile orgniac sulfur compounds, biosolids cake

1. Introduction

After stabilization, biosolids or wastewater sludges may be dewatered and disposed to farmland as fertilizer or soil conditioner if classified as class A or B biosolids (EPA, 1994). Although dewatered biosolids are rich in many nutrients, odor emissions from them are problematic. Odor concerns were voted as the number one issue regarding biosolids disposal in a survey of the Water Environment Research Foundation member agencies (Witherspoon *et al*., 2004). Among the malodorous chemicals, sulfur based odor compounds or volatile organic sulfur compounds (VOSCs) have been reported as major odor constituents of anaerobically digested and dewatered biosolids (Higgins *et al*., 2006 and 2008, Muller *et al*., 2004). VOSCs include methanethiol (MT), dimethyl sulfide (DMS), dimethyl disulfide and dimethyl trisulfide.

There are two VOSC generation mechanisms described in the literature. These are degradation of sulfur containing amino acids such as cysteine and methionine (Higgins *et al*., 2006; Kiene and Hines, 1995) and methylation of sulfide or methylated sulfur compounds such as MT (Higgins *et al*., 2006; Kiene and Hines, 1995; Lomans *et al*., 2002). Methylation of sulfide was observed to be a major pathway to produce VOSCs in natural water systems and lignin or humus was found to be the main methyl donors (Lomans *et al*, 2002). This may be applied to the wastewater treatment systems since Higgins *et al.* (2006) measured increase of VOSCs during an anaerobic incubation of dewatered biosolids spiked with the methoxylated aromatic compounds, syringate.

It is widely agreed that methanogens and sulfate reducing bacteria (SRB) can degrade

organic sulfur compounds. Higgins *et al.* (2006) concluded that methanogens were mainly responsible for VOSC degradation after observing a dramatic increase of VOSCs in the headspace of the bottles incubating bromoethanesulfonate (BESA) treated dewatered biosolids. BESA is a methanogenesis inhibitor. Tanimoto and Bak (1994) observed oxidation of MT and DMS to carbon dioxide and sulfide by sulfate reducers from thermophilic fermenter sludge with sulfate or nitrate as electron acceptors. However, Isa *et al*. (1986) suggested that SRBs might be less involved in VOSC metabolism in the anaerobic sludge digestion systems since these sulfate reducers are poor floc formers and organic rich environment of anaerobic digesters can offset SRB's greater substrate affinity.

Many studies showed that methylotrophic or aceticlastic methanogens can metabolize VOSCs in the freshwater sediments (Lomans *et al.*, 2002). However, these aceticlastic methanogens were observed to lose their capability in a high temperature environment such as thermophilic anaerobic sludge digestion condition. Wilson *et al.* (2008) observed retardation of thermophilic aceticlastic methanogenic activities during the anaerobic digestion at 57.5℃. They postulated that acetate oxidation and hydrogenotrophic methanogenesis were more thermodynamically favorable than aceticlastic methanogenesis under the thermophilic anaerobic digestion condition at the temperature greater than 55℃.

A similar thermal preference of methanogenic communities was also observed by Freeman *et al.* (2008). They found that methanogens utilizing various range of substrate (H_2/CO_2, formate and acetate) such as *Methanobacterium beijingense*, *Methanosarta*

harundinacea and *Methanosaeta concilii* were the most archaeal operational taxonomic units in the mesophilic anaerobic digestion (MAD) with excess sulfate (1.8 – 5.3 g/L). On the other hand, hydrogenotrophic methanogens such as *Methanothermobacter thermautortophicus* and *Methanobacteriales* were observed to be the primary archaeal species in the thermophilic anaerobic digestion with the same amount of sulfate in the system.

Studies at Virginia Tech, VA showed that thermophilically digested and dewatered biosolids could produce much greater VOSCs than mesophilically digested and dewatered biosolids (Kim and Novak, 2010). The partial source of the feed sludge was from the pulp and paper industry, which was high in sulfate and lignaceous materials. The result does not conform to the previous studies, where significantly high VOSCs were not observed from thermophilically digested and dewatered biosolids (Higgins *et al.*, 2008). However, it should be also noted that biosolids odors can be also affected by the dewatering method and types of sludge conditioning chemicals.

2. Objectives

This study was planned to determine the following:

a) Can lignin serve as a major methyl donor during the anaerobic incubation of dewatered biosolids? Since lignin is known to be one of the most recalcitrant materials in the nature, can it be degraded during thermophilic digestion?

b) Are methane and MT degraders active in thermophilic digestion? If their activity is reduced or few organisms are present, it is expected that MT will not degrade to sulfide in thermophilically digested and dewatered biosolids.

3. Methodology

The study was carried in two parts.

- The first part was to see if lignin, a highly methoxylated natural organic compound, can react with sulfide to form organic sulfur compounds during thermophilic or mesophilic anaerobic incubation of sludge centrates.
- The second part was to see if the same organic sulfur generation can be observed from dewatered biosolids cakes that were previously digested in either thermophilic or mesophilic condition with additional lignin and sulfate in the system.

3.1. Experiment setups

3.1.1. Role of lignin as methyl donor to form MT

The overall experiment was planned as Table 1. Digested thermophilic and mesophilic biosolids were collected from a previous study at Virginia Tech (Kim and Novak, 2010). Biosolids were from digesters operated under either thermophilic (55℃) or mesophilic (37℃) conditions with a 24 day retention time. The feed sludge used during the study was thickened waste activated sludge from Western Lake Superior Sanitary District in Duluth, MN, USA, in which a portion of the inflow was from pulp and paper industries. High sulfate is usually measured in this wastewater. These biosolids were collected and

stored in a 4℃ refrigerator until this study. Total storage time was about 1 year. At first, each digested biosolids sample was centrifuged at 2800 rpm (910 G) for 15 minutes and the supernatant was collected. This centrate was used for the study and the solids were discarded. Fifty milliliter (mL) glass vials and Teflon lined septa caps were used for the incubation. Each of 4 incubation bottles was filled with 30 mL of centrate from thermophilic biosolids. Two mL of 1M Na_2SO_4 solution was added to each of three incubation vials and 20 and 40 mg of alkali lignin (Aldrich, MO) were added to two incubation vials along with additional sulfate. Each bottle was purged with pure nitrogen for 15 sec before capping. The same preparation was done for the mesophilically digested biosolids. The thermophilic set was kept in a water bath (HAAKE DC 10, Thermo electric corporation, Germany) at 55℃ and the mesophilic set was held in a 37℃constant temperature room. The retention time was 12 days for both sets.

During the incubation, excess gas volume and composition (e.g. methane and MT) were monitored every 2 or 3 days.

3.2.2. Impact of sulfate and lignin on odor generation from dewatered biosolids

For the second part of the study, eight batch digestions (Table 2) were carried and their digested biosolids were centrifugally dewatered to study the impact of sulfate and lignin on MT generation from centrifugally dewatered biosolids. The first batch digestion set was prepared with primary and secondary sludges from the Christiansburg wastewater treatment facility, VA, USA. Primary and secondary sludges were mixed with either mesophilically or thermophilically digested biosolids (seed) from a previous study at

Virgnina Tech (Kim and Novak, 2010) and the mixing ratio was 2:2:1 (i.e. primary: secondary: seed) by volume. The second batch digestion was prepared with primary and secondary sludges from Pepper's Ferry wastewater treatment plant, VA, USA. The mixing ratio was 3:1:1 (i.e. primary: secondary: seed) by volume. The seed sludge was same to the one used for the first batch digestion. Both wastewater treatment plants have primary and secondary (i.e. activated sludge digestion system) wastewater treatment systems. Pepper's Ferry applies iron to suppress sulfide generation from their treatment facility.

Some of batch digestions were carried with additional sulfate and lignin (i.e. Numbers in parentheses are additional sulfate or lignin in mg/L). The target initial sulfate and lignin contents were 200 mg/L and 6 g/L, which were greater than those found in Duluth feed sludge, 171.3±3.7 mg/L and 5.9 g/L, respectively. All thermophilic batch digestions were done in a water bath (HAAKE DC 10, Thermo electric corporation, Germany) at 55℃ and mesophilic batch digestions were kept in a constant temperature room at 37℃. The overall batch digestion conditions are given in Table 2.

3.2. Analysis

3.2.1. Solids

Total solids (TS) and volatile solids (VS) were measured according to standard methods (APHA, 1998).

3.2.2. Anion

Solution sulfate (SO_4^{2-}) was analyzed using an Ion Chromatograph (Model No. DX120,

Dionex, CA) equipped with AS9-HC column (Model No. 051786, Dionex, CA). The eluent was 9.0 mM Na_2CO_3 and the flow rate was 1.0 mL/min.

3.2.3. Lignin

Lignin in biosolids sample was measured in accordance with ASTM E 1721 (2001). Three hundred mg of dried sludge (at 80℃ for overnight) was ground with a laboratory mortar and a pestle. After acid digestion with 3 mL 72% H_2SO_4 in a 35℃ water bath (HAAKE DC 10, Thermo electric corporation, Germany) for 1 hour, the digested sample was transferred to a 250 mL glass bottle (I-Chem, NY) along with 84 mL of de-ionized water. This bottle was autoclaved for an hour at 121℃ and 103.4 kPa. After autoclave, digestion was filtered through 1.2㎛ glass fiber filter (Fisher, PA). The filtered material and filter were dried at 105℃ for overnight and incinerated at 550℃ for 20 minutes. The weight loss by incineration is considered to be the amount of acid-insoluble material or lignin in the sample. For the quality purpose, two standards with 50 mg alkali lignin (Aldrich, MO) were treated as samples and incinerated amounts were used as correction factors.

3.2.4. Preparation for dewatered biosolids

Dewatering properties of digested biosolids from [Thermo + Sulfate + Lignin] and [Meso + Sulfate + Lignin] digesters for 2 batch sets were determined in accordance with Muller *et al.* (2004). Briefly 100 mL of biosolids was mixed with a certain volume of cationic polymer (1% Clarifloc 3275 by weight, Polydyne, GA) and sheared in a Waring blender (Model No.: 55A60VL22, General Electronics, IN) at 2300 rpm (120 G) for 30 sec. After shearing, biosolids and cationic polymer mixture was tested for dewatering

time by a capillary suction time apparatus (Model No.: W.R.C type 165, Triton Electronics Ltd., England) in accordance with standard method 2710G (APHA, 1998). The polymer dose at which the lowest CST was measured was determined as an optimum polymer dose and it was used for biosolids cake production.

The optimum polymer dose for [Thermo + Sulfate + Lignin] was applied to other thermophilic batch-digested biosolids to prevent change of odor generation potentials by applying different amount of cationic polymer (Muller *et al.*, 2004). After collecting about 500 mL of biosolids conditioned with optimum polymer dose, the mixture was centrifuged in a lab centrifuge at 10000 rpm (17700 G) for 15 min under a room temperature. The dewatered biosolids (biosolids cakes) were collected and pressed at 207 kPa for 15 min by a lab press. This process simulates a high-solids centrifuge system that causes pressed biosolids to produce the greatest organic sulfurs during anaerobic incubation (Muller *et al*., 2004). Five gram (g) of presses biosolids was collected in a 50 mL incubation bottle with Teflon lined septa cap. The incubation bottle was purged with pure nitrogen for 15 sec before capping. Total of 4 bottles were prepared for each batch digestion except for the digestion with no additional lignin and sulfate (2 bottles). Methanogenesis was inhibited in 1 of 4 bottles by adding 1 mL of 127 mM 2-bromoethanesulfonic acid (BESA, Acros, Netherlands) to 5 g dewatered biosolids and sulfate reduction was inhibited in another bottle by adding 1 20mM Na_2MoO_4-$2H_2O$ to 5 g dewatered biosolids. BESA is a methanogenesis inhibitor (Higgins *et al*., 2006) and molybdate is a sulfate reduction inhibitor (Isa and Anderson, 2005).

3.2.5. Gas volume and composition

Excess gas volume was checked by observing volume displacement of acidic salt solution as described in standard method 2720B (APHA, 1998). Gas volume drawn for analysis was taken into account to calculate total excess gas volume.

Headspace carbon dioxide and methane was measured by a gas chromatograph (Model No. GC-14A chromatograph, Shimadzu, Japan) and a chromatogram (Model No. CR501 chromatopac, Shimadzu, USA). Sample volume was 100 microliter (μL) and carrier gas was helium with a flow rate at 28 mL/min. The analysis was done with thermal conductivity detector (TCD) and 1.8 m column packed with HayseptD. Column temperature was 25℃ and injector temperature was 70℃. TCD was kept at 110℃. Total analysis time was 10 min.

MT was measured by the method of Glindemann *et al*. (2006). One hundred μL headspace gas was periodically collected and VOSCs in the gas sample was quantified by gas-chromatograph/mass spectrometry (GC 6890, MSD 5970, Hewlett-Packard, PA) with a cryo-trapping system. The cryo-trap was employed to accumulate gas samples and to generate narrow chromatographic peaks. The operational condition for GC-MS was a 30 m long and 0.25 mm I.D. column (Model No. 20751-01A, Supelco, PA); gas injection inlet (200 ℃); helium carrier gas at 2 mL/min; increase of oven temperature from 50 to 265 ℃ at a rate of 35 ℃/min. Total analysis time was 7.64 min. MT was of interest in this study since this organic sulfur compound was the most odorous compound from dewatered biosolids. Peak areas were integrated by the data analysis program, G1034C version C.03.00 (Hewlett-Packard, PA). The amount of MT in each

sample was quantified by comparing the sample peak area with the area of a standard gas mixture of known amounts of MT (Scott Specialty Gases Inc., PA).

4. Results

4.1. MT generation from centrate

This experiment was carried out to determine if lignin could serve as a methyl donor to sulfide and generate methylated sulfur compounds such as MT under either mesophilic or thermophilic anaerobic digestion conditions. In order to exclude any existing lignaceous materials, both thermophilically and mesophilically digested biosolids samples were centrifuged and their centrates were collected for the study.

A small amount of gas production (i.e. total volume is 1.5 to 2.5 mL) was measured from all incubation bottles. Since each incubation bottle was deprived of organics and biomass, no headspace methane was found. Carbon dioxide also comprised little portion of headspace.

However, very different MT generation was observed from incubation bottles under the two different temperature conditions. Little MT (< 5 ppmv as S) was generated in the mesophilic systems with or without lignin and sulfate in the system while 60 ppmv MT as S was generated from the thermophilic system with sulfate and lignin addition (Fig. 2). More lignin in the thermophilic system also resulted in about 25% more peak MT generation.

4.2. MT generation from dewatered biosolids

Two different sludges were batch-digested under different conditions and centrifugally dewatered to study the impact of excess sulfate and lignin on MT generation from dewatered biosolids. Two inhibitors were applied to some of biosolids cakes to observe the portions of MT that methanogens or SRBs can degrade during anaerobic incubation. Overall data is given in Table 3. Pepper's Ferry biosolids cakes generated much less peak MT than Christiansburg biosolids cakes. However, both biosolids cakes produced greater peak MT when added with sulfate and lignin during batch digestion.

