THE EFFECTS OF PHYSICAL STRESSORS ON BACTERIAL INACTIVATION RATES IN BIOSOLIDS

by

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ABSTRACT

Sanitation is fundamental to reducing disease and sustaining a high standard of living. The evolution of sewer systems and the modern engineering of wastewater treatment plants work to decrease health risk and manage environmental concerns associated with the reuse and disposal of treated effluent and solid wastes generated as byproducts. The recycling of treated solid wastes (biosolids) continues to be an environmental challenge due to the shear volume produced, and its potentially hazardous composition.

Solar drying of biosolids was studied in semi-arid regions as a sustainable method for reducing pathogens. The initial studies were performed with no intervening treatments. Average fecal coliform inactivation rates for digested biosolids during summer experiments were determined to be $0.17 \pm 0.03/day^{-1}$ and $0.17 \pm 0.04/day^{-1}$, respectively. *Salmonella* inactivation rates in aerobically digested biosolids were $0.11 \pm 0.08\ day^{-1}$ and $2.0 \pm 2.0\ day^{-1}$ for aerobically and anaerobically digested biosolids, respectively for the summer seasons. Solar drying during warm dry seasons was effective in reducing pathogens.

Microbial testing to verify the quality of biosolids can be expensive. Utilizing a mathematical model to predict pathogen density levels during the solar drying process can minimize such testing. The first order mathematical model, $N_t = N_o * 10^{-k_d t}$ where the inactivation constant, k_d, is further defined as a function of moisture (Θ) and temperature (T), i.e. $k_d = f(\Theta,T)$: $k_d = \left(\frac{k_1}{k_1 + \Theta}\right) * \left(\frac{T}{k_2 - T}\right) * k_3$, $k_1 = 0.112$, $k_2 = -$

41.88, and k_3 = -0.5357; for all T greater than or equal to 38ºC, T=38ºC provided a good estimate of the inactivation rate of fecal coliforms in biosolids. During subsequent field studies, treatments were employed to manage the drying cycle of biosolids—tilling increased the rate of drying, a covered solar drying bed increased the inactivation rate of fecal coliforms by 300%, and an automated rain shield was engineered to limit enteric bacterial regrowth due to rainfall.

Finally, since biosolids are to be considered a source of nitrogen when land-applied, temporal samples of biosolids from various solar drying experiments were analyzed to ascertain the levels of NH^{+}_{4}-N and NO^{-}_{3}-N throughout the drying process. Chemical analyses revealed that as much as 34-92% of nitrogen was lost via volatilization during the drying process.

CHAPTER 1. INTRODUCTION

1.1 BACKGROUND

Centralized wastewater treatment plants collect sewage from residences and pre-treated sewage from commercial and industrial sources in the United States. Sewage is comprised of suspended and dissolved organic and inorganic material containing a variety of pathogens (Cheremisinoff, 1995). The processes involved in sewage treatment include: (1) primary treatment- in which the solids are separated from the liquid by screening and gravity settling; (2) secondary treatment- commonly involving biological treatment such as activated sludge, trickling filters, and an aerobic oxidation ditch to further degrade suspended solids and reduce pathogens; and (3) tertiary treatment- involves advanced handling to further remove dissolved materials through filtration or chemical processing (Liu, 2000). These processes generate a secondary or tertiary treated effluent.

A second function of wastewater treatment plants is to stabilize the sludge or solid residuals generated from the treatment process. Measurement of sludge stability entails assessment of odor characteristics, determination of total and volatile solids, fraction of volatile solids destroyed (minimum requirement is 38%), presence of volatile fatty acids, oxygen demand, the presence of nitrate, pH values (values below 7 are indicative of instability), and the evolution of hydrogen sulfide whose presence also indicates instability, (Bruce, 1984). In general, stabilization of sludge is achieved by thickening, digestion and dewatering prior to final disposal. In specific terms, sludge stabilization can be achieved by various biological or chemical methods, including anaerobic and aerobic digestion, composting, and liming or via the addition of oxidizing agents

(Vesilind, 1979). At this point, the generated sludge still has the potential to contain a relatively high level of pathogens. These levels are dependent on the type of stabilization process utilized, see Table 1.

1.2 PROBLEM STATEMENT

In 2002, the National Science Academy reported that over 6 million dry tons of biosolids are produced annually in the United States alone. With an increasing human population, it is logical to assume that this amount will increase as well. The enormous volume produced nationally and worldwide poses a critical environmental problem to all countries. Wherever generated, biosolids must be disposed of or recycled in a manner that mitigates risk to the environment and the public at large in order to sustain sanitary health conditions.

In the 1972, the federal government enacted the Federal Water Pollution Control Act and later amended it to the Clean Water Act in 1977. The purpose of these pieces of legislation was to control water pollution levels and set wastewater standards. The Clean Water Act made it unlawful to dispose wastewater residuals into the nation's oceans, forcing municipalities to be more deliberate in their development of recycling programs for biosolids' reuse. During the early 1990's, the United States Environmental Protection Agency (US EPA) promulgated criteria classifying biosolids for their eventual reuse or disposal. The defining standards for biosolids were codified in 1993 and are included in the Code of Federal Regulations, Title 40, Part 503; the regulations are commonly referred to as "the 503 Rule". These regulations address chemical pollutant

Table 1. Pathogen reduction during sludge treatment.

	Log Reduction		
Treatment	Coliform Bacteria	Enteric Viruses	Parasites
Mesophilic anaerobic digestion	0.5 to 4	0.5 to 2	0
Aerobic Digestion	0.5 to 4	0.5 to 2	0
Composting	2 to > 4	2 to > 4	2 to > 4
Air-Drying	0.5 to 4	0.5 to >5	0.5 to > 4
Lime stabilization	2 to > 4	>4	0

Data from Godfree and Farrell, 2005

concentrations and loading rates, vector attraction requirements, and pathogenic levels for land application. Industrial pre-treatment programs to address point-of-source pollution have made the achievement of acceptable risk-based chemical pollutant concentration levels consistently attainable in the United States. However, the treatment and monitoring of biosolids in regards to achieving safe pathogen levels remains a demanding and complex challenge for the wastewater industry and many municipalities (Biocycle, 2000).

Sewage sludge harbors a large number of infectious pathogens which consist of enteric viruses, bacteria, and parasites. Examples of enteric viruses include Hepatitis A, Enteroviruses, Rotaviruses, Astroviruses, and Caliciviruses. Enteric bacteria include *Salmonella spp.*, *Shigella* spp., *Vibrio cholerae*, *Campylobacter jejuni*, and *Escherichia coli*. Parasites include protozoa- *Cryptosporidium parvum*, *Entamoeba histolytica*, *Giardia lamblia*; cestodes- *Hymenolepsis nana*, *Taenia saginata*; and nematodes- *Ascaris lumbricoides*, *Trichiuris trichiura*, etc. (Maier *et al.*, 1999 and Dumontet *et al.*, 2001). Secondary wastewater treatment typically produces Class B biosolids; characteristic levels of *Escherichia coli* and *Salmonella* spp. in wastewater and biosolids from the literature and Tucson area are listed in Table 2.

The microbial quality of biosolids is classified into two categories, Class B and Class A. Specific pathogen density level criteria for the classifications are shown in Table 3. Because of the higher pathogen density level, Class B biosolids have associated land-use restrictions to help mitigate risk to the public, animals, and the environment. Site restrictions include limits on crop harvesting, animal grazing and public access to land where Class B biosolids have been applied. Land application of Class A biosolids requires no further restrictions.

Table 2. Typical bacterial levels in sewage influent vs. levels in biosolids.

	E. coli (cfu/g)		*Salmonella*	
Country	Influent	Biosolids	Influent	Biosolids
Poland (1)	10^4 -10^8	10^2 - 10^5	10^2-10^3	n.d. - 10^2
United Kingdom (2)	10^5-10^6	3.36 log removal *	60-80/g DS	4 log removal**
Spain (3)	10^5	10^4	10^{8***}	8 log removal
United States (4)	10^7-10^{9+++}	10^6-10^{8+++}	10^2-10^4+	18 MPN/g++

Biosolids in this table refers to treated solid residuals which have been assayed after full retention time in secondary sludge digesters

(1) Mechanical-Biological Sewage Treatment Plant in Torun, Poland
n.d.= none detected. The level of *E. coli* in the influent flow of wastewater at the sewage treatment plant in Torun, Poland ranges from 10^8 to 10^4 (Paluszak *et al.*, 2003). The concentration of E. coli in the effluent after biological treatment was 10^5 to 10^2, representing log reductions between 1-4.

Paluzsek declares the main factor contributing to pathogen reduction in the sewage treatment plant in Torun to be biological treatment. However, the rate of reduction also varies seasonally. During the warmer months the survival rate of E. coli was reduced. Similarly, the greatest survival rate of *Salmonella* spp. is recorded below 10 C and at a %TS > 5 (Sobsey, M.D., 1989)

(2) United Kingdom (Horan *et al.*, 2004).
*E. coli was not spiked
**Feed sludge was spiked with *S. senftenberg* to 1.28 x 10^7 during primary sludge digestion and held for 12 days at 35 C. The sludge was then retained in a second digester for 15 days at 15 C (open to the atmosphere). A log of 2.23 occurred in the primary digester while a log reduction of 2 occurred in the second stage of digestion.

(3) WWTP at Gava-Viladecans, Spain (Moce-Llivina, L. *et al.*, 2003).
***Sludge was seeded with *Salmonella chloreraesuis* to 10^8. After temperature-time regimes of 80 C for 60 minutes and 60 C for 30 minutes, the *Salmonella* was reduced by 8 logs.

(4) United States
+Information for WWTPS in the United States. (Metcalf and Eddy, 2003).
++Number of *Salmonella* per gram of digested biosolids is 18 MPN/gram.
++ Number of *Salmonella* per gram of raw biosolids is 1800 MPN/g. (Epstein, Land Application of Sewage Sludge and Biosolids, 2003). Thermal destruction = death within 1 hour at 56C, death within 15 to 20 min at 60ºC, p. 115).
+++Metcalf & Eddy, 2003, p.111
++++Experimental data gathered from the summer of 2003 and 2004 (Choi, Pepper, and Gerba, Pathogen Reduction in Biosolids in Response to Stress Units, 2003 & 2004). 10^6 detected in Pima County WWTP, 10^8 detected from LA County biosolids trucked for 5 hours from California to Quartzsite, Arizona. MPN assays performed on treated anaerobic ally digested biosolids (Ina Road WWTP) prior to centrifugation have yielded results of 40 MPN/4g, 156 MPN/4g, 1440 MPN/4g, and 1920 MPN/4g.

To place these concentrations into perspective, the infectious doses of pathogens are- virus: 10-100; bacteria: 104- 109 depending on bacteria type (Glasmacher et al., 2003); and parasites: 1. (Maier et al., 1999 and Epstein, 1996). To reduce this pathogen level, EPA has published six (6) alternative methods to achieve Class A biosolids (40 CFR Part 503). Each alternative describes the criteria necessary to demonstrate Class A designation. The first alternative contains four (4) different time-temperature regimes. The second alternative requires raising the pH to a minimum level of 12 for a specified period of time. Alternatives 3 and 4 require testing for enteric viruses and Helminth ova in addition to enteric bacteria. Alternative 5 lists seven (7) different Processes to Further Reduce Pathogens (PFRP). These methodologies reduce pathogen to below detectable levels and thereby have further land-use restrictions associated with their application (Metcalf & Eddy, 2003).

Each alternative incorporates a particular type of heat or irradiation treatment. Alternative 6 allows "new" processes to be developed in producing Class A biosolids. The method must be equivalent to a PFRP and acceptable to the permitting authority. Wastewater treatment plants (WWTPs) utilizing any one of the six alternatives must also test for fecal coliform or *Salmonella* to verify that Class A levels have been met, see Table 2, and monitor and record test results.

The alternative utilized in the current research was Alternative 4- Sewage Sludge Treated in Unknown Processes (503.32)(a)(6). The disadvantage of this alternative is that it requires additional microbial testing for enteric viruses and helminth ova. Assays for enteric virus and helminth ova are expensive and additional time is required to ship samples, perform the assays and receive the results. In addition, the laboratory

performing the tests must be certified by the EPA or state department of environmental quality (Eljerdi, 2005).

Although the quality of biosolids produced by wastewater treatment plants across the United States appears to be improving and the onslaught of bans and ordinances restricting beneficial use seem to be abating, according to a survey published by BioCycle, 2000, only eight states utilize over 90% of their biosolids, and each municipality faces the pressures of odor control, public concern with pathogens and increasingly limited available sites for disposal.

Many small wastewater treatment plants in the United States and in developing countries utilize solar drying to dewater sewage sludge for economical transportation to landfills. Collaborative research has been accomplished with engineers and scientists in Jordan through the USAID/IALC Jordan- Biosolids' Project to perform methodical solar drying on biosolids for the purpose of reducing pathogen loads and developing protocols for use by domestic wastewater treatment plants throughout their country. This research has aided the Jordanians in taking steps to revise their national standards governing the production of Class A biosolids and will in the future assist in the development of best management practices for the handling of biosolids at WWTPs and for land application (Technical Report, RSS, 2004).

Table 3. Microbial classifications of biosolids.

Class B:	**Class A:**
Fecal Coliform Density Level: $< 2 \times 10^6$ MPN*/g total solids (dry wt.) In addition to the higher pathogen density level, Class B biosolids have associated land-use restrictions to help mitigate risk to the public and the environment. Site restrictions include limits on crop harvesting, animal grazing and public access on land where Class B biosolids have been applied	Fecal Coliform Density Level: $<10^3$ MPN/g of total solids (dry wt.) OR *Salmonella* **Density Level: < 3 MPN/4 g of total solids dry wt. AND Enteric viruses** Density Level: < 1 PFU/4 g, total solids dry wt. AND Viable Helminth ova level: < 1/4 g, total solids dry wt. These limits must be met at the time biosolids are disposed, or at the time biosolids are prepared for sale or given away in a bag or other container for application to the land.

Source: 40 CFR Part 50, United States Government Publications, 2004
*MPN refers to Most Probable Number and is a common method of estimating the number of bacteria present in water and wastewater samples
**Additional requirements necessary for Alternatives 3 and 4, 40 CFR 530.32 (a)(6)

Mexico is also in need of developing standard methods to convert their biosolids to safe reusable material. Defining a specific protocol using solar drying to ensure conversion of Class B to Class A biosolids is a cost effective method of pathogen reduction. Integrating engineering methods into the solar drying cycle can enhance dewatering and the management of solids handling to help the wastewater industry safely recycle biosolids onto agricultural fields or onto sites needing remediation such as hillside erosion or mine tailings. Integrating tilling to help enhance the desiccation rate of the biosolids may prove promising in reducing parasites in biosolids which have a high influent concentration and gravely affect public health (Dominguez-Sanchez, 2005).

1.3 LITERATURE REVIEW

1.3.1 BIOSOLIDS

Sewage sludge is the semi solid, solid, or liquid residue generated during treatment of domestic wastewaters (National Research Council, 2002). Biosolids is the term that describes sewage sludge that is treated to Class B or Class A levels as established by EPA, Table-2. Aerobically-digested biosolids refers to the treatment of waste-activated sludge using aerated sludge retention tanks. Anaerobically-digested biosolids refers to biosolids that are stabilized in the absence of molecular oxygen (Metcalf and Eddy, 2004). Anionic polymers were added to the biosolids produced at the Green Valley and Ina Road WWTP, studied here, to aid in flocculation and dewatering of the biosolids.

1.3.2 BIOTIC FACTORS AND PATHOGEN INACTIVATION IN BIOSOLIDS

Biosolids contain a large collection of indigenous living microorganisms (biotic component) which are interconnected with one another in terms of habitat, food web, and ecological relationships.

Significant biotic factors in waste-activated sludge include denitrifying bacteria, filamentous organisms, floc-forming bacteria, nitrifying bacteria and protozoa. Significant biotic factors in anaerobically-digested biosolids include acetogenic, fermentative, hydrolytic, methane-forming, and sulfur reducing bacteria (Gerardi, 2006).

Microorganisms have their own habitats or physical location within the biosolids and their specific role or niche. For example, aerobic bacteria will characteristically be found at the perimeter of a floc particle where the concentration of oxygen is highest.

Competition for nutrients and niches becomes a critical factor to the survival of organisms within the biosolids.

Likewise, predator-prey relationships exist among microorganisms in biosolids, the most notable is the protozoa-bacteria relationship. There are limited studies that demonstrate the significance of predator-prey and competition relationships between indigenous microflora in biosolids (alone). Information in Appendix E was the beginning of a short study to demonstrate the significance of biotic factors at a relatively high average daily temperature of 38ºC.

1.3.3 ABIOTIC STRESS UNITS AFFECTING PATHOGEN INACTIVATION IN BIOSOLIDS

There are a number of categorical abiotic stressors that impact pathogen survivability. These include physical stressors such as heat, desiccation, and cavitation as well as chemical stressors such as compounds producing exothermic energy or inducing oxidation and reduction reactions (Reimers, 2005).

Numerous physical stressors are identified in the literature as environmental parameters affecting the survivability of pathogens and pathogen indicators in soil and in sewage sludge mixed with soil. The primary factors are moisture, temperature, and competing soil organisms (Trevisan, *et. al*., 2001, Pietronave, 2002, Redlinger, 2001, Straub, 1992). The desiccation rate of biosolids has also been cited as a factor affecting pathogen survivability (Ward *et al.*, 1981 and Zaleski *et al.*, 2005). Other factors that affect pathogen survival are the degree of virus adsorption to soil, soil levels of resin-extractable phosphorus, soil pH, and exchangeable aluminum.

Many microorganisms survive better when they are associated with solids than when they are suspended in water. In wetlands, sediments prolong the survival of bacteria (Karim *et al.*, 2004). Raw sludge was found to be protective of poliovirus while stabilized sludge had variable effects on the survival of virus (Ward *et al.*, 1976). Enteroviruses are efficiently retained by the sludge-soil matrix and their association with sludge solids plays a significant role in the restriction of their movement through soils (Bitton *et al.*, 1984). Because the 503 Rule addresses the three categories of pathogen level in its criterion for Class A biosolids, the following sections provide specific citation to stressors impacting their inactivation.

1.3.3.1 VIRAL INACTIVATION

In 1979, Yeager and O'Brien showed that viruses survived longer in septic liquor than in ground water or surface water. Studies by Hurst et al. (1980) demonstrated that the main stressors affecting virus inactivation were soil moisture, temperature, presence of aerobic microorganisms, degree of virus adsorption to soil, soil levels of resin-extractable phosphorus, soil pH, exchangeable aluminum, the rate of desiccation, and soil type to a lesser extent.

According to Bitton *et al*. (1984) viruses have an affinity for aerobic sludge and embed in the sludge flocs or adsorb onto the floc surfaces. Embedding can aid in virus retention during sludge application to land. Virus survival in sludge-amended soil is mainly affected by desiccation and soil temperature. Bitton *et al.* (1984) showed that virus decline more rapidly during warm and dry seasons than in warm and wet seasons. Straub *et al.* (1992) studied the persistence of polio virus type I, and bacteriophages –MS-2 and PRD-1, in desert soils amended with anaerobically-digested sewage sludge. In a

controlled study, they determined that the key factors involved in viral reduction were moisture, temperature, rate of desiccation, and to a lesser degree, soil type. When the moisture levels were less than 5%, viruses were inactivated within 7, 3, and 2 days respectively, at temperatures of 15°C, 27°C and 40°C regardless of soil type. In laboratory studies, viruses in stabilized sewer sludge were inactivated quicker than viruses in raw sludge at temperatures of 45ºC, however at temperatures around 60°C, the inactivation rate in both types of sludges is similar. Full inactivation takes place in 20 minutes at this higher temperature (Dumontet *et al.*, 2001).

1.3.3.2 BACTERIAL INACTIVATION

Bacterial studies include field investigations performed by Redlinger *et al.*(2001) where fecal coliform reduction was analyzed over time in prefabricated, dry-composting toilets installed along the U.S.-Mexico border. Their findings showed that reduction occurred due to desiccation rather than other processes. There was a significant correlation between classification rating and moisture; drier samples had a greater proportion of Class A material. Solar exposure was also critical for achieving maximum Class A biosolids end-product. Pepper *et al.* (1993) amended soil with anaerobically-digested sludge, 4-6% total solids, in both laboratory and field study settings. The laboratory experiments demonstrated that indicator organisms, fecal coliforms, decreased over time, while the rate of reduction increased with increasing temperature. Trevisan *et al.* (2002) investigated the survival and leaching of fecal bacteria after spreading slurry on mountain hay meadows. The conclusions of these studies show that the major factors impacting bacterial reduction were the frequency of dry days, the depth in soil, the height of plant canopy, the amount of biomass, microbial competition, and soil type (which

affected percolation rate/leaching). Bacteria in the soil are affected by soil structure, texture and soil moisture level, and predation by protozoa.

As mentioned previously, temperature is also a key factor in bacterial reduction. According to Yeager and Ward, thermal destruction of bacteria occurs within 1 hour at 56ºC, and within 15 to 20 minutes at 60°C. In laboratory studies by Hong *et al.* (2004) the effects of microwave radiation were compared to the application of external heat on the level of fecal coliforms in sludge. External heating allowed more growth in fecal coliforms until a temperature range of 48°C to 57°C was reached. After this point, cellular membrane destruction of fecal coliforms occurred causing a rapid decrease in their levels. Fecal coliforms were not detected in waste activated or anaerobically-digested sludge after heating to 65ºC. Microwave irradiation disrupted DNA in fecal coliform cells at lower temperatures than external heating.

1.3.3.3 PARASITES: HELMINTH OVA

Parasites are the most resistant of enteric pathogens in sewer sludge (Yanko, 1988) and require temperatures near 51ºC for die-off (Farrell, 1979) and prevention of embrionization of *Ascaris lumbricoides* eggs (Dumontet *et al.*, 2001) or alkaline treatments of high pH and varying time regimes (Pecson and Nelson, 2005). Sedimentation is the primary means of inactivation for helminth ova in biosolids in lagoon pond treatments. However, chemical treatments in laboratory studies have been successful in reducing helminth ova (Jiminez, *et al.*, 2000). Once inactivated, virus and helminth ova do not become viable again. Influent levels of helminth ova in the Tucson metropolitan wastewater system are null to very low (Gundy, 2005). Helminth ova

assays were performed at the beginning and the end of the experimental process for all control beds in the solar drying experiments in accordance with EPA/625/R-92/013.

1.3.4 BACTERIAL REGROWTH AND RECOLONIZATION

It is important to include a discussion of bacterial regrowth in studies involving solar drying of sludge, as such information can be a method of determining whether the Class A designation is permanent or not. In the latter case, more stress units (heat or chemicals) may need to be applied. Bacterial regrowth, especially in the case of *Salmonella*, is a concern for public owned wastewater treatment works (POTWs) storing biosolids onsite and for contractors land applying the material. According to a review and laboratory experiments by Zaleski *et al.* (2005) survival, growth, and regrowth of enteric indicators and pathogenic bacteria in biosolids are dependent on favorable conditions of moisture, temperature, substrate availability, and competition and predation from indigenous microorganisms. Although regrowth in composted biosolids is well documented, reactivation of enteric bacteria in biosolids-amended-soil is minimal. Similar findings were also reported by Gibbs *et al.* (1997).

