Recurrence of Fecal Coliforms and *Salmonella* Species in Biosolids Following Thermophilic Anaerobic Digestion

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ABSTRACT: The U.S. Environmental Protection Agency (U.S. EPA) Part 503 Biosolids Rule requires the fecal coliform (indicator) or *Salmonella* species (pathogen) density requirements for Class A biosolids to be met at the last point of plant control (truck-loading facility and/or farm for land application). The three Southern Californian wastewater treatment plants in this study produced biosolids by thermophilic anaerobic digestion and all met the Class A limits for both fecal coliforms and *Salmonella* sp. in the digester outflow biosolids. At two plants, however, a recurrence of fecal coliforms was observed in postdigestion biosolids, which caused exceedance of the Class A limit for fecal coliforms at the truck-loading facility and farm for land application. Comparison of observations at the three plants and further laboratory tests indicated that the recurrence of fecal coliforms can possibly be related to the following combination of factors: (1) incomplete destruction of fecal coliforms during thermophilic anaerobic digestion, (2) contamination of Class A biosolids with fecal coliforms from external sources during postdigestion, (3) a large drop of the postdigestion biosolids temperature to below the maximum for fecal coliform growth, (4) an unknown effect of biosolids dewatering in centrifuges. At Hyperion Treatment Plant (City of Los Angeles, California), fecal coliform recurrence could be prevented by the following: (1) complete conversion to thermophilic operation to exclude contamination by mesophilically digested biosolids and (2) insulation and electrical heat-tracing of postdigestion train for maintaining a high biosolids temperature in postdigestion. *Water Environ. Res.,* **78,** 1005 (2006).

KEYWORDS: Class A biosolids, thermophilic anaerobic digestion, postdigestion processing, fecal coliforms, reactivation and growth, 40 *CFR* Part 503.

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Introduction

In 1998, approximately 60% of the total United States sludge production of 6.26 million dry metric tons was beneficially used in land application (Bastian, 1997; U.S. EPA, 1999). Production and land application of biosolids is regulated by the U.S. Environmental Protection Agency (U.S. EPA) in 40 *CFR* Part 503 (U.S. EPA, 1993, 1994). Anaerobic digestion is one of the processes most widely used by wastewater treatment plants for the treatment of wastewater solids, with most digesters currently being operated at mesophilic temperatures for the production of Class B biosolids (Goldstein, 2000; U.S. EPA, 1999). However, in response to local bans and ordinances on biosolids land application and growing public concern on the safety of biosolids, an increasing number of plants are implementing thermophilic anaerobic digestion for the production of Class A biosolids according to 40 *CFR* Part 503 requirements (Drury et al., 2002; Iranpour, Cox, Starr, Fan, Mundine, Abkian, and Haug, 2004; Iranpour et al., 2006; Mittsdörffer et al., 1990; Oh et al., 2005; Wilson and Dichtl, 1998).

Recent investigations have indicated the recurrence of fecal coliforms in postdigestion biosolids at some wastewater treatment plants that meet the Class A limits at the discharge of the thermophilic anaerobic digesters (Iranpour et al., 2002; Palacios et al., 2005). After digestion, biosolids are typically processed (e.g., screening, dewatering, and storage) before being transported to the farm. The Class A indicator and pathogen density requirements need to be met at the last point of control by the plant (U.S. EPA, 1993), which typically is at the truck-loading facility or the farm for land application. Therefore, increases of indicator and/or pathogen densities during postdigestion and storage in the plant could result in exceedance of the Class A limits. The federal regulations stipulate that either the fecal coliform or the *Salmonella* sp. density need to be met. The City of Los Angeles (California) has land-applied its biosolids in Kern County, California, since 1994. This county has adopted an ordinance that, since January 2003, has only allowed the land application of Class A biosolids that meet both Class A limits for fecal coliforms and *Salmonella* sp. Thus, recurrence of fecal coliforms would result in noncompliance with the Kern County ordinance, even though the biosolids comply with federal requirements for Class A biosolids by meeting the limit for *Salmonella* sp. (Iranpour, Cox, Kearney, Clark, Pincince, and Daigger, 2004).

As in digested biosolids, increasing densities of indicators and pathogens have sometimes been also observed during storage of composted biosolids (Burge et al., 1987; Hussong et al., 1985; Millner et al., 1987; Russ and Yanko, 1981; Sidhu et al., 2001; Soares et al., 1995). In either case, the underlying mechanisms are still not clearly understood. In general, the recurrence of indicators and pathogens in thermophically digested biosolids would require the presence of these bacteria and conditions that allow their reactivation and/or stimulate their growth in biosolids.

The overall objective of this study was to evaluate the bacterial quality of biosolids during full-scale, postdigestion operations. Therefore, the studies aimed at comparing different processes at three plants in Southern California to identify possible mechanisms of the recurrence of indicator (fecal coliforms) and pathogenic (*Salmonella* sp.) bacteria. The specific objectives were the following:

- In full-scale tests, to determine the profiles of fecal coliform density and temperature in postdigestion biosolids at Terminal Island Treatment Plant (TITP) and Hyperion Treatment Plant (HTP), of the City of Los Angeles, and Regional Plant No.1 (RP-1) of the Inland Empire Utilities Agency (Ontario, California).