Cake solids of dewatered biosolids were somewhere between 27 and 35% but they do not seem to affect MT generation. However, dewatered biosolids from [Thermo + Sulfate + Lignin] produced much greater peak MT than those from [Meso + Sulfate + Lignin] (Fig. 3).

Within thermophilic sets, dewatered biosolids from batch digestion with additional sulfate and lignin produced more peak MT than other dewatered biosolids without one or both additives (lignin and sulfate). It seems clear that thermophilic digestion condition can worsen organic sulfur based odors if associated with extra sulfate and lignin in the feed sludge compared to mesophilic sludge digestion systems. If not associated with lignin in the influent sludge, additional sulfate in the thermophilic digestion system did not result in greater MT generation from thermophilic biosolids cakes compare to ones without sulfate addition.

The [Thermo+Sulfate] biosolids cake for Pepper's Ferry plant was not included for inhibition analysis since its MT generation pattern with inhibition (e.g. BESA and molybdate) was much different from other thermophilic biosolids and it seems to be caused by bad preparation.

It was observed that, with methanogenesis inhibition by BESA, most of thermophilic biosolids cakes produced less peak MT than those without BESA addition. However, mesophilically digested and dewatered biosolids produced much greater peak MT with methanogens inhibited by BESA.

Much lower peak MT generation was measured from dewatered thermophilic biosolids treated with molybdate, a sulfate reduction inhibitor, than ones without SRB inhibition. However, it is not clear if SRBs removed significant portions of MT from mesophilic biosolids cakes or not. Since mesophilic biosolids cakes with molybdate produced greater peak MT than the ones without SRB inhibition for the first set while much less peak MT was measured from SRB inhibited mesophilic biosolids cakes than the one without additives for the second set.

Poor methanogenesis was observed from all the dewatered biosolids (Fig. 4). Less than 20% of headspace was measured as methane from most of thermophilic biosolids cakes. Low methane in the headspace was also observed from mesophilic biosolids cakes but methanogenic activities were developed in a relatively short incubation time around 13 days. Molybdate did not impact methanogenesis of any dewatered biosolids (data not shown).

5. Discussions

In the first part of study, lignin was found to be a methyl donor to sulfide for MT generation under thermophilic anaerobic digestion condition. With lignin and extra sulfate in the system, MT was measured from the thermophilically incubated centrate of thermophilic biosolids only. This implies that lignin could be solubilized under thermophilic condition at 55℃ and solubilized lignin could bind with sulfide to produce headspace MT via enzymatic reactions. Two times greater lignin in the thermo system resulted in about 25% greater peak MT generation, which also supports the assumption that lignin can serve as a methyl donor to sulfide to form MT. Degradation of cysteine or methionine can be excluded from MT generation pathways in this study since no MT was generated from thermophilic centrate without additives. Methylation may not be the major MT generation pathway for mesophilic biosolids cakes since little MT was measured from all the mesophilic centrates with or without sulfide and lignin.

Based on the finding from the first part of the study, the second part of study was carried to determine if lignin and excess sulfate could result in higher MT generation from thermophilically digested and centrifugally dewatered biosolids. It seems clear that excess lignin and sulfate in the thermophilic sludge digestion system can cause biosolids cakes to produce greater MT than mesophilic systems. In this study, greater MT generation was observed from [Thermo + Sulfate + Lignin] biosolids cakes than [Meso + Sulfate + Lignin] biosolids cakes. However, different sludges resulted in different amount of MT increase under same anaerobic digestion and biosolids cake

preparation conditions. Although the overall MT generation pattern did not change, the amount of MT generated by thermophilic batch digestion for Christiansburg sludge (plant 1) was very different from Pepper's ferry sludge (plant 2).

Even with methanogenesis inhibition by BESA, most of thermophilic biosolids cakes generated less peak MT than those without BESA in the system. Since MT can be degraded by methylotrophic or aceticlastic methanogens and thermophilic batch digestion environment is not thermodynamically favorable for these MT degraders (Wilson *et al*., 2004), little MT can be removed by methanogenic activities if biosolids cake is generated from a thermophilic sludge digestion. In this regard, if high MT generation condition is developed by high sulfate and lignin in the influent sludge, much greater MT generation can be expected from thermophilically digested and dewatered biosolids. In fact, many studies found that the dominant methanogens in thermophilic condition were hydrogenotrophic methanogens (Kiene *et al.*, 1986; Lomans *et al.*, 2002; Wilson *et al.*, 2008). However, MT degraders in mesophilic biosolids cakes were well inhibited by BESA thus resulting in much greater peak MT generation. Wilson *et al.* (2008) also observed that most of mesophilic methanogens were aceticlastic methanogens.

It seems that SRBs compete against methanogens for MT as substrate during an anaerobic incubation of thermophilic biosolids cakes. A huge reduction of peak MT (i.e. 50 to 76 %) was observed from SRB inhibited thermophilic biosolids cakes compared to ones without SRB inhibition. On the other hand, 6 to 39% less peak MT was observed from methanogenesis inhibited thermophilic biosolids cakes compared to ones without

BESA. These findings imply that methanogens can remove much greater MT than SRBs from thermophilically digested and dewatered biosolids and methanogenic removal of MT may be enhanced by SRB inhibition. The competition between methanogens and SRBs was not clear for dewatered mesophilic biosolids. Since [Meso + Sulfate + Lignin] with molybdate in the system produced greater peak MT than the dewatered mesophilic biosolids without any additives for the first set while much less peak MT was observed from dewatered mesophilic biosolids with molybdate than from the one without additives.

Poor methanogenic activities were measured from all biosolids cakes. However, different development pattern for methanogenesis was observed from mesophilic and thermophilic biosolids cakes. Headspace methane from mesophilic biosolids cakes was measured at earlier incubation time than thermophilic biosolids cakes. This observation also supports poor MT degradation in thermophilically digested and dewatered biosolids cakes since methanogens are major MT degrader in the dewatered biosolids (Higgins *et al.*, 2006). In addition, SRBs did not impact methanogenic activities even though they were found to be involved in MT degradation from thermophilic biosolids cakes. Their portions in biosolids cake odor reduction seem to be much smaller than methanogens.

6. Engineering implications

In this study, excess sulfate and lignin in the sludge inflow were studied to evaluate their impact on dewatered biosolids odors in association with different sludge digestion temperatures. There were several important engineering implications were drawn from

the study and they are:

- Thermophilic anaerobic digestion can produce a much odorous dewatered biosolids if associated with high sulfate and lignin in the inflow sludge. This would be commonly found for some industrial discharges. In this study, sulfate at 200 mg/L and lignin at 5 g/L or greater in the thermophilic batch digestion system resulted in much greater MT generation from dewatered biosolids.
- MT degrading microbes (e.g. methanogens) did not function after thermophilic anaerobic digestion of sludge.
- SRBs can remove MT from thermophilically digested and dewatered biosolids but the amount of removed MT is much smaller than methanogens can. Their capability to reduce MT is not clear for mesophilic biosolids cakes.

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Table 1. Experiment sets for the first part of study

Thermophilic at 55℃	Mesophilic at 37℃
Thermo[a]	Meso[b]
Thermo[a] + Sulfate[c]	Meso[b] + Sulfate[c]
Thermo[a] + Sulfate[c] + Lignin[d]	Meso[b] + Sulfate[c] + Lignin[d]
Thermo[a] + Sulfate[c] + Lignin[d] x 2	Meso[b] + Sulfate[c] + Lignin[d] x 2

a. Supernatant of centrifuged thermophilically digested biosolids (Duluth, MN) at 2800 rpm

b. Supernatant of centrifuged mesophilically digested biosolids (Duluth, MN) at 2800 rpm

c. 2mL of 1M Na2SO4, d. 20mg of alkali lignin

Table 2. Characteristics of anaerobic sludges before and after batch digestion

		pH	Volume (L)	Source of sludge	TS / VS (% / %)	Sulfate (mg/L)	Lignin (g/L)	Temper-ature (℃)	Incubation time (day)
Plant 1	Thermo	6.4 (0.0)	3	Christians -burg	1.8 / 1.5	30.6	-	55	24
	Thermo + Sulfate	-	3	Christians -burg	-	200 (169.4)	-	55	24
	Thermo + Sulfate + Lignin	-	3	Christians -burg	-	200 (169.4)	(1.6)	55	24
	Meso + Sulfate + Lignin	6.6 (0.0)	2	Christians -burg	1.4 / 1.2	200 (132.5)	(1.8)	37	24
Plant 2	Thermo	7.3 (0.0)	3	Pepper's Ferry	1.6 / 1.2	29.8	5.4	55	19
	Thermo + Sulfate	-	3	Pepper's Ferry	-	200 (170.2)	5.4	55	19
	Thermo + Sulfate + Lignin	-	3	Pepper's Ferry	-	200 (170.2)	7.0 (1.6)	55	19
	Meso + Sulfate + Lignin	7.2 (0.0)	3	Pepper's Ferry	1.6 / 1.2	200 (146.1)	6.7 (1.8)	37	19

* Thermo: Thermophilic anaerobic batch digestion (55℃); Meso: Mesophilic anaerobic batch digestion (37℃)

* Numbers in parentheses are additional sulfate or lignin in mg/L.

* - : Data not collected.

Table 3. MT generation from dewatered biosolids inhibited with different chemicals

Plant 1 (Christiansburg)			**Plant 2 (Pepper's Ferry)**		
Name	Cake Solids (%)	Peak Methanethiol (ppmv as S)	Name	Cake Solids (%)	Peak Methanethiol (ppmv as S)
Thermo + Sulfate + Lignin	31.0	1058	Thermo + Sulfate + Lignin	27.1	280
w/ BESA	-	993	*w/ BESA*	-	170
w/ Molybdate	-	524	*w/ Molybdate*	-	63
Thermo + Sulfate	29.5	896	Thermo + Sulfate	32.0	140
w/ BESA	-	543	*w/ BESA*	-	-
w/ Molybdate	-	270	*w/ Molybdate*	-	-
Thermo	34.2	917	Th	35.4	188
Meso + Sulfate + Lignin	30.1	556	Meso + Sulfate + Lignin	34.7	204
w/ BESA	-	873	*w/ BESA*	-	513
w/ Molybdate	-	774	*w/ Molybdate*	-	112

* Thermo: Thermophilic anaerobic batch digestion (55℃); Meso: Mesophilic anaerobic batch digestion (37℃); -: Data not collected

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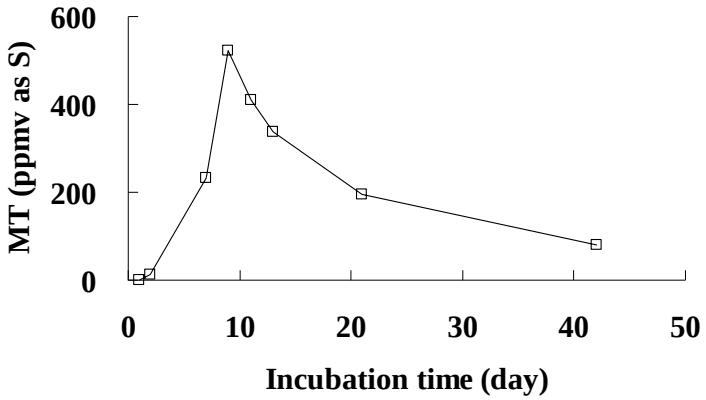

Figure 1. An example of methanethiol generation from dewatered biosolids.

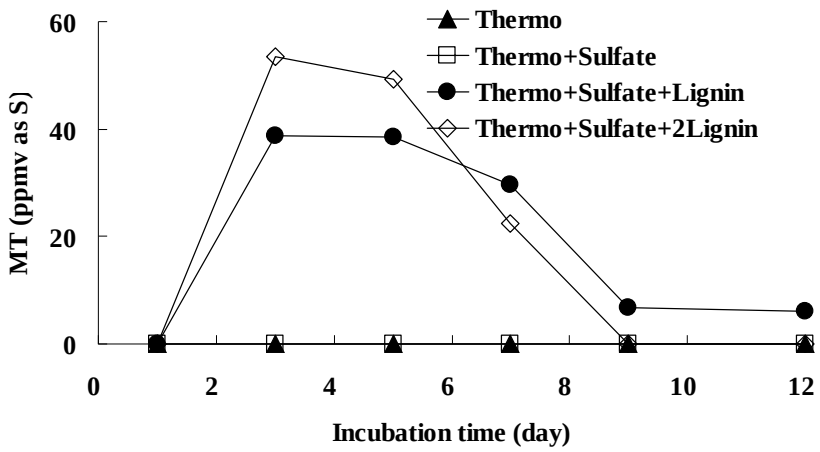

Figure 2. Methanethiol generation from thermophilically incubated thermophilic biosolids centrates with different sulfate and lignin additions.

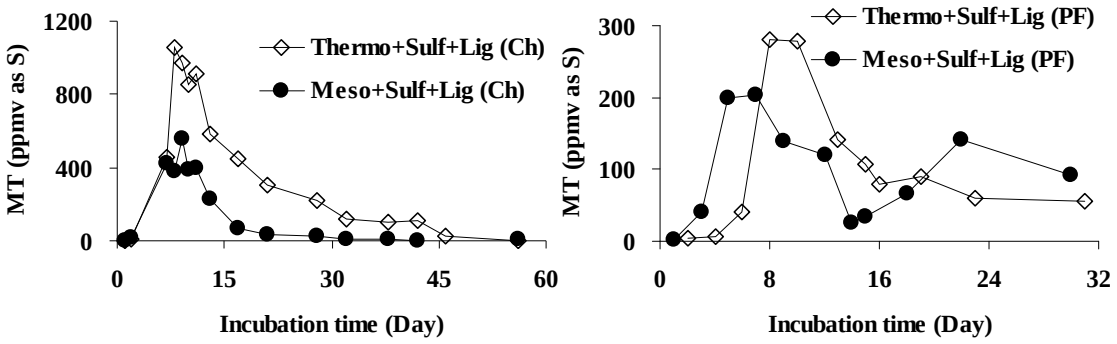

Figure 3. Methanethiol generation from [Thermo + Sulfate + Lignin] and [Meso + Sulfate +Lignin] biosolids cakes for plants 1 and 2.

