

Multistaged Anaerobic Sludge Digestion Processes

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Abstract: Two multistaged anaerobic digestion systems, a four-stage thermophilic anaerobic digestion (4TAD), all at 55°C, and a four-stage anaerobic digestion with a tapered temperature configuration (4ADT) at 55, 49, 43, and 37°C, 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 biosolids cakes from the 4ADT system generated fewer organic sulfur compounds than those from the 4TAD system. Both multistage 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 mesophilic system. Both multistage anaerobic digestion systems failed to dramatically remove DNA of the indicator organism, *E. coli*, quantified by real time polymerase chain reaction, even though the indicator organism densities measured by standard culturing methods satisfied EPA Class A biosolids requirements. DOI: 10.1061/(ASCE)EE.1943-7870.0000372. © 2011 American Society of Civil Engineers.

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Introduction

The demand for better municipal sludge handling strategies has resulted in various advanced anaerobic sludge digestion methods, including thermophilic sludge digestion (50–60°C), 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 (USEPA 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 owing to their

gluelike characteristics, and the composition of these materials determines 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 lectinlike proteins, and these lectin proteins are interconnected by the divalent cations calcium and magnesium. The fate of EPS after various sludge digestion processes is as important as the composition of EPS in sludge flocs. Novak and Park (2004) observed that greater reduction in solids 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). These sulfur based volatile organic compounds are primarily responsible for odors from anaerobically digested and dewatered sludges (Erdal et al. 2008; Higgins et al. 2006, 2008; Muller et al. 2004). The generation of organic sulfur compounds from anaerobically digested biosolids has been detailed by Higgins et al. (2006). The primary sources of volatile organic sulfurs from dewatered biosolids cakes appear to be the sulfur-containing amino acids cysteine and methionine, which can be degraded to hydrogen sulfide (H₂S) and MT, respectively, under anaerobic conditions. MT can be also generated by methylation of H₂S. DMS is a methylated form of MT that can result from the oxidation of MT. 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 in wastewater sludges, the indicator organisms *E. coli* and fecal coliform can enter a nonculturable state after thermophilic anaerobic digestion, so that standard culturing methods tend to underestimate their population in biosolids. Moreover, these nonculturable organisms increase

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further during cake storage and can be reactivated by centrifugal dewatering of digested sludge.

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, several field reports describe superior solids and pathogen reduction by thermophilic digestion using up to 4 stages.

Study Background

The Western Lake Superior Sanitary District (WLSSD) facility at Duluth, MN, was considering converting their anaerobic digestion system to a multistage system to produce Class A biosolids (USEPA 1994). The overall project design was based on a report regarding the successful operation of a four-stage thermophilic anaerobic digestion system at Annacis Island Wastewater Treatment Plant in Vancouver, Canada (Krugel et al. 1998). To obtain performance data for different multistage sludge digestion systems, WLSSD sludge was digested in two multistage sludge digestion systems [e.g., four-stage thermophilic anaerobic digestion (4TAD) and four-stage anaerobic digestion with a tapered temperature configuration (4ADT)] and two single-stage sludge digestion systems [e.g., thermophilic anaerobic digestion (TAD) and mesophilic anaerobic digestion (MAD)], and their digested biosolids characteristics were collected to evaluate their comparative digester performances. The current system has four digestion tanks with an overall detention time of 24 days. The specific objectives of this study are as follows:

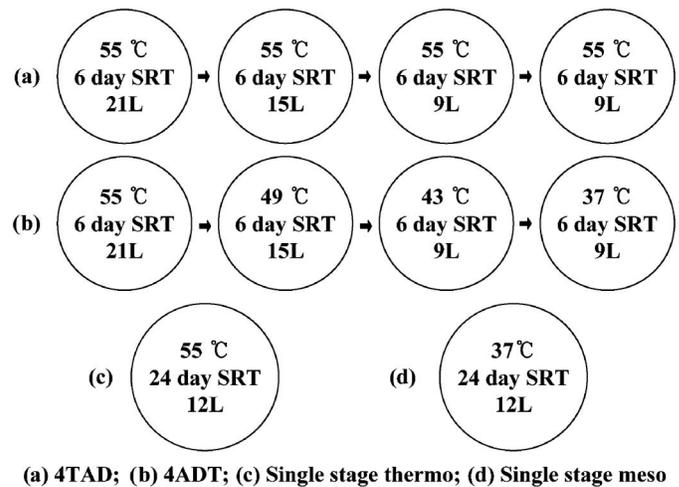
1. To compare the digester performances (e.g., solids reduction, dewatered biosolids odors, indicator organism reduction, and biosolids dewatering properties) between 4TAD and 4ADT and to single-stage thermophilic and mesophilic digesters with the same overall retention time; and
2. To relate effluent biopolymer properties (e.g., soluble and extractable) to digester performances and sludge characteristics (e.g., solids reduction, dewatered biosolids odors, and biosolids dewatering properties).

Methodology

Digestion Systems

Four anaerobic digesters were constructed of high density polyethylene (HDPE) brewer tanks (Model No. f6.5, 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°C and the four reactors of the 4ADT system were operated at 55, 49, 46, and 37°C. The operational temperature of the single-stage TAD system was 55°C and the single-stage MAD system was operated at 37°C. Gas mixing was applied to each of the multistage anaerobic reactors by circulating headspace 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. Because all reactors were kept completely mixed throughout the study, solids retention



(a) 4TAD; (b) 4ADT; (c) Single stage thermo; (d) Single stage meso

Fig. 1. Overall experimental settings

time (SRT) of each reactor was equal to hydraulic retention times. The retention time of each stage of the multistage systems was six days and the SRT of each single-stage reactor was 24 days, so the overall retention times of multi- and single-stage systems were the same. The operational volumes of the four-stage anaerobic reactors were 21, 15, 9, 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 approximately 1 L of waste sludges per day for analysis. 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 TAD systems were operated side by side with the same feed sludge. These were followed by operation of the 4ADT and single-stage MAD system, also in a side by side mode with the same feed sludge.

The inocula for the 4TAD and the single-stage TAD system were thermophilically digested anaerobic sludge from the WLSSD at Duluth, MN. Seeding was not needed for the 4ADT or the single-stage MAD system because 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 approximately 2/3 of wastewater flow into WLSSD is from pulp and paper companies. The 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 and 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 in Fig. 2, when the standard deviation for the volatile solids reduction data was $8.0 \pm 1.4\%$.

TS and volatile solids (VS) were determined according to standard methods (APHA 1998). 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. Samples were filtered through 0.45 μm membrane filter (Catalog No. 09 719 2D, Fisher Scientific) before testing. Solution sulfide was measured by the iodometric method (APHA 1998).

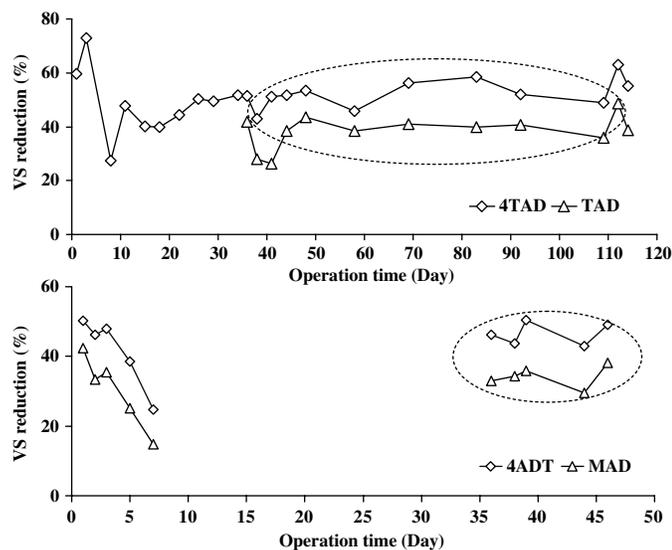


Fig. 2. VS reduction by date (circled data used for steady state analysis)