1.3.5 EFFECTS OF RAINFALL

It is well documented that fecal coliform regrowth in open solar drying beds occurs after exposure to rainfall (Trevisan *et al.*, 2001; Gibbs *et al.*, 1997; Hay, 1996; Soares and Cardenas, 1995) . Regrowth numbers can climb beyond influent values after the first rainfall event. Monsoon rains (precipitation generally happening from June through

August with a sustained dew point temperature of 55ºF) occurring during the summer months in arid and semi-arid locations like Arizona can cause Class A material to "fall out" of compliance with the required microbial criterion. Treatment, retesting, and rescheduling for hauling of biosolids can be expensive and cumbersome.

1.3.6 USE OF FECAL COLIFORM TO PREDICT PATHOGEN INACTIVATION IN BIOSOLIDS

Coliforms are non-spore forming, facultatively anaerobic, gram-negative rods, which ferment lactose to acid and gas within 48 hours at 35ºC. Fecal coliforms are naturally found in the intestines of warm blooded animals. They are differentiated from non-fecal coliforms by incubating in broth or selective media at 44.5ºC (Madigan, 2003).

Fecal coliforms are recognized as indicator microorganisms whose presence point toward the existence of pathogenic microorganisms. The US EPA uses bacterial indicators as standards for water quality and sewage sludge. The criteria for ideal indicator microorganisms are (1) the organism should be present whenever enteric pathogens are present; (2) the organisms should have a reasonably longer survival time than the hardiest enteric pathogen; (3) the organism should not grow in water; (4) the organism must be easily isolated and quantified by simple assay methods; (5) the density of the indicator should have some direct relations to the degree of fecal pollution, and (6) the organism should be a member of intestinal microflora of warm-blooded animals (Maier *et al.*, 1999). Die-off patterns of both fecal coliforms and *Salmonella* were monitored during the open solar drying field experiments. However, influent *Salmonella* levels in the majority of the field experiments were very low (< 5.0 MPN/4 g dry wt.) and

die-off was quick (within 7 to 14 days). Therefore, analysis of inactivation rates and the prediction modeling was focused mainly on fecal coliforms.

1.4 PROJECT OBJECTIVES

There is an immediate niche for solar drying to be utilized as a method of reducing pathogen loads in biosolids in many small wastewater treatment plants in the United States and in developing countries that have established "dewatering" infrastructure. Although, it is currently common practice for sewage sludge to be dewatered in sand drying beds for economical transportation to landfills, minimal drying is being conducted for the purpose of pathogen reduction. In Arizona and much of the United States, the majority of treated sewer sludge is recycled as Class B biosolids and land-applied (Fondahl, 1999). However, the national trend is progressing towards exclusive acceptance of Class A quality biosolids for land application. Pre-treatment programs implemented by publicly-owned wastewater treatment works (POTWs) successfully abate heavy metal concentrations, but the conversion from Class B to Class A microbial levels can be an expensive process involving large dollar investments for capital equipment and high energy demands. Current typical methods for producing Class A biosolids require intensive energy use, such equipment includes electric dryers, heat exchangers, thermophilic heating, composting, liming, and irradiation (Chen *et al.*, 2002). Solar drying of sludge can be developed as an economical method for producing Class A biosolids for the small wastewater treatment plants in the United States.

In addition, in the near future, many more developing countries will have to address the handling, treating and recycling of their biosolids as they continue to transition from waste

stabilization ponds to mechanical treatment of wastewater (e.g. Mexico, Egypt, Jordan, etc.). In these countries, biosolids can be an important and affordable resource for agricultural fertilizer and used to enhance nutrient poor soils. What is critical is that the final product be safe for land application and home-garden use and yet contain micro-and macro-nutrients that support crop growth and enhance soil quality.

Integrating engineering methods into the solar drying cycle such as tilling and enhanced passive solar heating, can improve the rate of dewatering and pathogen reduction in biosolids. This will have the effect of increasing drying bed capacity at existing WWTPs, where drying time and bed capacity have become an issue. This is the case at the Wadi Mousa WWTP, in Jordan and at the Green Valley Wastewater treatment plant in Arizona.

Experimental solar drying beds were set-up in semi-arid locations in Arizona to study pathogen inactivation for the purpose of developing an economically feasible method of converting Class B biosolids to Class A material while addressing the aforementioned issues pertaining to small wastewater treatment plants and to the challenges faced by developing countries. The overall goal of this project can be separated into the following objectives and their justifications:

1. Establishment of a web-based monitoring and data collection system at the solar drying site.

   Justification: Wastewater treatment plants and open solar drying fields (e.g. Quartzsite, Arizona) are typically located in remote locations. By establishing a web-based remote data collection and data communication system, onsite monitoring of the environmental parameters critical to pathogen inactivation during solar drying can be measured in a cost-effective manner.

2. Determine the efficacy of solar drying on aerobically and anaerobically-digested biosolids and sand drying beds versus imperviously lined beds with drainage in a semi-arid region.

   Justification: Solar drying has proven to be an effective method of converting Class B to Class A biosolids in an arid region (Choi *et al.*, 2005) and to some extent in a semi-arid region (Zaleski *et al.*, 2005). The question is "Can solar drying consistently be an effective method of pathogen load reduction in a semi-arid region utilizing sand drying beds for the two different types of biosolids, aerobically and anaerobically-digested?" Also, "How much inactivation occurs during cooler and wetter periods?"

3. Develop a mathematical model to predict pathogen density levels during the conversion process from Class B to Class A biosolids.

   Justification: Microbiological testing for verification of meeting Class A criteria can be relatively expensive and biosolids must be retested if the levels are not in compliance with Class A criteria. If a mathematical model can be developed to predict pathogen levels in biosolids, WWTP operators can be relatively certain of the proper sampling time for laboratory verification of Class A criteria. Future studies may allow for the development of an approved PFRP that eliminates microbial testing altogether.

4. Apply tilling to improve the desiccation rate of biosolids, a covered structure to increase heat stress units, and engineer an automated cover to shield drying biosolids from the deleterious effects of rainfall.

Justification: The effectiveness of solar drying of biosolids in reducing pathogen loads is related to a number of factors, one important factor is the depth of the biosolids in the drying beds. The typical depth of placement is between 15 and 20 cm. Therefore, solar drying requires a relatively large investment in land for drying biosolids, and in some cases land or the funds to increase drying bed capacity are limited. The incorporation of tilling treatments will increase the desiccation rate of biosolids but the effect of tilling on fecal coliform inactivation in a semi-arid location is relatively unknown. Covering a drying bed with material that allows for transmission of light energy but traps infra-red wavelengths has the ability to increase the temperature of the biosolids. However, unless the humid air is pulled out of the structure, the biosolids will not readily dry. The question of interest is whether high enough temperatures can be reached inside the drying bed to inactivate the fecal coliforms at an accelerated rate. Finally, rainfall can interfere with the conversion process and therefore developing a shield that automatically covers the biosolids as soon as it rains but is otherwise open to gain the full advantage of solar radiation and the convective heat drying effects of the wind may prove useful in efficiently managing the conversion process.

5. Quantitatively determine the degree of ammonia gas volatilization from biosolids during the open solar drying process.

Justification: Biosolids are an important agro-based product because of their N content. However, ammonia volatilization is known to occur based on studies performed on manures and biosolids that have been land applied. It is therefore prudent to determine how solar drying affects ammonia volatilization.

1.5 DISSERTATION FORMAT

This dissertation is presented using the Agricultural and Biosystems Engineering Departmental paper-option. In place of chapters submitted, manuscripts describe self-contained portions of this work.

1.5.1 PROPOSED PAPER CONTRIBUTION 1: APPENDIX A: REDUCTION OF PATHOGENS IN BIOSOLIDS IN OPEN SOLAR DRYING BEDS IN A SEMI-ARID CLIMATE – BASELINE STUDIES

This study embraced the first two objectives of the proposal. Seasonal solar drying experiments were performed at two different wastewater treatment plants within Pima County, Arizona. A remote weather station and data collection system was sited near the drying beds and continuous monitoring of the environmental parameters such as solar radiation, relative humidity, wind speed, air temperature, and the temperature and moisture content of the biosolids were measured. These measurements aided in calculating the physical stress units of temperature and moisture of the biosolids during the conversion process from Class B to Class A.

Specifically this Appendix addresses:

- The inactivation rates of fecal coliform and *Salmonella* in aerobically and anaerobically-digested biosolids as a function of time and moisture content.
- The use of sand lined drying beds versus impervious (concrete) lined drying beds on the inactivation rate of fecal coliform and *Salmonella*.
- The fate of fecal coliform and *Salmonella* in open solar drying beds during warm dry seasons in comparison to cool wet seasons.

- The general effectiveness of solar drying in reducing pathogen loads in a semi-arid region.

1.5.2 PROPOSED PAPER CONTRIBUTION 2: APPENDIX B. A MATHEMATICAL MODEL TO PREDICT PATHOGEN DIE-OFF IN BIOSOLIDS BASED ON MOISTURE AND TEMPERATURE STRESS UNITS

This appendix addresses the third objective of the project which is to develop a mathematical model that predicts the pathogen level in the biosolids during the conversion process from Class B to Class A.

Specifically this appendix:

- Quantifies the level of significance that the main physical stressors- moisture, temperature and the combination of the two- have on the inactivation rate of fecal coliform.
- Provides a model that predicts the rate of inactivation of fecal coliforms in biosolids during solar drying based on the average moisture and temperature of the biosolids through non-linear regression, using the Gauss Newton approach.
- Provides verification that moisture has a major role in the inactivation of fecal coliform in biosolids in the semi-arid region of Green Valley, Arizona.

1.5.3 PROPOSED PAPER CONTRIBUTION 3- APPENDIX C: MANAGING THE BIOSOLIDS' CONVERSION PROCESS FROM CLASS B TO CLASS A USING SOLAR DRYING TECHNOLOGIES

This appendix addresses objectives #1 and #4. The purpose of this contribution was to integrate engineering techniques- tilling, a covered structure to increase the heat gain of the biosolids, and an automatic retractable roof that shields a solar drying bed from rainfall in order to prevent regrowth of pathogens.

Specifically, this appendix:

- Analyzes the impact that tilling has on the inactivation rate of fecal coliforms in a semi-arid region.
- Demonstrates that above a minimum threshold value, as temperature increases in the biosolids, it governs the inactivation rate of fecal coliforms regardless of the moisture content of the biosolids.

Results confirm that preventing rainfall from wetting biosolids in drying beds helps to maintain Class A levels.

1.5.4 PROPOSED PAPER CONTRIBUTION 4- APPENDIX D- VOLATILIZATION OF AMMONIA IN OPEN SOLAR DRYING BEDS

This appendix addresses the last objective. The intent of this contribution is to analyze the N content of the solar dried Class A biosolids during the drying process to ascertain how solar drying affects the quality of biosolids for use as an agricultural fertilizer.

The findings in this appendix discuss the effect of solar drying on the nutrient content of biosolids. More specifically this appendix describes:

- The percent loss of N from biosolids during the solar drying process.
- The mechanism for the loss of N.
- The effects of tilling on N and water loss
- Provides examples of the monetary loss of the organic N in relation to the synthetic N that would have to be applied to provide adequate fertilization to crops.

- Recommends changes to the design of solar drying beds in order to recapture volatized ammonia while achieve Class A fertilizer.

1.5.5 APPENDIX E: SHORT STUDY BIOTIC EFFECTS ON PATHOGEN REDUCTION IN BIOSOLIDS

This section details a short laboratory experiment that was run to investigate the biotic effects on the inactivation rate of fecal coliforms in biosolids.

CHAPTER 2. PRESENT STUDY

2.1 REDUCTION OF PATHOGENS IN BIOSOLIDS IN OPEN SOLAR DRYING BEDS IN A SEMI-ARID CLIMATE – BASELINE STUDIES

The primary goal of this study was to investigate the impacts of different physical variables on pathogen reduction in biosolids when utilizing solar drying methods. These variables included the use of two different types of biosolids, aerobically-digested and anaerobically-digested and two different types of drying beds- concrete lined and sand lined beds. The solar drying experiments were carried out in semi-arid locations within Pima County, Arizona. Semi-arid locations typically receive low annual rainfall of 25 – 50 cm. Pima County experiences two distinct seasonal rainfall events, the summer monsoon season (normally begins in early July, after 3 days of dew point temperature at 55ºF) and the winter rains occurring in November to late January. The interest in performing solar drying of biosolids in semi-arid regions was the fact that similar climates can be found in parts of Jordan and Mexico where collaborative efforts regarding pathogen reduction in biosolids between these two countries and the University of Arizona (funded by the Water Quality Center, USAID/IALC- Drylands project and the TIES, Training Internship, Exchanges and Scholarship program) were planned. Jordan and Mexico have limited monetary resources to support the safe generation and management of biosolids. Therefore, use of solar drying without treatments was first studied to learn the effects with minimal intervention or investment in capital equipment. These baselines studies provided the temperature and moisture ranges to be investigated under controlled conditions and helped to develop boundary conditions in determining the coefficients for the non-linear regression model to predict pathogen inactivation.

2.2 MATHEMATICAL MODEL TO PREDICT PATHOGEN DIE-OFF IN BIOSOLIDS BASED ON MOISTURE AND TEMPERATURE STRESS UNITS

The primary objective of this study was to isolate the two main stressors that impact pathogen survivability- temperature and moisture. By isolating these factors, it was possible to quantify their impact on pathogen die-off in solar drying beds. The controlled study aided in the elimination of complexities and interactions between other environmental parameters that also impact pathogen inactivation. These parameters include solar radiation, wind speed, vapor pressure deficit, and introduction of bacteria by birds.

2.3 MANAGING THE BIOSOLIDS' CONVERSION PROCESS FROM CLASS B TO CLASS A USING SOLAR DRYING TECHNOLOGIES

The goal of this study was to incorporate treatments into the solar drying experiments to improve the biosolids drying cycle by increasing the rate of pathogen inactivation and preventing regrowth of pathogens. These two objectives were investigated by using tilling treatments to increase the rate of desiccation and a covered roof to increase the average daily heat units of the biosolids. An automatic cover was engineered to shield the biosolids from rewetting due to rainfall. Uncomplicated technologies (tilling with a light weight gas tiller and utilizing polyethylene on a simple poly vinyl chloride frame for the covered structure) were employed to maintain the sustainability of the converting the biosolids to Class A material and also to allow for technology transfer to developing countries where monetary resources are limited but human labor is readily available.

2.4 VOLATILIZATION OF AMMONIA IN OPEN SOLAR DRYING BEDS

The goal of this study was to investigate the effects of solar drying and treatments incorporated into solar drying on N loss. This investigation is important because the major benefit of biosolids application onto agricultural fields is the delivery of N at a reduced cost as compared to synthetic fertilizers. Alternative drying bed designs can be developed to scrub the ammonia and recover the N while continuing to allow the conversion process from Class B to Class A biosolids to be achieved.

2.5 OUTDOOR EXPERIMENTAL SETUP

The remote weather station, data collection system and in-situ sensors were the same for each outdoor experimental location.

2.5.1 METROLOGICAL MEASUREMENTS: REAL-TIME DATA ACQUISITION

The development of a stress unit concept is key to quantifying and predicting the reduction and inactivation of microorganisms in biosolids for conversion of Class B to Class A and eventual land use application. The information on stress units encountered in the field will be provided by heat and moisture sensors used in the biosolids' drying beds to aid in the development of a predictive model for pathogen inactivation. Remote monitoring is beneficial in the case of outlying treatment plants in the United States and other countries. Sampling of biosolids can be estimated from the real time data thereby saving time and money in travel costs. Remote monitoring can also aid WWTP operators in planning the release of Class A biosolids to the applicator.

2.5.2 CALIBRATION AND INSTALLATION OF SENSORS

Thermocouples were made by stripping the ends of type T, 20-gauge thermocouple wire, copper and constantan, hand twisting and soldering the end with a carbon tip

welder. Each thermocouple wire was calibrated in ice water and the offset value was placed into the CR10x datalogger® (Campbell Scientific, 2002) program.

Remotely measuring the moisture content of biosolids in the drying beds as well as solar radiation, wind, air temperature and relative humidity enables remote qualitative and quantitative moisture monitoring during the drying process.

The water content reflectometry probes function similarly to TDR systems. Both systems are typically used to measure the volumetric moisture content of soil. TDR probe readings measure the "apparent" dielectric constant (K_a) of a material. K_a can be thought of as the level of conductivity of a medium. The greater the moisture content of a medium, the greater its K_a value. Salinity also increases the K_a of a medium and is part of the calibration factor. The salinity of aerobically-digested biosolids from the Avra Valley WWTP was on the average 6.8 mS/cm. Moisture content reflectometry probes were utilized for qualitative drying analysis of the biosolids in the drying beds. Variation in the bulk density of the biosolids during the solar drying process from 0.94 g/cm3 (influent biosolids of ~ 8%TS) to 0.064 g/cm3 (dried biosolids of ~95%TS), and random cracking prevented reliable calibration necessary for quantitative measurements. See Figure- 1 for the probe stand utilized to fix the location of the thermocouples and moisture probe in the drying beds.

2.5.3 WEB-BASED REMOTE DATA ACQUISITION SYSTEM

The goal for this portion of the project is to be able to remotely monitor the environmental factors significant for pathogen inactivation in the drying beds. These parameters included: ambient temperature, solar radiation, wind speed, relative humidity, and

temperature and moisture content of the drying beds. A remote weather station and data acquisition unit was tested at the University of Arizona, and sited at the location of the experimental drying beds, Figure-2. Remote data access was possible via a combination of Radio Frequency (RF) telemetry at the Avra Valley and Green Valley sites and telephone communication to the University of Arizona. The data was retrieved from the remote datalogger and stored on a base computer. A managed file was then uploaded to a server for posting on a website, Figure -3.

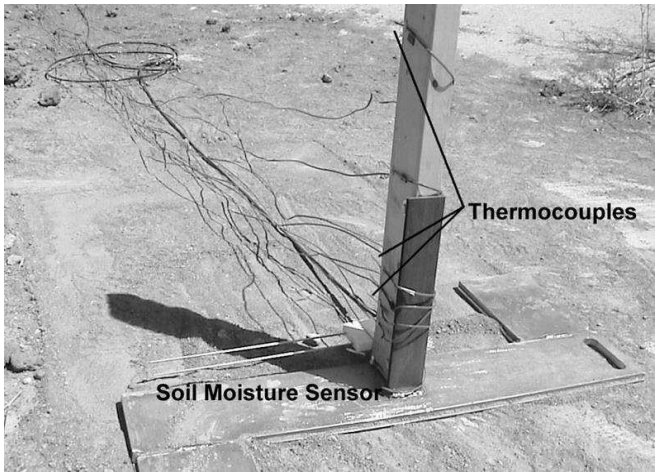

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Figure -1. Water content reflectometry probe and thermocouple wires.

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Figure -2. Remote weather station and data acquisition system

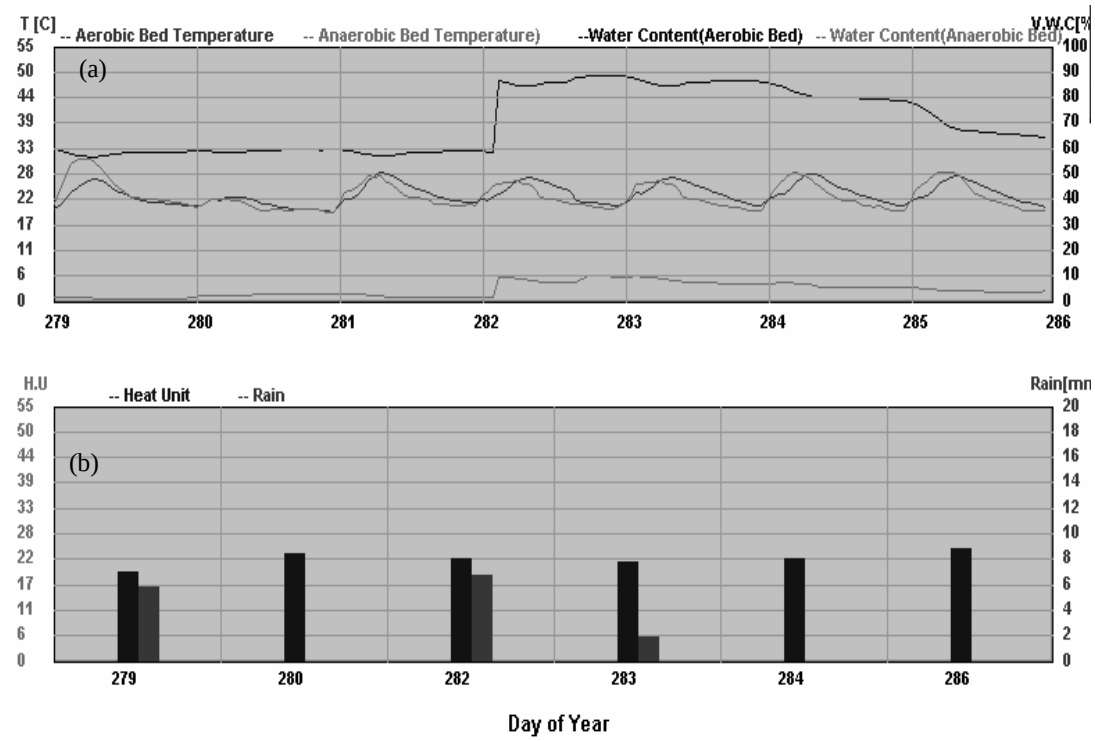

Figure-3. Data posted on the website: (a) Real-time data indicating temperature and moisture content of biosolids' beds; (b) Posts daily rainfall and heat units for a 7 day period of time.

2.5.4 ENVIRONMENTAL SAMPLING AND MICROBIAL ASSAYS

Environmental sampling of the biosolids was performed in accordance with Publication PB93-227957, POTW Sludge Sampling and Analysis Guidance Document US EPA-Office of Water Office of Wastewater Enforcement & Compliance, August 1989. At the Avra Valley WWTP, two composite samples were taken from each bed, one at the north end and the other at the south end. Four grab samples from each north and south location were taken at a radial distance of at least 0.5 meters from the position of the moisture probes located at either end of each experimental bed.

At the Green Valley WWTP, each experimental container was divided into quarters; grab samples of approximately 25 g were taken from the center of each quarter and combined to form a composite sample of the total bed. Approximately 3 cm of the upper crusted portion of the biosolids was removed prior to retrieving each grab sample. Removal of the top layer was performed to reduce the effects of solar radiation on pathogen die-off, which is considered to be a surface phenomenon. This allowed worse case sampling throughout the experiments. Each sample then included the entire depth of the remaining biosolids (to the top of the sand layer). For each experimental bed, four grab samples were collected to make one composite sample. A clean hand trowel was used to retrieve the grab samples and each set of grab samples was placed in its own sterile container and mixed for homogeneity. The composite sample was then stored in a sterile Nalgene® bottle or plastic bag and transported on ice to the University of Arizona campus. The analysis for percent total solids and the microbial assays for fecal coliforms and *Salmonella* were performed within 6 hours of sampling from the field.