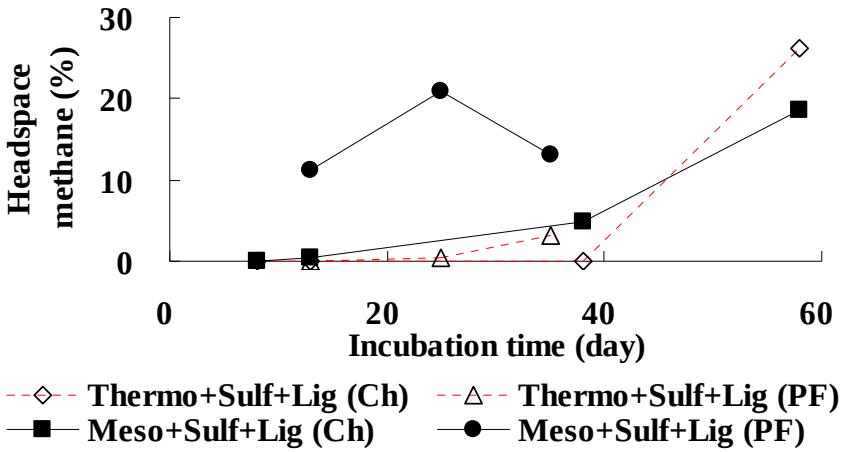

Figure 4. Headspace methane from [Thermo+Sulfate+Lignin] and [Meso+Sulfate+Lignin] biosolids cakes for two different sludges.

Chapter 6. A study of a combined anaerobic/aerobic system: enhanced nitrogen removal by a continuous aeration in the aerobic digestion system (Working paper)

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ABSTRACT

A combined anaerobic/aerobic sludge digestion system was studied to test its solids and nitrogen removal efficiencies. After a steady performance of the laboratory anaerobic digester (20 day retention time) was confirmed, effluent from the anaerobic digester was fed to aerobic digesters that were operated at retention times from 2 to 5 days. The anaerobic system was fed with a mixture of primary and secondary sludge from a local wastewater treatment plant. Feeding was done once per a day. The aerobic reactor was continuously aerated with ambient air to maintain a peak dissolved oxygen concentration at 1.1±0.3 ppm. More solids and ammonia reduction was observed from the aerobic system operated for the longer retention time but higher effluent nitrite/nitrate was also measured. Most of total Kjeldahl nitrogen was removed via ammonia oxidation but less than 10 % of removed ammonia was measured as oxidized nitrogen throughout the continuous aeration study. Aerobic denitrification was suspected as a major ammonia removal in the continuously aerated digestion system operated at low dissolved oxygen level.

KEY WORDS: *combined anaerobic and aeration digestion, continuous aeration, solids reduction, nitrogen removal*

1. INTRODUCTION

Excess nitrogen (N) in watersheds is one of the major causes of eutrophication and water quality deterioration. In this regard, the U S Environmental Protection Agency has regulated the effluent nitrogen level in treated wastewater and this has generated a great driving force for research to biologically remove nitrogen during treatment processes. Nitrogen in wastewater primarily exists as organic and ammonia-N and organic-N is converted to ammonia-N during wastewater treatment. Once organic-N is converted to ammonia, it can be removed by nitrification in the aerobic digestion system followed by denitrification in the anoxic digestion system Recently, simultaneous nitrification and denitrification systems have drawn interests (Grady *et al.*, 1999; Van Niel *et al*., 1992 and Wang *et al*., 2007) since this system does not require an additional anoxic zone for denitrification and organic matter in wastewater can be used as an organic source for denitrifiers.

Grady *et al.* (1999) proposed that the performance of sequencing batch reactors (SBR) could be optimized for greater ammonia removal and low nitrate in the effluent by changing the aeration fraction. It was concluded that the optimum aeration fraction was 60-70% of the total cycle time of the SBR. Zeng et al. (2004) also reported that simultaneous nitrification and denitrification was possible in a lab-scale SBR operated in alternating an anoxic-aerobic mode with a low dissolved oxygen (DO) environment.

In their study, only 2% of oxidized ammonia was found as nitrite or nitrate while 98% was emitted from the system as gas. These authors also observed that most nitrogen removal occurred via nitrite not nitrate, and the end product was mostly nitrogen oxide rather than nitrogen gas.

Some research has shown that microbes such as *Alcaligenes faecalis*, *T. pantotropha* and *Pseudomonas sp*. are capable of heterotrophic nitrification and denitrification in aerobic condition (Van Niel *et al*., 1992). *A. faecalis* is a nitrifier commonly found in soil and activated sludge. The difference between the amount of oxidized ammonia and the amount of oxidation products may be explained by the abundance of these heterotrophic nitrifiers that are also capable of aerobic denitrification. Greater specific growth rate and ammonia concentration along with low dissolved oxygen concentration are three observed requirements for active nitrification / denitrification by the heterotrophic denitrifiers (Van Niel *et al*., 1992). Moreover, experimental results showed that about 6–12 % nitrite in the aerobic zone could be removed by denitrification in the same aerobic system operated at the DO concentration smaller than 1 ppm and small ratio of chemical oxygen demand to total Kjeldahl nitrogen (COD/TKN ~2.9) (Wang *et al*., 2007).

A study performed by Kumar (2006) showed that the sequential anaerobic/aerobic (ANA/AER) sludge digestion system removed more than 60% overall volatile solids (VS) reduction. Among the overall VS reduction in the combined system, 10 to 15% additional VS reduction was from the AER system operated for 3 to 9 day retention times, respectively. Along with additional VS reduction, the AER system removed near

50% total N (TKN + oxidized N) from the ANA effluents. The author speculated that the most of nitrogen removal in the AER system was done via simultaneous nitrification and denitrification since little oxidized N was measured from the AER effluent. Similar result was also reported by Novak *et al. (2009)* who observed 62% overall VS reduction from the combined ANA/AER system with a 15 day anaerobic and a 5 day aerobic retention time along with 64.5% TKN removal from the combined digestion system.

2. OBJECTIVES

The combined ANA/AER digestion system was studied to evaluate the following objectives:

- To compare additional solids removal efficiencies in the aerobic digestion systems operated under continuous aeration for different retention times.
- To compare nitrogen removal efficiencies in the aerobic digestion systems operated under continuous aeration for different retention times.

3. METHODOLOGY

3.1. DIGESTION SETUP

The picture of overall digester setup is shown as Fig. 1.

The ANA system was prepared using a high density polyethylene brewer tank (Model No. f15b, The Hobby Beverage Equipment Company). Rubber gasket and silicone were used to ensure gas-tight sealing. Continuous gas mixing was applied by circulating

headspace gas to the bottom of the reactor using a peristaltic pump (Model No. 7553-70, Cole Parmer). A gas bag was also installed on the top of the reactor to alleviate excess gas pressure. The total sludge volume was 30 L and retention time was 20 days. Inoculation was done with anaerobically digested biosolids from a wastewater treatment plant in Philadelphia, PA. The reactor was kept in a constant temperature room at 37℃ throughout the study. The feed sludge was mixture of primary and secondary sludges from the Christiansburg wastewater treatment plant in VA, USA. The target total solids (TS) of feed sludge were 2%. Once steady state (standard deviation of VS reduction ≤ 10%) was confirmed after about 3 SRTs, feeding to the subsequent aerobic reactor was started.

A 10 L AER system built with acrylic plastic was used for the aerobic sludge digestion. Ambient air was supplied to the reactor by placing two bubble stones on the bottom of the reactor. Ambient air was provided to bubble stones by commercial electrical air diffusers. Tap water was added to the AER system right before wasting in order to maintain proper retention time. Continuous aeration was applied to find the effect of the solids retention time (SRT) of AER to solids reduction, ammonia removal and NO_x generation. One liter of effluent from the ANA was fed to the AER daily after wasting 1 L from the AER. Each SRT was simulated by changing sludge volume in the AER. For instance, a 3 L sludge volume was maintained to make a 3 day SRT of the AER.

3.2. ANALYSIS

Acidity of each biosolids sample was measured by a pH probe (Model No. 13-620-287, Accumet) while gentle mixing is applied. The pH meter (Model No. 910, Accumet) was

standardized against reference solutions of pH 4 and pH 7. The dissolved oxygen (DO) in the AER was measured periodically with a DO probe (Model No. YSI 5739, Yellow Springs Instrument Co., Inc.) and a DO meter (Model No. YSI 57, Yellow Springs Instrument Co., Inc.). TS, VS, COD, TKN and ammonia-N data were collected according to Standard Methods (APHA, 1998). TKN is a sum of ammonia-N and organic-N.

NO_2 and NO_3 (NO_x) were analyzed by an Ion Chromatograph (Model No. DX120, Dionex) equipped with AS9-HC column (Model No. 051786, IonPac). The eluent was 9.0 mM Na_2CO_3 and the flow rate was 1.0 mL/min. NO_2 comprised most of effluent NO_x in the study.

4. RESULTS

4.1. STEADY STATE PERFORMANCE OF ANAEROBIC DIGESTER

A steady state performance was observed from the ANA system throughout the study (Table 1). pH of the effluent was maintained at near neutral and a stable VS removal was observed throughout the study (standard deviation ~ 5.4 % of average VS removal). Small fluctuation was also observed for COD removal data (standard deviation ~ 2.4 % of average COD removal). Effluent COD was about 11239±3901 mg/L resulting in high COD/TKN at about 14. In the effluent from the ANA system, ammonia-N comprised about 58% of total-N.

4.2. CONTINUOUS AERATION STUDY

pH and solids removal data are given in Table 2. pH greater than 7 was observed for all

the effluents from the AER system operated for different SRTs. The pH range of this study fell in the pH range (6.0 – 9.0) that was observed to be suitable for complete nitrification in the aerobic system (Wang *et al.*, 2007). In addition, operation in the continuous aeration resulted in additional VS reduction, resulting in 60 to 74% overall VS reduction, as the SRT increased from 2 to 5 days. Although total VS reduction is different, additional VS reduction in the AER system treating ANA effluent fell in the values observed by other studies (Kumar, 2006; Novak *et al.*, 2009).

Along with greater solids removal, more ammonia and TKN were removed in the AER system operated for longer SRTs (Fig. 2). At 5 day retention time, ammonia and TKN removal from the effluent of the ANA system were near 97% and 56%, respectively in the continuously aerated AER system. Most of TKN removal in the AER system was from ammonia-N removal (Fig. 3) since overall organic-N removal in the AER was less than 5% of total-N removal. Considering that organic-N is indirect measure of total proteinaceous materials, less soluble protein release during an aerobic digestion of sludge (Novak *et al.*, 2003) may have caused little change of net organic nitrogen in the AER system operated for different SRTs.

More NO_x was measured from the AER system operated for longer retention time (Fig. 4). Three days or longer SRT seem to be the threshold for active nitrification in the AER system of this study since large increase of NO_x was observed in the AER system operated for greater than 3 day SRT. However, the amount of oxidized nitrogen in the AER system was less than 10% of removed ammonia-N regardless of SRTs of the AER system.

The peak DO level data are given in Fig. 5. All DO data were the peak values taken from the AER systems operated for different SRTs since measurement was done right after the feeding. Usual DO in the AER system decreases after feeding until soluble organics are depleted then increases until feed sludge is added. Similar trend was observed from oxidation and reduction potential (ORP) data Studies conducted by Kumar (2006) and Novak *et al.* (2009) showed a sudden decrease of ORP after feeding followed by an increase of ORP until the next feeding.

5. DISCUSSIONS

In this study, CO_2 stripping by continuous air injection along with hydrogen ion generation by nitrifiers resulted in pH decrease in the AER system operated for 4 day or longer SRTs. DO profile was also very similar to pH trend in the AER system with lower DO level in the AER system operated for longer SRTs, which indicates that more DO was consumed by nitrifiers at longer retention time. However, overall pH range in the AER system operated for different SRTs was suitable for complete nitrification in accordance with some researchers (Wang *et al.*, 2007).

The longer aeration time in the AER system resulted in more additional solids reduction. The amounts of additional VS reduction in the AER system were very similar to those observed by Kumar (2006), who also studied the combined ANA/AER system for the retention time from 3 to 9 days using continuous aeration and periodic slug feeding pattern. According to Novak *et al.* (2004), there are four pools of VS in wastewater

sludge such as a) aerobically degradable fraction, b) anaerobically degradable fraction, c) a fraction degradable regardless of oxygen level, and d) a non-degradable fraction. Among four different VS fractions, the combined ANA/AER system can remove fractions a) to c), which cannot be achieved by single ANA or AER reactor systems.

Greater total and ammonia-N removal was also observed from the AER system operated for longer SRT. However, the majority of removed TKN was ammonia-N since little variation (2.3% of the average organic-N concentration) was observed for organic-N contents in the AER system regardless of retention times. A study by Novak *et al.* (2004) seems to provide a reasonable explanation for little change of organic nitrogen in the AER system. According to the study, biosolids tends to release more soluble protein during an anaerobic digestion than an aerobic digestion. Since large portion of organic nitrogen is proteinaceous materials, less amount of solubilized protein in the AER system may have resulted in small variation of organic N contents in the AER system operated for different SRTs. In order to achieve more organic-N removal in the combined anaerobic/aerobic sludge digestion system operated with a continuous aeration mode, placing either high performance ANA system (e.g. thermophilic anaerobic digestion system) or a filtration after nitrification is highly recommended. Kumar (2006) found that post aeration for 6 days removed 15% more total nitrogen from thermophilically digested biosolids than from mesophilically digested biosolids.

More NO_x in the effluent was observed from the AER system operated at 4 day or longer SRTs. More ammonia removal in the AER system operated for longer SRT resulted in greater effluent NO_x (Fig. 6). However, the effluent NOx concentration was

accounted for less than 10.1% of ammonia removed in the AER system operated with low DO concentration for the retention time between 2 to 5 days.

Since pH of the AER system was much lower than the pKa (~9.2) of ammonia, ammonia stripping was less likely to have removed the rest of ammonia that was not measured as NO_x in the AER system. Kumar (2006) measured stripped ammonia gas and concluded that majority of nitrogen loss occurred via denitrification not ammonia stripping since stripped ammonia accounted for less than 10% of nitrogen loss during the post aerobic digestion of ANA biosolids. Nitrogen that was not measured as oxidized forms may have been removed via biomass generation and aerobic denitrification. Since increase of organic-N was not observed in the AER system, biomass generation can be excluded from the nitrogen balance. If taking into account of low DO levels in the AER system at around 1.0 ppm and huge organic supply (i.e. COD/TKN ~ 14), denitrification was possible even during the continuous aeration in the AER system. In fact, aerobic denitrification by heterotrophic nitrifiers is widely observed phenomenon in activated sludge digestion systems (Van Niel *et al*., 1992).

6. CONCLUSIONS

The combined ANA/AER system was studied to monitor additional solids reduction and nitrogen removal in the subsequent AER system operated for 5 different retention times from 2 to 5 days. Findings from this study are listed as follow:

- More than 90% ammonia-N and about 50% TKN can be removed from the

ANA system by applying post continuous aeration for longer than 3 day SRT and periodic feeding once per a day. Along with high nitrogen removal, 2 to 14% additional volatile solids were also removed from effluents of the ANA system by the continuous aeration. The peak DO level in the AER system was about 1 ppm throughout the study. However, longer aerobic retention time can result in more effluent NO_x.

