EFFECTS OF DEWATERING ON BACTERIA INACTIVATION: Centrifuge Simulation And Field Tests at The Hyperion Treatment Plant

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ABSTRACTS

The Hyperion Treatment Plant produces Class A biosolids by thermophilic anaerobic digestion according to the EPA 40 CFR Part 503 Biosolids Rule. Additional studies were performed to determine the effect of post-digestion processing on the regrowth of pathogens in biosolids. Potential sources of contamination by pathogens during post-digestion processing are in-plant transfer (pipelines), de-watering (diluted polymer addition and centrifugation), and storage. Temperature profiles along the plant's post-digestion train showed that cooling of biosolids was sufficient to allow for regrowth of pathogens. Laboratory experiments focused on re-growth patterns of *Salmonella* and fecal coliform in digested sludge (immediately after digester) and in wetcake (immediately after centrifuge) at ambient temperature. *Salmonella* regrowth was not observed in any of the experiments, nor in the field. Fecal coliform regrowth was observed in wetcake stored in silos at the truckloading facility, but not in laboratory experiments with digested sludge and centrifuge wetcake. Fecal coliform regowth studies were performed by centrifuging digested sludge in the laboratory under conditions that simulated the field dewatering system. These experiments showed that polymer and secondary effluent additions prior to dewatering of digested sludge are probably not significant factors that stimulate fecal regrowth.

KEY WORDS

Class A biosolids, thermophilic, regrowth, fecal coliform, pathogen

INTRODUCTION

Sludge processing after digestion typically consists of dewatering (diluted polymer addition and centrifugation), in-plant transfer (pipes or conveyor belts), and storage. Equipment details, such as water removal, in-plant transfer, and exposure to the air, etc. during storage may all be sources of contamination by microbes/pathogens. Thus, it is necessary to be sure that the post-digestion steps preserve the disinfection achieved by

thermophilic anaerobic digestion. The specifications in 40 CFR Part 503 Rule include regulations for sludge processing, biosolids standards and land application of biosolids. As pathogen regrowth affects the final biosolids quality, it is important to understand the underlying mechanism and the factors that determine regrowth (Ward *et al.*, 1999; Iranpour *et al.*, 2000b).

Storage of biosolids provides an opportunity for growth by any organism that either survived digestion or was introduced subsequently by contamination, as observed in several studies. Regrowth, however, does not always happen. For instance, Ward *et al.* (1999) reported a long and complex study of apparent self-disinfection effects in biosolids produced by applying an initial pasteurization step before mesophilic digestion. Likewise, studies at Hyperion Treatment Plant (HTP) consistently observed that as time passed there were decreases in densities of *Salmonella* spiked into thermophilically digested sludge.

This paper will discuss the effects of post-digestion processing at HTP, with focus on several issues: a) results of regrowth tests of digested sludge centrifuged in the laboratory to simulate the effect of the dewatering system on *Salmonella* and fecal coliforms that may have survived digestion; b) results of regrowth tests of wet cake samples collected in the field to determine regrowth/contamination after digestion; c) QA/QC procedures to minimize or eliminate regrowth/contamination, e.g., process walkthrough, reviews of protocols, sampling and delivery of samples, lab analyses, etc.; d) review of work by others on microbe/pathogen destruction and regrowth/contamination.

BACKGROUND

According to 40 CFR Part 503 Rule, the general requirement of all alternatives require either the fecal coliform or the *Salmonella* density limit to meet the standard for Class A biosolids (US EPA, 1993, 1994). However, as the City of Los Angeles Bureau of Sanitation seeks the best quality and safest biosolids, it has decided to look at both groups of microorganisms throughout the post-digestion train. The Bureau has decided to follow time and temperature requirement (Alternative 1) for the compliance.

The densities of fecal coliform in the digested sludge were always found to be less than the limit of 1000 Most Probable Number (MPN) per dry gram of Total Solids (TS). The densities of fecal coliform in the wet cake collected directly from the centrifuge was consistently very low, but an elevated density of fecal coliform was observed from the wet cake collected from silo or truck at Hyperion Treatment Plant (HTP). High densities of fecal coliform observed at the truck can be attributed to regrowth of organisms that survived the digestion or the growth of organisms subsequently introduced by contamination between the centrifuge outlet and the silo at the truck loading facility.

Post-digestion processing at HTP consists of several steps: in-plant transfer (pipelines), dewatering (diluted polymer addition and centrifugation), and storage. These steps are recognized as the potential sources of contamination. The long pipeline to the centrifuge,

the centrifuge itself, the post-centrifuge transport, and the storage facilities were all examined as possible sources of contamination and for conditions that would promote bacterial growth. In particular, polymer addition before centrifugation and the usage of small amounts of secondary effluent or High Pressure Effluent (HPE) to lubricate passage of the wet cake through the pipe from the centrifuge to the silo were suspected to be potential sources of contamination or regrowth.

Temperature drop in the post-digestion train is also another factor that promotes bacterial growth. Pipes transporting the sludge between the centrifuge outlet and the silo are located outside of the building without insulation. As a result, the biosolids temperature decreases and this drop in temperature may establish favorable conditions for bacterial growth.

The Bureau of Sanitation has been very active in implementing projects on thermophilic anaerobic digestion to obtain Exceptional Quality (EQ) Class A biosolids (Iranpour *et al.*, 2000a, 2000b, 2001a, 2001b, 2001c, 2002a, 2002b, 2002c, and 2002d).