The Most Probable Number test (MPN) 9221 B, Standard Total Coliform Fermentation Technique is the typical method of practice for assaying indicator organisms in sewer sludge (American Public Health Association, 1998). This method was utilized during the present study. A modified version of EPA Method 1682: Salmonella in Biosolids by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium, Draft Document 2003 was utilized to determine the Salmonella density levels of the biosolids in each container. Helminth ova assays were conducted in accordance with EPA/625/R-92/013: Environmental Regulations and Technology, Control of Pathogens and Vector Attraction in Sewage Sludge. Protocols for each assay method are located in Appendix F.

CHAPTER 3. CONCLUSIONS

Solar drying is an economically feasible method of converting Class B to Class A biosolids. Integrating engineering methods into the solar drying cycle can enhance dewatering and disinfection of the sewer sludge for the purpose of safely recycling the biosolids onto agricultural fields or land application onto sites needing remediation such as mine tailings or burned forest land. Class B biosolids were converted to Class A criteria using open solar drying beds with no intervention in time frames varying from 21 to 35 days. Experimental treatments such as a covered structure to intensify the cumulative heat units increased the inactivation rate during warm periods by as much as 36%. This time savings is important in cases were drying bed space is limited and solar drying is the only method of disinfection.

While tilling did not significantly increase the pathogen indicator inactivation in the field experiments, it did significantly increase the %TS of the biosolids in the drying beds. Further studies need to be conducted to investigate whether the interaction of tilling and relatively high temperatures impact pathogen indicator inactivation. Although tilling can be of benefit in managing the biosolids drying cycle, chemical analysis of the biosolids revealed that as much as 34-92% of nitrogen was lost via volatilization during the drying process. This reduction of nitrogen represents a resource loss of fertilizer and energy. However, these issues may be mitigated by altering current bed designs to include a covered unit that allows for scrubbing of captured air to collect ammonia and increase the average daily heat units, affecting pathogens in the biosolids.

The seasonal field studies suggest that best management practices associated with the drying and disinfection of biosolids is to provide drying beds with a large pervious surface area for drainage (e.g. a sand bed) and an enclosed overhead structure with a retractable roof. The roof should be kept closed during the first 28 days of drying and the air scrubbed for ammonia gas to allow for the maximum recapture of N and water as a resource for fertilizer and moisture when land applying the biosolids. Afterwards the roof can be left open to the air, especially if condensation is an issue. The automated shield can be positioned over the bed during rainfall events. The biosolids should be tilled uniformly throughout the year, however, the biosolids should not be considered disinfected if the average temperature of the biosolids is less than 25ºC.

The results from the solar drying field experiments demonstrated that integration of a covered structure or an automated rain shield into the drying cycle mitigates the regrowth of indicator pathogens in biosolids, tilling treatments enhance the desiccation rate of biosolids and yield a safe and economical means for conversion to Class A material.

CHAPTER 4. EXECUTIVE SUMMARY

Pathogen reduction of solid residuals produced by wastewater treatment is a critical environmental issue for the wastewater industry and municipalities. The safe production of a Class B or Class A biosolids is necessary to minimize the health risks for wastewater treatment operators and applicators who are likely to come in contact with the material, and minimize potential infection of the public and farmers who may be exposed to biosolids based-fertilizers and land-applied products.

The author of this dissertation was involved in the experimental set up and the microbiological assays for solar drying experiments conducted in Quartzsite, Arizona (an arid region), Avra Valley and Green Valley Wastewater Treatment Plants (semi-arid regions), Wadi Hassan, Jordan (a semi-arid region) and Cuernavaca, Mexico (a semi-tropical area). In each case the layout at the experimental site was similar--equivalent electronic sensors and remote data collection systems; the environmental sampling protocol was the same, and the microbiological assays using EPA standards were performed. During the studies, the inactivation patterns of fecal coliform, *Salmonella* spp., and Helminth ova, in the case of Mexico, was performed. The scope of this dissertation is limited to the studies performed at Avra Valley and Green Valley WWTPs.

These studies demonstrated that solar drying of biosolids reduces pathogens to relatively safe levels (Class A criteria) during warm dry periods when biosolids are exposed to a minimum average ambient air temperature of approximately 25ºC for approximately 21 days or more. Drying biosolids to > 90%TS is an excellent method of assuring Class A bacterial quality biosolids have been achieved.

Of key importance is the statistical analysis that demonstrated the physical stressors of temperature and moisture significantly impact fecal coliform inactivation rates. Of equal importance is the short study that demonstrated that there are biotic factors (competition and predation) that also significantly impact fecal coliform inactivation rates in biosolids at different moisture contents.

Furthermore, these studies describe management techniques that can benefit the drying and treatment cycle of biosolids when converting it from a Class B to a Class A material. These practices include tilling, intensifying passive solar heating and preventing the biosolids from rainfall after Class A is achieved.

Tilling increases the rate of desiccation of biosolids and allows for greater exposure of pathogens to inactivation by solar radiation. However, tilling does not increase the inactivation rate of fecal coliform or *Salmonella* when solar drying in a semi-arid climate.

Covering a solar drying bed with a greenhouse-like cover will intensify the amount of heat stress units impacting pathogens in biosolids to and rendering their numbers to below detection levels. This treatment was the most effective method of producing a "permanent" Class A material.

Studies described within this dissertation are important as they demonstrate: (1) solar drying biosolids in a semi-arid region, during warm dry periods, is an effective method of reducing pathogens; (2) the main physical stressors impacting enteric bacterial pathogens are temperature and moisture; (3) the inactivation constant, k_d, for fecal coliforms (pathogen indicator), can be predicated using measurable physical parameters; (4) permanent Class A material can be achieved using a covered structure to increase the average temperature of the biosolids; (5) it is possible and prudent to prevent bacterial

regrowth in biosolids from rainfall events using an automated cover; and finally (6) solar drying of biosolids does reduce the N (nitrogen) content of biosolids and is heavily dependent on the evaporation of moisture from the biosolids.

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APPENDICES

APPENDIX A: REDUCTION OF PATHOGENS IN BIOSOLIDS IN OPEN SOLAR DRYING BEDS IN A SEMI-ARID CLIMATE – BASELINE STUDIES

ABSTRACT

This study investigated the effects of solar drying on bacteria reduction in biosolids in a semi-arid region during extreme seasons, using two different types of sludge (aerobically and anaerobically-digested) and two different types of drying beds, i.e. sand and impervious lined beds during summer and winter seasons. Average fecal coliform inactivation rates for aerobically and anaerobically-digested biosolids for the summer experiments were determined to be $0.17 \pm 0.03/day^{-1}$ and $0.17 \pm 0.04/day^{-1}$, respectively. An ANOVA indicated that neither the type of biosolids ($p = 0.63$) nor the type of drainage ($p = 0.67$) had a significant impact on the inactivation rate and there was no significant interaction between these two factors on fecal coliform inactivation. *Salmonella* inactivation rates in aerobically digested biosolids were 0.11 ± 0.08 day^{-1} and 2.0 ± 2.0 day^{-1} for aerobically and anaerobically digested biosolids, respectively for the summer seasons. Due to low temperatures and rainfall, winter solar drying proved ineffective in achieving Class A biosolids.

This research demonstrated that solar drying is an effective means for reducing pathogens in biosolids and sand drying beds are beneficial in maintaining a Class A status during the summer drying season. Experimental land application of the solar-dried Class A biosolids demonstrated minimal regrowth of *Salmonella spp*. Regrowth of fecal coliforms did occur but was dependent on soil moisture content.

Keywords. Biosolids, fecal coliforms, heat units, sewer sludge, *Salmonella*, solar drying beds

1. INTRODUCTION

Solar energy has been utilized as a simple and reliable method to pasteurize water in developing countries (Burch and Thomas, 1998 and Joyce *et al.*, 1996). Moist heat treatments of *Cryptosporidium* to temperatures between 45ºC and 65ºC for a time period between 10 to 15 minutes rendered inocula non-infective (Anderson, 1985). Time-temperature regimes are also utilized in reducing pathogens in sludge (40 CFR Part 503, 1993).

Secondary treated sludge produced from municipal wastewater treatment plants during the stabilization process does reduce the density level of indigenous pathogens in sewage, however the biosolids produced to this point are typically reduced to only Class B levels (fecal coliform levels < 2 x 10^6 MPN /g dry weight) and if land-applied, specific restrictions which include limits on crop harvesting, animal grazing and public access must be applied. However, Class B biosolids can be converted into a higher-quality product, Class A biosolids, which are generally regarded as safe for immediate use as a renewable resource fertilizer when applied to lawns, home gardens, and reclaimed land. The typical conversion processes involved in the production of a Class A product includes the use of mechanical dryers, radiation, or chemical disinfectants. These are relatively expensive treatment methods. However, solar drying of biosolids would be a relatively inexpensive means to reduce pathogens in biosolids. The reasons vary, drying beds are commonly used as a method for dewatering sludge in the United States, operational and maintenance costs are low, and the technology is uncomplicated (Metcalf and Eddy, 2003). Thereby, it is logical to study the use of open solar drying in a semi-arid region to promote pathogen reduction in biosolids. The present study was conducted

to determine if Class B biosolids can be converted to Class A biosolids by the use of open solar drying beds in a semi-arid climate.

2. MATERIALS AND METHODS

The present investigation was performed in two phases: (1) drying and disinfection of biosolids; and (2) experimental land application of biosolids to determine regrowth potential. Freshly-digested biosolids were exposed to the elements with no treatment or turning. Environmental parameters including air temperature, relative humidity, solar radiation, wind speed, and rainfall were monitored by a remote data collection and communication system sited at the location of the field experiments. The moisture content and temperature of the biosolids in the drying beds were also monitored. *Salmonella* and fecal coliform levels were assayed weekly to determine their trend towards Class A levels.

This first phase of the experiment was performed at the wastewater treatment plant in Avra Valley (AVWWTP), Arizona using existing concrete-lined drying beds sectioned off by soil berms to the dimensions shown in Table A-1. The experiment was conducted during the months of June and July, from Day of Year (DOY) 169-207 in 2003. Information pertinent to the experimental setup is shown below:

Table A-1. Experimental set-up using drying beds with minimal drainage, summer 2003.

Drying Beds	1	2
Type of Biosolids	Aerobically-digested	Anaerobically-digested
Initial %Total Solids	1.2% (origin: AV WWTP)	7.43% (origin: Ina WWTP)
Depth of Biosolids	50 cm	40 cm
Bed Dimensions:	3.1 m x 16.8 m x 0.6 m	
Drainage	<1%- concrete lining	
Moisture Sensors	3 Water Content Reflectometry Probes, located 5 cm from FFE	
Temperature Sensors	3 sets spatially located at either end and middle of drying bed- 2 cm, 7cm and 10 cm from FFE	

The experiment was repeated at the GVWWTP (Green Valley Wastewater Treatment Plant) utilizing medium-sized experimental beds lined with sand and high-density polyethylene liners (to imitate a smooth impervious surface). All beds were sloped to drain at a 3% slope in 12 inches to a drainage portal (5.0 cm in diameter) located at the north end of each bed. This experiment was performed in 2004 during the months of May and June, DOY 131-166.

Freshly-produced biosolids from the GVWWTP were placed onto beds 1b and 2a and anaerobically-digested biosolids were imported from the Ina Road WWTP and pumped onto beds 1a and 2c. Each bed was constructed of steel, the bottom was insulated from the concrete pad by an 8" layer of aggregate base course and R-11 extruded polystyrene board was permanently attached to each of the beds' four sides, see Figure A-1.

For the summer 2005 baseline experiments conducted during the months of June and July, DOY 174- 202, only containers 1 and 2 as described in Table A-2 were utilized for baseline drying studies of anaerobically (2.99%TS) and aerobically (12.98%TS) digested biosolids. Beds 3 and 4 were not utilized for baseline studies during this experimental period since the difference in inactivation rates between the sand and HDPE liners were insignificant.

2.1 INACTIVATION RATES AND HEAT UNITS

Often times, microbial inactivation is described in terms of the first order equation, $\log (N_t/N_o) = -k_d*t$, known as the Chick-Watson law where N_t = microbial number at time (t), N_o= initial microbial number, k_d = inactivation rate as a function of a parameter, and

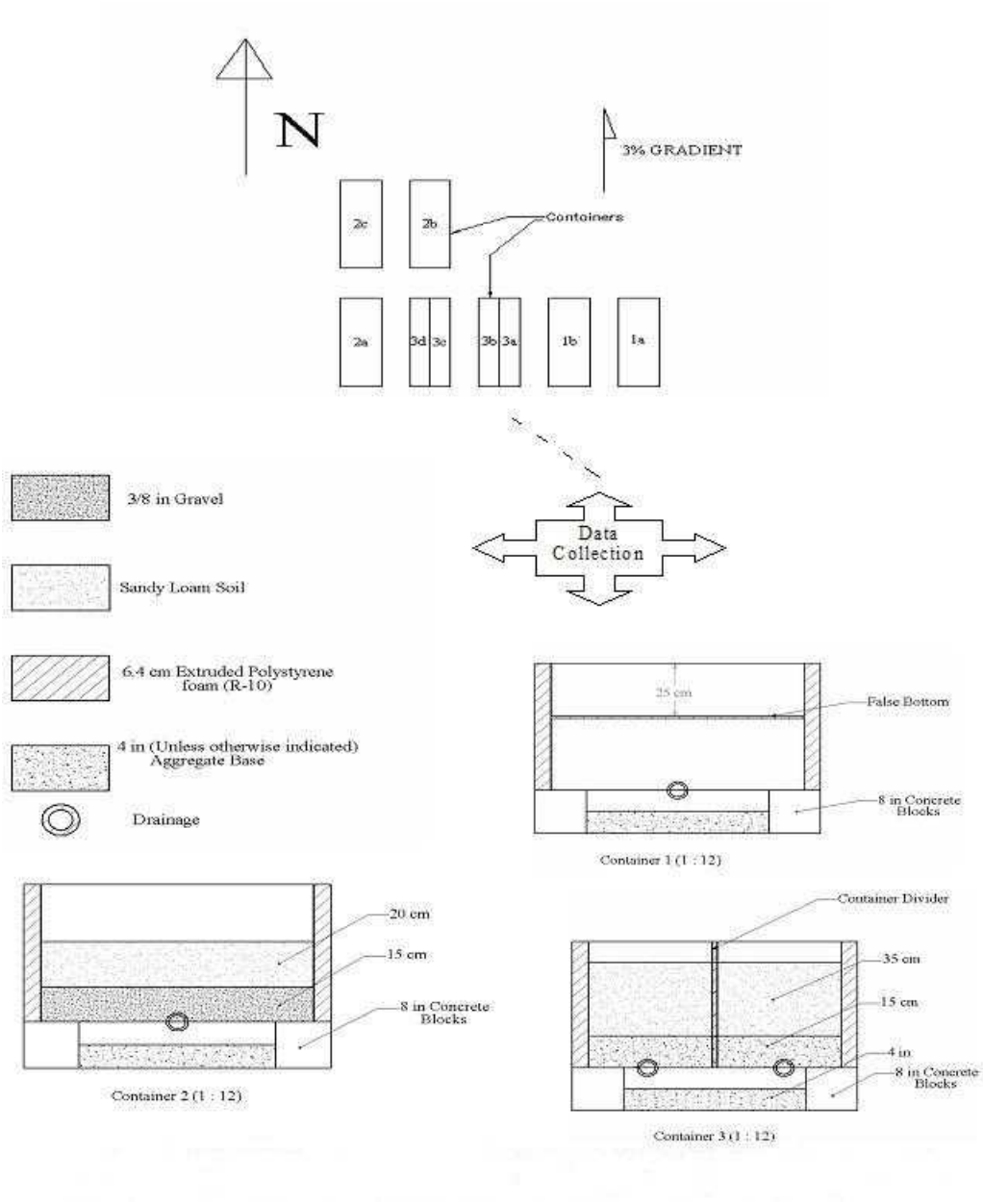


Figure A-1. Experimental layout at the Green Valley WWTP in Pima County, Arizona. Experiments were conducted here in the summers of 2004-5 and winters of 2005-6.

Table A-2. Experimental set-up drying beds with drainage, summer 2004.

Containers	1a	1b	2a	2c
Biosolids	Anaerobically-digested	Aerobically-digested	Aerobically-digested	Anaerobically-digested
Initial %TS	6.57%	16.37%	16.37%	6.57%
Drainage Type	HDPE* Liner	HDPE Liner	Sand	Sand
Depth of Biosolids	20 cm			
Dimensions	1.0 m (width) x 3.0 m (length) x 0.6 m (depth)			
Moisture Sensors	1 Water Content Reflectometry Probe, located 2 cm from FFE			
Temperature Sensors	3 sets spatially located at different depths of drying bed- 1 cm, 2cm and 3 cm from FFE			

*HPDE-high density polyethylene

t = time. The inactivation rate k_d was calculated as a function of time. Daily heat units were used for determining the amount of heat stress units that affected pathogens in solar drying beds. Daily heat units were derived by averaging the hourly temperature readings from the remote data collection system during the drying experiments for the biosolids over a 24-hour period of time, daily heat unit (DHU) = $T_i/24$. Cumulative daily heat units are a summation of daily heat units; CHU = DHU. Calculating heat units allowed normalization of field temperature data and the development of set-point temperatures for the microcosm study.

2.2 ENVIRONMENTAL SAMPLING

Environmental sampling of the biosolids was performed on a weekly basis (in accordance with Publication PB93-227957, POTW Sludge Sampling and Analysis Guidance Document US EPA-Office of Water Office of Wastewater Enforcement and Compliance, August 1989). At the Avra Valley WWTP, two composite samples were taken from each bed, one at the north end and the other at the south end. Samples were taken at a radial distance of at least 0.5 meters from the position of the moisture probes located at either end of each experimental bed. Four grab samples were taken to make one composite sample.

At the Green Valley WWTP, each experimental container was divided into quarters; grab samples of approximately 25 g were taken from the center of each quarter and combined to form a composite sample of the total bed. Approximately 3 cm of the upper crusted portion of the biosolids was removed prior to retrieving each grab sample. Each sample then included the entire depth of the sludge (down to the top of the sand layer). For each experimental bed, four grab samples were collected to make one composite

sample. A clean hand trowel was used to retrieve the grab samples and each set of grab samples was placed in its own sterile container and mixed for homogeneity. The composite sample was then stored in a sterile Nalgene® bottle or plastic bag and transported on ice to the University of Arizona campus. The analysis for percent total solids and the microbial assays for fecal coliforms and *Salmonella* were preformed within 6 hours of sampling from the field.

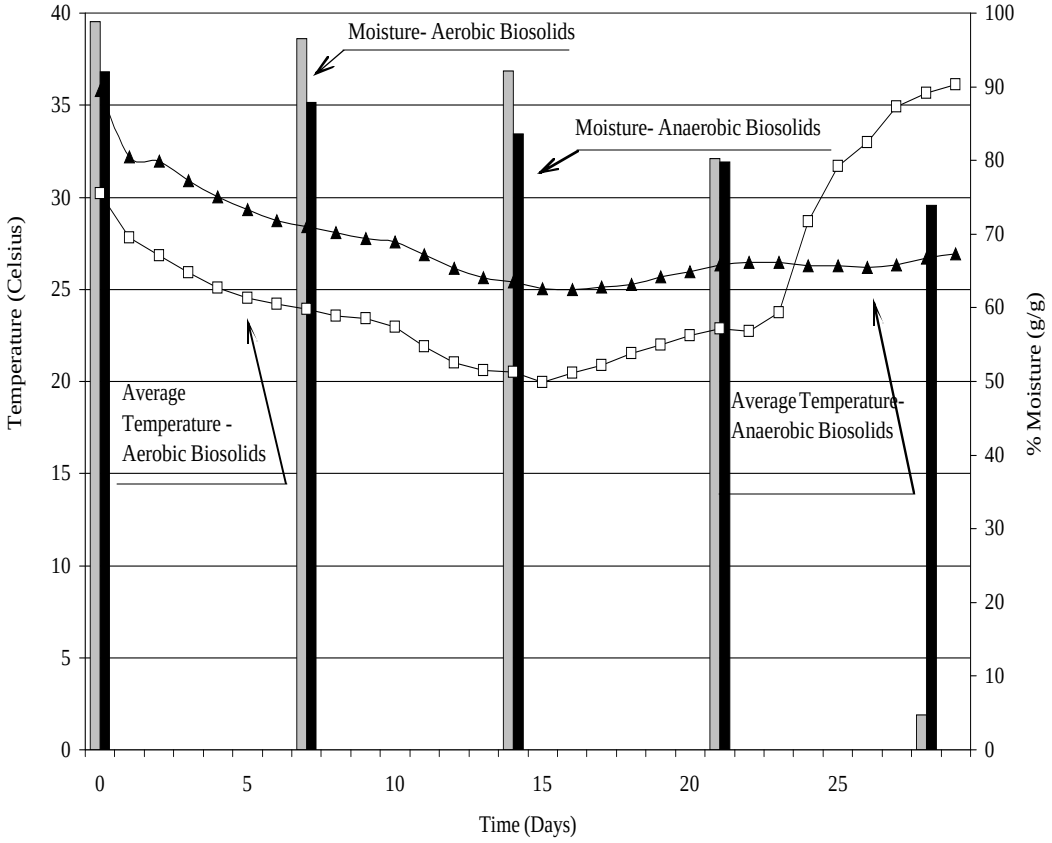
2.3 MICROBIAL ASSAYS

The Most Probable Number test (MPN) 9221 B, Standard Total Coliform Fermentation Technique is the typical method of practice for assaying indicator organisms in sewage sludge (American Public Health Association, 1998). This method was utilized during this experiment. A modified version of EPA Method 1682: *Salmonella* in Biosolids by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium, Draft Document 2003 was utilized to determine the *Salmonella* density levels of the biosolids in each container. When assaying the soil, rocks were discarded from the sample and it was essential that biosolids were present in the material assayed.