- Little change was observed for net organic-N concentrations in the effluents of the ANA systems operated for different retention times. Most of nitrogen was removed via ammonia-N reduction.
- The majority of nitrogen loss in the combined ANA/AER system occurred by heterotrophic denitrifiers that can simultaneously nitrify and denitrify in the AER system under low DO environment.

7. ACKNOWLEDGEMENT

I thank the Christiansburg wastewater treatment facility, VA, USA, for allowing me to collect sludges for this study.

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Table 1. Characteristics of the effluent from the anaerobic system

	Value
pH	7.1 (0.2)
TS (%)	1.01 (0.11)
VS Removal (%)	59.9 (3.3)
COD Removal (%)	56.9 (1.4)
TKN (mg-N/L)	784.1 (143.1)
Ammonia (mg-N/L)	451.0 (90.1)

* Numbers in parentheses are standard deviations.

Table 2. Data for the aerobic system during continuous aeration study

	SRT of AER with continuous aeration mode (days)				
	2	2.5	3	4	5
pH	7.9 (0.1)	8.1 (0.1)	7.9 (0.1)	7.6 (0.2)	7.3 (0.2)
Additional VS removal (%)	2.3 (3.8)	5.6 (2.9)	9.1 (3.2)	11.3 (2.5)	14.4 (2.3)
Additional COD removal (%)	11.6 (1.6)	14.7	17.6	17.9 (5.4)	19.1(6.0)

* Numbers in parentheses are standard deviations.

Figure captions

- Figure 1. Overall digester setup
- Figure 2. Total and ammonia-N removal in the continuously aerated aerobic system
- Figure 3. Nitrogen removal in the aerobic system receiving anaerobic biosolids
- Figure 4. NO_x in the effluent of the aerobic system
- Figure 5. Peak dissolved oxygen in the aerobic system
- Figure 6. Ammonia-N removal and % oxidized N from removed ammonia

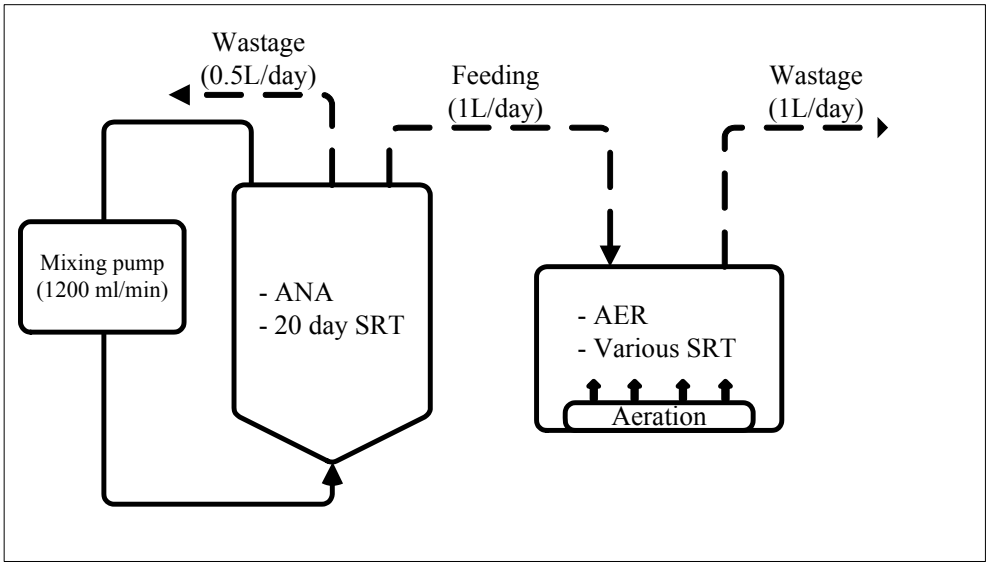

Figure 1. Overall digester setup

* Feeding and wasting were done once per a day.

* Feeding and wasting was done once per a day.

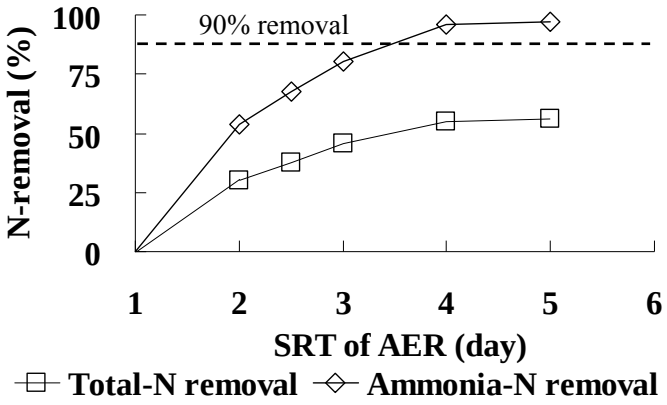

Figure 2. Total and ammonia-N removal in the continuously aerated aerobic system

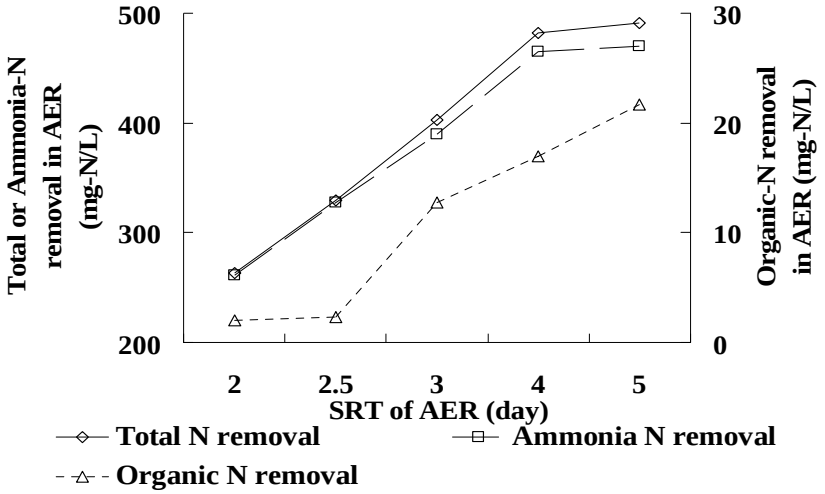

Figure 3. Nitrogen removal in the aerobic system receiving anaerobic biosolids

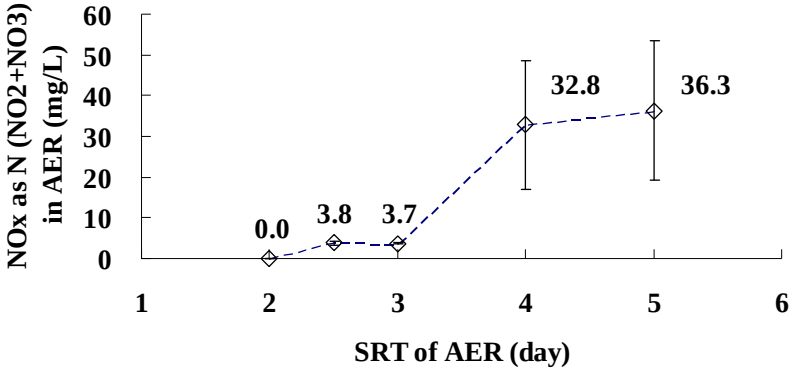

Figure 4. NO_x in the effluent of the aerobic system

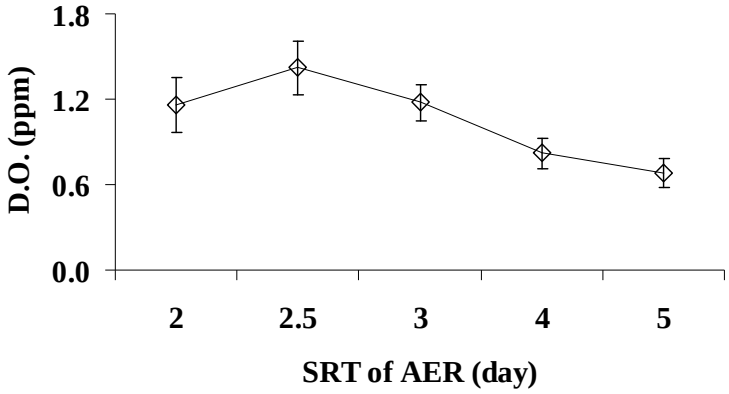

Figure 5. Peak dissolved oxygen in the aerobic system

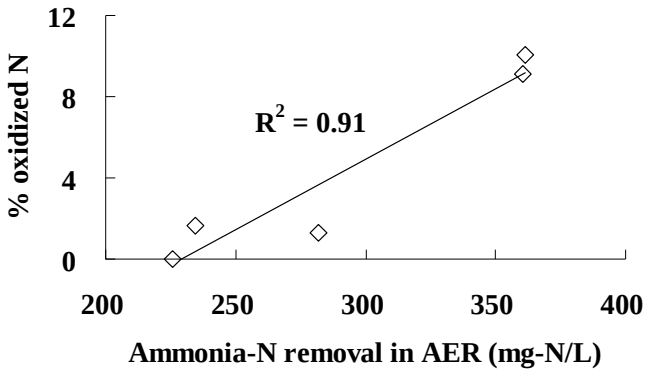

Figure 6. Ammonia-N removal and % oxidized N from removed ammonia

Chapter 7. A study of a combined anaerobic/aerobic system: enhanced N removal by alternating aeration modes in the aerobic digestion system (Working paper)

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ABSTRACT

A combined anaerobic/aerobic sludge digestion system was studied to determine the optimum aeration condition in the aerobic system for maximum solids and nitrogen removal with lower effluent oxidized nitrogen. The study was carried for two phases. After a steady performance was observed from the anaerobic digestion system, continuous aeration was applied to the subsequent aerobic system operated for the retention time from 2 to 5 days. Feeding was done once per a day. More solids and ammonia reduction was observed from the aerobic system operated for the longer retention time but higher effluent oxidized nitrogen was also measured. Three days or longer retention times were chosen for the following study since 90% or greater ammonia removal was achieved at the retention times longer than 3 days. As the second half of study, combinations of six aeration modes (50% aeration time for 30 minutes cycle, 66.7% aeration time for 45 minutes cycle, 75% aeration time for 60 minutes cycle, 50% aeration time for 40 minutes cycle, 66.7% aeration time for 60 minutes cycle, and 50% aeration time for 60 minutes cycle) and three retention times (3, 4, and 5 days) were applied to the aerobic system to determine the optimum aeration on/off condition

for maximum solids and ammonia removal and lower effluent oxidized nitrogen. Feeding was done continuously. Among 18 alternating aeration modes, 66.7% aeration time (60 minutes cycle) for 4 day retention time was determined as the optimum, which achieved about 7% additional volatile solids reduction, 90% ammonia removal and generated 2.6 mg /L effluent oxidized nitrogen.

KEY WORDS: c*ombined anaerobic and aeration digestion, nitrogen removal, sequential aeration*

1. INTRODUCTION

Nitrogen (N) removal in the wastewater flow has been one of major topics among environmental scientists and engineers since this element is a direct cause of eutrophication and water quality deterioration. Anthropogenic activities have been known to be the major source of nitrogen discharge into the nature. It is recent year that the US Environmental Protection Agency forced more strict effluent nitrogen regulation to the treated wastewater and this inevitably generated a strong driving force toward research for advanced wastewater treatments to biologically remove N from wastewater. There are roughly two kinds of N in wastewater with one as organic N and the other as inorganic N such as ammonia and nitrogen oxides. During anaerobic treatment of wastewater, organic N is converted to ammonia and this inorganic N can be removed by providing enough oxygen in the aerobic digestion system. As a result of aeration, nitrogen oxides are generated and they can be removed by denitrification in the anoxic condition with organic supply.

There are several studies where aerobic denitrification was seen for low dissolved oxygen environment (Grady *et al.*, 1999; Wang *et al.*, 2007; Zeng *et al.*, 2004). This aerobic N removal is also called simultaneous nitrification and denitrification (SND) and it has drawn great interests since this system does not require an additional anoxic zone for denitrification and organic matter in wastewater can be used as nutrients for denitrifiers. In addition, energy cost can be saved since aeration can be intermittently turned off.

Grady *et al.* (1999) observed greater ammonia-N removal and low effluent nitrate in a sequencing batch reactor (SBR) system by changing aeration fraction to 60-70% of the total cycle of the SBR. SND was also reported from a lab-scale SBR operated in an alternating anaerobic-aerobic mode under a low dissolved oxygen (DO, ~ 0.5 mg/L) condition (Zeng *et al.*, 2004). In this lab-scale SBR study, 2% of removed ammonia-N was measured as nitrite and the rest was converted to nitrogen oxide rather than nitrogen gas. The aerobic denitrification in a single aerobic system was also observed, from which 6-12 % nitrite was converted to nitrogen gas under low DO environment (< 1 ppm) and low chemical oxygen demand to total Kjeldahl nitrogen ratio (COD/TKN ~ 2.9) in the feed sludge (Wang *et al.*, 2007).

Heterotrophic nitrifiers were widely studied for their capability to denitrify in an aerobic condition. Among them, *Alcaligenes faecalis* is a heterotrophic nitrifier that is commonly found in soil and activated sludge systems (Van Niel *et al.*, 1992). Aerobic denitrification is thought to be a cause for the nitrogen loss that is not measured as

oxidized N in the aerobic digestion systems. In addition, high ammonia and low DO environment is found to be required for active SND in an aerobic condition (Van Niel *et al*., 1992).

The combined anaerobic/aerobic (ANA/AER) sludge digestion system can provide benefits from both sludge treatment methods. Park *et al.* (2006) found that the combined reactor system could tackle fractions that were solely anaerobically digestible as well as fractions that were solely aerobically digestible thus achieving additional solids removal. Along with additional solids reduction, the combined ANA/AER system can achieve greater N reduction that may not be possible by ANA or AER system alone. In other words, high ammonia-N can be generated in the ANA system and reduced N can be oxidized and converted to gaseous form via SND in the subsequent AER system.

In order to validate advantages of the combined system, it has been studied by several researchers. A study performed by Kumar (2006) showed that the sequential anaerobic/aerobic (ANA/AER) sludge digestion system removed more than 60% overall volatile solids (VS) with 10 to 15% VS reduction in the subsequent AER system operated for 3 to 9 day retention times, respectively. Near 50% total N (TKN + oxidized N) removal from ANA effluents was also achieved in the AER system. The author speculated that the most of N removal in the AER system was done via SND since little oxidized N was measured from the AER effluent. Similar result was also reported by Novak et al. (2009) who observed 62% overall VS reduction and 64.5% TKN removal from the combined ANA/AER system with a 15 day anaerobic and a 5 day aerobic retention time. The subsequent AER system of these two studies (Kumar, 2006; Novak

et al., 2009) were operated at a DO level greater than 3 ppm and periodic feeding as once per a day was practiced for the study.