Table 1—Overview of full-scale thermophilic anaerobic digestion processes.

Process/parameter	HTP, Phase I	HTP, Phase II	HTP, Phase III		TITP	RP-1
Digester volume (m^3)	9460	9460	9460		5190	3220–6440
1st stage digestion	Thermophilic	Thermophilic	Thermophilic	Mesophilic	Thermophilic	Mesophilic
– Number of digesters	4	4	15	6	3	1
– Mode of operation	Continuous	Continuous	Continuous	Continuous	Sequential batch	Continuous
– HRT (days)	13	13	10.9	39	22	2.5 to 3.5
– Holding time (hours)					24	
– Temperature (°C)	57.8	57.7	54.4	35	55	32 to 40
2nd stage digestion	Thermophilic	Thermophilic	Thermophilic		NA	Thermophilic
– Number of digesters	2	2	2			1
– Mode of operation	Batch	Batch	Continuous			Continuous
– HRT (days)			1.3			14 to 16
– Holding time (hours)	13	24				
– Temperature (°C)	54.4	53.5	51.4			50 to 52
3rd stage digestion	NA	NA	NA		NA	Thermophilic/mesophilic
– Number of digesters					1	
– Mode of operation					Continuous	
– HRT (days)					5 to 6	
– Holding time (hours)						
– Temperature (°C)					46 to 49	
Post-digestion processing						
– Dewatering		Centrifuge			Centrifuge	Filter belt press
– Coagulant		Mannich polymer			Mannich polymer	Ciba 7818
– Dosage (kg/dry ton)		10			25	5 to 6
– In-plant transfer		Closed pipes/Abel pumps			Conveyor belt	Conveyor belt
– Total solids in concentrated biosolids (%)		~30			~30	~16

- In laboratory-scale tests, to determine the potential of selected biosolids samples from postdigestion to support growth of fecal coliforms and *Salmonella* sp. during incubation at controlled laboratory conditions.
- In laboratory-scale centrifuge simulation tests, to evaluate the effect of biosolids dewatering in centrifuges and the additions of Mannich polymer and high-pressure effluent on fecal coliform recurrence.
- In laboratory- and full-scale tests, to determine the minimum temperature required for preventing the recurrence of fecal coliforms.

Materials and Methods

Plant Descriptions and Process Operations. Table 1 summarizes digestion and postdigestion parameters of the processes in the plants that were selected for this study.

Hyperion Treatment Plant Phases I, II, and III. The HTP's average daily flowrate is approximately 1.4 million m^3/d (360 mgd), and full secondary treatment was established in 1998 (Clark et al., 2002). The HTP produces approximately 700 to 800 metric tons of wet biosolids per day. Since 2000, several tests were conducted to convert HTP to thermophilic anaerobic digestion. During phase I (late 2001) and phase II (early 2002), a two-stage, continuous-batch process was investigated in a battery of six thermophilic digesters and a designated thermophilic postdigestion train that was isolated from mesophilic plant operations (Iranpour et al., 2006) (Figure 1a). Phase III (August through September 2002) was a test of a two-stage continuous process for digestion of the plant's total sludge production of 11 000 m^3/d (3.0 mgd) of primary sludge and 3000 m^3/d (0.8 mgd) of thickened waste activated sludge (Iranpour, Cox, Starr, Fan, Mundine, Abkian, and Haug, 2004) (Figure 1b). The first stage consisted of 15 thermophilic digesters (receiving 90% of the sludge) and 6 mesophilic digesters (receiving 10% of the sludge). Mesophilic and thermophilic sludges were blended in two second-stage digesters, also operated in a continuous mode. Postdigestion at HTP consists of screening, dewatering in centrifuges, biosolids transfer by pumps through pipes, and storage in silos up to a maximum of 1 day. Between phases II and III, the postdigestion train between the digesters and the silos at the truck-loading facility were insulated and provided with electrical heat-tracing.

Terminal Island Treatment Plant. The TITP treats, on average, 64 000 m^3/d (17 mgd) of wastewater containing approximately 30 to 50% domestic wastewater, 40 to 60% industrial wastewater (mainly from oil refineries and food processing), and 10% seawater. The mixed inflow causes high sulfide and salinity concentrations in the influent and large fluctuations in pH, biological oxygen demand, suspended solids, and ammonia. The biosolids production at TITP amounts to 50 wet metric tons/d, which are produced in three egg-shaped digesters (one digester is standby). Since July 2001, the digesters have been operated in a single-stage sequential batch process to meet the time-and-temperature requirement of Alternative 1 of 40 *CFR* Part 503 (Oh et al., 2005; Shao et al., 2002) (Figure 1c). Each digester is operated on complementary 3-day cycles of sludge feeding, holding, and withdrawal of 24 hours each. Postdigestion processing consisted of dewatering in centrifuges, transport of concentrated biosolids over conveyor belts, and storage of biosolids in silos up to a maximum of 1 day.