3. RESULTS

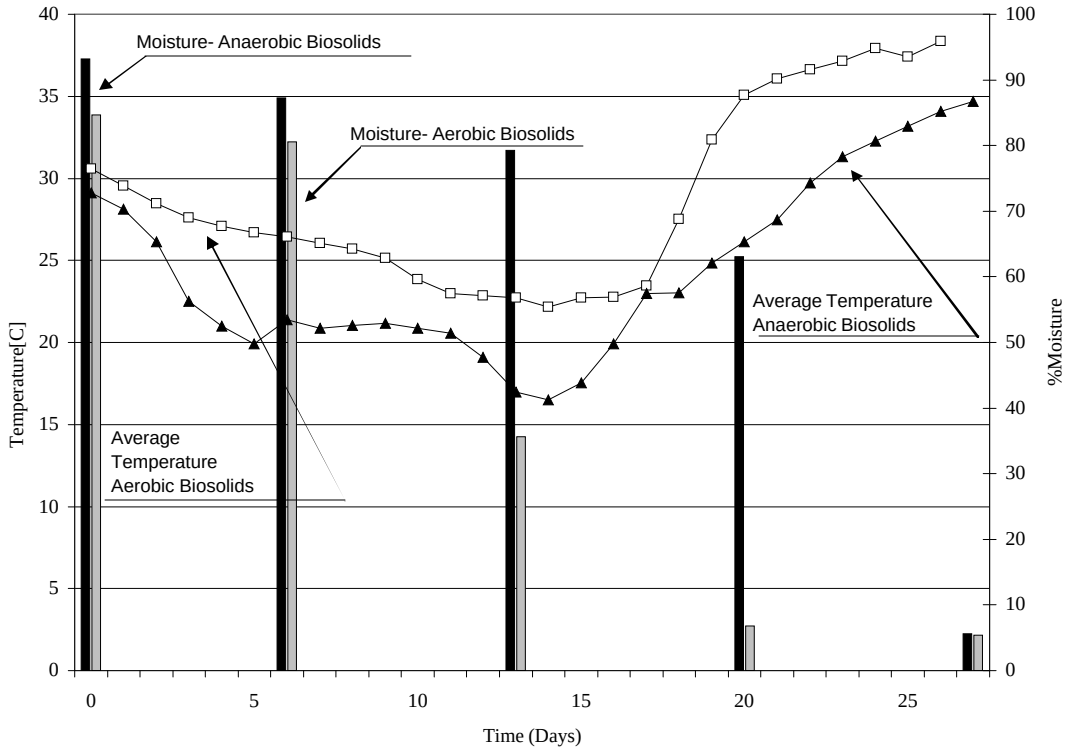
3.1 SUMMER ENVIRONMENTAL

Table A-3 indicates that the average daily ambient air temperature was relatively high in the summer 2005 experiment (however not significantly so at an alpha level of 0.05, p =1.00) as compared to the summer 2003 and 2004 experiments. The summer 2005 experiment also ran for a shorter period of time. The graphs in Figure A-2

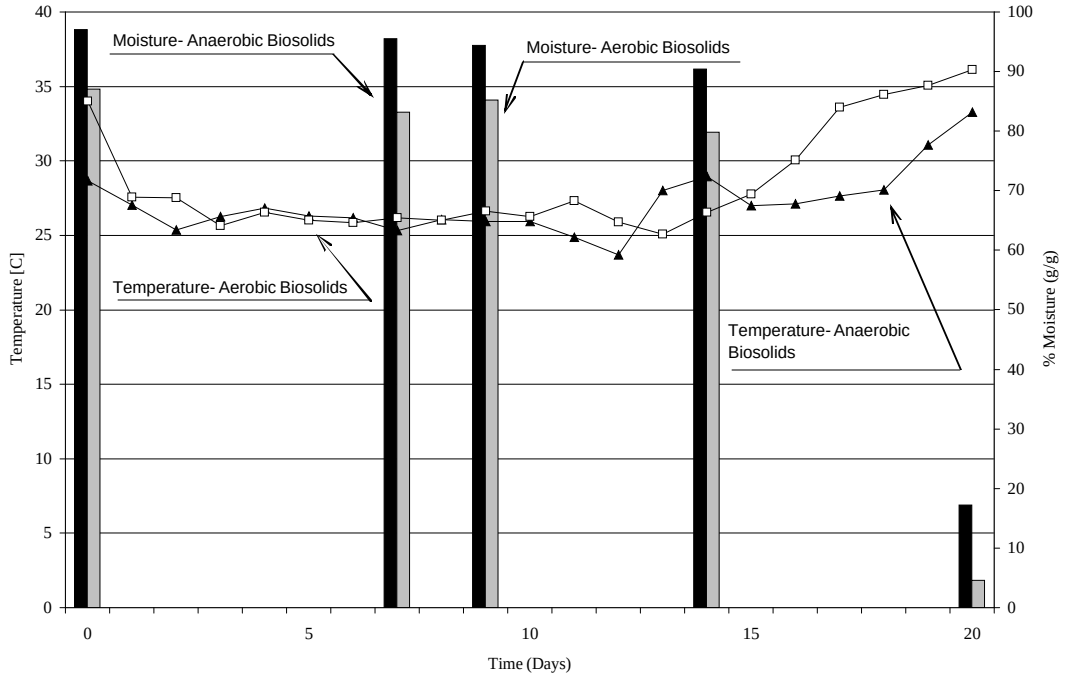


(a) Summer 2003 experiment, Avra Valley, Arizona

Figure A-2. Graphs of average daily temperatures of biosolids as compared to moisture content during solar drying experiments: (a) summer season 2003, Avra Valley, Arizona; (b) summer season 2004, Green Valley, Arizona; and (c) summer season 2005, Green Valley, Arizona.



b) Average temperature of biosolids and moisture content, summer 2004 experiment.



c) Average temperature of biosolids and moisture content, summer 2005 experiment.

demonstrate that as the water concentration in the biosolids is reduced, less cooling due to evaporation occurs, and the high porosity of the dried biosolids readily allows for heat conduction, causing the internal temperature of the biosolids to rise. The interaction between the increasing temperature and decreasing moisture content of the biosolids may impact pathogen inactivation.

3.2 WINTER ENVIRONMENTAL

Winter 2006 was warmer and drier than the winter solar drying period in 2005 (Table A-3). The average temperature during the winter 2006 experiment was 13ºC with an average relative humidity of 29.96% while in the 2005, the average temperature was approximately 12ºC, but the relative humidity was 50.65%. Rainfall during the winter 2006 experiment was approximately ¼ of that in 2005.

3.3 EVAPORATION RATES

The dewatering process for all field experiments was dependent on evaporation; no significant drainage occurred due to the impervious bed material (concrete and HDPE lined) and the presence of a polymer in the generated biosolids.

Table A-3. Environmental data for summer season experimental field sites.

Experiment	Ambient Air Temperature (Celsius)			RH (%)			Wind speed (m/sec)		
	Average	Max	Min	Average	Max	Min	Average	Max	Min
Summer 2003 DOY 169-207	27.63	44.1	14.47	17.63	95.6	5.39	2.63	9.38	0.16
Summer 2004 DOY 131-166	26.70	41.37	10.96	17.80	54.94	4.17	3.02	7.84	0.30
Summer 2005 DOY	32.4	42.7	20.04	21.69	78.8	5.46	2.48	7.37	0.115

Rainfall: summer 2003 = 2.03 mm, summer 2004 = 0.00 mm, summer 2005 = 11.94 mm

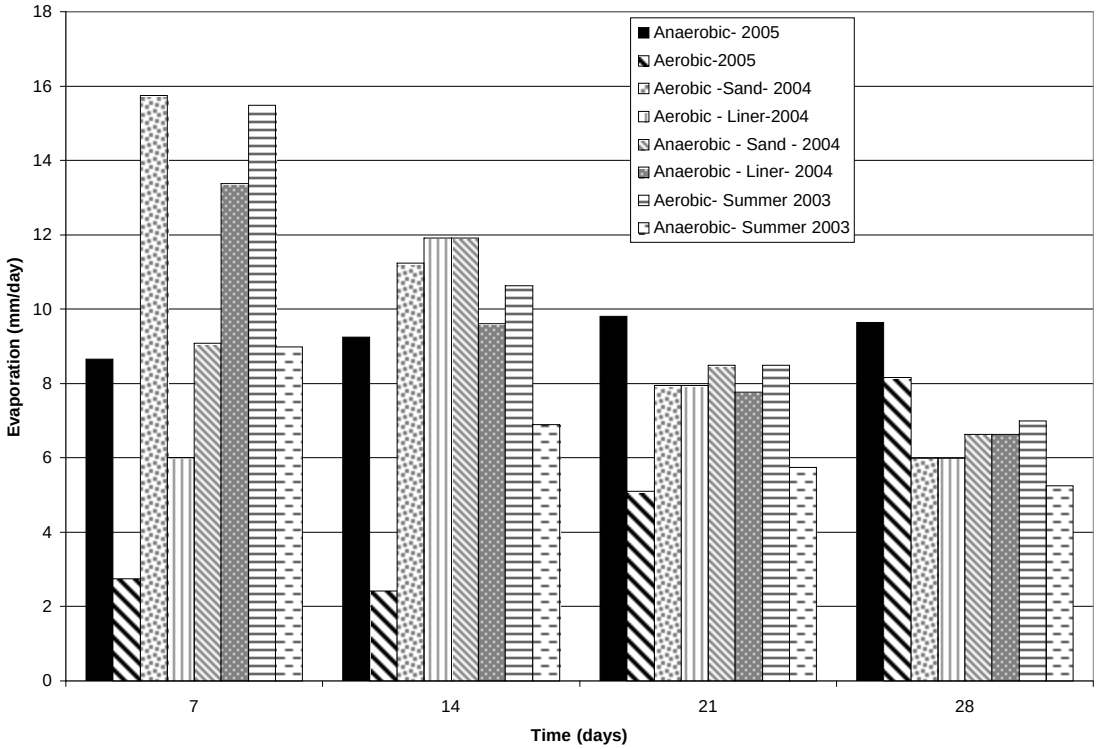
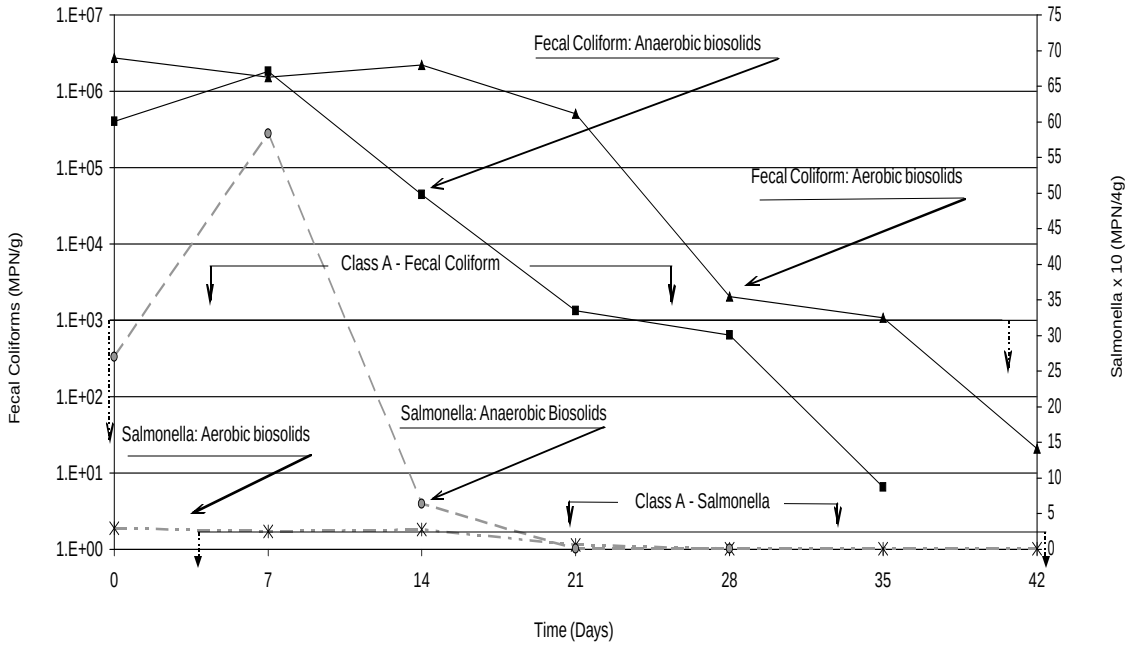


Figure A-3. Evaporation rates (mm/day) from biosolids' beds, summer solar drying experiments.

Figure A-3 shows the variability in the evaporation rates for each experimental bed during the summer seasons. Evaporation was dependent on wind speed, air temperature, solar radiation and the vapor pressure deficit between the surface boundary layer and the ambient air. There is a general trend with time towards a decreasing rate of evaporation in all the solar drying beds with the exception of the anaerobically-digested biosolids in the summer of 2005, which remains relatively constant. The static rate is likely due to the high initial moisture content (~96% g/g) of the anaerobically-digested biosolids and the presence of a polymer which retards evaporation (Metcalf and Eddy, 2003). This graph also indicates that the rate of evaporation was highest during the summer 2004 experiment as compared to the summers of 2003 and 2005. It is suspected that the uncharacteristic high peaks of evaporation are due to shrinking and random cracking of the biosolids, exposing more surface area and allowing for increased evaporation.

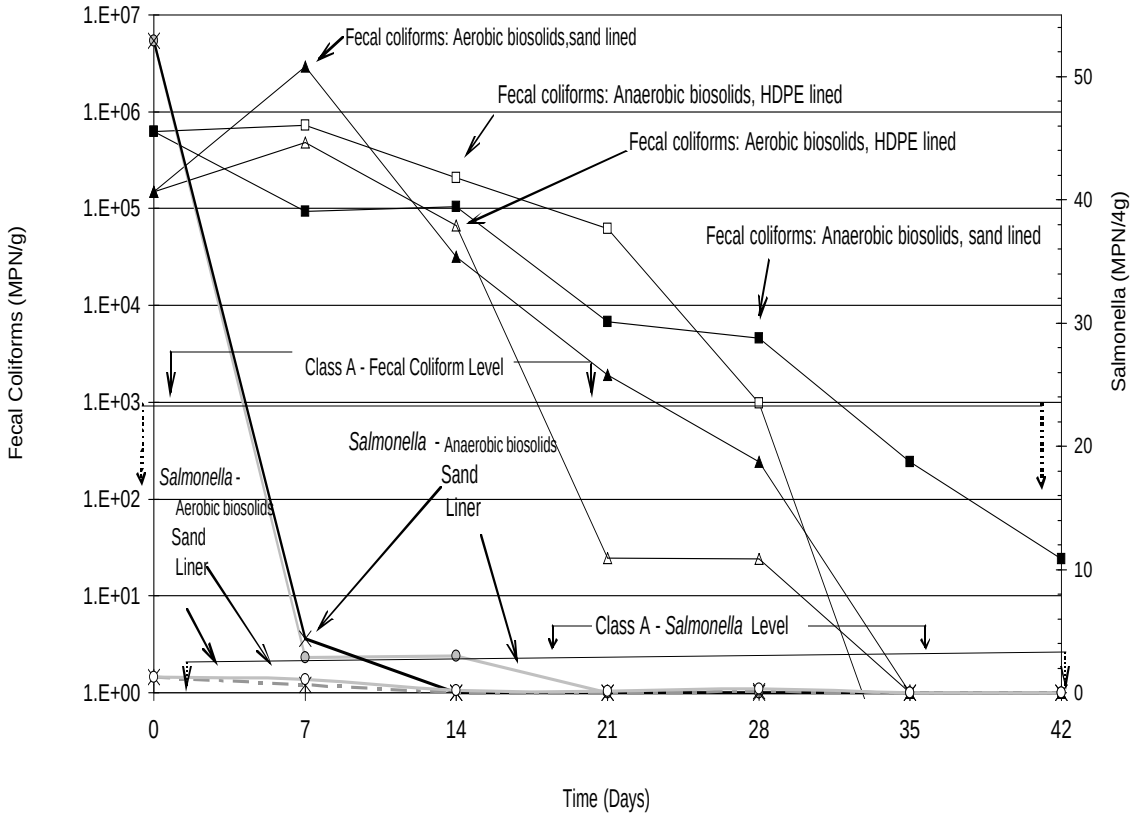
3.4 MICROBIAL RESULTS

Figure A-4 demonstrates the die-off pattern of fecal coliforms and *Salmonella spp*. in the open solar drying beds during the dry periods for the three summer solar drying seasons. In the summer of 2003 experiment, the fecal coliform levels in the anaerobically-digested biosolids reached Class A quality in approximately 21 days; aerobically-digested biosolids reached Class A levels after 35 days. Class A *Salmonella* levels were obtained by Day 21 and 28 of the experiment for anaerobically and aerobically-digested biosolids, respectively. The shorter time difference for inactivation in the anaerobically-digested biosolids may be due to substrate limitation and microbial competition (Smith *et al.*, 2005).

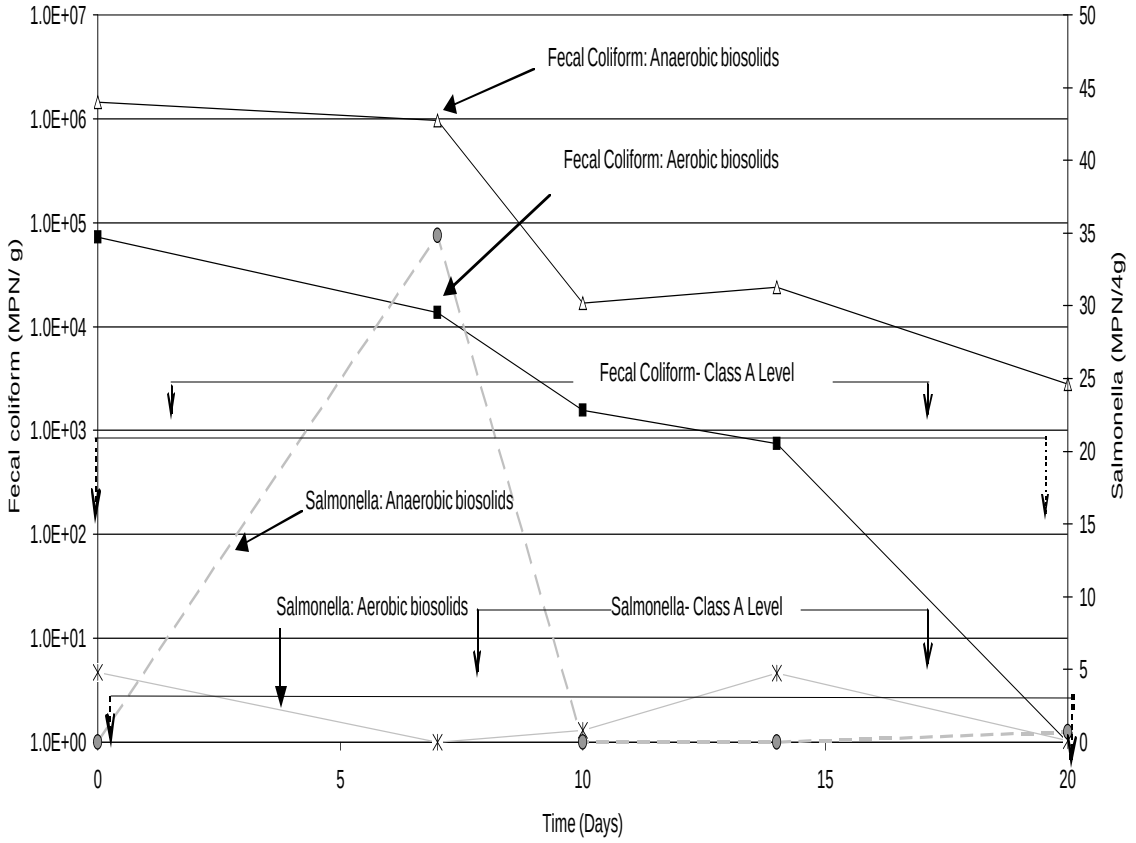


(a) Summer 2003, Avra Valley WWTP.

Figure A-4. Fecal coliform and Salmonella density levels during field solar drying experiments: (a) summer 2003, Avra Valley; (b) summer 2004, Green Valley; and (c) summer 2005, Green Valley.



(b)Summer 2004, Green Valley, Arizona.

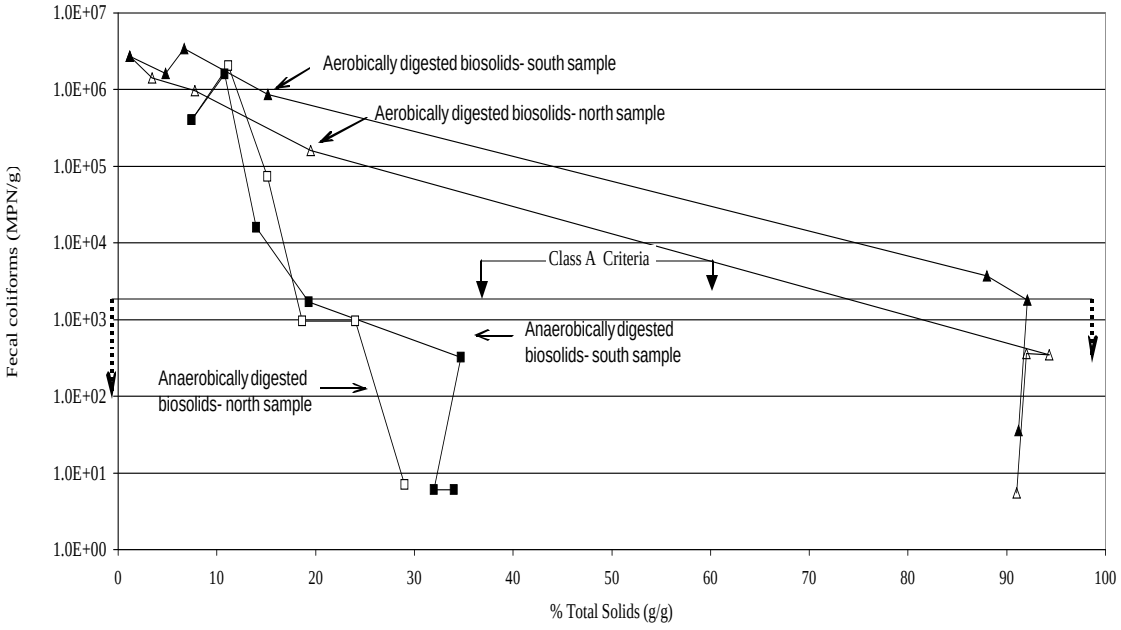


(c) Summer 2005, Green Valley.

The average daily ambient air temperature during the first six weeks of the summer 2004 experiment was 27.6°C. Fecal coliform levels for both of types of digested biosolids reached Class A levels within a range of 21 to 35 days. This time frame is similar to the baseline data obtained for the summer 2003 experiment. Likewise, the biosolids with the higher water content took longer to attain Class A criteria.