2. OBJECTIVES

In this study, the combined ANA/AER system was studied under various aeration conditions to determine the followings:

- The retention time of the continuously aerated AER system to achieve 90% or more ammonia reduction from anaerobically digested biosolids;
- The optimum alternating aeration mode (aeration on/off time in minutes) in the combined anaerobic/aerobic system for maximum solids and ammonia-N removal along with less effluent oxidized N.

3. METHODOLOGY

3.1. DIGESTION SETUP

The picture of overall digester setup is shown as Fig. 1.

A 15-gallon high density polyethylene tank (Model No. f15b, The Hobby Beverage Equipment Company) was used to build the ANA system. Mixing was applied by circulating headspace gas to the bottom of reactor by a peristaltic pump (Model No. 7553-70, Cole Parmer). Excess gas generation was captured in a gas bag attached to the top of the reactor. The total sludge volume was 30 L and solids retention time (SRT)

was 20 days. Initial inoculation was done with anaerobically digested biosolids from a wastewater treatment plant in Philadelphia, PA. The reactor was kept in a constant temperature room at 37 ℃ throughout the study. The feed sludge was mixture of primary and secondary sludges from the Christiansburg wastewater treatment plant in VA, USA. The target total solids (TS) of feed sludge were 2%. Periodic feeding to the ANA system was done once per a day. A steady performance of the ANA (standard deviation of VS removal ≤ 10%) was confirmed in about 3 SRTs (~ 60 days). Then feeding to the subsequent AER system was started.

A 10 L AER system was built with 0.9 cm-thick acrylic plastic panels. Air was supplied by injecting ambient air through two bubble stones connected to electrical air diffusers. Since fine air bubbles were released from the bottom of reactor, the AER system was well mixed during aeration is on. This AER system was also kept at 37 ℃ throughout the study.

For the first part of the study, the AER system was continuously aerated to find the effect of different retention times (2, 2.5, 3, 4, and 5 days) to the ammonia removal and oxidized N generation in the AER system. Periodic feeding with 1 L of ANA effluent was done once per a day after wasting 1L of the AER biosolids. Desirable SRTs were simulated by changing sludge volume in the AER system. For example, 4 L of sludge volume was maintained for 4 day SRT of the AER system. In order to make up for evaporation, proper sludge level was maintained by adding tap water.

For the second part of the study, aeration was alternately turned on and off for three

SRTs. Six different alternating aeration modes were studied per each retention time so total of 18 combinations were operated in the AER system (Table 1).

A 4-outlet circuit controller (Model No. XT-4, ChronTrol) was used to schedule intervals and durations of feeding, wasting and aeration. Continuous feeding and wasting was done by two peristaltic pumps (Model No. 100MDC, Stenner). Wasting was scheduled at the end of the aeration period while feeding was carried out right after the aeration cycle (Fig. 2). Feeding was needed to provide organics for denitrification while the air was off. During the alternating aeration mode, a total of 1 L AER biosolids was wasted daily while a total of 1 L ANA effluent was added. The SRT of the AER with alternating aeration was chosen based on the result of the continuous aeration study. Retention times were maintained by the method used for the first part of the study. Most of the aerobic reactors showed a steady performance (standard deviation of effluent VS $\leq$ 10%) after 1 retention time, but some erratic sets were rerun to provide additional data.

3.2. ANALYSIS

The pH was measured by placing a probe (Model No. 13-620-287, Accumet) in biosolids samples while slow mixing was applied. The pH meter (Model No. 910, Accumet) was standardized with reference solutions of pH 4 and pH 7. The DO in the AER was measured periodically with a DO probe (Model No. YSI 5739, Yellow Springs Instrument Co., Inc.) and a DO meter (Model No. YSI 57, Yellow Springs Instrument Co., Inc.). DO was measured right before the feeding during the continuous aeration study while DO was continuously measured for the alternately aerated AER systems. All the alternately aerated AER systems showed a similar DO profile as Fig. 3

where the highest DO was reached at the end of the aeration cycle while the lowest was right before the aeration started.

TS/VS, COD, TKN and ammonia-N data were collected according to Standard Methods (APHA, 1998). TKN is a sum of ammonia-N and organic-N.

Oxidized N (NO_3/NO_2) was analyzed using an Ion Chromatograph (Model No. DX120, Dionex) equipped with AS9-HC column (Model No. 051786, IonPac). The eluent was 9.0 mM Na_2CO_3 and the flow rate was 1.0 mL/min.

4. RESULTS

4.1. STEADY STATE PERFORMANCE OF ANAEROBIC DIGESTER

A steady state was assumed for the ANA system after three retention times (~ 60 days) and it continued throughout the study (Table 2). pH of the reactor was maintained at near neutral and solids reduction was very stable (standard deviation ~ 5.4 % of average VS reduction). High COD/TKN ratio (~ 14) was also measured in the effluent and this could drive denitrification in the subsequent reactor.

4.2. CONTINUOUS AERATION STUDY

Overall data for the continuous aeration study are given in Table 3. Operation in the continuous aeration mode resulted in improved solids reduction and ammonia-N removal as the aerobic SRT increased from 2 to 5 days.

More than 96 % of the ammonia-N in the anaerobic biosolids feed was removed in the AER system operated for 4 day retention time or longer. Along with good ammonia-N removal, greater solids, COD and TKN removal were achieved at the longer retention times under continuous aeration. However, the longer SRT resulted in more nitrification in the AER system thus lowering DO and pH. All DO data were collected right before the feeding and these measurements corresponded to the peak DO in the system. Usual DO decreases after feeding until soluble organics are depleted and increases until the next feeding. Low DO condition at 1 ppm or lower was maintained throughout the study, which is amenable for SND (Van Niel *et al*., 1992). The pH changes during continuous aeration in the AER system were not big enough to suppress nitrification. Observed pH range for complete nitrification is 6.0 to 9.0 in accordance with Wang *et al.* (2007).

Since the AER system achieved greater than 90% ammonia removal from the anaerobically digested sludge at a SRT greater than 3 days, SRTs of 3 to 5 days were chosen for the second phase of study using 18 alternating aeration modes.

4.3. OPTIMIZATION OF ALTERNATING AERATION MODE

4.3.1. pH

Three different pH ranges were observed for different retention times as 7.9 to 8.1 for 3 day SRT, 7.3 to 7.9 for 4 day SRT, and 7.1 to 7.6 for 5 day SRT. Similar pH ranges were also measured from the continuously aerated AER system operated for the same retention times. The pH ranges observed in the second phase of study were found to be appropriate for nitrifiers in accordance with Wang *et al*. (2007).

4.3.2. VS REDUCTION

The additional VS reduction in the alternately aerated AER ranged from 1 to 10% above the VS reduction (Fig. 4) that the ANA system achieved at 20 day SRT. However, the additional VS reductions by alternating aeration were about 5 to 8 % less than those done by continuous aeration at the same aerobic retention times. Less VS reduction seems to be caused by relatively smaller aeration imparted during alternating aeration study. Above all, the greatest additional VS reduction in the AER system was observed from the 75% aeration time for 60 minute cycle modes regardless of the SRT while no specific VS reduction pattern was found from other alternating aeration modes.

4.3.3. COD REDUCTION

Additional COD reduction was anywhere between 12 to 19% (Fig. 5). It was very close to the additional COD reduction measured from the continuously aerated AER system operated for same retention times. The AER systems operated with the same aeration modes showed greater additional COD reduction for longer SRTs. However, the amount of COD removed varied for different aeration modes. The greatest COD removal from anaerobic biosolids was observed for the AER system operated with 66.7% aeration time for 60 min cycle at 4 day SRT. On the other hand, the smallest removal was measured from the AER of 50% aeration time for 30 min cycle at 3 and 4 day SRTs.

4.3.4. TKN AND AMMONIA-N REDUCTION

Greater TKN removal was observed from the AER systems operated for longer SRTs (Fig. 6). Overall TKN removal was between 30 and 56 %, which was very close to

those achieved by the continuously aerated AER system operated for the same retention time. It was observed that 5 day retention time was the threshold for 50 % or more TKN removal in the AER system regardless of the aeration modes in this study. However, no specific removal trends were found for the different alternating aeration modes.

Longer aerobic SRT also resulted in greater ammonia-N removal. In this study, 47 to 93% ammonia-N removal was observed (Fig. 7), which was roughly 7 to 10% lower than those removed by the continuously aerated AER system operated for the same retention time. However, ninety percent or greater ammonia-N removal was achieved by 5 alternately aerated AER systems with 2 systems for 4 day SRT (e.g. 75% aeration time for 60 min cycle and 66.7% aeration time for 60 min cycle) and 3 systems for 5 day SRT (e.g. 75% aeration time for 60 min cycle, 66.7% aeration time for 60 min cycle and 50% aeration time for 60 min cycle).

4.3.5 OXIDIZED NITROGEN IN THE EFFLUENT

Effluent oxidized N was also measured to evaluate the nitrification efficiencies of each aeration mode for different SRTs (Fig. 8). The major oxidized N was nitrite. Most of alternating aeration modes in the AER system generated much less effluent oxidized N than those generated from the continuously aerated AER system for the same retention time. Only three aeration modes produced greater effluent oxidized nitrogen, which were 50% and 66.7% aeration time for 60 min cycle at 3 day SRT and 75% aeration time for 60 min cycle at 4 day SRT. All the aeration modes for 5 day SRT generated less effluent oxidized nitrogen than the continuously aerated AER system for 5 day retention time. However, no specific pattern was observed for different alternating aeration modes.

Overall, there were 3 aeration modes with 2 systems for 4 day SRT (e.g. 50% aeration time for 40 min cycle and 66.7% aeration time for 60 min cycle) and 1 system for 5 day SRT (e.g. 50% aeration time for 60 min cycle) that generated effluents with smaller oxidized N contents at 0.1 to 4.1 mg-N/L.

It was found that an increase of peak DO in the alternately aerated AER system caused more effluent oxidized (R^2 = 0.59 by linear correlation). Higher DO environment is essential for greater nitrification in the AER system. Even though no anoxic zone was practiced in the study (i.e. no denitrification was planned.), more than 85% of removed ammonia-N was not measured as oxidized N regardless of aeration modes. Aerobic denitrification was thought to have caused high N loss in the alternately aerated AER system. In order to validate this, DO profiles of each aeration mode were investigated to find the relationship with % N loss in the AER system (Fig. 9) since low DO environment is required for heterotrophic nitrifiers to denitrify in an aerobic condition (Van Niel *et al.*, 1992). Percent N loss was calculated by the following calculation.

$$\%\ \ N\ \ loss = \frac{(Ammonia - N\ \ removal) - (Effluent\ \ Oxidized\ \ N)}{(Ammonia - N\ \ removal)} \times 100$$

The longer the low DO (< 1 ppm) condition lasted, the greater % N loss was observed for the alternately aerated AER system. On the other hand, the opposite trend was observed for the high DO (> 1 ppm).

4.3.7 OPTIMUM ALTERNATING AERATION MODE

Overall performances of the alternately aerated AER systems were evaluated in order to determine the optimum aeration mode. Selection was done by applying the following procedure:

a) Rank 6 alternating aeration modes per each of 3 aerobic SRTs for their VS reduction, COD reduction, TKN reduction, Ammonia-N reduction and low effluent NOx.
b) Select first three modes per each of 5 parameters.
c) Assign 1 - 3 (low – high) to selected modes.
d) Sum assigned numbers for each of selected aeration modes (Totally 9 modes).
e) The alternating aeration modes with the highest sums per each retention time are determined as ones showing the best performance.

The performance assigned to each AER system is shown in Fig. 10. For example, the AER system of 66.7% aeration time (60 min cycle) mode for 4 day SRT ranked as first for COD removal and second for VS, TKN and ammonia-N removal and low effluent NOx, which gave this system 11 (= 3+2×4) for its overall performance. The numbers marked above columns in Fig. 9 are for the AER systems that showed the best performance for the given retention time. The highest performance (= 11) was shown for the 75% aeration time (60 min cycle) and 66.7% aeration time (60 min cycle) modes for the 4 day SRT. Due to high effluent oxidized N (44.1 ± 6.6 mg-N/L) in the 75% aeration time (60 min cycle) mode for 4 day SRT, the AER system operated at 66.7% aeration time (60 min cycle) mode for 4 day SRT was chosen as the optimum alternating aeration mode that realized high solids reduction, greater than 90% ammonia

removal and low effluent NO_x.

5. DISCUSSIONS

This study was consisted of two sub-studies using the combined ANA/AER system.

As the first part of the study, the subsequent AER system was continuously aerated for different SRTs to maximize ammonia-N removal. Greater than 90% ammonia-N in anaerobic biosolids was removed in the subsequent AER system operated for the SRTs longer than 3 days. Since ammonia-N removal in the AER system is mostly via nitrification, aerobic biosolids should be high in oxidized N such as nitrite/nitrate. However, more than 90% of removed ammonia-N was not measured as oxidized N. It was speculated that low DO environment (peak DO ~ 1.0 ppm) and high COD/TKN ratio (~ 14) enhanced aerobic denitrification in the continuously aerated AER system of this study causing most of oxidized-N to be converted to nitrogen gas. In fact, similar phenomenon was observed by several studies (Grady *et al.*, 1999; Kumar, 2006; Van Niel *et al.*, 1992; Wang *et al.*, 2007 and Zeng *et al.*, 2004), where they concluded that aerobic denitrification or SND was responsible for the ammonia-N loss that was not measured as oxidized N in the continuously aerated AER system.

As the second part of the study, the subsequent AER system was intermittently aerated for 3, 4 and 5 day SRTs. These retention times were chosen since the continuously aerated AER system removed more than 90% ammonia-N from anaerobic biosolids at the retention times greater than 3 days. Some of

Additional VS reduction in the alternately aerated AER system ranged from 1 to 10%, which was somewhat lower than additional VS reduction achieved by the continuously aerated AER system for the same aerobic retention times. Additional VS reduction by aerating anaerobic biosolids was achieved by removing aerobically digestible solids that were thought to be different from anaerobically digestible sludge solids (Novak *et al.*, 2004). In this regard, the combined ANA/AER system removed more solids, which could not be done by the ANA or AER system alone. Additional COD reduction and TKN removal by the alternately aerated AER systems were very similar to those removed by the continuously aerated AER system for the same aerobic retention times. Ninety percent or greater ammonia-N removal from anaerobic biosolids was observed from 5 alternately aerated AER systems operated for 4 and 5 day SRTs. Overall ammonia-N removal by the intermittently aerated AER system was also smaller than that achieved by the continuously aerated AER system. It seems that greater total aeration time of the continuously aerated AER system oxidized more ammonia-N than the alternately aerated AER system. Although more effluent oxidized N was observed from the alternately aerated AER system operated for longer SRT, its amount was 10% or less of removed ammonia-N. Similar to the continuously aerated system, majority of N loss was done by aerobic denitrification. Considering that the range of pH was much lower than pKa of ammonia (~ 9.2), most of ammonia should be protonated (in NH_4^+ form) and this had ammonia stripping excluded from the possible explanation for the N loss that was not measured as oxidized forms. A study by Kumar (2006) found that less than 10% of ammonia was stripped during the post aerobic digestion of anaerobic biosolids and concluded that majority of N loss occurred by aerobic denitrification. As

observed by several studies (Van Niel *et al.*, 1992; Wang *et al.*, 2007 and Zeng *et al.*, 2004), low DO environment is required for active aerobic denitrification. Since continuous feeding was practiced for the second part of study and DO in the AER system fluctuated more often than the continuously aerated AER system, the durations of low DO condition (< 1 ppm) per a cycle were investigated to find its relationship with N loss. It was found that the longer duration of low DO condition per a cycle resulted in greater N loss that was not measured as oxidized forms in the alternately aerated AER system. In addition, anaerobic biosolids showed high COD to TKN ratio (~ 14) supporting active denitrification in the subsequent AER system. In fact, aerobic denitrification by heterotrophic nitrifiers is widely observed phenomenon in activated sludge digestion systems (Van Niel *et al.*, 1992).