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 Frlund et al. (1996). Bovine serum albumin (Prod No. 23209, Thermo Scientific) was used as a standard. Biopolymer 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 as follows:

1. CER extraction was adopted from the method of Park and Novak (2007). One hundred mL of sludge sample was centrifuged at 10,000 rpm (17,700 G) for 15 min at 4°C. The sludge pellet was resuspended in 200 mL of phosphate buffer solution (PBS; 10 mM NaCl + 6 mM Na₂HPO₄ + 1.2 mM KH₂PO₄) and the mixture was put into a plastic mixing beaker with 4 baffles where previously PBS-washed CER (Dowex 508, Sigma-Aldrich) was placed as 60 g resin/g TS. A shearing by a mixing paddle at 600 rpm (8 G) was applied for one hour and extracted material was separated from the resin beads by filtering the extraction through a 1.5 μm glass fiber filter (Catalog No. 1827 055, Whatman). The filtrate was centrifuged at 10,000 rpm (17700 G) for 15 min at 4°C and the centrate was filtered through 0.45 μm membrane filter (Catalog No. 09 719 2D, Fisher Scientific). The filtrate was stored in a freezer until biopolymer analysis.
2. Base extraction was also carried out in accordance with the method of Park and Novak (2007). The sample preparation was mostly the 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, N₂ atmosphere was maintained to prevent pH change by CO₂ in atmosphere. The rest of the extraction procedure was identical to that of CER extraction.
3. Shearing extraction was applied to sludge pellets that were resuspended in PBS buffer, as described in the CER extraction procedure. After resuspension of the sludge pellet in the PBS, 1 min of mechanical shear was applied to the resuspended sample, followed by a 1 min rest and reshearing for 1 min. 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.

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

The method described by Muller et al. (2004) was used for the sludge dewatering testing and for the preparation of dewatered biosolids 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 s and centrifuged in a lab centrifuge at 10,000 rpm (17,700 G) for 15 min under 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 biosolids cakes were used for measurement of volatile organic sulfur compounds.

Volatile organic sulfur compounds were measured by the method of Glindemann et al. (2006). Twenty five g 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 the 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-inside diameter (i.d.) column (Model No. 20751-01A, Supelco) was connected to the gas injection inlet (200°C) and helium was used as a carrier gas (2 mL/min). The oven temperature was increased from 50 to 265°C at a rate of 35°C/min. Total analysis time was 7.64 min. Odorous compounds measured in the study were H₂S, MT, DMS, and DMDS. Peak areas of each organic sulfur compound were integrated by the data analysis program G1034C v.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 H₂S, MT, and DMS (Scott Specialty Gases). DMDS was quantified using DMS as a reference.

Microbial Test for Pathogen

Feed sludge and effluents from each digestion system (500 mL each) were collected and shipped overnight in ice to the Bucknell University Environmental Engineering and Science Laboratory in Pennsylvania. The indicator organisms in these sludge samples, *E. coli* and fecal coliform, were quantified by Standard Method 9221F and 9221E (APHA 1998). The test was performed twice per sample and the averages are presented. 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 each) were centrifuged at 10,000 rpm (17,700 G) for 15 min at 4°C and pellets were shipped overnight in dry ice to 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 polymerase chain reaction (rt-PCR) was used to enumerate the specific *E. coli* gene, glutamate decarboxylase (*gadA/B*) in the extracted DNA samples. In brief, the extracted DNA was amplified

with a forward (50—GCGTTGCGTAAATATGGTTGCCGA—30) and a reverse primer (50—CGT CAC AGG CTT CAA TCA TGC GTT—30) by the Brilliant SYBRs Green QPCR Master Mix (Stratagene). The specific *E. coli* DNA was quantified by the Stratagene MX3005P rt-PCR system. Serially diluted *E. coli* DNA (2 to 7,620 copies) was used as an external DNA standard for each real time PCR analysis. DNA quantification was performed 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 multistaged systems were compared to each other and to those of single-stage systems to quantify and compare each reactor system's solids removal efficiencies in accordance with their unique digestion conditions. All the multistaged reactors removed more solids than single-stage reactors. A greater solids reduction associated with higher temperatures was also observed from the single-stage reactors. For the single-stage systems, the VS reduction of the single TAD system (39.9–2.6%) was approximately 6% greater than that of the single MAD system (34.1–3.1%).