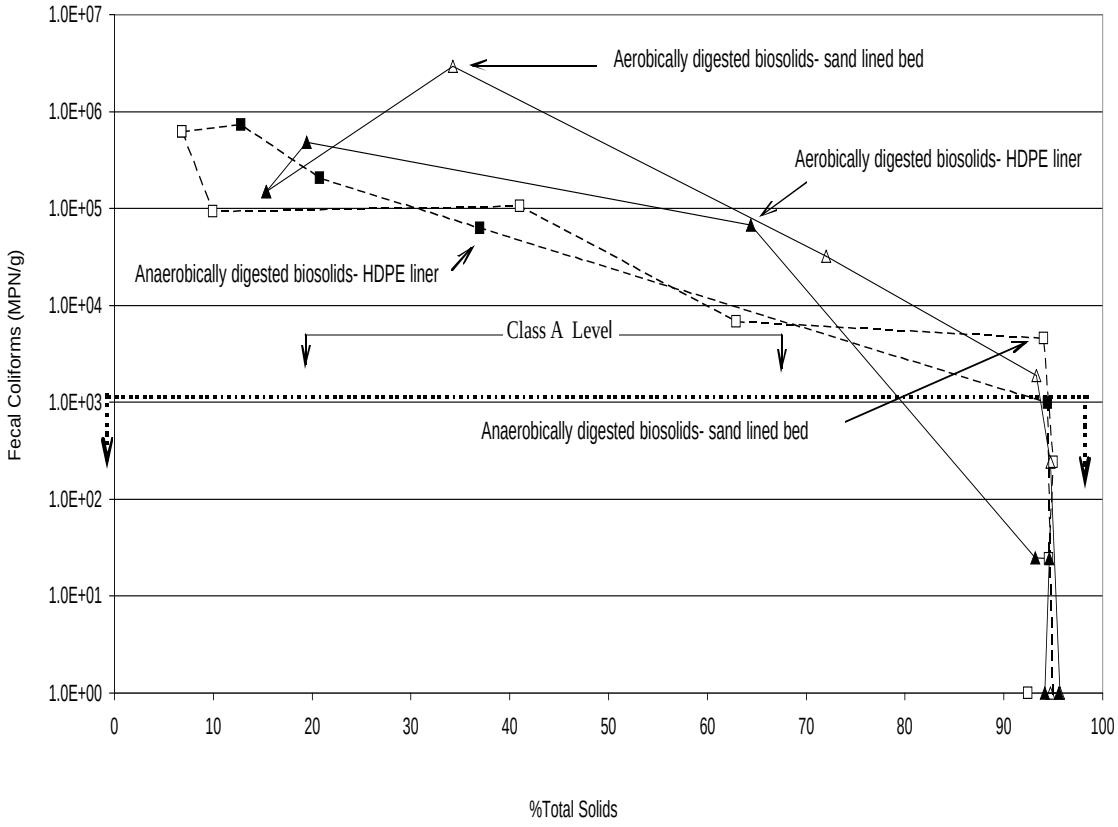
Ambient air temperatures were higher during 2005 and the fecal coliforms reached Class A level on Day 14. The anaerobically-digested biosolids did not reach Class A during this drying season because of their high initial moisture content (97%) and the early monsoon rainfall. The monsoon rains wet the beds and sustained high levels of both *Salmonella* and fecal coliforms in the mesophilic anaerobically-digested biosolids. Influent *Salmonella* levels were low for both types of biosolids, <3 MPN/4 g dry weight (anaerobically-digested) and 4.8 MPN/ 4 g dry weight (aerobic digested). However, *Salmonella* levels in the anaerobic biosolids spiked around Day 7, the same type of event was seen in the summer 2003 experiment and in the solar drying experiment performed in Quartzsite, Arizona (Choi *et al.*, 2005). This spike may be due to adaptation of the bacteria to a new environment.

Figure A-5 demonstrates the existence of a critical minimum threshold of moisture content for fecal coliform survival. The graph for data during the summer of 2003 indicates the critical value to be less than 10% moisture (g/g) for aerobically-digested biosolids. The decline of fecal coliforms in anaerobically-digested sludge may be moisture-dependent; however, the threshold level occurs much sooner,

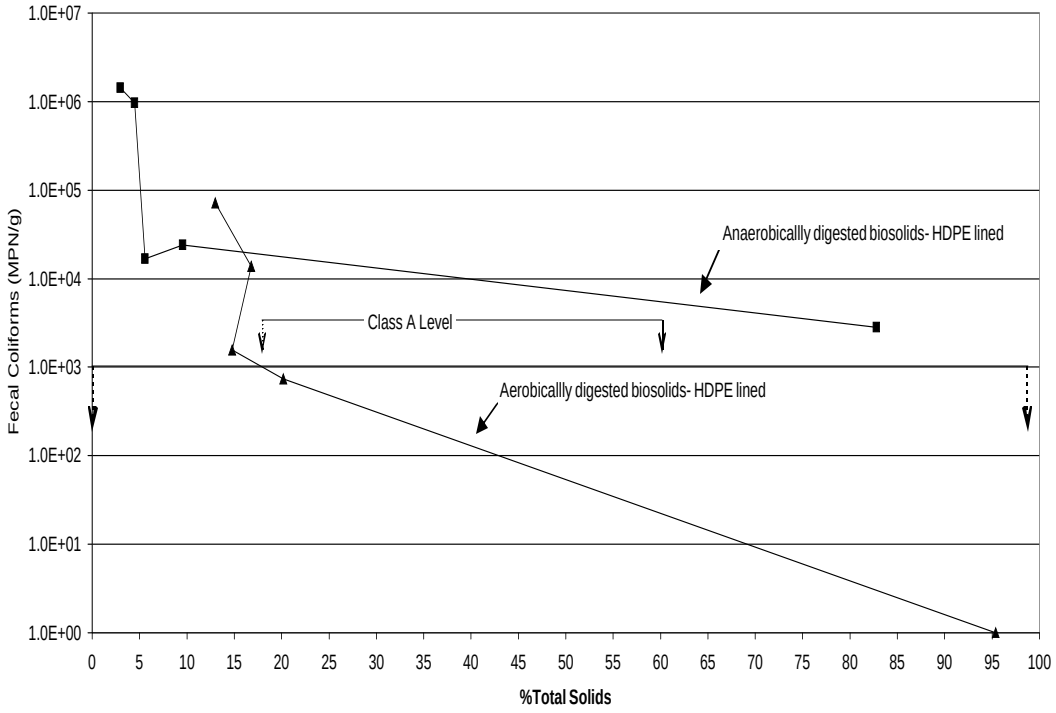


(a) Summer 2003, Avra Valley

Figure A-5. Fecal coliform levels in relation to %Total Solids (g/g) for warm solar drying experiments: (a) summer 2003, Avra Valley, Arizona; (b) summer 2004, Green Valley, Arizona; and (c) summer 2005, Green Valley, Arizona.



(b) Summer 2004, Green Valley, Arizona



(c) Summer 2005, Green Valley

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i.e. at 30% TS (g/g) during this experiment. Data from the summer 2004 experiment also indicates the presence of a critical moisture level for obtaining Class A pathogen levels; i.e., at approximately 95% TS (g/g) for both types of biosolids (Figure A-9).

The results from summer of 2005 cannot be used to characterize this same trend; fecal coliform inactivation rates fell sharply between 15-20 %TS in the aerobically-digested biosolids, (the anaerobically-biosolids did not reach Class A criteria during the experiment, Figure A-10, due to the monsoon rains).

The average inactivation rates for fecal coliforms in aerobically-digested biosolids were 0.17 ± 0.03 day^{-1} and 0.17 ± 0.04 MPN day^{-1}. There was no significant difference between the type of drainage and type of biosolids utilized. Table A-4 lists the various inactivation rates for fecal coliforms as a function of time and a function of %TS. Note that the correlation factor, R^2, is relatively high for both variables with the exception of the anaerobically-digested biosolids (Control bed) for summer 2005.

It is important to make a distinction between %TS (which addresses moisture level) and rate of moisture loss (change in moisture). There is no demonstrable correlation of inactivation to the rate of desiccation (Δ moisture/Δ time) of the biosolids. Fecal coliform inactivation in biosolids at the Avra Valley and Green Valley WWTPs was affected by moisture level not the rate of moisture loss. Figure A-6 demonstrates the low correlation factor (R^2) for k_d as a function of the rate of change of moisture; i.e. Δ moisture/Δ time.

3.5 *SALMONELLA* DENSITY LEVELS

Salmonella is of major epidemiological concern in the wastewater treatment industry as it can readily make people ill (Horan *et al.*, 2004). The presence of *Salmonella* in wastewater effluent is also used as an index for sanitation in Europe. The infectious dosages for bacteria are 10^4- 10^9 organisms, depending on the type of bacteria (Maier *et al.*, 1999).

In general, the inactivation rates for *Salmonella* were inconsistent, partially due to highly-varied influent levels (very low levels from the GVWWTP and log 2 levels from the Ina Road WWTP). *Salmonella* levels in the anaerobically-digested biosolids during the summer 2003 experiment started at a relatively high level of 270 MPN/4g dry weight. The initial amount of *Salmonella* detected in the aerobically-digested biosolids was 30 MPN/4 g. Within 21 days of open solar drying, the *Salmonella* in the anaerobically-digested biosolids reached non-detectable levels, i.e. < 3 MPN/4 g dry weight; the percentage of total solids was 18.64%. Within 28 days, the *Salmonella* in the aerobically-digested biosolids reached non-detectable levels. The percentage of total solids in this case was 94.3%.

Table A-4. Inactivation rates for fecal coliform regressed over time and %TS for summer solar drying experiments.

Type of Biosolids	k_d(time)	R^2	k_d(%TS)	R^2
Aerobic- south sample, 2003	0.1266	0.85	0.043	0.98
Aerobic- north sample, 2003	0.1806	0.95	0.033	0.99
Anaerobic- south sample, 2003	0.183	0.95	0.25	0.78
Anaerobic – north sample, 2003	0.1793	0.97	0.16	0.81
Aerobic, liner, 2004	0.1642	0.90	0.05	0.83
Aerobic, sand, 2004	0.1796	0.87	0.04	0.83
Anaerobic, liner, 2004	0.1955	0.84	0.03	0.99
Anaerobic, sand, 2004	0.1009	0.95	0.03	0.93
Aerobic, liner, 2005	0.204	0.83	0.05	0.86
Anaerobic, liner, 2005	0.19	0.95	0.02	0.49

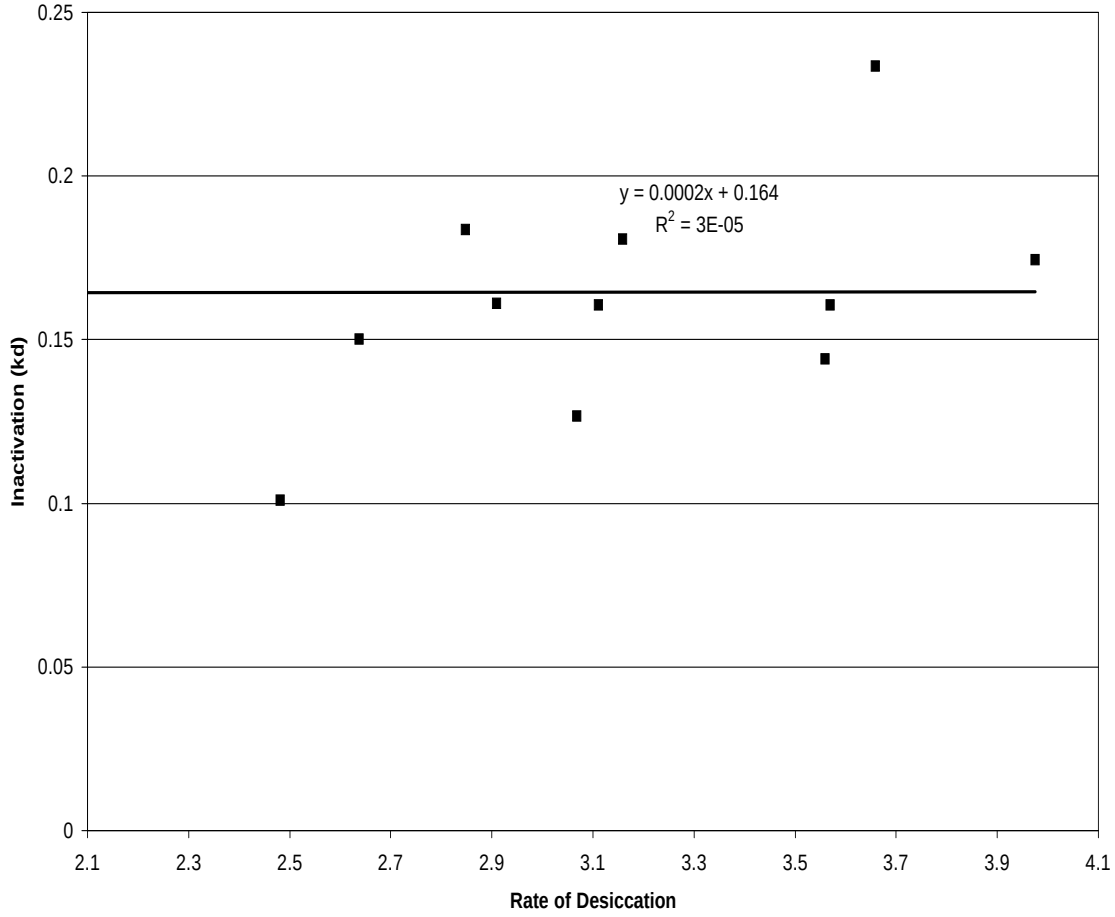


Figure A-6. Fecal coliform inactivation rates, kd, versus the rate of desiccation (Δ moisture/Δ time) for all baseline solar drying studies performed in Pima County, during the dry summer seasons, 2003, 2004, 2005.

Initial levels of *Salmonella* detected in the two different types of biosolids during the summer 2004 experiments were 52.91 MPN/4g and 1.3 MPN/4g for anaerobically and aerobically-digested biosolids, respectively. Both beds reached Class A levels on Day 20 of the experiment. The origin of the aerobic biosolids for this experiment was different that of the summer 2003 experiment (Green Valley WWTP vs. Avra Valley WWTP). The literature describes typical levels of *Salmonella* in raw sewer sludge in the United States to be at levels in the range of 10^2-10^4MPN/g (Metcalf and Eddy, 2003). Epstein *et al.*, 2003 indicates that treated biosolids prior to land application have a typical level of 18 MPN/g. Possible explanations for the relatively low levels of *Salmonella* in the aerobically-digested biosolids from the GVWWTP are that:

1) The population served by the wastewater treatment is a relatively healthy population extrapolated from reasoning provided by Dumontet *et al.*(2001), as they report that the type of pathogen most commonly found in sewage sludge and its derivatives depend on the state of public health, as well as on the presence of hospitals, tanneries, and meat processing factories; or

2) The Green Valley WWTP was recently upgraded from aeration ponds to a modern activated sludge system that employs an oxidation ditch, aerobic digesters and a belt press for solids handling.

Inactivation rates for *Salmonella* are listed in Table A-5 for all summer solar drying experiments.

3.6 WINTER EXPERIMENTS

Both experimental winter seasons were relatively warm. However as shown in Figures A-7 and A-8, winter 2005 had a high amount of rainfall as compared to winter 2006. Fecal coliforms in the anaerobically-digested biosolids for both winter experiments did not reach Class A levels. The %TS for these beds did not climb above 20% in winter 2005 and was less than 40% during winter 2006. The relatively lower temperatures and decreased solar radiation retarded the evaporation rate of these biosolids. However, the fecal coliform density level in the aerobically-digested biosolids during the winter 2006 trial did reach Class A criteria on Day 43 of the experiment, but then rose slightly above 10^3 MPN/g by Day 50 of the experiment, possibly due to an earlier rainfall event on Day 47. It has been shown that Class A biosolids are typically not achieved during cool and wet seasons (Choi *et al.*, 2005 & Al-Hmoud *et al.*, unpublished data, 2006). Class A criterion in this latter case was only met during the late portion of the field experiment after a prolonged dry period and only after the temperature of the biosolids had climbed to approximately 23ºC.

Table A-5. Inactivation rates of *Salmonella*, summer solar drying experiments

Type of Biosolids	k_d (time)	R^2
Aerobic, 2003	0.05	0.78
Anaerobic, 2003	0.11	0.83
Aerobic, liner, 2004	0.21	0.75
Aerobic, sand, 2004	0.03	0.75
Anaerobic, liner, 2004	3.6	0.75
Anaerobic, sand, 2004	3.8	0.81
Aerobic, liner, 2005	0.13	0.16
Anaerobic, sand, 2005	0.46	0.05

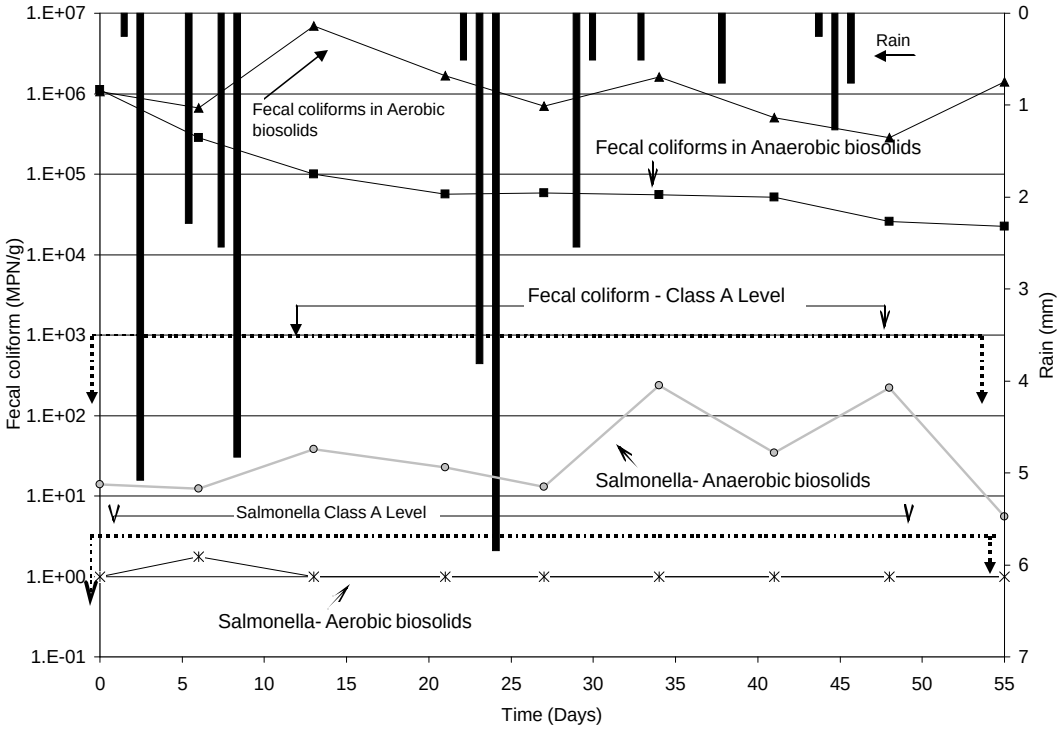


Figure A-7. Fecal coliform and *Salmonella* levels during the winter 2005 experiment, GVWWTP.

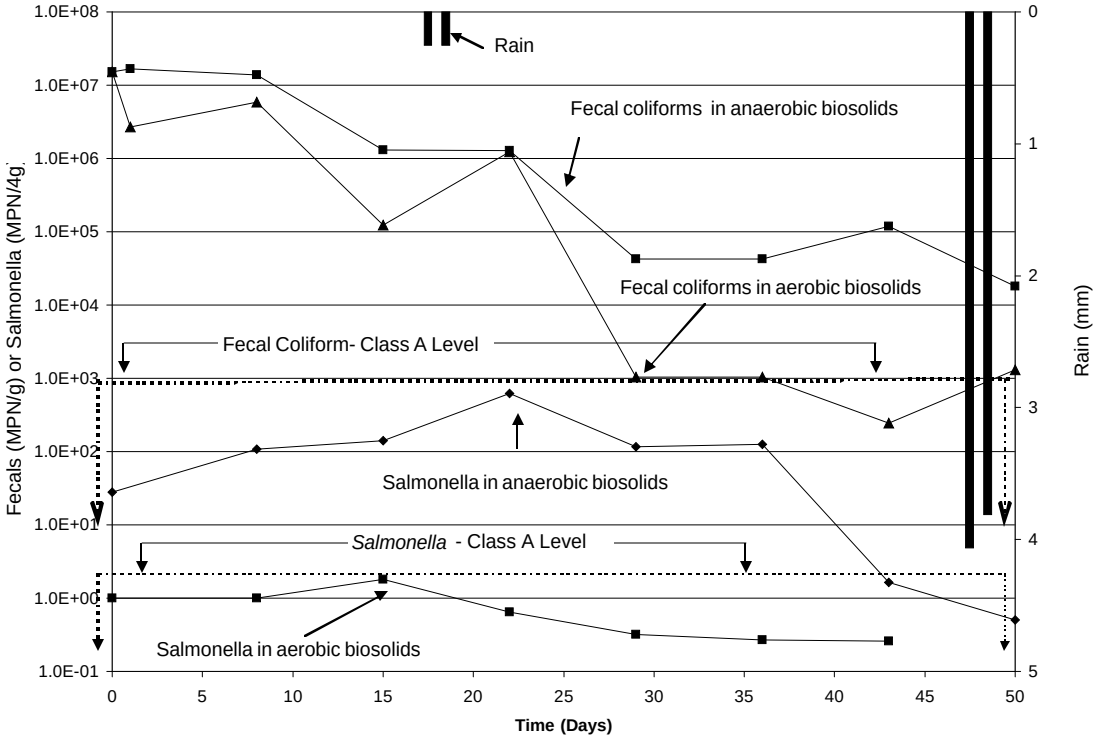


Figure A-8. Fecal coliform and Salmonella levels during the winter 2006 experiment, GVWWTP.

3.7 PHASE II- LAND-APPLICATION OF CLASS A BIOSOLIDS

Solar-dried biosolids that had reached Class A microbial quality were land-applied onto experimental soil beds at the GVWWTP site at a rate of 50Mg/ha. The soil beds were 0.5m (width) x 3.0 m (length) and the biosolids were hand-tilled into the soils to a depth of 20 cm. The control bed was also hand-tilled for consistency. These beds were labeled 6b (application of aerobically-digested biosolids), 7a (control- no application of biosolids), 7b (application of anaerobically-digested biosolids in summer 2004 and heat treated aerobically-digested biosolids in summer of 2005), see Figure A-1. During the summer of 2004 (see Figure A-14) fecal coliform regrowth in soil containers receiving aerobically and anaerobically-digested Class A biosolids was observed on Day1 after applying the biosolids to the soil containers. Regrowth levels in the anaerobically-digested biosolids exceeded initial fecal coliform levels for Day 0 of the drying experiment. Both soil containers exceeded Class A fecal coliform levels on Day 1 of the land-application experiment. However, fecal coliform levels returned to Class A criteria within a range of 5-7 days. During this period of time, the soil moisture in the experimental containers decreased to less than 5% due to natural evaporation. Regrowth occurred a second time after the first rainfall event on DOY 189 or Day 10 of the Phase II experiment. These results are similar to results obtained by Pepper *et al* (1993) following the application of sewer sludge onto different types of soil under field conditions; fecal coliforms decreased with decreasing soil moisture and increased after rain fall events.