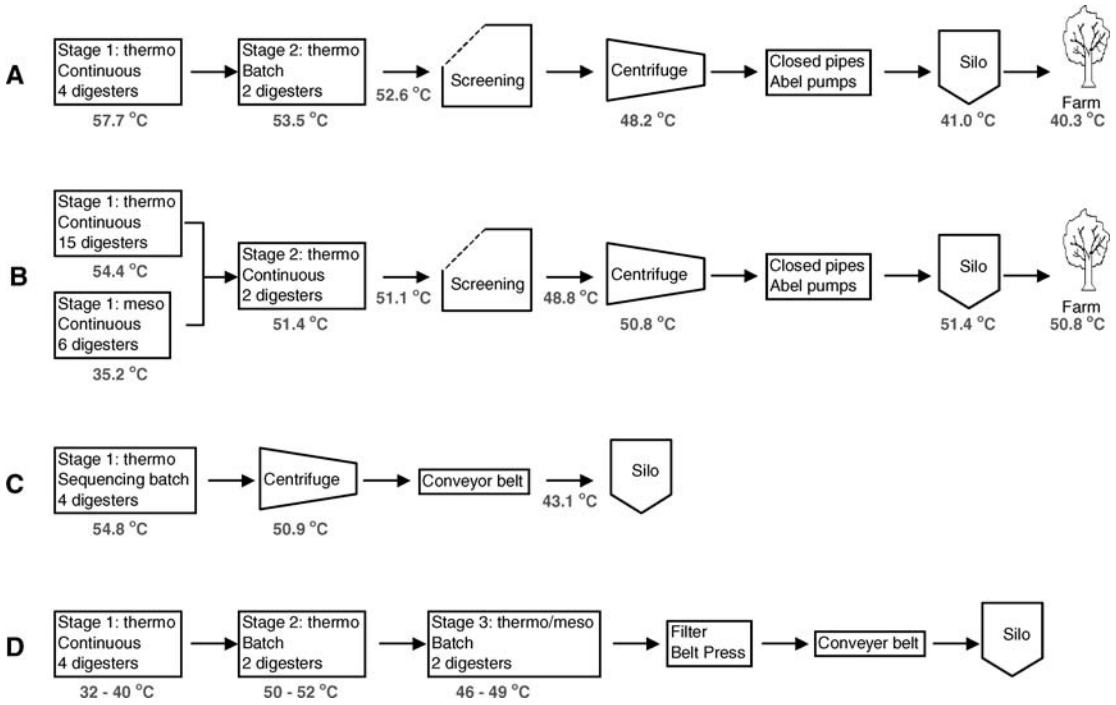

Figure 1—Process diagrams with biosolids temperature profiles at (a) HTP, City of Los Angeles, phases I and II; (b) HTP, phase III; (c) TITP, City of Los Angeles; and (d) RP-1, Inland Empire Utilities Agency, Ontario, California.

Regional Plant No. 1. The RP-1 is an advanced tertiary treatment facility with a capacity of 167 000 m^3/d (44 mgd). The daily biosolids production amounts to approximately 105 wet metric tons. The Inland Empire Utilities Agency investigated several processes for production of Class A biosolids (Drury et al., 2002). A three-phase continuous process was recently approved by U.S. EPA to use Alternative 3 of 40 *CFR* Part 503 for demonstrating Class A pathogen reduction at RP-1 (Figure 1d). After digestion, the biosolids are concentrated in filter belt presses and transported over conveyor belts to the truck-loading facility, where the biosolids are immediately loaded into trucks.

Sampling and Laboratory Procedures. *Field Sampling and Analytical Procedures.* Samples were aseptically taken from various locations in the postdigestion train and the farm for land application. Biosolids samples from the plants were collected in sterile bottles and bags and immediately transported to the laboratory for analysis or for further testing (as discussed in next section). Farm biosolids were stored in cooled boxes for up to 5 hours before arrival at the laboratory. At each sampling event, the biosolids temperature was measured immediately after sample collection. Microbial analyses were performed by the Environmental Monitoring Division at HTP and BioVir Laboratories (Benicia, California), according to the requirements of 40 *CFR* Part 503. Fecal coliform densities were determined by the most probable number (MPN) procedure as described by APHA et al. (1992) in Parts 9221-B and 9221-E1. The analysis of *Salmonella* sp. was according to the procedure of Kenner and Clark (1974), and total solids were determined according to Part 2540-G in APHA et al. (1992).

Laboratory-Scale Tests. Biosolids were aseptically collected from the postdigestion train; transferred to sterile, capped bottles; and incubated in the laboratory at constant temperatures in the range 21 to 55°C for up to 7 days. At regular time intervals, samples were aseptically withdrawn and analyzed for total solids and fecal coliforms or *Salmonella* sp. Samples that were suspected to be completely disinfected were spiked in the laboratory with primary sludge as a source of fecal coliforms or with a pure culture of *Salmonella typhimurium* ATCC 14028. Spiked samples were then processed the same way as unspiked samples.