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

Overall, the four-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 the TAD systems operated at temperatures greater than 49°C generated more gas volume per g VS removed (Table 2). However, high VS reduction in the first reactors of both multistage systems did not result in greater gas volume generation. All the total gas volume data fell in the range of 0.6 to 1.6 L/g VS removal, which was suggested by Gerardi (2003).

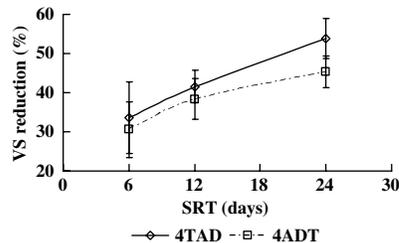


Fig. 3. VS reduction of each multistage reactor

Table 2. Gas Generation Data

Reactor	L-gas/day	g volatile solids reduction (VSR)/day	L-gas/g VSR	Total L-gas/g VSR	
4TAD	1	7.9 (1.7)	20.5	0.4	
	2	8.6 (1.3)	6.4	1.3	
	3	2.3 (0.4)	1.4	1.7	
	4	3.1 (2.4)	2.8	1.1	0.7
	Single TAD	4.1 (0.5)	4.4	0.9	0.9
4ADT	1	7.9 (2.0)	20.3	0.4	
	2	6.9 (2.1)	4.4	1.6	
	3	1.4 (0.2)	2.2	1.3	
	4	1.5 (0.5)			0.7
	Single MAD	2.5 (0.7)	3.3	0.8	0.8

Note: Numbers in parentheses are standard deviations.

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 (USEPA 1994) are met, digested sludges can be dewatered and land-applied as a disposal method without restrictions. The single-stage TAD system is listed as a sludge treatment method to attain Class A biosolids (USEPA 1994). Standard culturing data for both of the multi-stage anaerobic digestion systems and the single TAD system showed near complete removal of *E. coli* and fecal coliform, which meets the Class A biosolids requirement of less than 1,000 MPN/g DS (Fig. 4). Most of the indicator organism removal was achieved in the first thermophilic anaerobic digester of the multistage digestion systems (Fig. 5). Regardless of their digestion temperature conditions, subsequent reactors of multistage systems provided little additional indicator organism destruction. The single MAD system removed a smaller percentage of indicator organisms than the multistage systems and the single TAD system, even though all were operated for the same overall retention time.

However, DNA data generated by rt-PCR failed to confirm the dramatic reduction of *E. coli* DNA. In general, either single-stage or

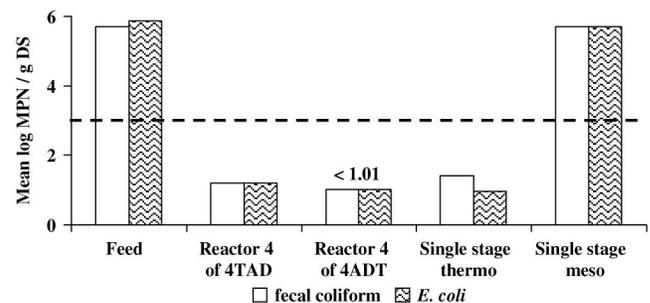


Fig. 4. Mean log most probable number (MPN) of indicator organisms in single and multistage digestion systems (line indicates EPA Class A biosolids requirement)

Table 1. Statistical Analysis of Percentage of VS Reduction of Multistage Systems ($\alpha = 0.05$)

	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

Note: Equal variance was assumed for all *t*-tests.