EXPERIMENT

The following experiments were performed in order to evaluate the extent of regrowth in wetcake obtained from the thermophilic post-digestion process and to define the effect of temperature and contamination sources on the growth of *Salmonella* and fecal coliform:

1. Literature review regrowth phenomena.
2. Temperature profile study.
3. *Salmonella*/fecal coliform regrowth at ambient laboratory temperature.
4. Fecal coliform regrowth under centrifuge simulated conditions

1. Literature review: Pathogen regrowth in digested biosolids in general requires:

- The presence of pathogens (either those surviving disinfection, or those introduced by contamination after digestion).
- Environmental conditions that allow/stimulate growth (e.g., substrate, temperature, no toxic digestion products).
- Absence of inhibition by the indigenous population (e.g., substrate competition, predation, antibiotics products).

From a practical point of view, it is important to first establish the effectiveness of pathogen inactivation during digestion. The 40 CFR Part 503 Rule specifies the use of Processes to Further Reduce Pathogens (PFRP) to produce Class A biosolids with levels of pathogenic organisms (fecal coliform, *Salmonella*) below the detection limit (US EPA, 1993). This raises the issue of recovery of pathogens by the recommended analytical procedures. These are classic microbiological procedures that have been used for many years. The analytical principle is that biosolids are mixed with nutrient broths specific for the growth of *Salmonella* and fecal coliform. If growth occurs, then the conclusion is that the sample contained pathogens and, vice-versa, absence of growth would point to

absence of pathogens. It is however questionable whether the recommended procedures provide complete recovery of pathogens. It is widely known that classic microbial techniques are able to detect only a small portion of the great variety of bacteria in Nature. In addition, digestion does not necessarily kill the pathogen. It is quite conceivable that (part of) the pathogens are not killed but merely inactivated, and thus unrecoverable during the analysis. A typical example is the effect of toxic concentrations of toluene on the toluene-degrading bacterium *Pseudomonas putida*. When cells are exposed to high toluene concentrations, they loose their ability to grow on toluene but they remain alive (Mirpuri *et al.*, 1997; Villaverde *et al*, 1997). Thus, the toluene nutrient broth selective for P. putida would be unable to detect the species.
A similar situation may exist with respect to fecal coliform and *Salmonella* disinfection in biosolids, in particular because digestion temperatures (~55^{0}C) are much lower than temperatures typically used for sterilization (121^{0}C) (i.e., inactivation versus killing). As also recommended by Gibbs *et al.* (1997), detection methods may need to be more sensitive and/or they need to be able to also detect non-culturable pathogens.

Another important factor to consider is the temperature. Post-digestion processing causes cooling of biosolids, and the temperature may drop to a level that allows the growth of pathogens. In this respect it should be realized that Salmonella and fecal coliform are relatively thermotolerant microorganisms. The EPA recommends Part 9221 of Standard methods for the detection of fecal coliform (APHA, 1992; US EPA, 1993). This procedure uses an incubation temperature of 44.5^{0}C for fecal coliform growth. Some Salmonella species may even grow at temperatures up to 49^{0}C (Neidhardt, 1987). Clearly, only little cooling is sufficient to reduce the temperature from levels typical during digestion to a level that allows growth during post-digestion processing.
Another factor to consider is the water content. Gibbs *et al.* (1997) observed that pathogens regrew during storage of biosolids during the winter. This was attributed to more rainfall, which increased the water content of the biosolids.

By a series of pasteurization and digestion experiments, Ward *et al.* (1999) provide valuable information on pathogen regrowth in Class A biosolids. Most interestingly, they observed that the presence of the microbial population indigenous in digesters suppresses the growth of Salmonella. Without the indigenous population, Salmonella would grow rapidly when added to pasteurized (70^{0}C) digested biosolids. This observation would also explain why pathogen reduction is observed during mesophilic digestion, i.e., at temperatures that do not inactivate pathogens (e.g., Berg and Berman, 1980; Carrington *et al.*, 1991; Watanabe *et al.*, 1997). Likewise, anaerobic digestion is listed by the EPA as a Process to Significantly Reduce Pathogens (PSRP) (US EPA, 1993). The mechanism of microbial antagonism is not clear, and it may depend on the particular sample and conditions. Research on the storage of composted and digested biosolids and pathogen regrowth suggests a number of possibilities (Burge *et al.*, 1987; Hussong *et al.*, 1985; Millner *et al.*, 1987; Russ and Yanko, 1981; Sidhu *et al.*, 2001; Yeager and Ward, 1981): inhibition by digestion products (ammonia, volatile fatty acids), competition for limited nutrient availability, inhibition by microbial products from the indigenous population (e.g., antibiotics), and predation.

2. Temperature profile study: *Salmonella* and fecal coliform require a drop in the sludge temperature along the post-digestion train in order to attain a favorable temperature for bacterial growth. Thus, the sludge temperatures along the post-digestion thermophilic train were measured in order to locate the most probable sections of the train where bacterial growth may occur due to a drop in temperature.

Sludge temperatures were measured at the same locations where samples were collected for *Salmonella* and fecal coliform analyses and also at some additional locations. Temperature was measured using three types of thermometers: conventional mercury, dial, and digital. Figure 1 shows the measurement locations.

Table 1 shows the temperature profile obtained on December 5, 2001 along the post-digestion thermophilic train. Reported values represent only the measurements obtained with the digital thermometer, which provided the most reliable absolute measurements. Additionally, the calculated difference in temperatures between a given pair of points was similar regardless of the type of thermometer used. The difference in temperature between a given sampling location and the previous one is indicated in Table 1 as T. A negative value indicates a drop in temperature and a positive value indicates an increase. Changes in temperature at locations 2, 3, 4 are small, around 1°C, and they compensate each other. Therefore, overall drop in temperature from location 1 (digester) to location 4 (wetwell) is minimal ($\Delta T = -0.6^{\circ}C$). A larger drop in temperature occurs between locations 4 and 5 (mixing with polymer, $\Delta T = -2.3^{\circ}C$). However, this drop is partially compensated by an increase in temperature between locations 5 and 7 ($\Delta T = 1.8^{\circ}C$). The highest drop in temperature, $\Delta T = -6.8^{\circ}C$, was observed between the outlet of the centrifuge (Location 7) and the top of the silo (Location 8). The total drop in temperature between the digester outlet and the silo at the truck loading facility was 7.8 °C.