Laboratory Dewatering Simulations. Biosolids samples were collected from the outflow of TITP digesters and mixed with the following combinations of high-pressure effluent (HPE) and Mannich polymer: (1) blank (no addition); (2) addition of 10% (v/v) HPE; (3) addition of 10% (v/v) 0.2% Mannich polymer in sterile water; and (4) addition of 10% (v/v) 0.2% Mannich polymer in HPE. These additions reflected actual conditions of dewatering at HTP (dosage of approximately 10 kg Mannich polymer as a 0.2% solution in HPE to 1 metric ton of dry biosolids). For each test, approximately 10 L of digester biosolids with the additions as indicated above were concentrated in a laboratory centrifuge to a total solids concentrations of approximately 10%. The concentrated biosolids of each test were subsequently divided into four portions; transferred to sterilized 300 mL bottles; and incubated in water baths at 25.6, 37.0, 46.1, and 55.0°C. Samples of 30 g wet weight were periodically withdrawn over a period of 6 days and analyzed for total solids and fecal coliforms. Sample collection, transfer, centrifugation, and incubation were done aseptically.

Results

Digester Outflow Analyses. At the time of these investigations, the fecal coliform and *Salmonella* sp. densities in the biosolids from the digester outflows of the three plants were, in all cases, below the limits for Class A biosolids (1000 MPN/g dry wt and 3 MPN/4 g dry wt, respectively).

Hyperion Treatment Plant. *Phase I.* As shown in Figure 2, biosolids from the centrifuge contained very low levels of fecal

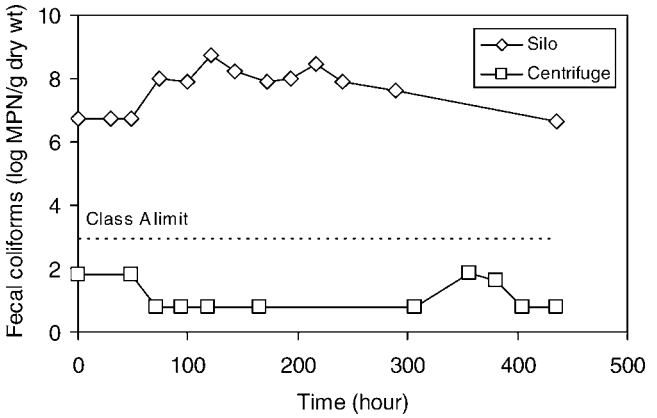

Figure 2—Hyperion Treatment Plant phase I: fecal coliform growth in centrifuge and silo biosolids during incubation at 21°C.

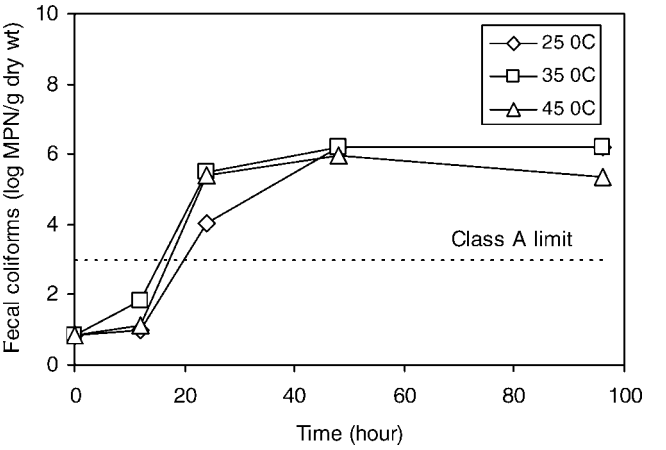

Figure 4—Hyperion Treatment Plant phase II: fecal coliform growth in spiked centrifuge biosolids during incubation at 25, 35, and 45°C.

coliforms at the time of sampling, and incubation of these biosolids at ambient temperature did not result in increasing densities of fecal coliforms. Biosolids from the silos contained fecal coliform densities well above the Class A limit of 1000 MPN/g dry wt. Further growth occurred up to a density of 10^9 MPN/g dry wt after incubation for 120 hours, after which a slow decline was noted (Figure 2). These results indicated that either contamination of biosolids occurred between HTP's centrifuges and silos or fecal coliforms, which were injured during digestion, but not killed, were reactivated during postdigestion. These tests also indicated that silo biosolids were able to support the growth of fecal coliforms. In contrast, growth was not observed in digester outflow biosolids, even after spiking of these biosolids with fecal coliforms from primary sludge, because a rapid decline of the fecal coliform density was observed during incubation at temperatures of 25, 35, and 45°C (Figure 3).