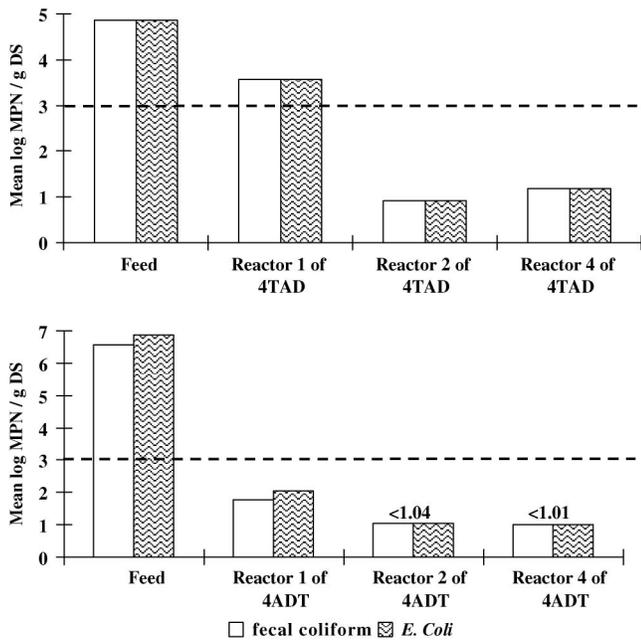


Fig. 5. Effect of temperature to mean log MPN of indicator organisms in multistage digestion systems (lines indicate EPA Class A biosolids requirement)

four-stage systems showed approximately one log *E. coli* reduction from the feed sludge, from which 6.9 ± 0.1 *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., Reactors 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 Biosolids Cakes

Odors from sludge biosolids were voted as the top odor problem associated with wastewater treatment at a Water Environment Federation workshop, held in Anaheim, CA (Forbes et al. 2004). Because proteins usually comprise 50 to 70% of volatile solids of wastewater sludges (Forbes et al. 2004), and sulfur based odors are primarily 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 4TAD, the dewatered sludge from the fourth reactor of the 4TAD system ($376.4\text{--}260.8$ ppmv as *S/g VS*) produced a much greater peak volatile organic sulfur concentration than the dewatered sludge from the fourth reactor of 4ADT system ($29.4\text{--}13.2$ ppmv as *S/g VS*) and the single MAD system ($4.9\text{--}2.5$ ppmv as *S/g VS*). The single-stage TAD system also produced as much peak organic sulfur ($334.8\text{--}74.3$ ppmv as *S/g VS*) as the fourth reactor of the 4TAD system. The lowest peak total organic sulfur compounds were measured from the dewatered biosolids cakes of the single MAD system, which removed the least VS. Moreover, neither the solids ($R^2 = 0.02$ from the linear regression) nor the solution biopolymer profiles ($R^2 = 0.18$ from the linear regression) showed a reasonable relationship with volatile organic sulfur compound data.

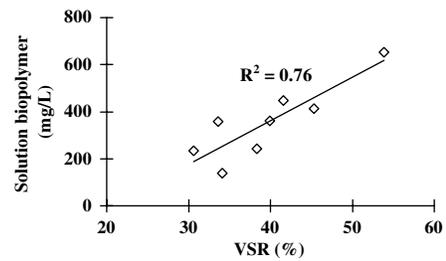


Fig. 6. VS reduction and solution biopolymer

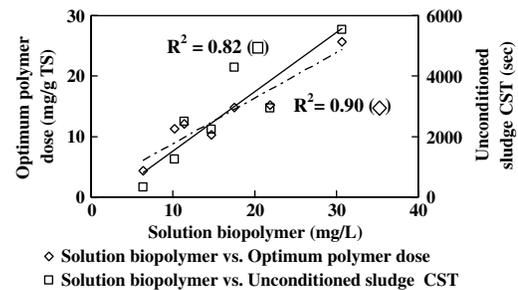


Fig. 7. Relationship among solution biopolymer, optimum polymer dose, and dewaterability of unconditioned sludge

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 multistaged digestion systems (649.6 ± 147.1 mg/L for 4TAD and 413.4 ± 56.6 mg/L for 4ADT) than in the single-stage systems (405.0 ± 100.0 mg/L for single TAD and 139.4 ± 33.8 mg/L for single MAD) were less VS was removed than in the multistage systems. Likewise, thermophilic systems accumulated more solution biopolymer than mesophilic systems.