Table 2 shows the temperature profile at the truck loading location obtained on December 6, 7 and 10, 2001. This profile was measured in wetcake when being discharged into the truck in order to evaluate the temperature gradient existing inside the silo. Temperatures of the wetcake obtained at the front of the truck correspond to wetcake located at the lower level of the silo whereas temperatures measured at the rear of the truck correspond to wetcake located at a higher level in the silo. The data indicates that there is a decrease in temperature along the depth of the silo that ranges from 1.2 °C to 4.9 °C.

3. Salmonella/fecal coliform regrowth at ambient laboratory temperature: An initial evaluation of fecal coliform regrowth was performed in wetcake samples obtained at two locations on October 11 (at the truck) and October 16 (after the centrifuge), 2001. Several individual samples were left at room temperature for over 400 hrs and analyzed for fecal coliform density at predefined time intervals. Results are shown in Figure 2. Fecal coliform counts in the centrifuge wetcake samples were low ($<10^1$ MPN/g dry wt) with a minor deviation to about 10^2 MPN/g dry wt at t = 350 h. The deviation to 10^5 MPN/ g dry wt at t = 24 h was due to a dilution error in the laboratory. No regrowth was observed in the centrifuge wetcake samples at ambient temperature even after approximately 400 hrs. Counts were high (10^6 MPN/g dry wt) and regrowth was

observed (up to 10^8 MPN/g dry wt) in wetcake samples from the truck. After approximately 400 hrs, counts decreased to the initial levels. These results confirm that either regrowth or contamination with an external microbial source occurred between the centrifuge and the silo. *Salmonella* regrowth was also evaluated in wetcake samples obtained on October 11 from the truck. In contrast to fecal coliform, the results in Table 3 shows that no regrowth of *Salmonella* occurred in the sample from the truck.

4. Fecal coliform regrowth under field simulated conditions: Fecal coliform inoculant may originate from bacterial contamination introduced during or after the centrifugation process. Thus, the objective of this study was to evaluate fecal coliform regrowth in digested sludge centrifuged in the laboratory under conditions that simulate the field dewatering system. Possible sources of bacterial contamination tested included the polymer and the high pressure effluent (HPE). Also two other factors affecting regrowth, i.e., temperature and storage time, were considered and incorporated in the test.

Digested sludge samples were collected from Terminal Island Treatment Plant (TITP) on November 15, 2001, since thermophilic samples from HTP were unavailable at that time. However, HPE, concentrated polymer, and field diluted polymer samples were collected from HTP on the same day. Sludge and various combinations of HPE and polymer were mixed to prepare four batches. Batch 1 or Blank was prepared to be used as a control.

Batch 1 (Blank, no additions):	digested sludge
Batch 2 (Blank + HPE):	digested sludge + HPE
Batch 3 (Blank + polymer):	digested sludge + lab diluted polymer (with fresh sterile water)
Batch 4 (Blank + HPE + polymer):	digested sludge + field diluted polymer (with HPE)

Polymer and HPE were added in amounts to simulate actual field condition. For example, the amount of polymer was added based on plant's dosage, which was 20 pounds per ton of dry wetcake. HPE was also added according to the amount the plant uses for dilution of the polymer.

The batches were centrifuged to a density of approximately 10% total solids in laboratory centrifuges. The laboratory tried to obtain 30% of TS wetcake but only 10% of TS wetcake was obtained due to the capacity of instrument. After centrifugation, the relatively concentrated solids were transferred to sample bottles that were incubated at four temperatures, 25, 37, 44.5, and 55°C (78, 99, 115, 131°F). All sample preparation operations were performed following strict QA/QC procedures to avoid bacterial contamination. The samples from each bottle were analyzed according to the schedule in Table 4.

Negligible regrowth, well below the legal limit, was observed after 96 hrs at 25, 37°C in all four digested sludge batches (Figures 3, and 4). Small regrowth was also observed in some samples at 44.5°C after 72 hrs (Figure 5). However, the fecal coliform counts decreased to the initial levels at 96hrs. At the highest temperature tested 55°C (131°F), all counts were at or below the threshold and no regrowth was observed (Figure 6).

This study showed that negligible regrowth was observed in digested sludge dewatered in the laboratory. The increase in fecal coliform densities was well below the legal limit, indicating that the amount of bacteria that may have survived digestion was not sufficient to cause considerable regrowth to the numbers as observed in the wetcake samples at the truck. It also indicates that the HPE and the polymer are not significant sources of fecal coliform contamination.

It should be mentioned that total solid content of the dewatered sludge obtained by centrifugation in the laboratory was only about 10%, which is one third of the content present in dewatered biosolids in the plant centrifuge. Therefore, regrowth results from this study should be interpreted with some caution.

DISCUSSION

It is clear from all the studies presented in this section that *Salmonella* regrowth is not an issue in wetcake samples at any point of the post-digestion train.