Phase II. Similar to phase I, fecal coliform densities in phase II at HTP were below the Class A limit at all locations in the postdigestion train, except for the silos where densities of approximately 10^6 MPN/g dry wt were found. Fecal coliform densities in farm biosolids were, on average, 10^7 MPN/g dry wt, indicating that further growth of fecal coliforms occurred during transport to the farm. Laboratory-scale tests were performed with centrifuge biosolids spiked with fecal coliforms, as shown in Figure 4. These tests demonstrated a rapid increase of the fecal coliform density during incubation at 25, 35, and 45°C. Hence, centrifuge and silo biosolids had a similar ability of supporting fecal coliform growth, which was not present in digester outflow biosolids.

Comparable tests performed with *Salmonella* sp. demonstrated a rapid decline of the density of this pathogen during incubation of spiked centrifuge biosolids (Figure 5). Therefore, whereas centrifuge biosolids could support fecal coliform growth, the same samples were not able to support growth of *Salmonella* sp.

Phase III. The biosolids at the truck-loading facility and the farm always complied with the Class A limit for *Salmonella* sp. Fecal coliforms in silo biosolids at the truck-loading facility were below the Class A limit in 30 out of 32 samples. Farm biosolids complied with the Class A fecal coliform limit in 7 out of 8 samples. Laboratory-scale tests performed with silo and farm biosolids did not show any growth during incubation of either sample at 21°C for up to 150 hours (Figure 6).

Temperature Profiling. During phases I and II without insulation of the postdigestion train, the average biosolids temperature

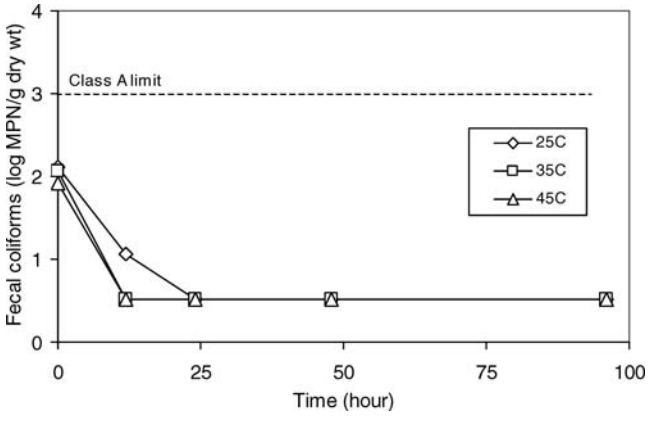

Figure 3—Hyperion Treatment Plant phase I: fecal coliform growth in spiked digester outflow biosolids during incubation at 25, 35, and 45°C.

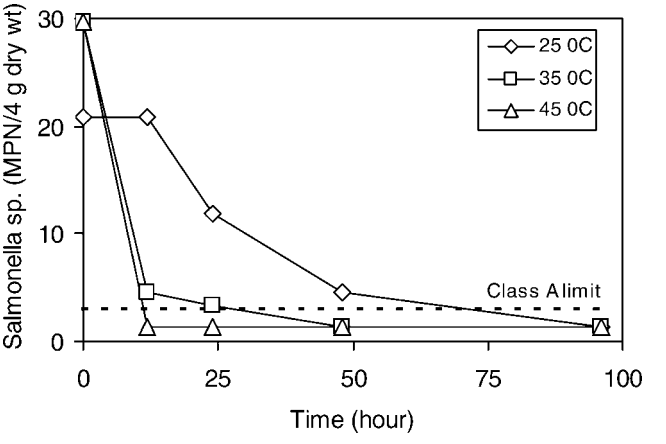

Figure 5—Hyperion Treatment Plant phase II: *Salmonella* sp. growth in spiked centrifuge biosolids during incubation at 25, 35, and 45°C.

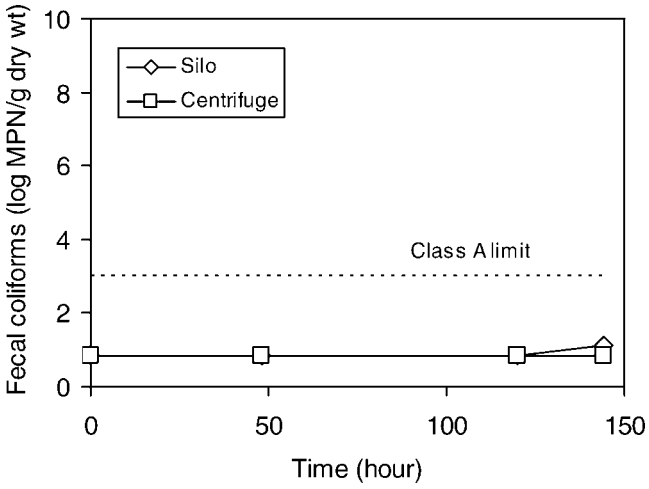

Figure 6—Hyperion Treatment Plant phase III: fecal coliform growth in silo and farm biosolids during incubation at 21°C.