The land application experiment was repeated in the summer of 2005(see Figure A-10). Again, fecal coliform regrowth occurred on Day 1 after land application. This time, the background level of fecal coliform in the Control bed was relatively high at ~ 10^4

MPN/g dry wt. of soil. Fecal coliform levels decreased in all three soil beds as a function of time. Rainfall occurred on Day 7 of the experiment causing a slight increase in soil moisture and the density of fecal coliform in bed 6b.

Assays for *Salmonella* from all three soil containers in the summer 2004 experiment, showed no regrowth throughout the duration of the land application experiment. However, during the summer of 2005, *Salmonella* regrowth was evident but only to very minimal levels; i.e. < 1.0 MPN/4g dry wt. of soil. A study by Gibbs *et. al* (1997) where biosolids with an initial *Salmonella* level of 0.09 MPN/g were land-applied, indicated non-detectable levels of *Salmonella* during weeks 8 through 29 of the experiment. *Salmonella* regrowth did occur to the level of 0.72 MPN/g during week 36 of the experiment. This regrowth occurred after eleven consecutive rainfall events which began in week 25. Accordingly, Yates and Yates, (1988) in a review of published articles report that soil moisture appears to be the dominant factor affecting the chances of survival of *Salmonella typhi* in soil. *S. typhi* could be recovered from moist soil for 70 days, while in dry soil it could only be recovered for 2 weeks. Gibbs *et al.* (1997) reports that upon reviewing several studies involving *Salmonella* regrowth in biosolids after land-application, there had been no reported regrowth of *Salmonella*.

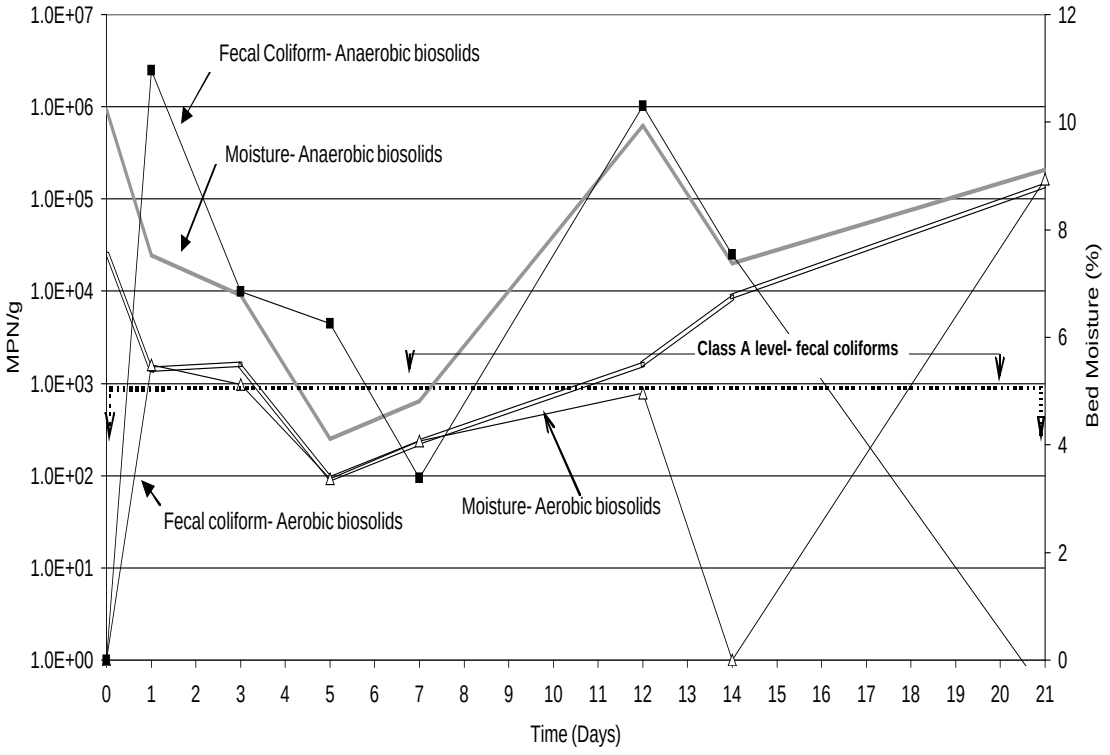


Figure A-9. Fecal coliform regrowth after land application; no Salmonella regrowth was detected, summer 2004.

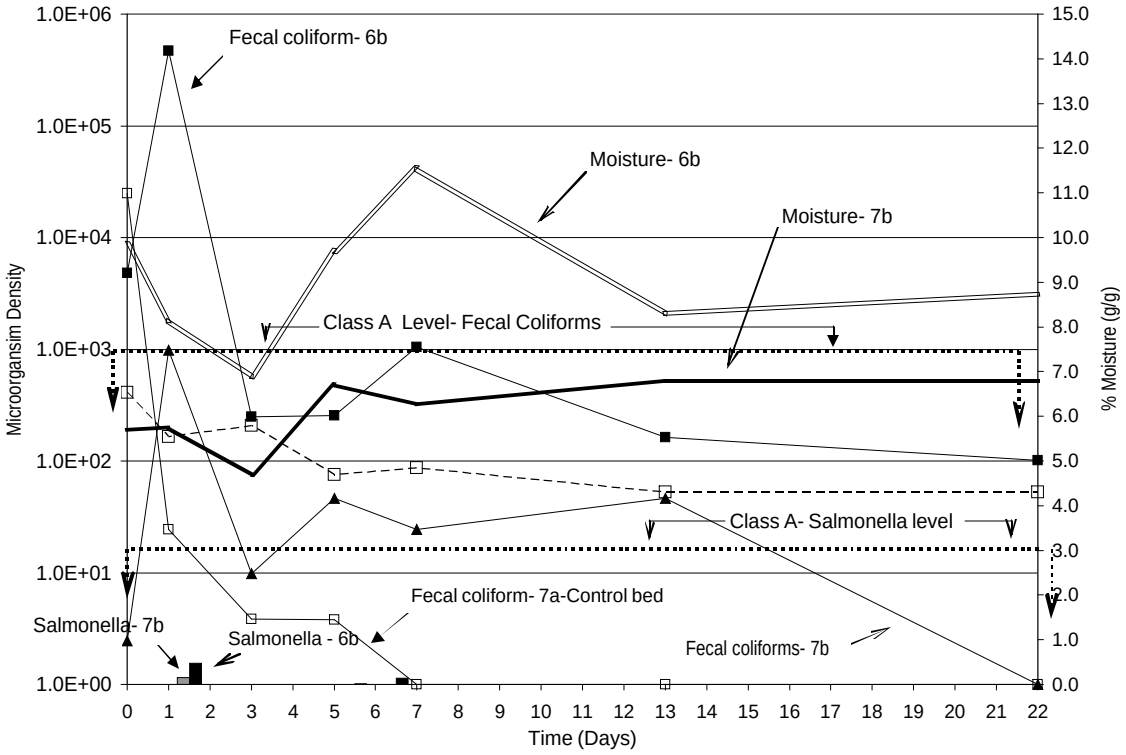
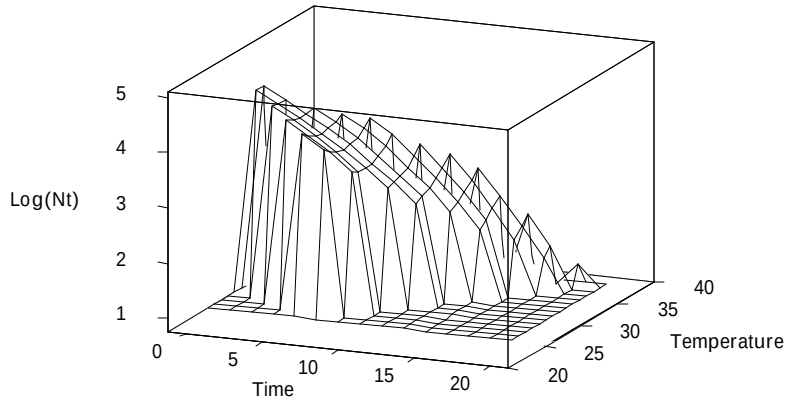


Figure A-10. Fecal coliform and Salmonella regrowth and inactivation after land application, summer 2005.

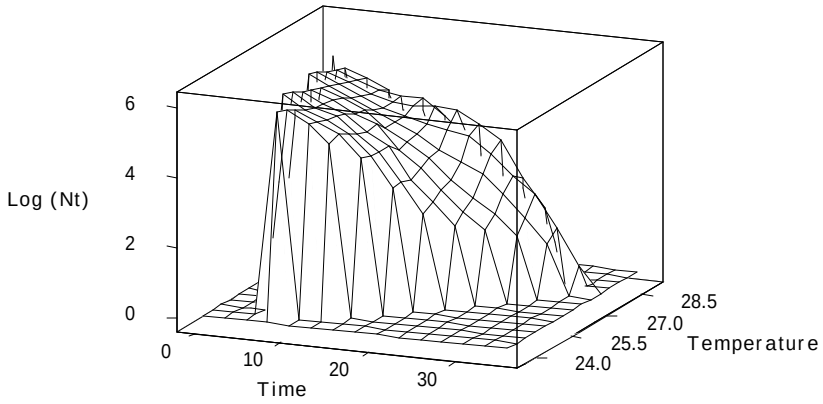
4. CONCLUSIONS

The results indicate that using open solar drying during warm dry seasons is a feasible method for converting Class B biosolids to Class A material. However, there is no significant difference between inactivation rates for aerobically and anaerobically-digested biosolids nor type of drainage bed design. However, it has been observed that the use of sand lined drying beds is more effective in sustaining low levels of bacteria and bacterial indicator pathogens after rainfall. Sand drying beds provide greater surface area for drainage and aid in the prevention of ponding due to rainfall, thereby reducing regrowth levels after rainfall events. Although removing biosolids from a sand lined bed can be difficult without disturbing and removing a large portion of sand, private enterprises have designed a cellular confinement system that holds the sand in place but allows a small loader to remove the dried sludge (Banks and Lederman, 1990).

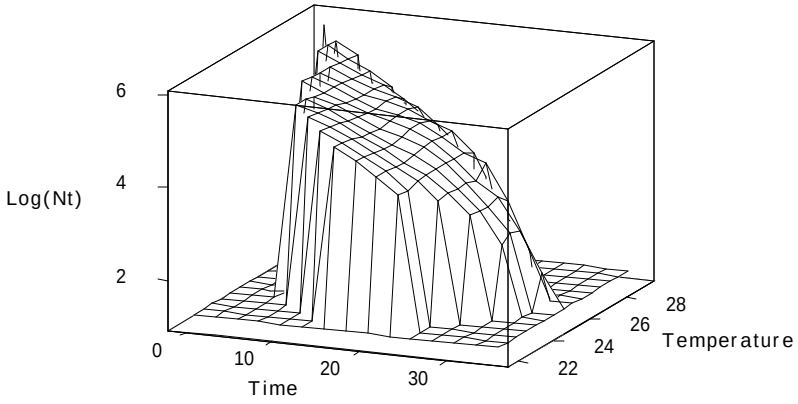
Figures A-11 and A-12 are three-dimensional graphs that indicate the effects of moisture and temperature over time on fecal coliform inactivation. The inactivation rate of fecal coliforms can be discussed in terms of %TS, which may in the future be calculated as function of heat and radiation units. Using heat units as a basis for calculating the moisture level in biosolids over time can be very specific to a given area and therefore can be more predictive of the inactivation rate of pathogens.



a) Summer 2005, anaerobically-digested biosolids

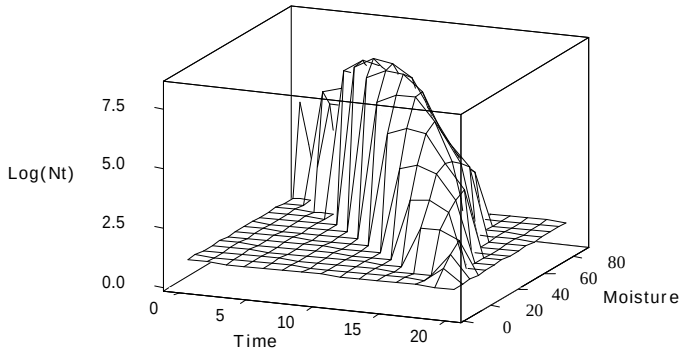


b) Summer 2004, aerobically-digested biosolids

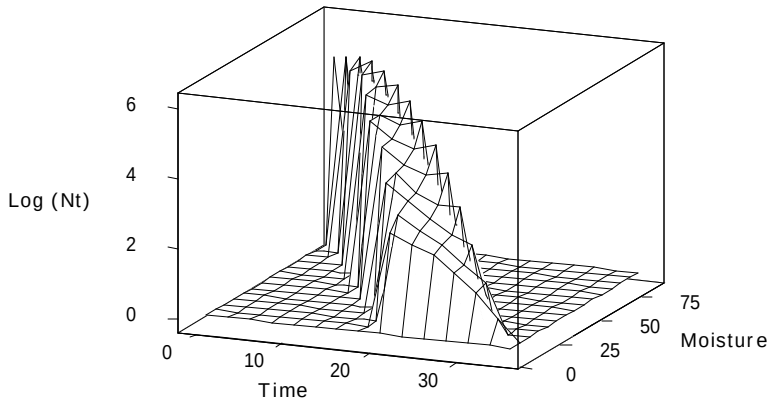


c) Summer 2004, anaerobically-digested biosolids

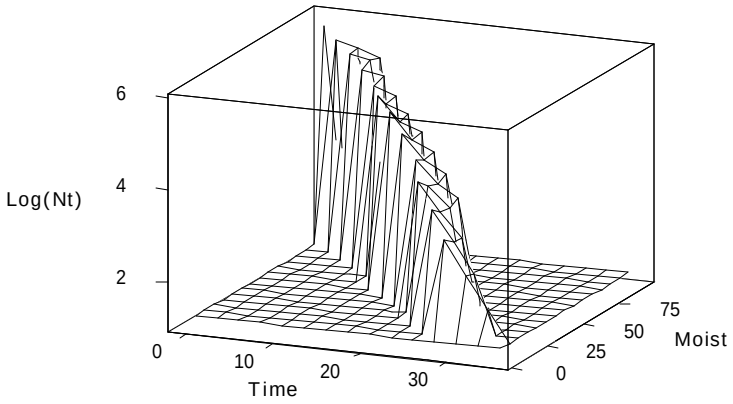
Figure A-11. Fecal coliform levels (Nt) vs. time and temperature for non-tilled biosolids (a) anaerobically-digested biosolids, summer 2005; (b) aerobically-digested biosolids; and (c) anaerobically-digested biosolids, summer 2004.



(a) Summer 2003, aerobically-digested biosolids



(b) Summer 2004, aerobically-digested biosolids



(c) Summer 2005, anaerobically-digested biosolids.

Figure A-12. Fecal coliform levels (Nt) vs. time and moisture for: (a) aerobically-digested Control Bed, Summer 2005; (b) aerobically-digested biosolids, summer 2004; and (c) anaerobically-digested biosolids, summer 2004.

These current studies indicate that Class A biosolids produced by open solar drying beds are vulnerable to regrowth while in the drying beds. However, the land application studies suggest that the addition of low levels of *Salmonella* to healthy soil does not exhibit high potential for regrowth. For the sake of practicality, serious consideration should be given to correcting the perception that fecal coliform are satisfactory indicators of pathogens in biosolids, once land applied.

Investigation of fecal coliforms and *Salmonella* regrowth was performed in the field using Class A solar-dried biosolids that had been land applied at a rate of 50 tons/ha during the summers of 2004 and 2005. Biosolids dried to 95%TS were hand tilled into sandy loam soil beds to a depth of 10 cm. Subsequent microbial analysis over a two-week period of time indicated that fecal coliform regrowth did occur but was dependent on the moisture level of the soil. *Salmonella* did not regrow in soil during the summer of 2004 but regrowth to minimal levels after land application did occur in the summer of 2005 experiment.

Although regrowth of fecal coliforms occurred in soil after land applying Class A biosolids, their levels were not indicative of *Salmonella* levels in the soil. This loss of parity upon land application may be a consequence of competing micro flora in the soil (Gibbs *et al.*, 1997; Hay, Jonathan, 1996; Sidhu *et al.*, 2001,Soares and Cardenas, 1995, Yeager and Ward, 1981), high levels of coliforms or the improbability of a small inoculated population to reestablish itself (Zaleski *et al.*, 2005).

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APPENDIX B: MATHEMATICAL MODEL TO PREDICT PATHOGEN DIE-OFF IN BIOSOLIDS BASED ON MOISTURE AND TEMPERATURE STRESS UNITS

ABSTRACT

The objectives of this study were to quantitatively determine the degree to which temperature and moisture influence the inactivation of fecal coliforms in biosolids and develop a mathematical model that predicts pathogen inactivation in biosolids while exposed to solar drying during the conversion from Class B to Class A levels. Aerobically-digested biosolids were amended to four (4) different moisture levels and exposed to three (3) different temperature levels. Samples were inoculated with *E. coli* and sacrificed over a 10 to 20 day period depending on the temperature. Results from the experiment demonstrated that under controlled conditions the main factors of temperature and moisture as well as their interaction significantly influenced the inactivation rate of fecal coliforms at an alpha level of 0.05, $p << 0.05$. For each temperature, the average inactivation rate at the 10% moisture level was always greater than the average inactivation rate at the other moisture levels. Similarly, inactivation rates at the temperature level of 38°C were greater than inactivation rates at the lower temperatures for each moisture block of samples. A mathematical model developed from this study predicted the inactivation rate of fecal coliform levels in biosolids in open solar drying beds with an average error rate of 13.5%.

Keywords: biosolids, fecal coliforms, inactivation, moisture content, sewer sludge, solar drying, temperature

1. INTRODUCTION

Experimental field studies have demonstrated that solar drying in semi-arid and arid locations during warm dry seasons can successfully convert Class B to Class A biosolids (Choi *et al.*,2005), but it is unclear as to whether the mechanisms for pathogen inactivation are due to temperature, moisture level, a combination of these two factors, or the rate of drying. Studies from the literature indicate that there are numerous factors which affect the inactivity of pathogens and pathogen indicators in soil and in sewer sludge mixed with soil. The primary factors can be narrowed to moisture, temperature, to rate of desiccation, and competing microorganisms in the soil (Bitton *et al.*, 1984; Pepper *et al.*, 1993; Straub *et al.*, 1992; Redlinger, 2001; Trevisan, 2002; Sidhu *et al.*, 2001). The ecology of the media also plays a role in pathogen survival. Studies by Yeager and O'Brien (1979) indicated that virus adsorption is dependent on soil pH, CEC (Cation Exchange Capacity), organic matter content, and clay content. Viruses survived longer in the septic liquor than in the ground water and surface water, more than likely due to the organic content of the sludge and water adsorption properties.

There is limited literature that addresses enteric pathogen inactivation in the (sole media of) biosolids. In this controlled laboratory study, temperature and moisture were held constant while the inactivation rate of the pathogen indicator organism, fecal coliform, was assessed over time. Fecal coliforms are gram negative bacteria that serve as pathogen indicators for enteric pathogens and are frequently assayed for the purpose of designating the bacterial microbial quality of biosolids (40 CFR Part 503) in lieu of *Salmonella*.

2. METHODS AND MATERIALS

2.1 PRELIMINARY ANALYSIS

In an effort to maintain constant moisture levels throughout the duration of the experiment, the moistened biosolids were placed in air-tight sterile Nalgene® jars. To establish if the air-tight jars would significantly impact fecal coliform inactivation, a comparison was made between fecal coliform survival rate in biosolids in open jars stored at room temperature versus biosolids in closed jars stored at room temperature. Freshly collected aerobically-digested biosolids (~85.6% TS), from the Green Valley Wastewater treatment plant(GVWWTP) were packed into sterile Nalgene® jars; two of the sets were tightly capped, while the remaining two sets were left uncapped. All jars were placed into an incubator with high relative humidity at a temperature of approximately 25°C. Two replicates from each set were sampled on a schedule of days 0,1,3,5,7,10, 15 and 20. The inactivation rates were calculated and compared using a two-sample t-test and assuming equal variances. The analysis indicated that the inactivation rate of fecal coliforms in biosolids in the closed jars was not significantly different from the inactivation rate in the open jars, at an alpha level of 0.05, $p = 0.318$. The decision was therefore made to move forward with the microcosm study and maintain constant moisture of biosolids by capping the Nalgene® jars.

2.2 EXPERIMENTAL DESIGN AND PREPARATION

The controlled study was designed as a factorial experiment to investigate the significance of temperature and moisture and their interaction on the inactivation rate of fecal coliforms (Table B-1). Three temperature levels (25°C, 32°C and 38°C) and four

Table B-1. Experimental design.

Average Temperature ± std deviation	*Moisture Levels (H_2Og/g total mass)*	*Duration of Experiment (days)*	*Biosolids per sample (g)*
25 ± 1.7	10-35-65-80%	20	10
32 ± 2.4	10-35-65-80%	15	10
38 ± 2.1	10-35-65-80%	9	10

moisture levels, 10%, 35%, 65%, and 80% (g water/g wet sample) were investigated. These values represent the median to upper temperature ranges measured during summer field experiments, while the moisture levels span nearly the full range of water content of the biosolids during the drying process. Solar dried, aerobically-digested and belt-pressed biosolids were collected from the Green Valley Wastewater Treatment Plant in Pima County, Arizona. Samples were prepared by placing 10.0 g of the collected dry biosolids into each Nalgene® jar, and amending with sterile distilled water to the desired moisture level. Each jar was then inoculated with 1.0 ml or less of *Escherichia coli* ATCC 15592 (obtained from the Department of Soil, Water and Environmental Science, University of Arizona). Prepared samples, as described above were placed into a convection oven (Isotemp vacuum oven 282A, Fischer Scientific International, Inc., Hampton, N.H.) which was placed into a walk-in refrigerator at 4°C. A total of three temperature runs were conducted, each at the separate temperatures of 25°C, 32°C, and 38°C. The temperatures were chosen to parallel a range of average heat units measured during the warmer field drying experiments conducted at the Green Valley WWTP in 2004 and 2005.

One thermocouple each was secured to the top and bottom racks of the convection oven and a CS500 RH and temperature sensor (Campbell Scientific, Logan, Utah, 2003)

was positioned on the middle rack inside the oven. Continuous data-logging indicated the following oven temperature runs and their corresponding standard deviations:

2.3 SAMPLE PREPARATION

Solar dried (from June 2005 through September 2005) biosolids from the GVWWTP were collected from experimental drying beds in October 2005 and stored at 4°C until utilized. 10 grams of the biosolids were placed into each of the 84 sterile tared and labeled Nalgene® jars. Samples were amended to moisture levels of 10%, 35%, 65%, and 80% (g H_2O /g total mass) with the combination of sterilized de-ionized water and the amount of injected inoculum. Seven sampling days were scheduled for each temperature run and three replicates of each moisture level were prepared. Samples were retrieved from the oven and weighed prior to sacrificing. These weights were compared to the initial weights recorded at the time the Nalgene® jars were prepared. The inoculum was prepared by isolating a single colony of *E. coli* 15592 from a TSA plate and inoculating a 100 ml flask of Tryptose Soy Broth (TSB) for approximately 18 hours in an incubator at 38°C. The initial titer levels were enumerated by plating and dilution on m-Endo Agar; the approximate titer levels at the time of inoculation were $\log 10^8$ CFU/ml.