Conflicting results of fecal coliform regrowth were obtained from the several studies presented in this section, making it unclear whether regrowth, contamination or both are responsible for the high fecal coliform counts observed at the truck. Therefore, it is important to discuss and define experimental protocols to re-evaluate the role of contamination and regrowth. The following are issues to be considered in future protocols:

1) A thorough evaluation of the fecal coliform contribution from the HPE and the polymer needs to be conducted. The fecal coliform density in the diluted polymer needs to be evaluated since diluted polymer is prepared by diluting concentrated polymer with HPE and the small amounts of fecal coliform carried over with the HPE may increase during the temporal storage of the diluted polymer in the field. In addition to the fecal coliform densities in HPE and polymer, frequency and quantity of application of both have to be considered.
2) Find the effect of substrate of digested sludge in wetcake for fecal coliforms regrowth. When the fecal coliform regrowth test was performed with digested sludge and centrifuge wetcake, there was no sign of significant regrowth but the wet cakes collected from the silo showed regrowth. A change of the biosolids chemical composition before and after dewatering (water content, substrate content) may be important for fecal coliform regrowth. The following procedures will be used to test this hypothesis.

a. Wetcake collected from silo will be diluted with centrate in the laboratory to achieve 5, 10, 15, 20 and 25% of wetcakes.
b. Digested sludge (2%) and wetcake (30%) from silo will be used as controls.
c. Samples will be well mixed to obtain homogeneous matrix and stored at room temperature.
d. Samples will be analyzed every day for one week to find the changes in fecal coliform densities.

3) For simulation of dewatering in the laboratory, experiments need to be performed using wetcake samples with total solid concentrations similar as in field samples in order to obtain conclusions that can be fully extrapolated to the field conditions. This would first require investigation for laboratory procedures and equipment that produce wetcake of 20-30% TS.

CONCLUSIONS

Thermophilic anaerobic digestion is effective in producing Class A biosolids according to the EPA 40 CFR Part 503 Biosolids Rule. The potential sources of contamination by pathogens during post digestion processing are in-plant transfer (pipelines), de-watering (diluted polymer addition and centrifugation), and storage. Cooling of biosolids in post digestion train is sufficient to allow for regrowth of pathogens, however, *Salmonella* regrowth was not observed in digested sludge and in wetcake at ambient temperature. Fecal coliform regrowth was observed in the wetcake stored in silos at truckloading facility, but not in laboratory experiments with digested sludge and centrifuge wetcake. Polymer and secondary effluent additions prior to dewatering of digested sludge were not considered the significant factors that stimulate fecal coliform regrowth. Maintaining high enough temperature at storage or silo will suspend the regrowth regardless of wetcake contamination. Longer detention time at silo may promote the regrowth if there were a source of contamination.

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REFERENCES

APHA (1992). Standard Methods for the Examination of Water and Wastewater, 18th ed., Washington, D.C.

Berg, G., Berman, D. (1980). Destruction by anaerobic mesophilic and thermophilic digestion of viruses and indicator bacteria indigenous to domestic sludges. *Appl. Environ. Microbiol.* **39**:361-368.

Burge, W.D., Enkiri, N.K., Hussong, D. (1987). Salmonella regrowth in compost as influenced by substrate (Salmonella regrowth in compost). *Microb. Ecol.* **14**:243-253.

Carrington, E.G., Pike, E.B., Auty, D., Morris, R. (1991). Destruction of faecal bacteria, enteroviruses and ova of parasites in wastewater sludge by aarobic thermophilic and anaerobic mesophilic digestion. *Wat. Sci. Tech.* **24**:377-380.

Gibbs, R.A., Hu, C.J., Ho, G.E., Unkovich, I. (1997). Regrowth of faecal coliforms and Salmonellae in stored biosolids and soil amended with biosolids. *Wat. Sci. Tech.* **35** (11/12):269-275.

Hussong, D., Burge, W.D., Enkiri, N.K. (1985). Occurrence, growth, and suppression of Salmonellae in composted sewage sludge. *Appl. Environ. Microbiol.* **50**:887-893.

Iranpour, R., Oh, S., Kim, H.S. Ruiz, M., Marashi, S., Gardner, K., Eldridge, M., Shao, Y.J., Moghaddam, O., Garnas, G., Varsh, V., Langley, J. Wislon, J., Ahring, B.K. (2000a). Full Scale Thermophilic Digestion of Sludge Waste at City of LA's Terminal Island Treatment Plant: Success Story, *Proceedings of WEFTEC'2000 Conference, Anaheim, Calif.*

Iranpour, R., Moghaddam, O., Stenstrom M. K., (2000b). Of: Preventing Growth of Pathogens in Pasteurized Digester Solids, *Wat. Env. Res.*, 72(2), 251.

Iranpour, R., Zermeno, M., Paracuelles, R., Stenstrom, M.K., Ahring, B.K. (2001a) Successes and Failures of Thermophilic Anaerobic Digestion of Municipal Wastewater Sludges around the World, *Proceedings of WEF 2001 Joint Residuals and Biosolids Conference, San Diego, Calif.*

Iranpour, R., Oh, S., Kim, H., Eldridge, M., Marashi, C., Shao, Y.J., Wilson, J., Stenstrom, M., Ahring, B.K. (2001b). Startup and stabilization of thermophilic digestion at TITP, *Proceedings of WEF 2001 Joint Residuals and Biosolids Conference, San Diego, Calif.*

Iranpour, R., Oh, S., Kim, H., Shao, Y.J., Hagekhalil, A., Schafer, P., Stenstrom, M.K., and Ahring, B.K. (2001c). Legal Standards for Municipal Sewage Plant Biosolids Disinfection: Application to Production of Class A Exceptional Quality. *WEFTEC, 2001. Atlanta.*