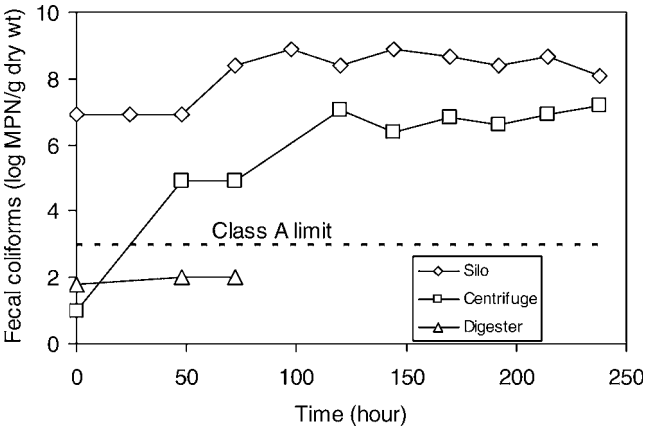

Figure 7—Terminal Island Treatment Plant: fecal coliform growth in digester outflow, centrifuge, and silo biosolids during incubation at 21°C.

declined from 53.5°C in the second-stage digesters to 41°C in the silos, with the major temperature drop occurring after the centrifuges (Figure 1a). Insulation and electrical heat-tracing of the postdigestion train was effective in maintaining a constant biosolids temperature during the phase III test. The biosolids temperature in the silos was almost the same as in the second-stage digesters, indicating that heat losses during postdigestion processing and transport through the plant were strongly reduced (Figure 1b).

Terminal Island Treatment Plant. *Bacterial Analyses.* Fecal coliform laboratory-scale tests with TITP biosolids are shown in Figure 7. No growth of fecal coliforms was observed in digester outflow biosolids, although fecal coliforms were present at a low density. Centrifuge biosolids also contained low densities of fecal coliforms; however, the numbers rapidly increased during the first 60 hours of incubation. This demonstrates that centrifuge biosolids from TITP, unlike centrifuge biosolids from HTP (e.g., Figure 2), already contained fecal coliforms that were able to grow. Silo biosolids contained high densities of fecal coliforms that further increased during incubation (Figure 7).

Temperature Profiling. A rapid decline of temperature occurred during transport of biosolids over the conveyor belt (Figure 1c). Like at HTP during phases I and II, this temperature drop was large enough to allow fecal coliform growth in the silo biosolids, because laboratory-scale tests had indicated that growth could occur at 45°C (e.g., Figure 4).

Centrifuge Dewatering Simulations. Fecal coliforms were not detected in HPE and Mannich polymer; hence, these additions could be ruled out as contamination sources. Consequently, any growth observed in TITP biosolids after dewatering in the laboratory would then be a result of fecal coliforms already being present in TITP biosolids at the time of sampling from the digester outflow. Very little growth of fecal coliforms was observed during incubation at 25, 37, and 45°C of centrifuged biosolids without or with the addition of HPE and/or Mannich polymer (Figures 8a, b, and c). At 55°C, all counts remained at or below the detection limit (Figure 8d), indicating that a high biosolids temperature would prevent fecal coliform recurrence. Overall, fecal coliform densities in TITP sludge after laboratory centrifugation remained well below the Class A limit of 1000 MPN/g dry wt and never reached the numbers observed after full-scale centrifugation (e.g., Figure 7).

Regional Plant No. 1. *Bacterial Analyses.* Fecal coliform densities were well below the Class A limit at all locations at the time of sampling. In laboratory-scale tests, growth of fecal coliforms was very low at 21°C, and the fecal coliform density remained well below the Class A limit during incubation of biosolids from the digester outflow, belt press, and truck-loading facility for up to 120 hours (Figure 9).

Temperature Profiling. Profiles of the biosolids temperature in postdigestion were not determined.

Discussion

None of the plants in this study experienced the recurrence of *Salmonella* sp.; hence, the main concern for plants that use thermophilic anaerobic digesters appears to be compliance with the Class A limit for fecal coliforms. Fecal coliform recurrence in postdigestion to the levels as observed in silo biosolids at HTP in phases I and II and TITP requires their initial presence in postdigestion biosolids and conditions that allow their proliferation. This may tentatively be attributed to operation of the digesters and conditions during postdigestion.

Incomplete Destruction During Thermophilic Treatment. Biosolids in the outflow from the thermophilic digesters at HTP (phases I and II) and TITP often contained detectable levels of fecal coliforms, typically in the range 10 to 1000 MPN/g dry wt. Surviving fecal coliforms could have increased in number as allowed by actual conditions during postdigestion. In contrast, *Salmonella* sp. were never detected in digester outflow biosolids; hence, their complete destruction during digestion eliminated the possibility of growth in postdigestion (*Salmonella* sp. at HTP were detected in raw sludge, typically in the range <2 to 20 MPN /4 g dry wt, but never in digester outflow biosolids). The RP-1 did not experience the recurrence of either fecal coliforms or *Salmonella* sp., for which several explanations may be postulated, including the following: (1) the relatively long residence time in the three-stage digestion process and the low pH in the acid-phase digester may have achieved complete destruction of both groups of microorganisms, (2) the use of filter belt presses instead of centrifuges for dewatering; and (3) a relatively short retention time in postdigestion.