The relationship between VS reduction of the digestion system and solution biopolymer content is shown 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). Fig. 7 shows that 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 (Forbes et al. 2004). 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 and potential amounts of degradable biopolymer under advanced or multistage digestion. Four biopolymer extraction methods were applied to sludge samples from each anaerobic digestion system. Details of the extractions are provided in the following.

The amount of extractable biopolymer in the feed sludges changed while shifting from the 4TAD and single TAD system

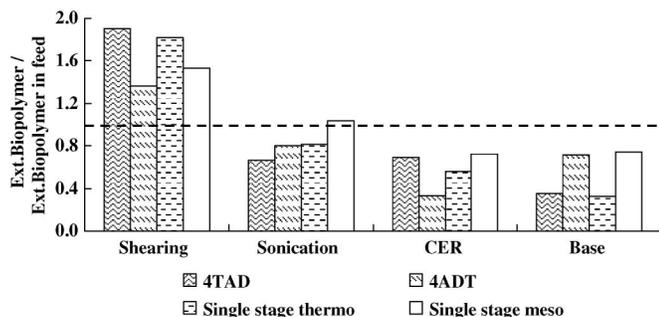


Fig. 8. Extractable biopolymer profiles of single- and multistage systems

to the 4ADT and single MAD 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.

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 multistage anaerobic digestion of sludges at different temperature conditions (e.g., thermophilic and mesophilic) for the same total retention time can 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 was also observed from mechanically sheared sludges after single- or multistage sludge digestions.

Discussions

Solids Reduction

Overall, the four-stage systems degraded more solids than the single-stage systems and reactors operated at a temperature greater than 49°C removed more solids than the reactors operated at a lower temperature. The different mixing configurations may 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 TAD systems resulted in greater gas volume generation than reactors operated at lower temperatures. Interestingly, greater VS removal in Reactor 1 of both multistage systems did not result in more gas volume generation. Most soluble organics generated from Reactor 1 of both multistage systems were consumed in the subsequent reactors, resulting in more gas production. More gas generation per g VS consumed was observed from thermophilic systems throughout the study. The combination of four different digestion temperatures was expected to offer advantages over the four-stage digestion system at the same digestion temperature, but that was not seen in the data.

E. coli DNA Reduction

On the basis of a successful full-scale operation, a four-stage thermophilic digestion system may 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 a dramatic reduction of *E. coli* DNA in the multistaged systems, although their effluents fulfilled Class A biosolids requirements (USEPA 1994). This implies that there is potentially a large population of nonculturable *E. coli* in both the single- and multistage systems, which could result in reactivation after centrifuge dewatering (Higgins et al. 2007). The data show that the reduction

in *E. coli* in the four-stage full-scale system is attributable to something other than the thermophilic temperatures and multiple stages at that facility.

Sulfur Based Odor

Greater solids removal by the multi-stage TAD system did not result in less sulfur based odor generation from dewatered biosolids cakes, as suggested by Krugel et al. (1998). This seems to be caused by the combination of less degradation and greater generation of sulfur odors. Aceticlastic or methylotrophic methanogens have been observed to degrade organic sulfur compounds from dewatered biosolids cakes (Higgins et al. 2006). However, their metabolisms on organic sulfurs are slowed under thermophilic digestion conditions (Wilson et al. 2008), and even greater suppression on their metabolism has been reported when the thermophilic anaerobic digester contains high sulfate (Freeman et al. 2008). Along with the thermal inhibition of methanogenic metabolism on organic sulfur compounds, high sulfate in the raw sludge stimulated sulfur based odor generation from dewatered biosolids. Within two known organic sulfur generation mechanisms, methylation of sulfur requires two prior conditions such as sufficient sulfate and high methylated aromatic compounds like humic and lignin (Higgins et al. 2006; Lomans et al. 2002). Because effluents of pulp and paper industries can supply enough sulfate and methylated aromatic compounds, favorable conditions for high organic sulfur production can be assumed during the incubation of thermophilically digested and dewatered biosolids. On the other hand, mesophilic sludge digestion conditions accelerated methanogenic metabolism on organic sulfur compounds, which resulted in high organic sulfur degradation from mesophilically digested and dewatered biosolids cakes.