Iranpour, R., Shao, Y. J., Oh, S., Kim, H., Soung, T., Kearney, R., Alatriste, F., Stenstrom, M. K., (2002a), "Experience with Several Modes of Full-scale Thermophilic Digestion Operations at Terminal Island Treatment Plant," *Proceedings of CWEA 2002*

Iranpour, R. *et al*, City of Los Angeles Bureau of Sanitation Class A Task Force (WESD Aapplied Research, HTP, TITP, EMD, RAD, EED, HRDD), (2002b). "Class A Biosoilds: Hyperion Treatment Plant" *Report prepared for Bureau of Sanitation.*

Iranpour, R. *et al*, City of Los Angeles Bureau of Sanitation Class A Task Force (WESD Aapplied Research, HTP, TITP, EMD, RAD, EED, HRDD), (2002c). "Class A Biosoilds: Terminal Island Treatment Plant" *Report prepared for Bureau of Sanitation.*

Iranpour, R., Shao, Y.J., Stenstrom, M.K., and Ahring, B.K. (2002b). Changing mesophilic sewage sludge digestion into thermophilic operation at the Terminal Island treatment plant. *Wat. Env. Res.*, in press.

Millner, P.D., Powers, K.E., Enkiri, N.K., Burge, W.D. (1987). Microbially mediated growth suppression and death of Salmonella in composted sewage sludge. *Microb. Ecol.* **14**:255-265.

Mirpuri, R., Jones, W., Bryers, J.D. (1997). Toluene degradation kinetics for planktonic and biofilm-grown cells of Pseudomonas putida 54G. *Biotechnol. Bioeng.* **53**:535-546.

Neidhardt, F.D. (1987). Escherichia coli and Salmonella typhimurium. Volume 1 and 2, *Am. Soc. Microbiol., Washington, D.C.*

Russ, C.F., Yanko, W.A. (1981). Factors affecting Salmonellae repopulation in composted sludges. *Appl. Environ. Microbiol.* **41**:597-602.

Sidhu, J., Gibbs, R,.A., Ho, G.E., Unkovich, I. (2001). The role of indigenous microorganisms in suppression of Salmonella regrowth in composted biosolids. *Wat. Res.* **35**:913-920.

US EPA (1993). The Standards for the Use and Disposal of Sewage Sludge (Title 40 of the Code of Federal Regulations, Part 503). Federal Register 58 FR 9248 to 9404.

US EPA (1994) Plain English Guide to the EPA Part 503 Biosolids Rule. EPA/832/R-93/003.

Villaverde, S., Mirpuri, R. Lewandowski, Z., Jones, W.L. (1997). Physiological and chemical gradients in a Pseudomonas putida 54G biofilm degrading toluene in a flat plate vapor phase bioreactor. *Biotechnol. Bioeng.* **56**:361-371.

Ward, A., Stensel, H.D., Ferguson, J.F., Ma, G., Hummel, S. (1999). Preventing growth of pathogens in pasteurized digester solids. *Wat. Environ. Res.* **71**:176-182.

Watanabe, H., Kitamura, T., Ochi, S., Ozaki, M. (1997). Inactivation of pathogenic bacteria under mesophilic and thermophilic conditions. *Wat. Sci. Technol.* **36(6/7)**:25-32.

Yeager, J.G., Ward, R.L. (1981). Effects of moisture content on long-term survival and regrowth of bacteria in wastewater sludge. *Appl. Environ. Microbiol.* **41**:1117-1122.

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Figure 1. Isolation of thermophilic from mesophilic train for temperature profile testing