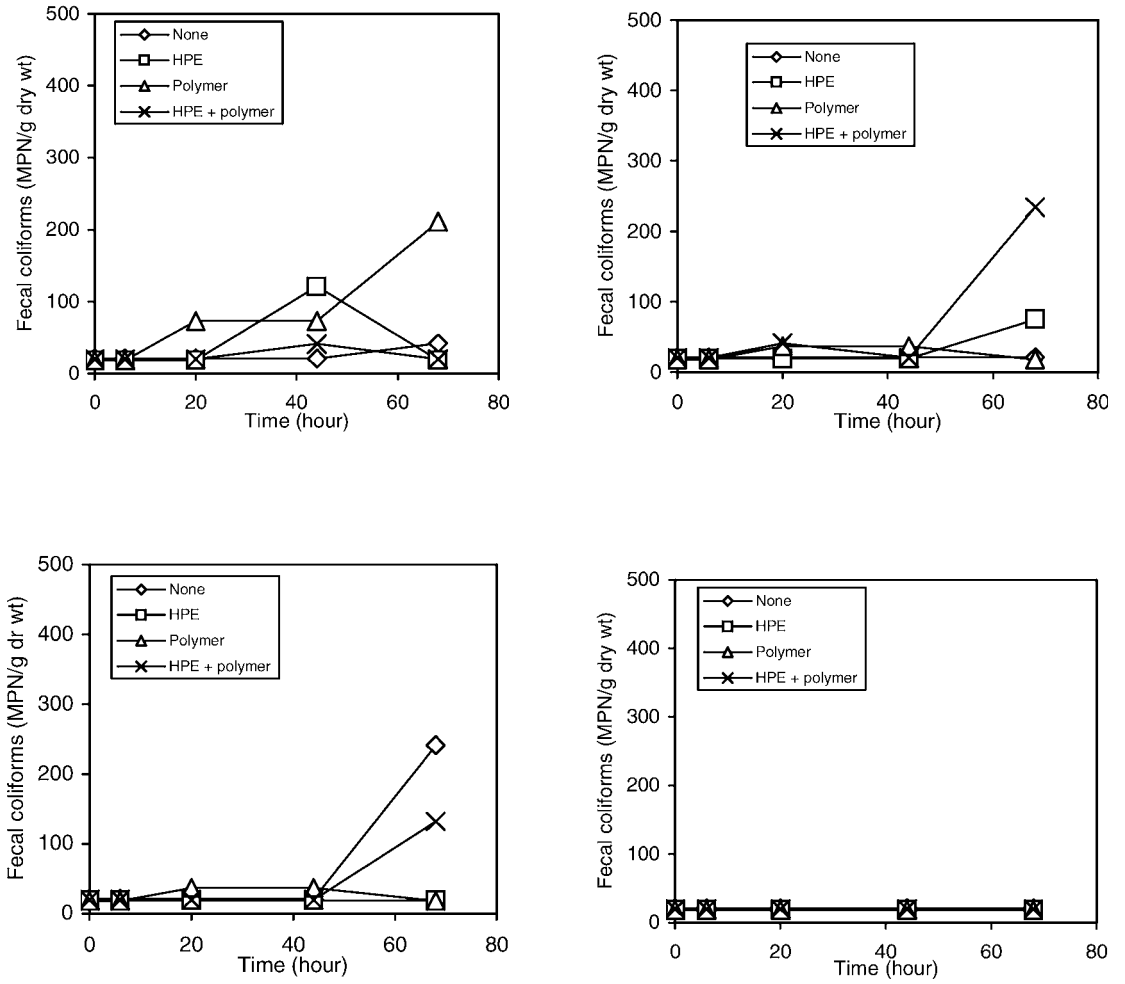

Figure 8—Terminal Island Treatment Plant: fecal coliform growth in digester biosolids after centrifugation in the laboratory with and without addition of polymer and/or HPE: (a) growth at 25°C; (b) growth at 37°C; (c) growth at 44.5°C; (d) growth at 55°C. Note that all growth curves are below the Class A limit of 1000 MPN/g dry wt.

Contamination in Postdigestion. Contamination could have been a contributing factor to the presence of fecal coliforms in postdigestion at HTP (phases I and II) and at TITP. Although all actions were taken to isolate the thermophilic train from mesophilic operations at HTP, contamination of thermophilically digested biosolids by mesophilically digested biosolids during postdigestion would have been difficult to prevent entirely. At TITP, biosolids transport over conveyor belts was probably a source of contamination. Visual inspection of the belts indicated buildup of spilled biosolids that were difficult to remove by the cleaning procedures that were used.