2.4 ASSAY FOR FECAL COLIFORMS

The dilution and plating method was used to enumerate fecal coliforms for the samples prepared at the 10%, 35%, 65%, and 80% moisture levels adjusted by utilizing sterilized deionized water. The dilution solution was comprised of sterile K_2PHO_4 and the agar utilized for plating was m-FC agar (Acumedia, Baltimore, MD). At the 10%

moisture level, the three-tube Most Probable Number (MPN) Method was used in addition to the plating technique to enumerate the biosolids prepared at the 10% moisture level (American Public Health Association, 1998). The reason for the two different techniques was to obtain readings below $\log_{10} 2$. The MPN method is particularly useful for low concentrations of organisms (<100/g) especially for those samples whose particulate matter may interfere with accurate colony counts (Peeler and McClure, 2003)

3. RESULTS AND DISCUSSION

The temperatures within the oven (at the different rack levels) were within ± 2.4 ºC from each other; the deviation was due mainly to a spatial gradient and the opening of the unit to retrieve samples for assay purposes. Samples were placed randomly within the oven to offset bias in temperature differentials. The moisture level of all samples was maintained by the capping of the Nalgene® jars as evidenced by the minimal variance between the mass of the samples on packing and the recorded mass upon sacrificing, (i.e. variance, $s^2 = 0.28$ g). Mold was found consistently in all biosolids' samples containing a moisture content of 35% for all temperature runs. The mold was white in color and filamentous in shape.

3.1 SIGNIFICANT PHYSICAL FACTORS

The first order inactivation rate constant, k_d, for fecal coliforms, $k_d = -\frac{\log(N_t / N_o)}{t}$ (Maier *et al.*, 2000) were determined for each replicate and each moisture block run at the three different temperature levels and are shown in Table B-2. The inactivation rates were calculated to the Class A level (10^3MPN/g or 10^3 CFU/g). An analysis of variance of these inactivation rates is shown in Table B-3 below. The small sum of squares for

temperature (T) and moisture (Θ) indicate that these factors significantly impact fecal coliform inactivation at the alpha level of 0.05. The ANOVA table also demonstrates that the interaction between temperature (T) and moisture (Θ) significantly impacts the reduction of fecal coliform in biosolids, $p << 0.01$.

In general, within the blocked moisture treatments, fecal coliform consistently demonstrated increased rate reduction as temperature levels increased. Within the temperature treatment blocks, inactivation tended to increase as moisture decreased. The largest inactivation rates were demonstrated at the highest temperature level (38°C) and the lowest moisture content (10% g/g) combination.

Figure B-1 indicates the pattern of fecal coliform inactivation for all temperature runs. In some cases the standard deviation was high, especially towards the latter days of the experimental runs as demonstrated in the graphs. Regrowth of fecal coliforms was a probable reason for these high levels of standard deviation. This can especially be noted at the 65% moisture level at both temperature runs of 32°C and 38°C. Only one data point was determined to be an outlier and therefore not considered in the calculation of k_d (Day 3, T=38°C, M=35%) as it was greater than two (2) standard deviations from the mean.

Tailing effects are evident with the majority of the inactivation patterns shown above and are especially obvious at the moisture level of 10%. This phenomenon has been found in a number of microorganisms and is generally regarded as a measure of a subpopulation's resistance or ability to produce heat shock proteins (Humpheson *et al.*, 1998). Figure B-2 clearly indicates the independent effects of moisture and temperature

on the inactivation rate of fecal coliform. As the physical stressor becomes more extreme (minimal moisture and higher temperature), the inactivation rate, k_d, changes significantly. Figure B-3 clearly shows the synergism between moisture and temperature; the greatest rate of inactivation occurs when temperature is at a high level and moisture is at a low level as evidenced by the peak shown along the positive z-axis.

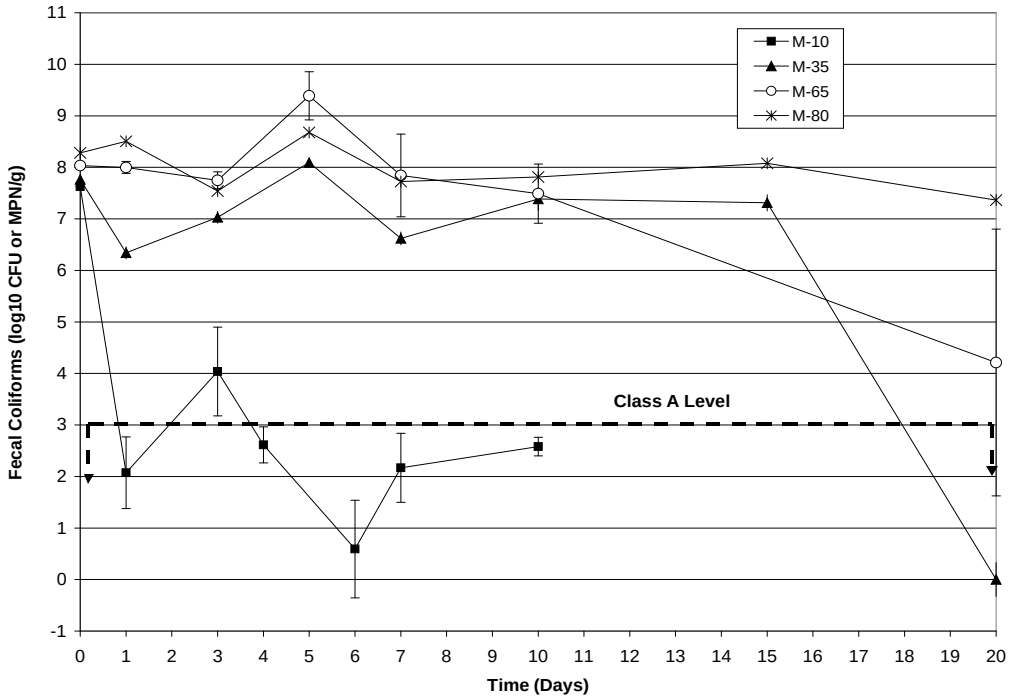
Table B-2. Inactivation rate constant, k_d, (3 replicates) for temperature and moisture matrix.

	Moisture				
Temperature	10%	35%	65%	80%	Means (T)
25°C	0.74,0.88, 0.91	0.25,0.26, 0.26	0.31, 0.02*, 0.25	0.02, 0.03, 0.05	0.33
32°C	0.69,0.66,0.51	0.02,0.39,0.29	0.70,0.57,0.41	0.30,0.30, 0.40	0.44
38°C	3.3,2.7, 2.6	1.47,0.41, 0.50	1.1,0.50,1.2	1.1,0.9,1.1	1.41
Means (Θ).	1.44	0.43	0.56	0.47	Total Avg = .73

*Value is an outlier, i.e. > 3σ

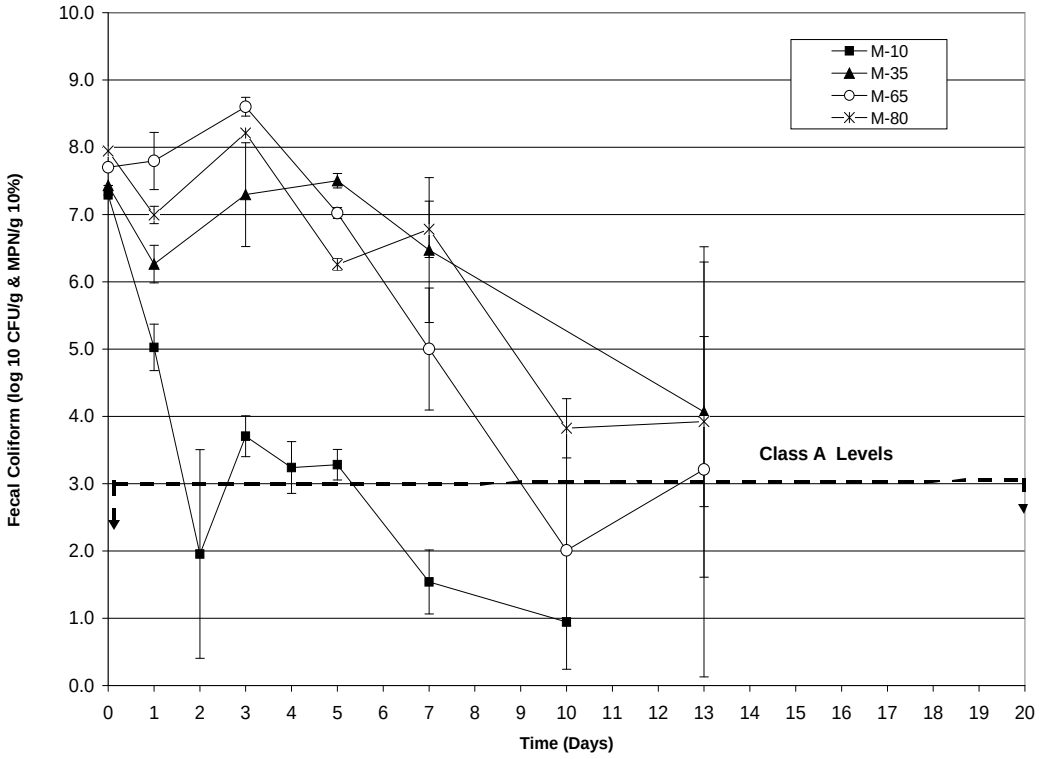
Table B-3. Two-way ANOVA: kd versus moisture and temperature.

Analysis of Variance for kd					
Source	DF	SS	MS	F	P
Moisture	3	6.2782	2.0927	33.59	0.000
Temp	2	8.4302	4.2151	67.65	0.000
Interaction	6	3.7646	0.6274	10.07	0.000
Error	24	1.4955	0.0623		
Total	35	19.9685			

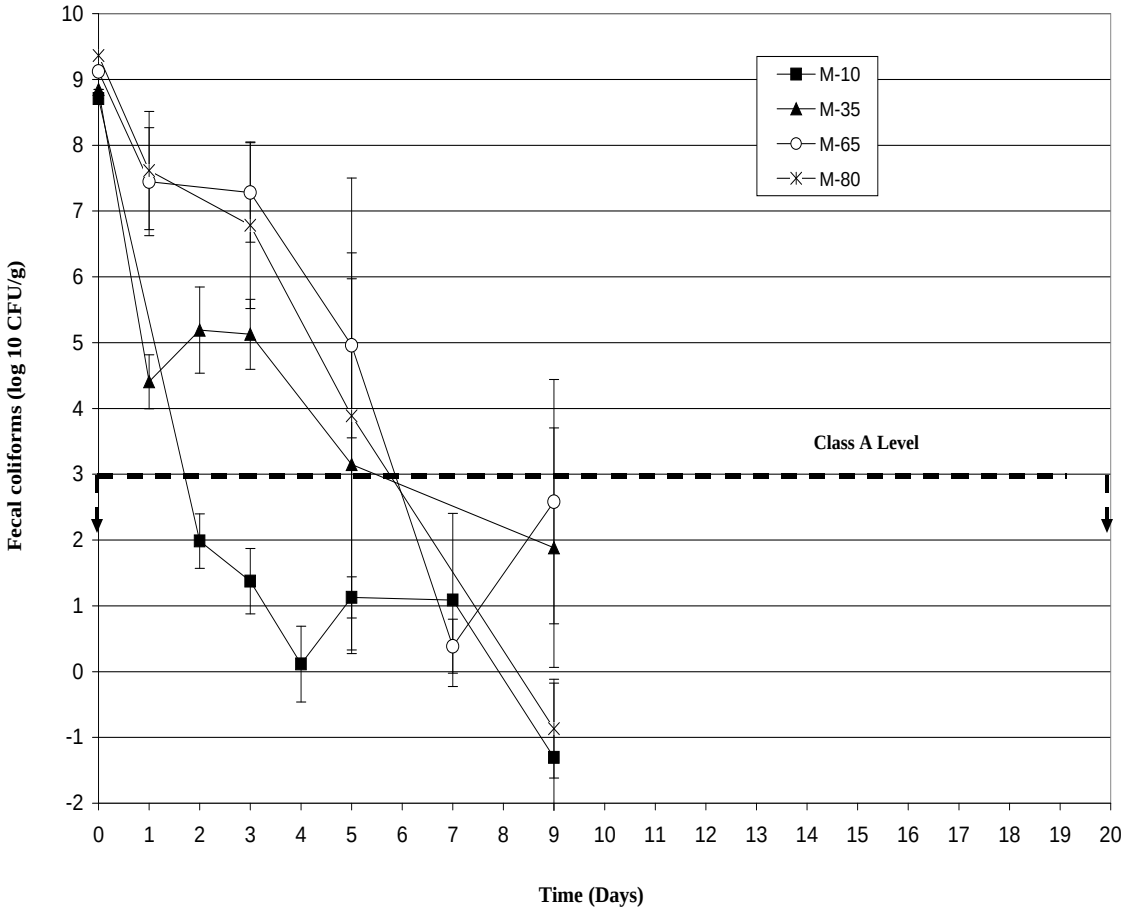


(a) Temperature = 25°C

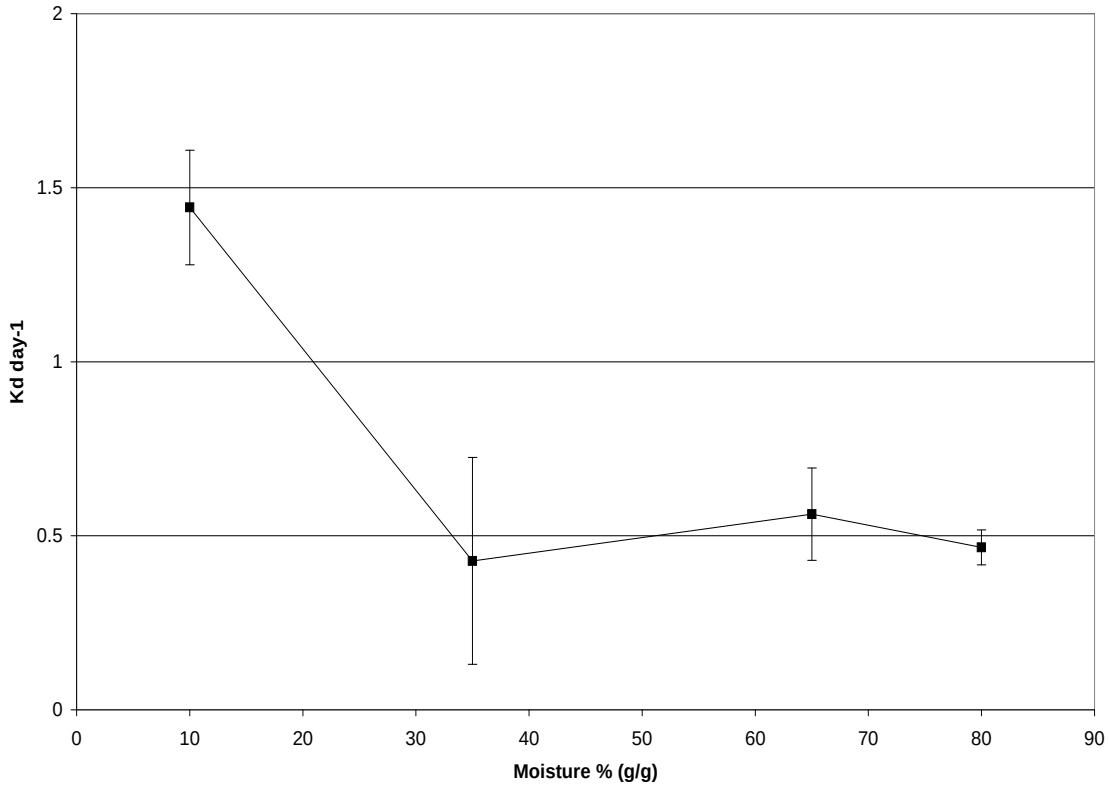
Figure B-1. Inactivation patterns for the fecal coliforms at the four moisture levels and three temperatures. Moisture is on a mass basis (g H20/g total mass) 10% (M-10), 35% (M=35), 65% (M=65%) and 80% (M=80). Temperature levels are: (a)T=25°C; (b) T= 32°C; and (c) T= 38°C. Each point represents the average of three replicates, the error bars represent the standard deviation from the mean.



(b) Temperature = 32°C

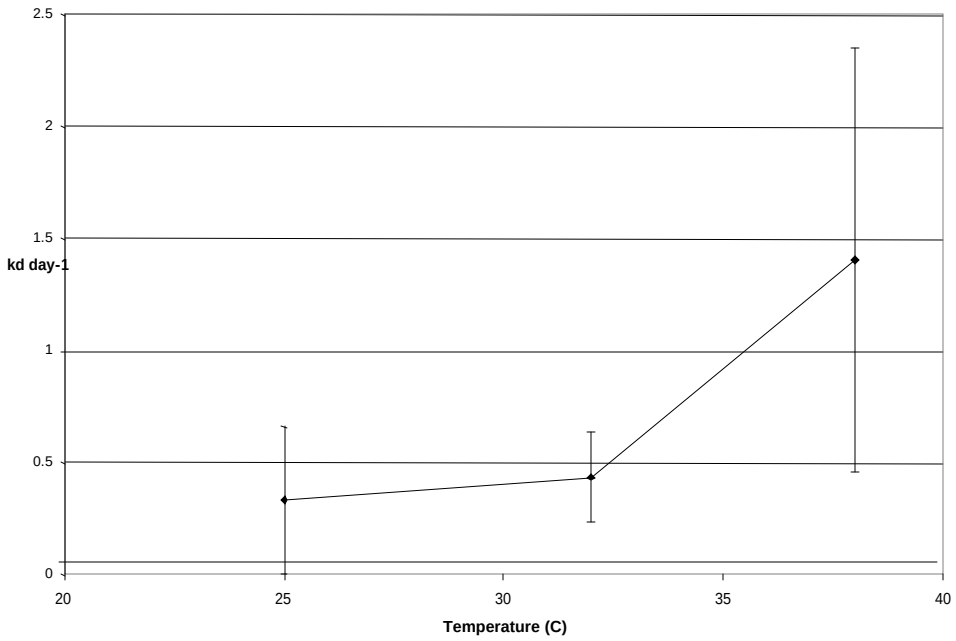


(c) Temperature = 38°C



(a) Inactivation as a function of moisture level in biosolids

Figure B-2. Fecal coliform inactivation, kd as a function of: (a) moisture for all data; and (b) temperature all data points of the microcosm study. Error bars represent the standard deviation from the mean.



(b) Inactivation as a function of temperature.

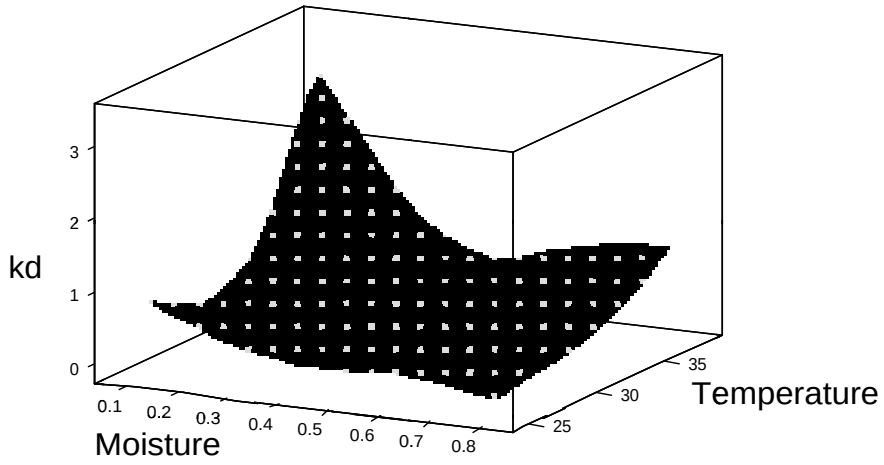


Figure B-3. Surface contour plot of fecal coliform inactivation versus temperature and moisture of biosolids.

3.2 MODEL DEVELOPMENT

In order to predict N_t , the density level of fecal coliform at time, t, during the drying process, it is necessary to formulate a model predicting k_d. SYSTAT® (Version 8.0, Copyright SPSS, Point Richmond, CA) software was employed to determine a feasible prediction model for N_t. The rationale behind using Equation B.2 is that patterns of fecal coliform inactivation rates as a function of temperature and moisture are similar to Michaelis Menten-type kinetics (Shuler and Kargi, 2002). The similar formation of Monod kinetics has been used successfully to summarize mineralization data from aqueous incubation, the biodegradation of organic compounds and substrate depletion (Koch *et al.*, 2001). Figure B-2 was utilized to form the basis for the modeling terms and aided in determining the initial estimates for the iteration process. Non-linear multiple regression analysis using the Gauss-Newton procedure was utilized to determine the prediction values of k_d. This method is the process most commonly used in software computing algorithms for finding the least squares estimator in a nonlinear model (Myers, 1990). Best-fit models of k_d using linear regression against the parameters of T and Θ yielded R^2 values less than 0.71 and were therefore abandoned for the non-linear approach.

Using the first order equation:

$$N_t = N_o * 10^{-k_d t} \qquad \text{(B.1)}$$

where $k_d = f(\Theta,T)$, where Θ = moisture (g H_2O/g total sample), and T = average daily temperature. Inactivation or k_d was determined to be:

$$k_d = \left(\frac{k_1}{k_1 + \Theta}\right) * \left(\frac{T}{k_2 - \mathrm{T}}\right) * k_3 \qquad \text{(B.2)}$$

where $k_1 = 0.112$, $k_2 = -41.88$, and $k_3 = -0.5357$; for all T 38ºC, T=38ºC.

Figure B-4 shows a comparison of predicted and observed inactivation values; data is constrained to the microcosm study. R^2 describes the amount of variation in the observed response values that is explained by the predictor(s) T and Θ. The largest deviation in the predicted versus observed values of k_d lie within the 35% moisture block at the 38ºC temperature level. The prediction level is greater than the observed for two of the three replicates. 35% moisture content may be an optimum moisture level for fecal coliform to thrive or perhaps the mold observed in the Nalgene® jars were competing with the fecal coliforms.

Inactivation rates of fecal coliform for the field experiments were calculated based on linear regression of $\log(N_t/N_o)$ over the time needed to reach Class A and are shown in Table B-4. The values for the predicted inactivation rate constants, k_d, were calculated using Equation (B.2) where temperature (T) and moisture (Θ) were the average daily heat units measured in the biosolids and the average moisture content of the biosolids, respectively. Comparison of the observed versus the predicted demonstrates a 13.5 % error rate on the predicted $\log(N_t)$ value. Figure B-5 demonstrates the relationship between predicted values of k_d and the measured values of k_d from the semi-arid and arid field experiments.