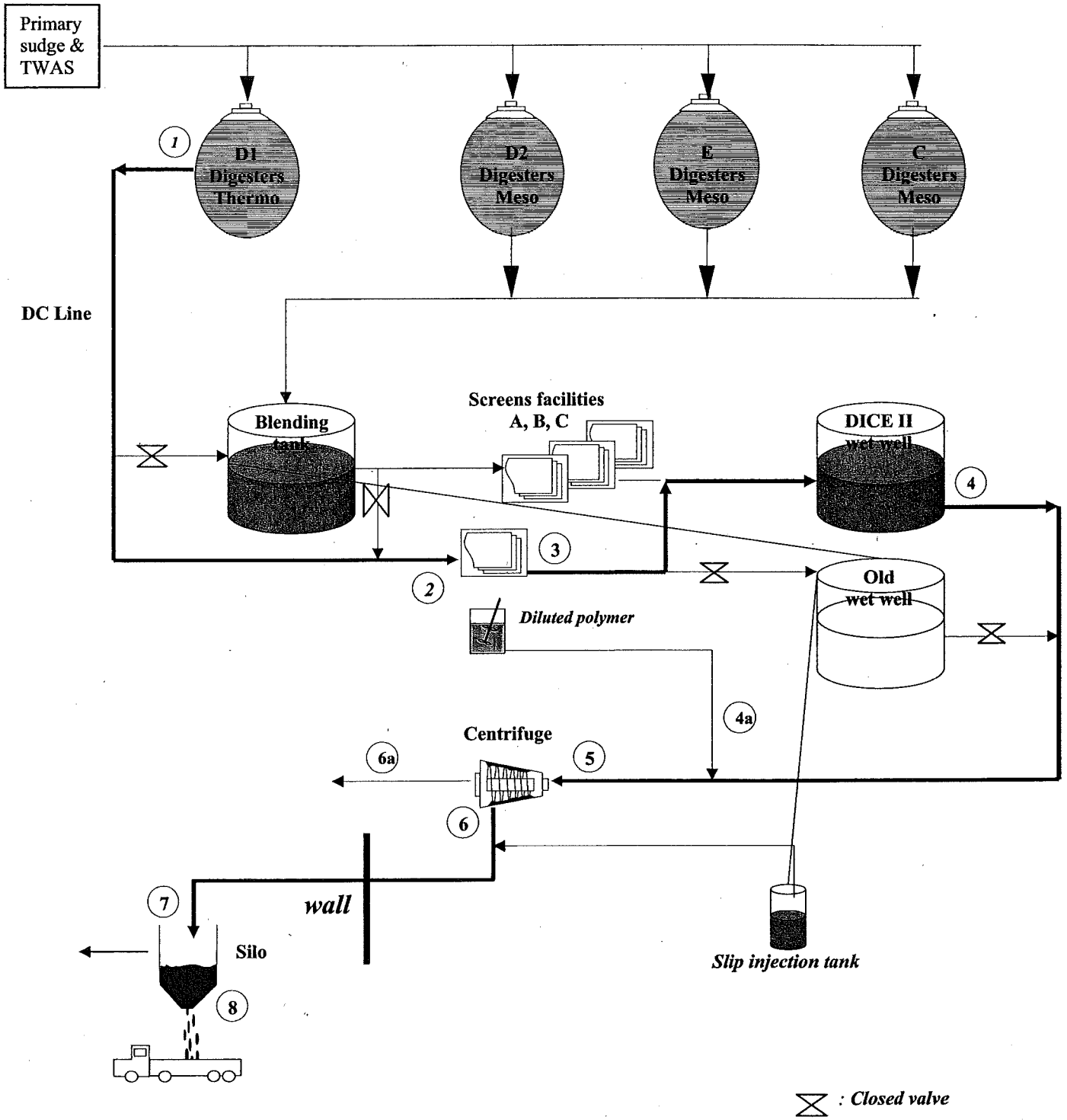

Table 1. Temperature profile data (Locations 1 to 7)

Digester Temperature: 5D1 = 51.1 °C

	Location	Temperature (°C)		Ambient
		Digital	ΔT (°C) **	Temp. (°C)
1	Digester 5D1	51.4	-	25.8
2	Before screen	50.8	-0.6	
3	After screening facility	50	-0.8	15.4
4	After Dice II wetwell	50.8	0.8	20.7
4a	(Diluted polymer)	24.4	-	
5	Mixture of digested sludge/polymer	48.6	-2.2	
6	Centrifuge outlet	50.4	1.8	
6a	(Centrate)	49.8	-	
7	Before falling into silo	43.6	-6.8	19.6

Notes
Digested sludge flow rate at Centrifuge # 6 and # 7 = 700 gpm
Test started on 12/05/2001 at 8:20 a.m.
* This temperature may not be representative of actual temperature due to difficulties in sample collection process
** Change in temperature (ΔT) is calculated with reference to the previous location.

Table 2. Temperature profile data (Location 8)

Date	Time	Time elapsed (hrs)	Truck Location	Thermometers (°C)		Silo level (ft)		Ambient Temp. (°C)
				Digital	ΔT*	before	after	
12/6/01 Thursday	7:20 AM	15	front	39.9		18.4	13.1	12.0
			rear	44.8	4.9			
12/7/01 Friday	9:25 AM	41	front	44.4		13.1	9.6	18.6
			rear	45.6	1.2			
12/10/01 Monday	10:00 AM	114	front	36.9		9.6	5.2	14.8
			rear	39.7	2.8			

Notes
* Differnece in temperature (ΔT) between the rear and front samples.