Temperature in Postdigestion. The analytical procedure required by 40 *CFR* Part 503 for detection of fecal coliforms uses an incubation temperature of 44.5°C (APHA et al., 1992; U.S. EPA, 1993). As fecal coliforms are relatively thermotolerant microorganisms, the drop of the biosolids temperature during postdigestion at HTP (phases I and II) and TITP was sufficiently large to reduce the temperature to below the maximum for growth. Hence, if fecal coliforms were present in postdigestion biosolids, either because of incomplete destruction during digestion or by contamination, the large temperature drop during postdigestion could have allowed growth of fecal coliforms to the density of approximately 10^7 MPN/g dry wt in silo biosolids at the truck-loading facility. It should be noted that silo biosolids at HTP and TITP were sampled at the time of unloading into the trucks (i.e., after a maximum of 1 day of storage in the silos).

Centrifuge Dewatering. A contributing factor to fecal recurrence at HTP (phases I and II) and TITP (phase III) may have been the dewatering centrifuges, as suggested by the following observations: (1) fecal coliform densities only increased after the dewatering centrifuges; (2) fecal coliforms were able to grow in centrifuge biosolids, but not in digester outflow biosolids (Figure 3 versus Figure 4); (3) other plants with dewatering centrifuges have reported similar problems (Request for Proposal for Water Environment Research Project 03-CTS-13T; Water Environment Research Foundation, Alexandria, Virginia); (4) recurrence of fecal coliforms did not occur after dewatering in filter belt presses at RP-1 (Figure 9). However, the laboratory simulations failed to confirm field observations, because no significant growth of fecal coliforms was observed during incubation of the biosolids that were centrifuged in the laboratory. It should be noted that the total solids content after laboratory centrifugation was only approximately 10%, which is approximately 2.5 to 3 times less than the content in dewatered biosolids from TITP's centrifuge. The results from the

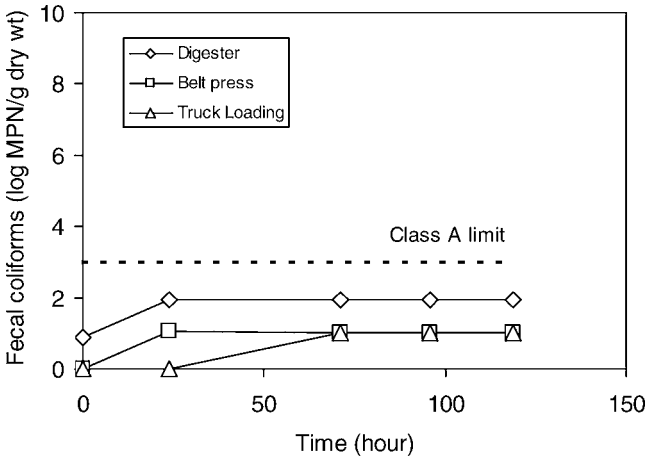

Figure 9—Regional Plant No. 1: fecal coliform growth in digester, belt press, and silo biosolids during incubation at 21°C.

laboratory simulations should, therefore, be interpreted with caution, as extrapolation to full-scale centrifuge dewatering may not be fully justified.

Compliance at Last Point of Plant Control. Fecal coliform recurrence at HTP did not occur in phase III, which can probably be attributed to the following: (1) preventing potential contamination by mesophilically digested biosolids by completing the conversion to thermophilic operation, and (2) insulation and electrical heat-tracing of the postdigestion train, thereby maintaining a biosolids temperature above the maximum for growth of fecal coliforms between the digesters and the silos at the truck-loading facility. Postdigestion train modifications and the conversion to thermophilic operation were done simultaneously because of time constraints to demonstrate compliance of HTP with the Class A standards by the end of 2002. Hence, it is not possible to determine whether fecal coliform recurrence at HTP in phases I and II was a temperature or contamination problem, or a combination of both.

Conclusions

The present investigation demonstrates that increasing densities of fecal coliforms in biosolids in full-scale postdigestion (TITP and phases I and II at HTP) may be attributed to one or more of the following factors:

- Incomplete destruction of fecal coliforms during thermophilic anaerobic digestion;
- Contamination of thermophilically digested biosolids with mesophilically digested biosolids (HTP, phases I and II) or spilled biosolids accumulating in the postdigestion train (TITP);
- A large drop of the biosolids temperature after the centrifuges, which could have allowed fecal coliform growth during storage in the silos; and
- An unknown effect of biosolids dewatering in centrifuges that may have increased the potential of biosolids to support growth of fecal coliforms.

It may be postulated that many factors are related to fecal coliform recurrence in thermophilically digested biosolids. These may include biosolids characteristics, conditions during digestion, and conditions during postdigestion. Further investigations are needed to elucidate the mechanism of fecal coliform recurrence and to determine whether it is a general or plant-specific problem.

Even though the conditions that lead to fecal coliform recurrence are yet not fully understood, it could be prevented by insulation and electrical heat-tracing of the postdigestion train. The HTP in phase III complied with the Class A limits for fecal coliforms at the last point of pant control. Likewise, TITP achieved Class A certification in mid-2004 after implementing similar modifications to the postdigestion train. Other plants that experience fecal coliform recurrence in postdigestion may consider insulation and electrical heat-tracing, because maintaining a postdigestion biosolids temperature above the maximum for growth of fecal coliforms will prevent their recurrence, irrespective of plant-specific conditions that may have caused it.

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