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 owing to the accumulation of additional biopolymer in solution

It has been reported that the CER extraction method is specific for divalent cation-associated biopolymer and the base-extractable fraction, although it is the least specific extraction method and includes the aluminum-associated biopolymer fraction (Park and Novak 2007). These extraction methods did not appear to extract clearly separate biopolymer pools, which in turn gave inconclusive results such as those in Fig. 8. None of the extraction data from these two methods correlated well with other data such as VS reduction, soluble biopolymer contents, or volatile organic sulfur compound (VOSC) generation from dewatered biosolids. However, the other two physical extraction methods showed a relationship, albeit a weak relationship, with characteristics of biosolids from different sludge digestion processes.

The generation of odor-causing organic sulfur compounds has been associated with high intensity shear in centrifuges (Muller et al. 2004), and the data are consistent with this observation. The increase in biopolymer, especially protein, as a result of mechanical shear is expected to lead to higher organic sulfur odors. The extractable protein profile from shearing extraction showed a correlation with peak total volatile organic sulfur from biosolids cakes (Fig. 9). The single MAD system was excluded from the correlation in Fig. 9 because it produced a much less odorous sludge and was the only system that did not include at least one thermophilic stage. More study is warranted to elucidate the mechanism

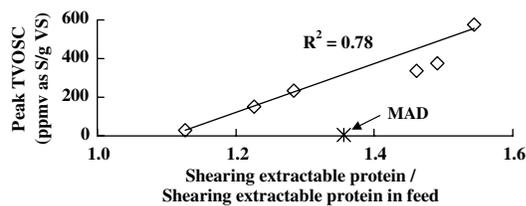


Fig. 9. Shearing extractable protein ratios and peak sulfur based odor compounds

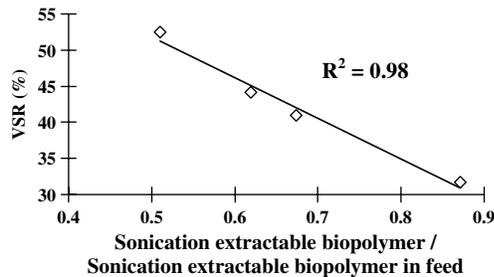


Fig. 10. Sonication extractable biopolymer ratio and VS reduction

for the lower organic sulfur odor generation from the single mesophilic system. 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 stages. This agrees with research suggesting that shearing within a centrifuge during dewatering may release materials that become bioavailable, which is then degraded to produce odorous compounds (Higgins et al. 2006).

The sonication extractable ratios correlated well with the VS reduction from the digestion systems (Fig. 10). The greatest VS reduction of the 4TAD system corresponded to the lowest sonication extractable biopolymer ratio and the lowest VS reduction of the single MAD system brought about the greatest sonication extractable biopolymer ratio. The single MAD system was the only system that generated biosolids containing more sonication extractable biopolymer than those in feed sludge. This indicates that the single MAD system removed the least amount of VS and also turned more biopolymer into sonication extractables in 24 days of retention time. Sonication could be integrated into sludge recirculation line to remove more biosolids organics from single MAD systems.

Conclusion

Usual anaerobic sludge digestion systems may not treat influent sludges with high sulfate contents owing to a partial pulp and paper wastewater stream (2/3 of total inflow) into their treatment facilities. However, the writers believe that this study still does provide some valuable guidelines for wastewater treatment facility designers and operators when they choose a combination for their multistage sludge digestion system as following:

1. The 4TAD removed more solids than 4ADT and both removed more volatile solids than single-stage digesters at the same overall retention time.
2. The multistage anaerobic digestion systems did not show dramatic reduction of indicator organism DNA, although they removed most indicator organisms in accordance with standard

culturing method data. This suggests that the systems may have reactivation potential.

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

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