Figure 2. Regrowth of fecal coliforms

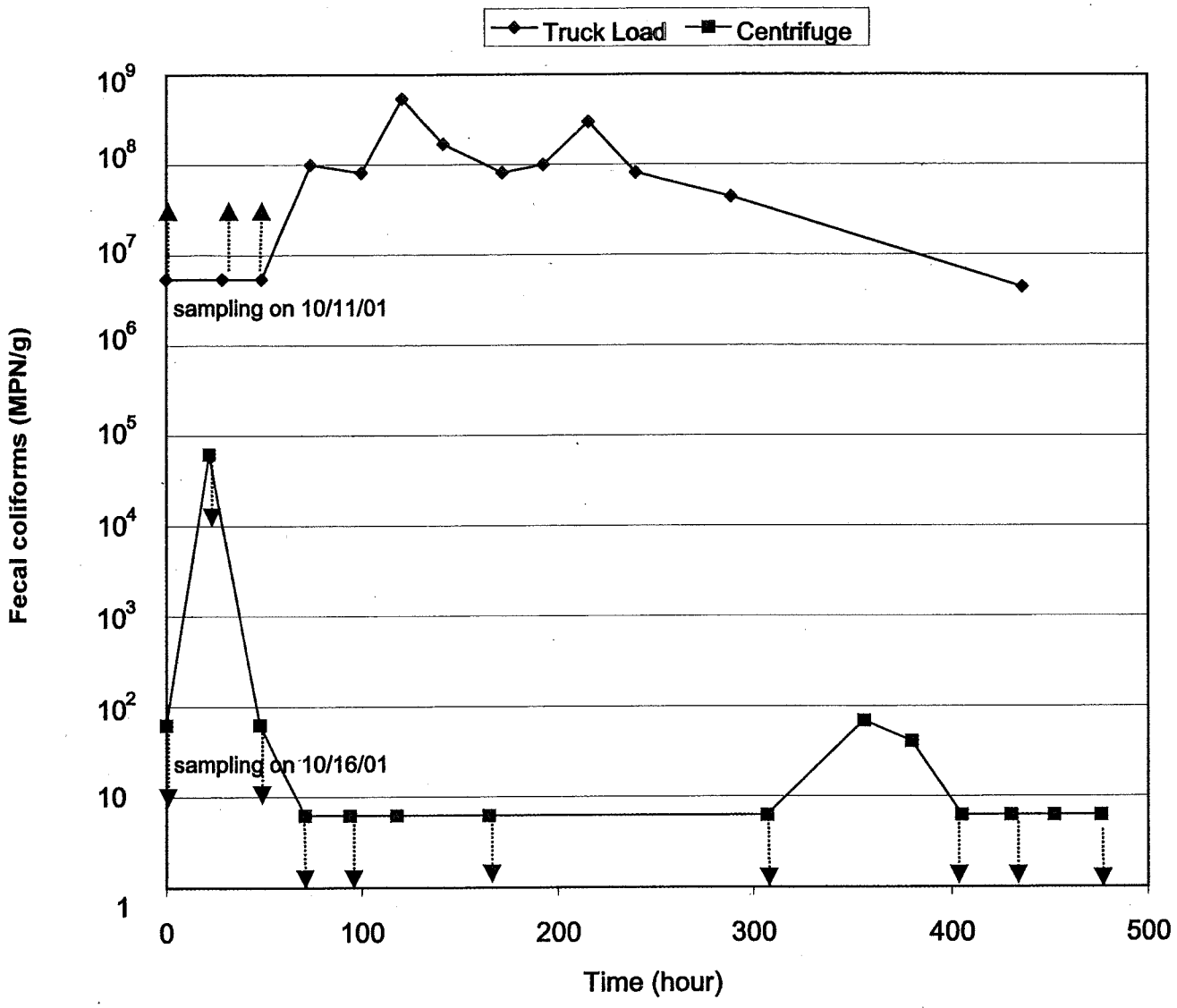

Table 3. Regrowth of *Salmonella* sp.

Sample Date	Location	Regrowth Hr	Elapsed Time (hr)	MPN/4 dry gram
10/11/01	4	11:35Hr (10/12/2001)	27	3.5
	4	9:35 Hr (10/15/2001)	97	<1.5
	4	15:18 Hr (10/17/2001)	151	<1.4

Note: Location 4 = Silo#4 (Truck Loading)

Table 4. Analysis schedule for fecal coliform regrowth test

Batch	Temperature		Time, hours							
	°F	°C	0	8	24	48	72	96	120	144
Batch 1	78	25.6	X	X	X	X		X		
	99	37.0	X	X	X	X		X		
	115	46.1	X	X	X	X		X		
	131	55.0	X	X	X	X		X		
Batch 2	78	25.6	X	X	X	X		X		
	99	37.0	X	X	X	X		X		
	115	46.1	X	X	X	X		X		
	131	55.0	X	X	X	X		X		
Batch 3	78	25.6	X	X	X	X		X		
	99	37.0	X	X	X	X		X		
	115	46.1	X	X	X	X		X		
	131	55.0	X	X	X	X		X		
Batch 4	78	25.6	X	X	X	X		X		
	99	37.0	X	X	X	X		X		
	115	46.1	X	X	X	X		X		
	131	55.0	X	X	X	X		X		

Note:
If the samples show continuously negative up to 48 hours, 72 hour analyses will be skipped and tests begin at 96 hour.
If the 96 hour samples still show negative, the further analyses will be halted.

: Optional

Figure 3. Regrowth results at 78 °F (25 °C)

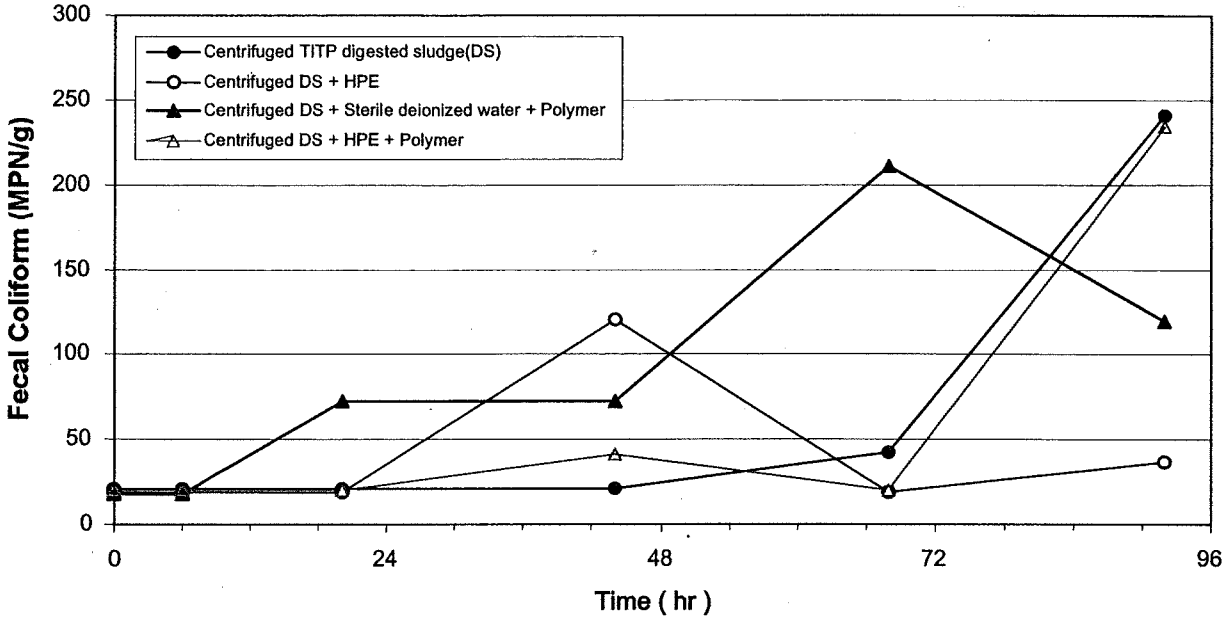

Figure 4. Regrowth results at 99 °F (37 °C)