Results of this study show that there exists the optimum alternating aeration mode (% aeration time in a cycle) for the given SRT in a single AER system for maximum solids and N removal. Longer duration of low DO environment may be an important condition to achieve low effluent oxidized N from the alternately aerated AER system that is continuously fed with anaerobic biosolids. This approach is likely to be more efficient from an energy standpoint than continuous aeration in the AER but may still provide decent digester performances

6. ENGINEERING IMPLICATIONS

- More than 90% ammonia removal is possible by applying continuous aeration to anaerobically digested biosolids for longer than 4 day SRT. However, longer

aeration can result in more effluent oxidized N.

- It is possible to find an optimum aeration mode that provides more than 90% ammonia-N removal and low effluent oxidized N.
- In this study, optimum performance was observed from the AER operated at 66.7% aeration time time of 60 minute cycle (20/40 aeration off/on in minute) for 4 day SRT.
- Majority of N loss that was not measured as oxidized N was done via aerobic denitrification. In addition, greater duration of low DO environment in the alternately aerated AER system was observed to be important for active denitrification.

7. ACKNOWLEDGEMENT

I thank the Christiansburg wastewater treatment facility, VA, USA, for allowing me to collect sludges for this study.

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Table 1. Combinations of alternating aeration mode

Retention time (day)	Cycle (min)	Aeration time (min)	% aeration time
3	30	15	50%
	45	30	66.7%
4	60	45	75%
	40	20	50%
5	60	40	66.7%
	60	30	50%

* Lines are indicating 6 combinations associated with 3 day SRT. Same combinations were made for other 2 retention times.

Table 2. Characteristics of effluent from the ANA system

	Value
pH	7.1 (0.2)
TS (%)	1.01 (0.11)
VS Removal (%)	59.9 (3.3)
COD Removal (%)	56.9 (1.4)
TKN (mg-N/L)	784.1 (143.1)
Ammonia-N (mg-N/L)	451.0 (90.1)

* Numbers in parentheses are standard deviations.

Table 3. Data for the continuously aerated AER system

	SRT of AER with continuous aeration (days)				
	2	2.5	3	4	5
pH	7.90 (0.13)	8.13 (0.10)	7.85 (0.12)	7.61 (0.21)	7.28 (0.16)
Additional VS reduction (%)	2.28 (3.81)	5.59 (2.87)	9.12 (3.17)	11.27 (2.46)	14.41 (2.30)
Additional COD reduction (%)	11.55 (1.56)	14.68	17.62	17.85 (5.42)	19.05 (5.95)
TKN reduction (%)	29.89 (7.35)	35.26 (3.98)	43.36 (6.77)	54.81 (2.27)	55.83
Ammonia-N reduction (%)	53.83 (7.58)	67.98 (4.92)	80.01 (5.21)	96.19 (1.17)	97.09 (1.29)
NO_3^- + NO_2^- (mg-N/L)	0.011 (0.016)	3.83 (0.41)	3.67 (0.22)	32.77 (15.88)	36.31 (17.01)
Peak dissolved oxygen (ppm)	1.10 (0.25)	1.42 (0.19)	1.18 (0.13)	0.84 (0.24)	0.68 (0.10)

* Numbers in parentheses are standard deviations.

Figure captions

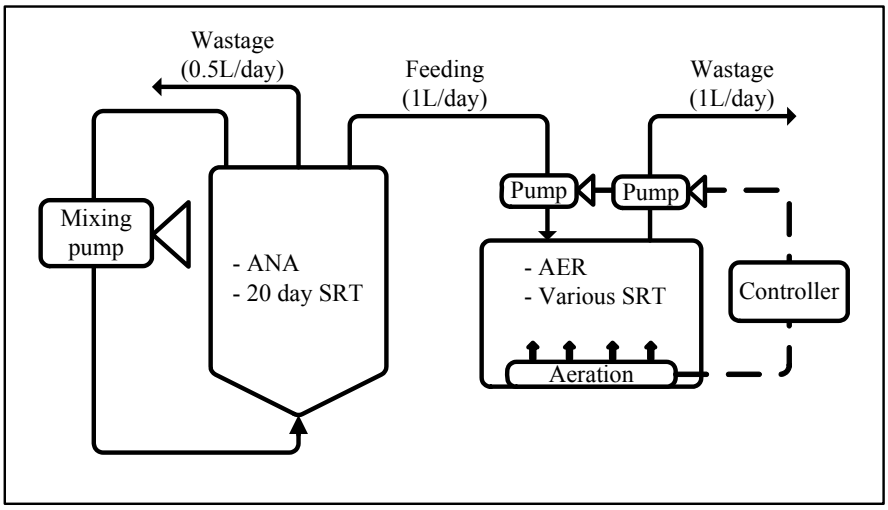

Figure 1. Overall digester setup

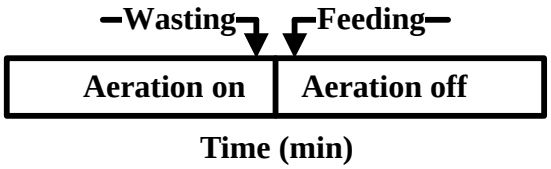

Figure 2. One cycle of alternating aeration mode

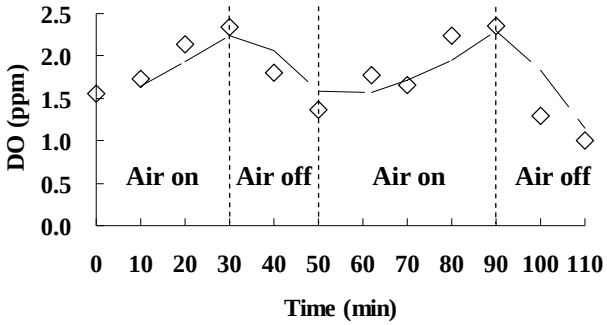

Figure 3. DO profile of the AER system with 66.7% aeration for 60 min cycle at 5 day SRT

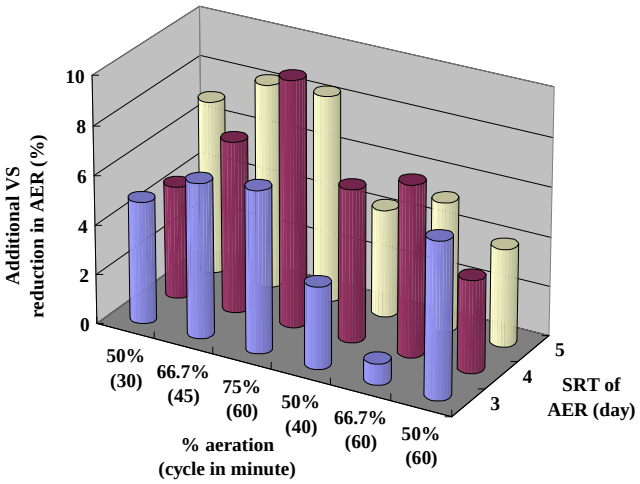

Figure 4. Additional VS reduction in the alternately aerated AER

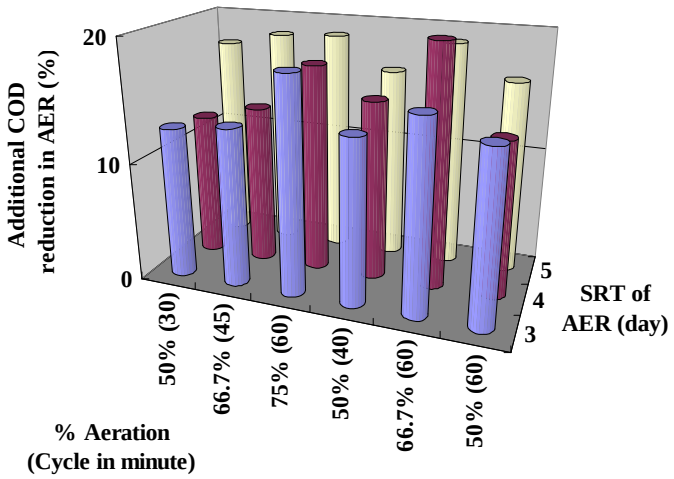

Figure 5. Additional COD reduction in the alternately aerated AER

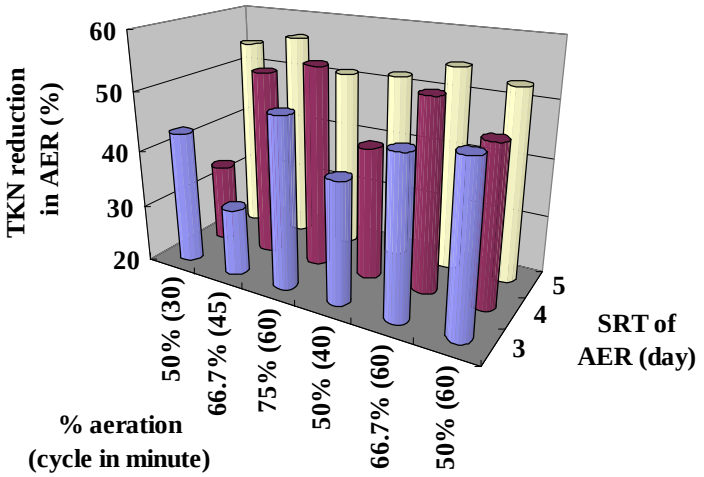

Figure 6. TKN reduction in the alternately aerated AER

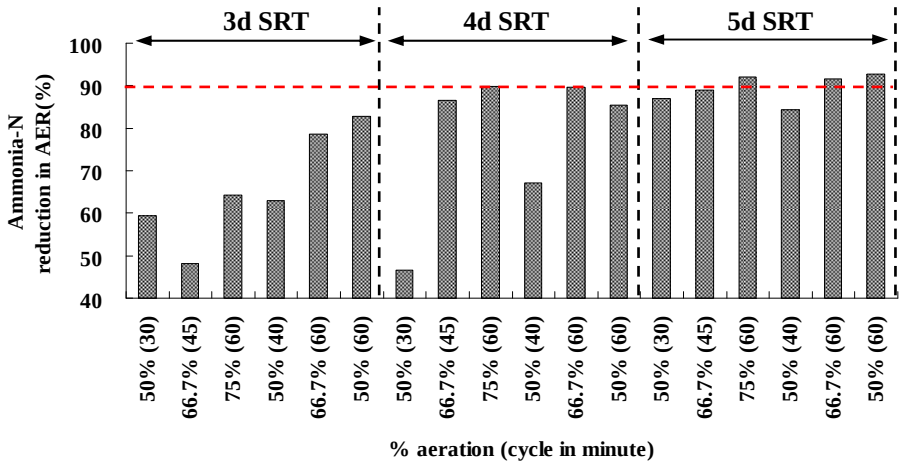

Figure 7. Ammonia-N reduction in the alternately aerated AER

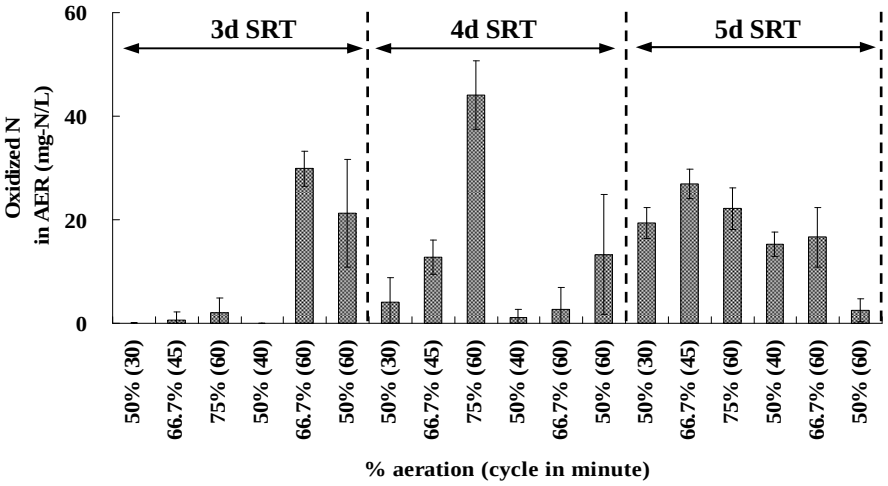

Figure 8. Oxidized N in the effluent of the alternately aerated AER

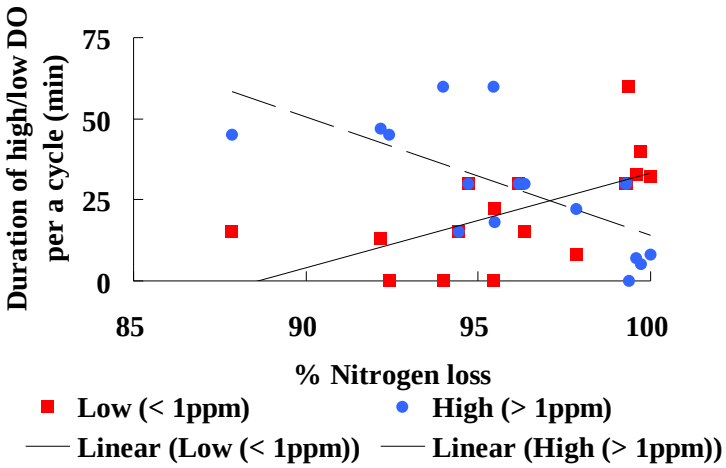

Figure 9. Duration of low or high DO per cycle and % N loss in the alternately aerated AER

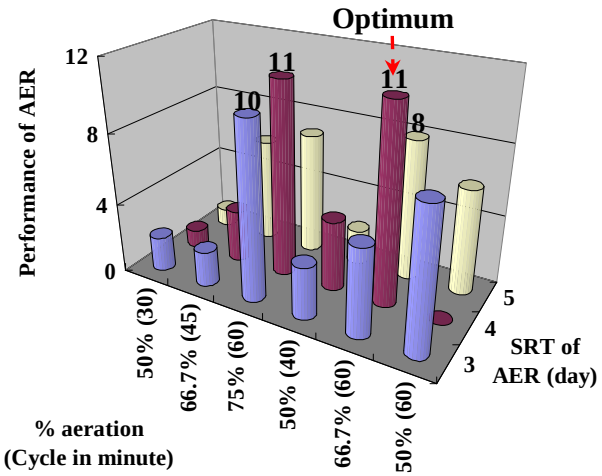

Figure 10. Overall performance of the alternately aerated AER

Appendix 1. Combination of coagulating agents (Aluminum sulfate and Cationic polymer) for biosolids dewatering and its impact to odors

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Abstract

A combination of two conditioning agents, aluminum sulfate and cationic polymer were applied to dewater anaerobically digested biosolids to study their impact to dewatering properties of biosolids and to sulfur based odor generation from dewatered biosolids. Lower sulfur based odor compounds were measured from dewatered biosolids conditioned with greater amount of aluminum sulfate (alum) while higher cationic polymer dose resulted in more sulfur based odors from dewatered biosolids. More alum deteriorated biosolids dewatering properties while more cationic polymer improved dewatering rates for biosolids. Overall data suggest that there exists an optimum combination of alum and cationic polymer dose for better biosolids dewatering characteristics and less sulfur-based odor generation from dewatered biosolids.