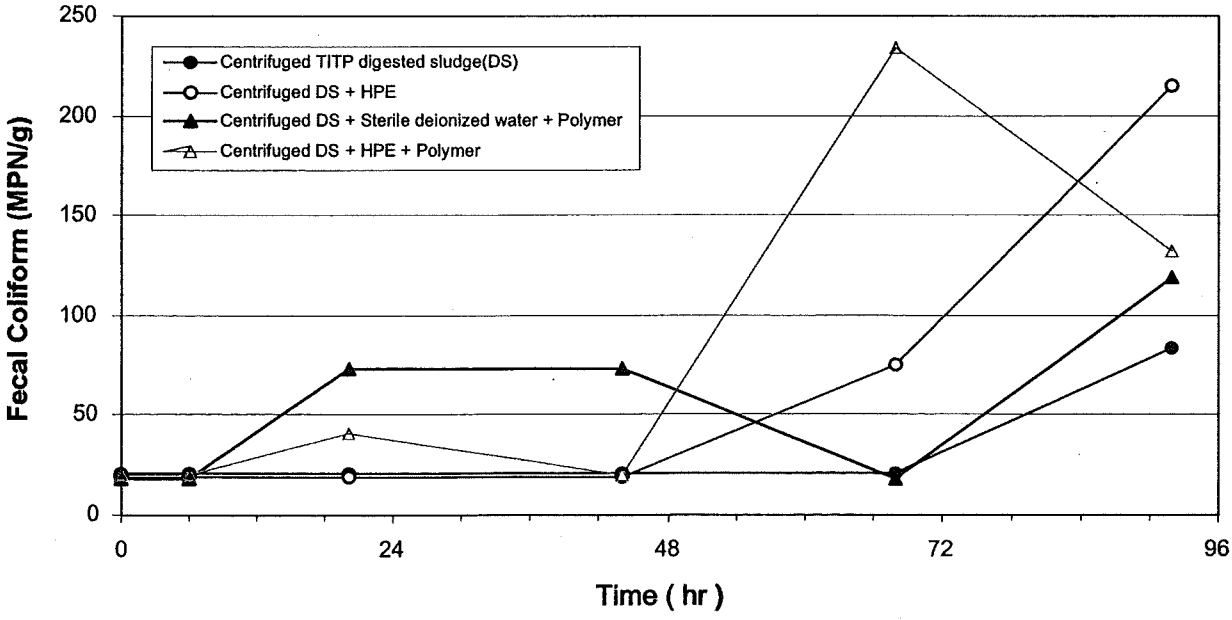

Figure 5. Regrowth results at 115 °F (44.5 °C)

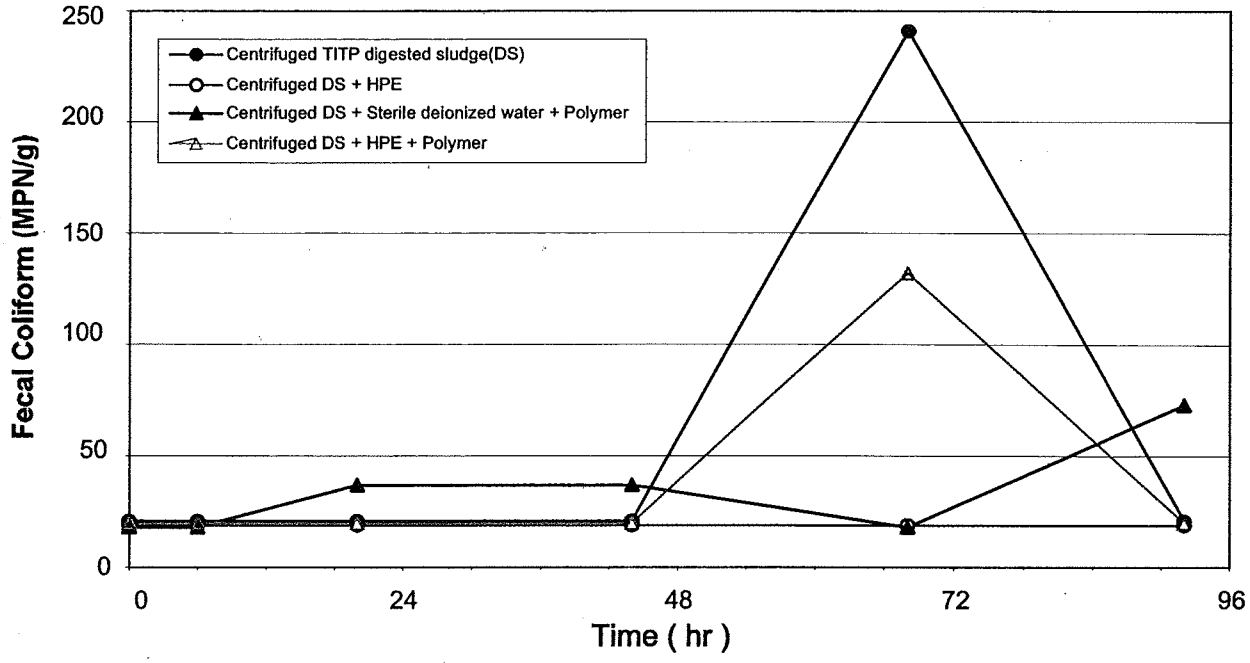

Figure 6. Regrowth results at 131 °F (55 °C)