Key words: *aluminum sulfate, cationic polymer, dewaterability, volatile organic sulfur compounds*

Introduction

Anaerobically digested biosolids are usually dewatered to be disposed to a landfill or applied to lands for agricultural use. During dewatering, chemical or polymeric conditioning agents are applied to improve biosolids dewatering rate and filtrate quality.

Since biosolids particles are negatively charged, cationic chemicals can neutralize biosolids particles, allowing coagulation and separation from water, if the appropriate amount is applied. Along with charge neutralization, bridging the solids particles by high molecular weight cationic polymers is also an important mechanism of biosolids conditioning (Spinosa and Vesilind, 2001). During the mixing process, weak connections of biosolids flocs are broken and they can re-flocculate with residual flocculants, resulting in stronger flocs.

Aluminum sulfate (alum) and various cationic polymers are widely used for biosolids conditioning and dewatering to facilitate charge neutralization and moisture exclusion from biosolids flocs (Wu and Wu, 2001). However, alum biosolids are notorious for their poor dewatering properties and liquid like behavior at much higher dosage due to Newtonian flow characteristics (Cheremisinoff, 1994). Yang *et al.* (2007) observed improved dewatering characteristics from a mixture of municipal wastewater and alum biosolids which were conditioned with cationic polymer. This co-conditioning method

also enabled additional phosphorus removal from reject water.

Many researchers (Erdal *et al.*, 2008; Higgins *et al*., 2006; Muller *et al*., 2004) have agreed that major malodorous chemicals from anaerobically digested and centrifugally dewatered biosolids are sulfur based volatile organic compounds or volatile organic sulfur compounds (VOSCs). These organic sulfur chemicals include methanethiol (MT), dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS). According to Higgins *et al*. (2006), the main sources of VOSCs from dewatered biosolids are sulfur-containing amino acids such as cysteine and methionine. These compounds can be degraded to hydrogen sulfide (H_2S) and MT, respectively, during anaerobic incubation of biosolids cakes. The methylation of H_2S is another way to generate MT from dewatered biosolids (Higgins *et al.*, 2006). DMS is a methylated form of MT and DMDS can result from the oxidation of MT. As a final step, VOSCs go through demethylation and mineralization by methanogens to form sulfide, methane and carbon dioxide (Lomans *et al.*, 2002).

Muller *et al.* (2004) observed higher biosolids odors from the dewatered biosolids cakes when anaerobically digested biosolids were subjected to greater shearing during biosolids dewatering process. In addition, greater odor generation was observed from biosolids cakes conditioned with greater amounts of cationic polymer. The authors speculated that higher shear intensity released a much greater amount of readily biodegradable proteins into the solution, which could be entrapped by polymers and become the sources of greater odors during biosolids cake incubation. On the other hand, aluminum tends to strongly bind to labile proteins, limiting their availability to odor

generating microbes even after high shear (Subramanian *et al.*, 2005).

In this study, two coagulant agents (alum and cationic polymer), separately and in combination, were used to produce dewatered biosolids cakes from anaerobically digested biosolids and the dewatering characteristics and VOSC generation from biosolids cakes conditioned with different combination of these coagulant agents were measured. The objectives of this study were;

a) To compare the dewaterability of biosolids cakes conditioned with different combinations of alum and cationic polymer;

b) To compare the VOSC generation pattern of biosolids cakes conditioned with different combinations of alum and cationic polymer; and

c) To determine the combination of biosolids conditioning dosages for optimum dewaterability and sulfur based odor generation from dewatered biosolids.

Methodology

Anaerobically digested biosolids were provided by a large municipal wastewater treatment plant in the USA. Digestion was carried for 20 days at 35℃. Average total solids (TS) and volatile solids (VS) of the sample biosolids were 2.08 ± 0.01 % and 1.26 ± 0.01 %, respectively.

TS and VS were measured in accordance with APHA (1998).

Dewatering testing was performed by the method described by Muller *et al*. (2004).

Initially, 10 % (w/w) aluminum sulfate solution (alum) was mixed with 100 mg biosolids sample and the mixture was sheared in a Waring laboratory blender (Model #: 55A60VL22, General Electronics) at 2300 rpm (120 G) for 30 sec. The sheared mixture was then tested in a capillary suction time (CST) apparatus (Model No.: W.R.C type 165, Triton Electronics Limited) with 17CHR (Whatman) filter paper to measure the dewatering time of the mixture. The optimum dose of alum was determined for the alum dose, where the lowest CST was measured. One percent (w/w) high molecular weight cationic polymer (Clarifloc 3275, Polydyne) was also tested for the optimum dose. The optimum dose for alum was found to be 3.2 mg/g TS while the lowest dewatering time was measured from biosolids conditioned with 0.04 mg-cationic polymer/g TS (Fig.1).

High-solids centrifuge conditions were simulated to prepare dewatered biosolids samples for sulfur based odor measurement (Muller *et al*., 2004). Both coagulants were added simultaneously before shearing. Initially, a biosolids sample (100 ml) was mixed with a combination of coagulants. A total of 16 combinations of coagulants were applied and the matrix of combinations is given in Table 1. Each alum dose was accompanied with each cationic polymer dose. There were 4 different alum doses and 4 different cationic polymer doses used for the study. The optimum alum dose (3.2 mg/g TS) was not used because no cake would form. The dewatered solids at this dose remained as a liquid so this alum dose was not used for further studies.

Each conditioned biosolids sample was sheared in the Waring blender at 2300 rpm (120 G) for 30 sec and centrifuged at 10000 rpm (17700 G) for 15 min under the room temperature. The biosolids pellet was collected and pressed at 207 kPa for 15 min by a

lab press. Twenty five gram of pressed biosolids pellet was incubated in a glass bottle (250 mL, I-Chem) with a Teflon lined septa for VOSC measurements.

VOSCs were measured by the method of Glindemann *et al*. (2006). The volatile sulfurs of interests were H_2S, MT, DMS and DMDS. The sum of MT, DMS and DMDS is referred to total VOSCs (tVOSCs) in the paper. The quantification of these compounds was periodically done by injecting 100 μL of headspace gas into gas chromatography (GC, GC 6890, Hewlett-Packard) - mass spectrometry (MS, MSD 5970, Hewlett-Packard). About 1 m of GC column (20751-01A, Supelco) was placed outside of GC and inserted to a dewar jar filled with liquid nitrogen. This cryo-trap was used to accumulate gas and to generate a narrow peak profile by gas chromatography. The injected gas was accumulated in the part of column that was dipped in liquid nitrogen. After 30 seconds, the column was removed from the liquid nitrogen and the data acquisition sequence was started. The GC column was connected to the gas injection inlet (temperature at 200℃) and helium gas at 2ml/min was used as a carrier gas. The oven temperature was increased from 50 to 265℃ at a rate of 35℃/min. Total analysis time was 7.64 min. Peak area estimation was done by the data analysis program, G1034C version C.03.00 (Hewlett-Packard). The concentration of organic sulfurs in the sample was calculated by comparing the sample peak area with the area of a standard gas mixture of known amount of H_2S, MT and DMS (Scott Specialty Gases Inc.). DMDS was quantified using DMS as a reference. An example of H_2S and VOSC measurement by GC-MS is shown in Fig. 2. The VOSC data are presented as peak tVOSCs (peak tVOSCs), which correspond to the highest total VOSCs generated from dewatered biosolids.

Results

The dewatering times (CSTs) of biosolids conditioned with different combinations of alum and polymer doses are shown in Fig. 3. The CST decreases as the amount of cationic polymer increases while the CST increased as biosolids samples were conditioned with more alum. Additional alum lowered dewatering rates only when biosolids were conditioned with 0.01 mg-cationic polymer /g TS.

It has been shown that dewatered biosolids with higher cake solids concentrations generated greater amount of organic sulfur based odors (Muller *et al.*, 2004). An increase in cake solids was observed from dewatered biosolids conditioned with greater amount of cationic polymer while alum had little impact on cake solids (Fig. 4). The cake solids prepared with 1.6 mg-alum/g TS was the highest for all cationic polymer doses.

More cake solids did not necessarily result in greater sulfur based odor generation when both of alum and cationic polymer were used as conditioners (Fig. 5).

However, a clear relationship was observed between the amount of conditioner and the peak tVOSCs generated from dewatered biosolids (Fig. 6). An increase in peak tVOSC was observed from biosolids cakes conditioned with greater amount of cationic polymer while additional alum showed a decrease in peak tVOSCs. This observation indicates that an optimum combination of alum and cationic polymer can be chosen to promote

reasonable odor reduction from dewatered biosolids.

The optimum combination of coagulants was chosen for the alum and cationic polymer dose that produced biosolids that were easier to dewater and generating less sulfur based odor after dewatering. In order to find the optimum combination, the ratios between biosolids CSTs to the greatest CST (biosolids CST / peak CST) and between peak tVOSCs to the highest peak tVOSCs (peak tVOSC of dewatered biosolids / the highest peak tVOSC of dewatered biosolids) were calculated and the sums of these two ratios were presented in Table 2. The peak CST (138 sec) was found from biosolids conditioned with 0.01 mg - polymer/g TS only while the highest peak tVOSCs (333 ppmv as S/g VS) were measured from the dewatered biosolids conditioned with 0.04 mg-polymer/g TS only. Better performance can be assumed for the combination of coagulants showing a lower sum of ratios.

For example, biosolids conditioned with 1.6 mg-alum/g TS and 0.01 mg-polymer/g TS were dewatered at the CST of 118 sec (86% of the peak CST of 138 sec) and generated a peak tVOSC of 15 ppmv as S/g VS (5% of the greatest peak tVOSC of 333 ppmv as S/g VS). Thus, the co-conditioning efficiency of biosolids conditioned with 1.6 mg-alum/g TS and 0.01 mg-polymer/g TS is 0.90 (= 0.86 + 0.04). Among 16 combinations of alum and cationic polymer, the lowest sum of the ratios (0.60) was observed from the biosolids conditioned with 1.6 mg-alum/g TS and 0.03 mg-cationic polymer/g TS.

Implications

The test results indicate that co-conditioning with cationic polymer and alum does not

improve biosolids dewatering properties (Fig. 3). The best dewatering was achieved for the biosolids conditioned with cationic polymer only. More alum always caused poorer dewatering properties.

However, a dramatic reduction of sulfur based odors was observed from dewatered biosolids conditioned with more alum regardless of the cationic polymer dose (Fig. 6). In addition, almost no odor was generated from dewatered biosolids conditioned with 1.6 mg-alum/g TS or more. It seems that the bridging effect of cationic polymer is much more susceptible to strong shear than the chemical binding effect of alum. Odor causing microbes appear to consume more labile proteins in biosolids that are conditioned with polymer only and processed through high shearing condition, resulting in high biosolids odors. On the other hand, alum can form strong chemical bonds in biosolids, which is less disrupted by high shear, making the biosolids less amenable to odor causing microbes. Co-conditioning of biosolids with alum and cationic polymer can make use of the best characteristics of both biosolids conditioners. Better dewatering properties by cationic polymer can be accompanied with less biosolids odor generation by alum.

The decision for optimum combination for alum and cationic polymer may vary in accordance with the need of different wastewater treatment plants. That is, plants that care more about biosolids odors may choose the combination of higher alum and lower polymer while locations, where biosolids dewatering is more important, may choose the combination of lower alum and higher polymer.

Since anaerobically digested biosolids were collected from only one source, results of

this study may not be universal. However, sulfur based odor generation patterns in accordance with different combinations of coagulant dosages can provide wastewater treatment plant operators with optional biosolids handling strategies that can promote better acceptable dewatering properties and biosolids odor control by the use of combined cationic polymer and alum for chemical conditioning.

Conclusions

Co-conditioning of anaerobically digested biosolids was studied and the results suggest that biosolids handling can be optimized by applying the right amount of alum and cationic polymer. The followings are the conclusions that were drawn from this study.

- An increase of cake solids was observed from biosolids cakes conditioned with greater amount of conditioning chemicals.
- Cationic polymer tends to improve biosolids dewatering properties while more alum makes biosolids harder to dewater when both chemicals are used for biosolids conditioning.
- A greater amount of sulfur based odor compounds was produced from dewatered biosolids conditioned with more cationic polymer while lower odor compounds were observed from dewatered biosolids conditioned with more alum.
- There exists the optimum combination of alum and cationic polymer that promotes better biosolids dewatering properties and less generation of sulfur based organic odors from dewatered biosolids.

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Table 1. Combination of coagulants

Alum (mg/g TS)	Cationic polymer (mg/g TS)
0	0.01
0.8	0.02
1.6	0.03
2.4	0.04

* Lines indicate 4 combinations of coagulants with 0% alum dose. Same combinations are applied to other alum doses.

Table 2. Co-conditioning efficiencies expressed as the sum of [biosolids CST / peak CST] and [peak tVOSC of dewatered biosolids / the highest peak tVOSC of dewatered biosolids]

	No alum	0.8 mg-alum /g TS	1.6 mg-alum /g TS	2.4 mg-alum /g TS
0.01 mg-polymer/g TS	1.06 (1.00 + 0.06)	0.91 (0.90 + 0.01)	0.91 (0.86 + 0.05)	0.76 (0.74 + 0.02)
0.02 mg-polymer/g TS	0.92 (0.52 + 0.39)	0.72 (0.58 + 0.14)	0.72 (0.65+0.07)	0.79 (0.77 + 0.02)
0.03 mg-polymer/g TS	0.87 (0.25 + 0.62)	0.77 (0.41 + 0.36)	**0.60** **(0.57 + 0.03)**	0.75 (0.72 + 0.03)
0.04 mg-polymer/g TS	1.23 (0.23 + 1.00)	0.84 (0.47 + 0.37)	0.70 (0.62 + 0.08)	0.94 (0.91 + 0.03)

* Numbers in the parenthesis are the ratio of sample CST to the greatest CST and sample peak tVOSC to the highest peak tVOSC, respectively. ** Shaded area represents the optimum combination of conditioners.

Figure Captions

- Figure 1. Optimum alum (a) and cationic polymer (b) doses
- Figure 2. An example of H_2S and VOSC measurement by GC-MS
- Figure 3. CST of biosolids conditioned with different combinations of alum and cationic polymer.
- Figure 4. Solids of dewatered biosolids conditioned with different combinations of alum and cationic polymer.
- Figure 5. Cake solids and peak tVOSCs from the biosolids cakes
- Figure 6. Peak tVOSC from dewatered biosolids conditioned with different combinations of alum and cationic polymer.

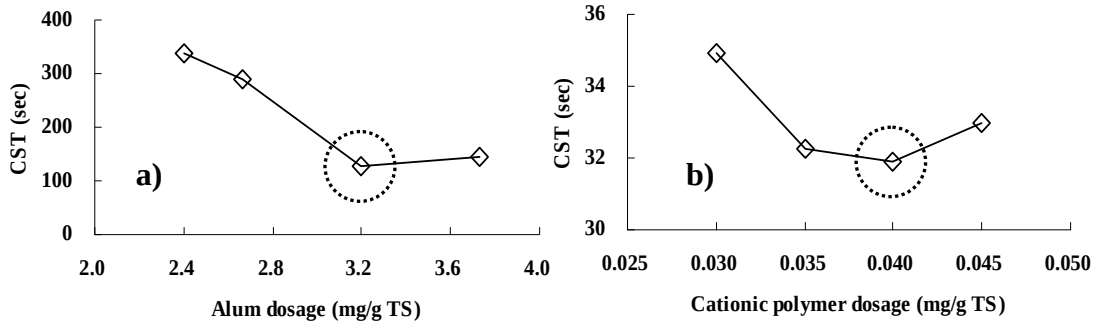

Figure 1. Optimum alum (a) and cationic polymer (b) doses

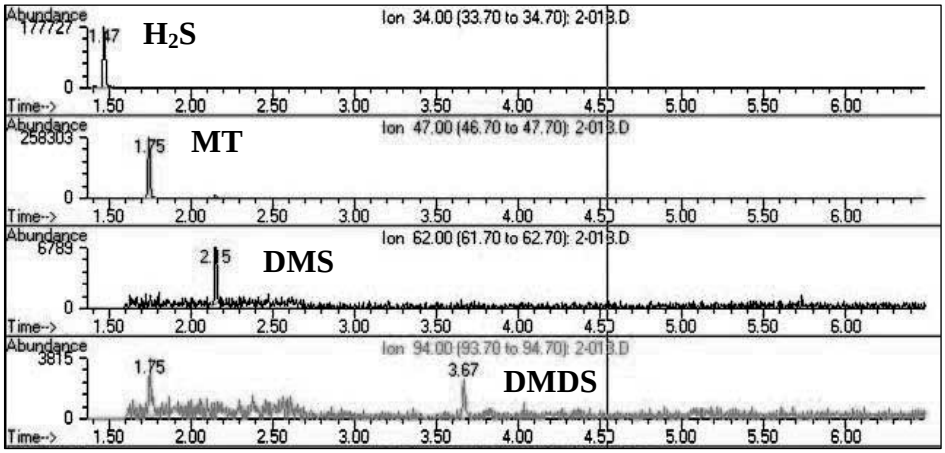

Figure 2. An example of H_2S and VOSC measurement by GC-MS

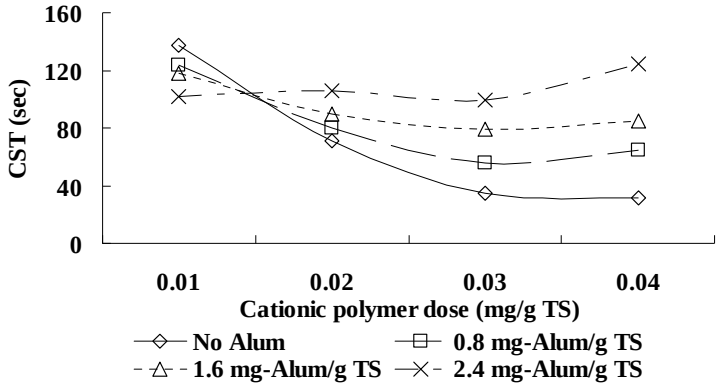

Figure 3. CST of biosolids conditioned with different combinations of alum and cationic polymer.

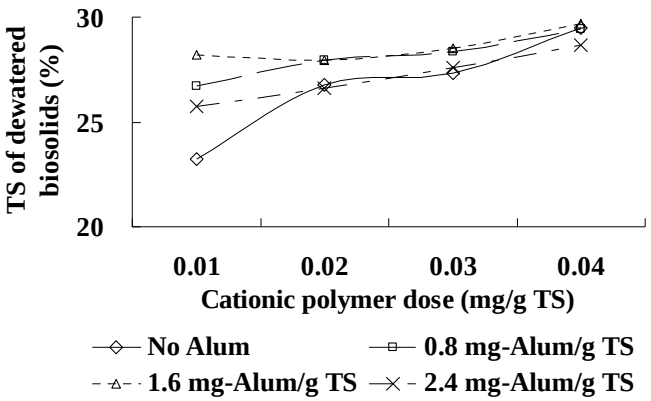

Figure 4. Solids of dewatered biosolids conditioned with different combinations of alum and cationic polymer.

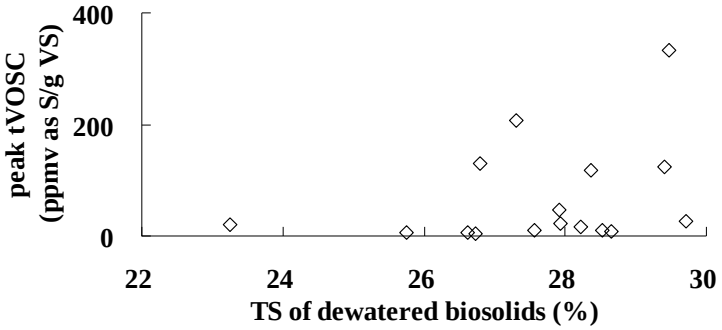

Figure 5. Cake solids and peak tVOSCs from the biosolids cakes

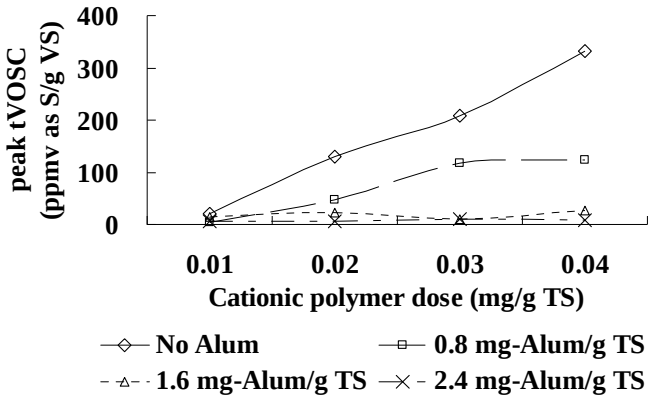

Figure 6. Peak tVOSC from dewatered biosolids conditioned with different combinations of alum and cationic polymer.

Appendix 2. terminal Restriction Fragment Length Polymorphism (tRFLP)

Better nitrogen removal efficiencies in the combined anaerobic/aerobic systems operated for 20 day anaerobic retention time and 4 day aerobic retention time seem to be associated with more even relative abundant but diverse microbial community distribution along with greater similarity of the community composition between different aeration modes (i.e. time for aeration off or on).

a. Methodology

Aerobic biosolids (50 mL per each) from the optimum alternating aeration modes for each SRT were centrifuged at 10000 rpm (12000 G) for 15 minutes at 4 ℃. DNA was extracted from 20 mg of each centrifuged biosolids pellet in accordance with manufacturer's manual of FastDNA spin kit for soil (Prod Code. 116560200, Qbiogene). Extracted DNAs were amplified by polymerase chain reaction (PCR) with a forward primer, ba27f (5'-AGAGTTTGATCMTGGCTCAG-3') and a 5'6-carboxy-fluorescein (FAM) labeled reverse primer, ba907r (5'-/56-FAM/CCGTCAATTCMTTTRAGTT-3'). Each PCR mixture was prepared by mixing 14.05 μL sterilized distilled water, 5 μL 5X buffer, 2.5 μL 10X buffer, 1.5 μL 25mM Mg^{2+}, 0.5 μL 5mM dNTP, 0.05 μL of each primer (5 μM), 0.35 μL Taq DNA polymerase (5 Prime) and 1 μL DNA sample. Thermal cycle was 1 cycle of denaturing at 94 ℃ for 2 minutes, 28 cycles of denaturing at 94 ℃ for 30 seconds, annealing at 52 ℃ for 45 seconds and extension at 72 ℃ for 30 seconds followed by 1 cycle of extension at 72 ℃ for 7 minutes. Each PCR reaction was checked by electrophoresis on 1% agarose gel in 1X TAE buffer stained with SYBR green followed by UV excitation. Successfully amplified DNA samples were

digested with an endonuclease, AluI. The digestion mixture was prepared by mixing 11 μL sterilized distilled water, 20 units of restriction enzyme, 2 μL 10X NE buffer and 5 μL PCR reaction and incubated at 37 ℃ for 6 hours. The final products were sent to Virginia Bioinformatics Institute, VA, USA, for DNA fragment (terminal restriction fragment, tRF) size analysis. GeneScan^{TM}-1000 ROX^{TM} (Prod No. 401098, Applied Biosystems) was used for the size marker of DNA fragment analysis. Data analysis was done with Peak Scanner Software 1.0 (Applied Biosystems). Baseline peak height (the lowest fluorescent unit) was set to 25 for the analysis. Three PCR products were prepared per each extracted DNA sample and replicate tRFs were standardized by the method of Dunbar et al. (2001). Only reproducible tRF profiles (i.e., tRFs that were shown for all three replicates) were used for bacterial community distribution analysis. Relative abundances (A_p) of tRFs were calculated in accordance with the method described by Schwarts et al. (2007).

b. Data analysis and Results

Biosolids from the anaerobic digester and four biosolids samples from the aerobic digesters showing the best performance for the given retention times were tested for tRFLP analysis. Standardized tRFs were analyzed using ecological analysis tools such as Shannon-Wiener indices (H'), Shannon's evenness (E), and Morisita indices (I_M) (Schwartz *et al*., 2007).

Shannon-Wiener indices (H') and Shannon's evenness (E_H) are the indices that are commonly used to characterize species diversity in a community. The equations for both indices are as follow:

a) $H' = -\sum_{i=1}^{S} p_i \ln p_i$ where, p_i = the proportion of tRF *i* abundance relative to the total abundance of tRFs.

b) $E_H = H' / \ln H'_{max}$ where, H'_{max} is the greatest H'.

More diverse microbial community distribution can be indicated by greater Shannon-Wiener indices (H'). More even distribution of microbial community can be interpreted if Shannon's evenness (E_H) approaches 1.

Greater Shannon-Wiener indices (H') were observed from the anaerobic digester and the aerobic systems operated for 4 day SRT (2.73 ± 0.13) than the aerobic systems operated for 3 or 5 day SRTs (2.15 ± 0.47), which indicates that more diverse microbial communities exist in the anaerobic digester and the aerobic systems operated for 4 day SRT than for the other digestion systems. In addition, fairly even distribution of microbial communities was observed from the anaerobic digestion system (E = 1.00) and aerobic digesters operated for 4 day SRT (E = 1.00) while less even distribution is observed from the aerobic digesters operated for 3 and 5 day SRTs (E < 1) regardless of the aeration schemes.

Morisita index (I_M) is a statistical measure of dispersion of individuals in a population. It is used to compare similarity among sample community compositions. This index uses Simpson's index (D), which is an ecological tool often used to quantify the biodiversity of a habitat. Equations for Morisita index (I_M) and Simpson's index (D) are as follow:

a) $I_M = \frac{2\sum_{i=1}^{S} x_i y_i}{(D_x + D_y)XY}$

where, x_i = Abundance of tRF i from sample x
X = total abundance of tRFs from sample x
y_i = Abundance of tRF i from sample y
Y = Total abundance of tRFs from sample y
D_x and D_y = Simpson's index for sample x, y

b) $D = 1 - \frac{\sum_{i=1}^{S} n_i (n_i - 1)}{N(N-1)}$

where, S = number of tRFs
N = total abundance of tRFs from sample x
n_i = Abundance of tRF i from sample x

If Morisita indices between two communities approach 1, both microbial communities can be assumed similar. The data are presented in Table A-1. The label indicates aeration mode, % aeration in 1 hour cycle and aerobic retention time. For example, O(75)3d means that the biosolids sample was collected from the aerobic digestion system while aeration was on for 75% aeration for 1 hour cycle with 3d retention time. On the other hand, X(75)3d indicates that the biosolids sample was collected from the aerobic digestion system while aeration was off for 75% aeration for 1 hour cycle with 3d retention time. Most of the aerobic systems operated for 3 or 5 day SRTs showed much less similarity ($I_M < 0.5$) in microbial community composition with other reactor systems regardless of aeration schemes. Higher similarities were observed between the AER systems operated for 4 day SRT and the ANA system ($I_M = 0.84 \pm 0.07$). Retention times in the aerobic digestion system seem to affect microbial community distributions more than the aeration scheme (i.e. different aeration on/off time).

It seems that more even and diverse microbial community distributions are closely related to better N removal in the AER systems operated at alternating aeration modes. Along with microbial community distribution, similar compositions of microbial communities in between different aeration mode (i.e. aeration on/off time) are also important for better performance of the AER systems.

Table A-1. Morisita indices (I_M)

ANA	O(75)3d	O(75)4d	O(67)4d	O(67)5d	X(75)3d	X(75)4d	X(67)4d	X(67)5d	
1	**0.28**	0.89	0.90	**0.23**	0.58	0.89	0.81	0.74	**ANA**
	1	**0.32**	**0.30**	**0.33**	0.57	**0.25**	**0.24**	**0.22**	**O(75)3d**
		1	0.94	**0.29**	0.65	0.82	0.76	0.57	**O(75)4d**
			1	**0.26**	0.61	0.83	0.77	0.56	**O(67)4d**
				1	**0.33**	**0.21**	**0.19**	**0.18**	**O(67)5d**
					1	0.52	**0.48**	0.42	**X(75)3d**
						1	0.89	0.80	**X(75)4d**
							1	0.75	**X(67)4d**
								1	**X(67)5d**

* ANA: anaerobic digester; O: Aeration on; X: Aeration off; ( ): % aeration in 1 hr cycle; #d: Aerobic retention time

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