Fecal coliform inactivation rates, the average moisture content and the average daily heat units at the time of sampling, were calculated from data sets from solar drying

field experiments conducted in Quartzsite, Arizona (Grabau, 2005) and Wadi Hassan, Jordan (Al-Hmoud, 2006) in addition to the data measured from the solar drying experiments at the Avra Valley and Green Valley Wastewater Treatment Plants. The data from Quartzsite required capping of the temperature of the biosolids to 38ºC. The fact that data sets from various locations yield good results for estimating fecal coliform inactivation favors the use of such a model to predict pathogen levels, N_t, at any time, t, from the predicted k_d values and known initial values of fecal coliforms in freshly-produced biosolids:

$$Nt = N_o * 10^{-k_d t}$$

Predicted rate constant values with a lesser % error are for those field experiments conducted in semi-arid locations with no additional treatments. Those indicating a larger % error are those experiments located in an arid region where tilling had been applied to the drying process, Table B-4.

With future studies and analyses it may be possible to predict the moisture content of the biosolids from the environmental data measurements made during each solar drying experiment. This will allow for full remote monitoring of pathogen levels in biosolids in solar drying fields.

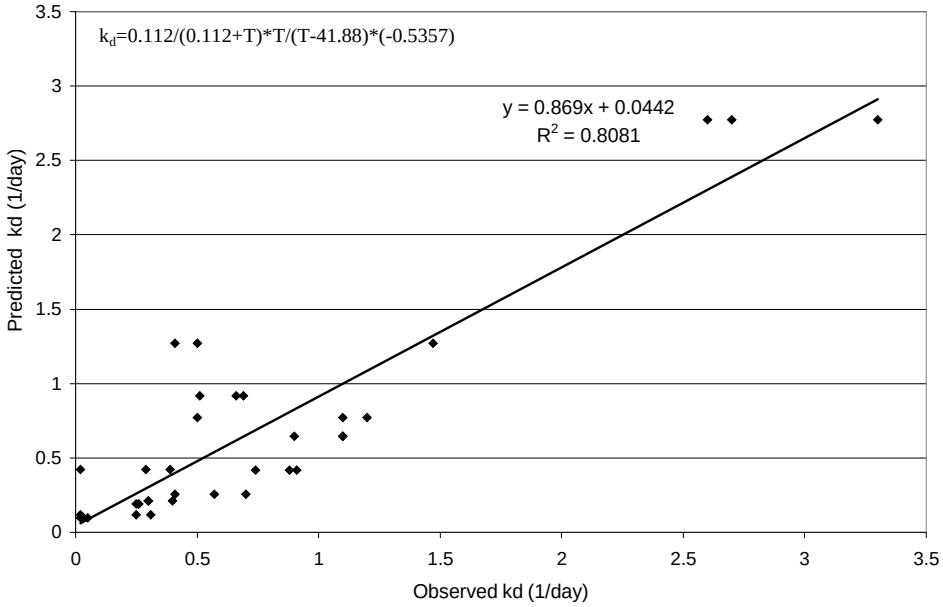


Figure B-4. Comparison of non-linear regressed predicted values versus observed values for inactivation of fecal coliform in biosolids under controlled conditions.

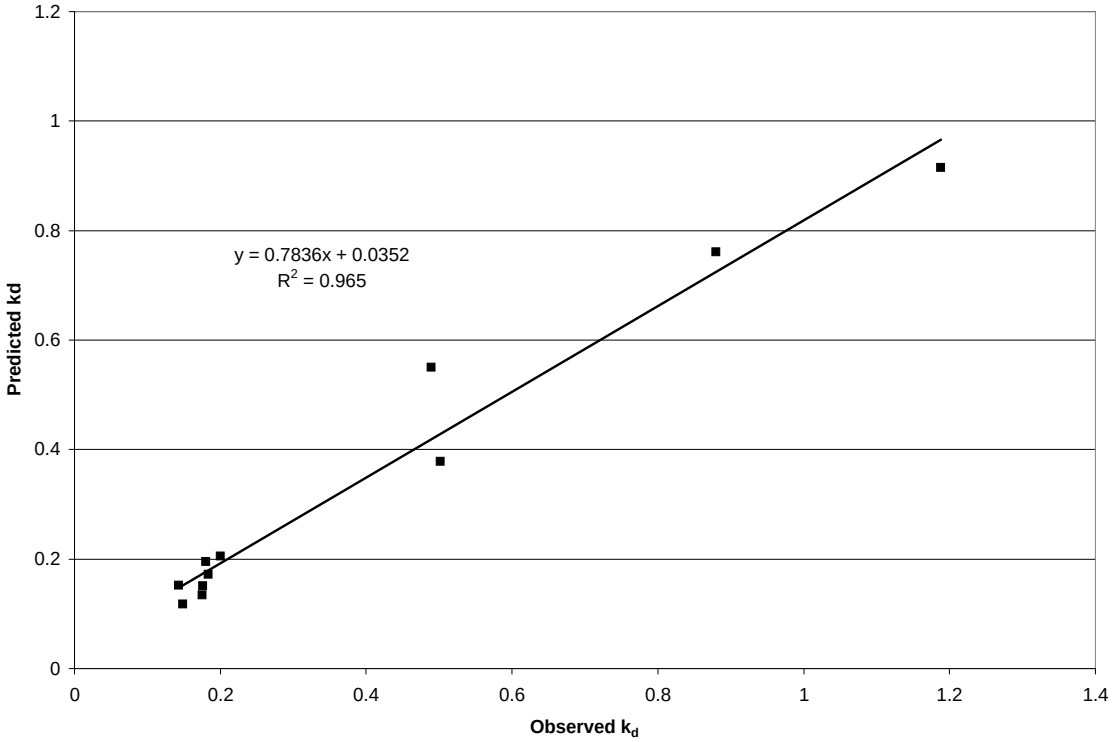


Figure B-5. Comparison of observed fecal coliform inactivation rates (field experiments) and predicted inactivation rates using Equation (B.2).

Table B-4. Comparison of predicted and observed inactivation rates (inactivation rates were based on obtaining Class A levels).

Experiment	Biosolids Type	k_d observed	% Average Moisture (g H2O/g total mass)	AVG Daily HU (ºCelsius)	k_d predicted	% Error
GVWWTP, Summer 2005	Aerobic no till	0.14	83.82	27.22	0.12	17.6
GVWWTP, Summer 2005	Aerobic-cover	0.49	84.54	37.59	0.55	12.5
GVWWTP, Summer 2005	Aerobic -till open, bed 4	0.18	83.55	29.47	0.15	14.7
GVWWTP, Summer 2005	Aerobic Till open, bed 5	0.18	81.53	30.44	0.172	6.3
GVWWTP, Summer 2004	Aerobic-Sand	0.14	51.91	25.75	0.151	5.6
GVWWTP, 2004	Aerobic No sand	0.20	38.07	26.3	0.205	2.5
Wadi Hassan-Summer 2004	Aerobic	0.17	67.25	26.64	0.133	21.8
Wadi Hassan - Spring 2005	Aerobic	0.18	66.35	29.83	0.191	6.1
Quartzsite, Summer 2004	Anaerobic-Control	0.501	67.55	34.85	0.38	24.6
Quartzsite, Summer 2004	Anaerobic-Moderately Tilled	0.88	65.65	38.00	0.76	13.5
Quartzsite, Summer 2004	Anaerobic-Intensively Tilled	1.188	53.44	38.00	0.92	23.0
Average %Error 13.46						

4. CONCLUSIONS

Pathogen inactivation in biosolids exposed to open solar drying is likely the result of a number of parameters and their complex interaction – physical, chemical, and biotic. This study demonstrated that temperature and moisture are two major physical factors impacting inactivation. Maintaining levels of known temperature and moisture over time, demonstrated that an increased inactivation rate for fecal coliforms occurs at a threshold temperature near 38ºC and also at a minimum threshold moisture level near 10% (g/g). A previously controlled study investigating the effects of temperature and moisture on virus survival in soil found similar results in that evaporation of soil moisture at higher temperatures accelerates virus (coliphage PRD-1) inactivation significantly and decrease in soil moisture to approximately 5% also increased the inactivation rate of the coliphage PRD-1 virus (Song *et al.*, 2005).

Heat is known to injure cells by damaging cell membranes when heat is transferred from surrounding water molecules (Woo *et al.*, 2000). At high temperatures, heat will impact microorganisms by damaging their cellular membrane and possibly intracellular proteins, DNA, and ribosomes (Humpheson *et al.*, 1998). As biosolids dry, they become very porous and the air replaces the water within the pores. The specific heat of air is much less than that of water (1.005 kJ/kg·K @ 300K compared to 4.179 kJ/kg·K @ 300K, respectively). Therefore it requires more energy to raise the temperature of wet biosolids than it does to raise the temperature of dry biosolids.

As water is lost from biosolids, the temperature increases 5 to10ºC above ambient air temperature, making cooling by evaporation no longer feasible at the surface (O'Shaughnessy *et al.*, 2005).

Low moisture levels affect cellular activity and it has been shown that reduced moisture levels affect the water activity of microorganisms in soil. West *et al*. (1992) showed via laboratory experiments that the respiration and metabolic activity of microorganisms in soil decreased linearly with the decline of volumetric moisture content of the soil. According to Stark and Firestone (1995), moisture may limit microbial activity in a wide range of environments including salt water, food, soil and biofilms.

Although *Salmonella* assays were not performed during this microcosm study, it is likely that moisture and temperature would have similar affects on their inactivation rates. *Salmonella* along with *Escherichia* are included in the large group of *Enterobacteriaceae* which are gram negative, non-sporulating, rod-shaped bacteria that share many biochemical and physiological properties (Madigan *et al*., 2003)

Physical factors are not the only parameters that significantly affect pathogen inactivation in biosolids. Further studies should be performed on the biotic effects on pathogen inactivation in biosolids as well as the impact of the desiccation rate of biosolids on pathogen survivability.

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APPENDIX C: MANAGING THE BIOSOLIDS' CONVERSION PROCESS FROM CLASS B TO CLASS A USING SOLAR DRYING TECHNOLOGIES

ABSTRACT

Seasonal solar drying experiments in medium sized experimental beds were conducted at the Green Valley Wastewater Treatment Plant in Pima County, Arizona for the purpose of determining the impact of tilling and a covered structure to intensive solar heating on the reduction of pathogens in aerobically-digested biosolids and to investigate the potential for an automated rain shield to abate bacterial regrowth in drying beds during rainfall events.

During summer solar drying periods, the covered structure increased the inactivation rate of fecal coliforms by 36% over all treatments and the automatic rain shield did abate fecal coliform regrowth from the summer rains. Also of significance was that tilling during a cool wet season increased the %TS of the biosolids and aided in pathogen reduction. Tilling during the summer season and dry winter season had no significant impact on pathogen inactivation. The advantages of a rain shield during a winter season were inconclusive.

Keywords: biosolids, solar drying, rain shield, tilling

1. INTRODUCTION

GVWWTP has a capacity of 1.2 million gallons per day (MGD) and is located in a semiarid region in southwestern Arizona. The plant was upgraded in 2004 from aerated lagoons to an activated sludge wastewater treatment plant with an oxidation ditch for biological nutrient removal (BNR) and aerobic sludge digesters for waste stabilization.

Aerobic digestion has been widely used in wastewater treatment plants for many years and is a common method of treatment for small to medium sized facilities (Turovskiy, 2001). Solids produced from the treatment of sewage are further treated in various manners to accomplish dewatering and disinfection. Treatments are geared towards disposal of the residuals and typically include chemical, mechanical, and thermal conditioning. At the GVWWTP, solids are released from the aerobic digesters to a belt filter and press for dewatering. The belt press is run approximately 3 days per week and produces a Class B biosolids that are typically in the range of 12-15% total solids (Pima County Wastewater Management data, 2006). The cake is then spread onto onsite concrete lined solar drying beds for dewatering and pathogen reduction. During the commercial drying process, a front-end loader is used to turn over the material at a rate of one time per week (ideally). Periodic turning of the sludge has been found to increase the drying rate, decrease the insect populations and reduce the odor problem through increased aeration and disturbing the incubation of insects (Chen *et al.*, 2002). After the 90 day period, the biosolids are piled onto a concrete lined holding bed in the configuration of a windrow until test results indicate that the material is at the Class A level. The required testing consists of analyses for the presence of heavy metals, compliance with vector attraction criteria, and microbial density levels (Eljerdi, 2006).

The solar drying of biosolids for dewatering purposes has been a common practice throughout the United States and other first world countries (Metcalf and Eddy, 2004). Also, the uses of covered structures have a historical place in managing biosolids. In 1938, glass covered structures were used on drying beds in the northern part of the United States when the climate became the limiting factor to dewatering biosolids (Carpenter, 1938). Recently, the University of Hohenheim and a private company, Thermo-System, developed a fully automated low temperature solar drying plant that allows for sludge stabilization and dewatering. The system has integrated automated tilling to improve the dewatering process (Bux *et al.*, 2002).

1.1 OBJECTIVES OF THIS EXPERIMENT

The goals of this experiment were to investigate the time required to produce a microbial Class A material at this site and to augment the current drying process by integrating (1) enhanced passive solar heat gain using a covered structure; (2) tilling; and (3) prevention of regrowth of pathogens and pathogen indicators using an automated retractable shield during the summer monsoon and winter rainy seasons.

2. METHODS

2.1 EXPERIMENTAL SET-UP

Four separate field experiments were conducted at this site:

(1) Summer of 2004 (May 5– July 26);

(2) Summer of 2005 (June 16-August 10);

(3) Winter of 2005 (January 16 – April 15); and

(4) Winter of 2006 (January 23- March 14).

During the summer of 2004, baseline studies were conducted on mesophilic anaerobically-digested biosolids and aerobically-digested biosolids by placing them on high-density polyethylene (HDPE) liners (which simulate concrete lining) and sand lined drying beds. The beds with HDPE liners were constructed with false bottoms so that the finished floor elevation of these beds was congruent to the sand lined beds. During the winter 2005, summer 2005, and winter 2006 experiments, the experimental controls were conducted in the HDPE lined beds. The sand drying beds were utilized for solar drying of biosolids receiving tilling treatments. Mixing sand with the biosolids was unavoidable during the tilling treatments. All beds measured 1.0 m (width) x 3.0 m (length) were sloped to the north and received a load of approximately 600 kg of biosolids placed to a depth of 20 cm (Figure C-1). All beds were tilled twice a week on the same days. Tables C-1, C-2 and C-3 provide detailed information on the experimental beds that were utilized during the different solar drying seasons and information regarding the type of biosolids used in each bed.

A remote data collection and communication system was set up within 3.0 m of the experimental beds. Environmental parameters such as outdoor temperature, relative humidity, solar radiation, wind speed, and the temperature of the biosolids and their moisture content were monitored continuously throughout the experiment. Data was collected every 20 seconds, averaged and stored hourly. Data was downloaded from the datalogger on a regular basis by a RF telemetry/land-line telephone link.

The retractable shield shown in Figure C-2 was designed and constructed for the present study. The circuitry for the automated control is shown in Figure C-3. The frame for the

shield was welded steel and a weather-proof tarp, riveted onto the frame. A direct current (dc) motor was connected to the rear shaft of the shield and a double-pole/double-throw switch allowed bi-directional movement. The shield had four grooved wheels (2 front-end and 2 rear-end) which were guided along a metal track placed on either end of the bed. Two separate wheels (one on each side) were attached to the shield frame by an extended bracket and positioned along the bottom of the top rail to prevent the shield from lifting up during high winds. Closure of the shield was activated by rain tipping the gauge switch inside the rain bucket (Campbell Scientific, Logan, Utah). The minimum amount of rain required for tipping was 0.254 mm. A switch closure was transduced into a signal that was fed to the CR10X datalogger (Campbell Scientific, Logan, Utah) which in turn sent a pulse to the relay driver (Campbell Scientific, Logan, Utah) which amplified the signal and triggered a mechanical relay providing power to the motor. An inner set of micro-switches provided a "sense of position" (covered or uncovered) for the shield. The outer set of micro-switches functioned as safety mechanisms (wired in the normally closed position) which opened the circuit if the shield contacted either one of these switches, dropping power to the motor. The latter circuitry was intended to prevent the shield from rolling off the track at either end during the course of experiment.

The covered structure shown in Figure C-4 covers the sand drying bed that is 1.0 m wide by 3.0 m long. The cover is a clear polyethylene plastic stretched over ½" pvc (poly vinyl chloride) pipe frame.

Table C-1. Experimental set-up for solar drying beds- Summer 2004

Containers	1a	1b	2a	2c
Biosolids	Anaerobically-digested	Aerobically-digested	Aerobically-digested	Anaerobically-digested
Initial %TS	6.57%	16.37%	16.37%	6.57%
Drainage Type	HDPE Liner	HDPE Liner	Sand	Sand
Depth of Biosolids	20 cm			
Dimensions	1.0 m (width) x 3.0 m (length) x 0.6 m (depth)			
Moisture Sensors	*Water Content Reflectometry probe, located 2 cm from finished floor elevation			
Temperature Sensors	T-type thermocouples spatially located at depths of 1 cm, 2cm and 3 cm from finished floor elevation			

*WCR- water content reflectometry probe (Campbell Scientific, 2002)

Table C-2. Experimental set-up for solar drying beds- winter 2005

Containers	1a	1b	2a	2b	2c
Biosolids	Anaerobically-digested	Aerobically-digested	Aerobically-digested	Aerobically-digested	Aerobically-digested
Initial %TS	8.03	14.14	14.14	14.14	14.14
Drainage Type	HDPE Liner	HDPE Liner	Sand	Sand	Sand
Depth of Biosolids	20 cm	20 cm	20 cm	20 cm	20 cm
Treatment	None	None	Covered – No Till	Covered-Tilled	Open-Tilled
Dimensions	1.0 m (width) x 3.0 m (length) x 0.6 m (depth)				
Moisture Sensors	*Water Content Reflectometry probe, located 2 cm from finished floor elevation				
Temperature Sensors	T-type thermocouples spatially located at depths of 1 cm, 2cm and 3 cm from finished floor elevation				

Table C-3. Experimental set-up for solar drying beds- Summer 2005 & Winter 2006

Containers	1a	1b	2a	2b	2c
Biosolids	Anaerobically-digested	Aerobically-digested	Aerobically-digested	Aerobically-digested	Aerobically-digested
Initial %TS	2.9/6.1	12.9/15.67	12.9/15. 7	12.9/15. 7	12.9/15. 7
Drainage Type	HDPE Liner	HDPE Liner	Sand	Sand	Sand
Depth of Biosolids	20 cm				
Treatment	None	None	Covered – Tilled	Retractable Roof- Tilled	Open-Tilled
Dimensions	1.0 m (width) x 3.0 m (length) x 0.6 m (depth)				
Moisture Sensors	*Water Content Reflectometry probe, located 2 cm from finished floor elevation				
Temperature Sensors	T-type thermocouples spatially located at depths of 1 cm, 2cm and 3 cm from finished floor elevation				

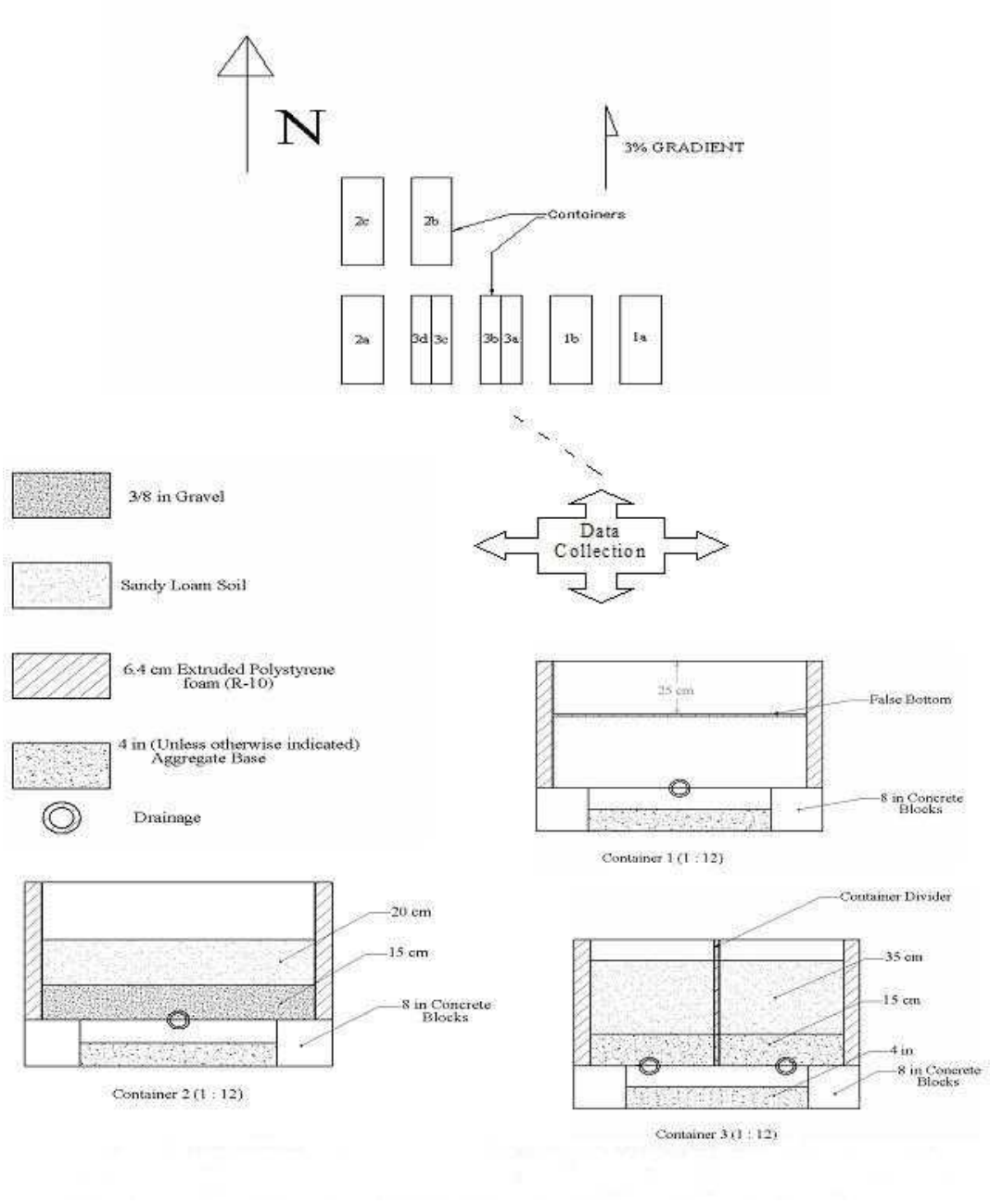

Figure C-1. Solar drying experimental layout at the Green Valley WWTP: containers 1a-b:HDPE liner, beds 2a-d:sand lined, and containers 3a-d, used for land application.

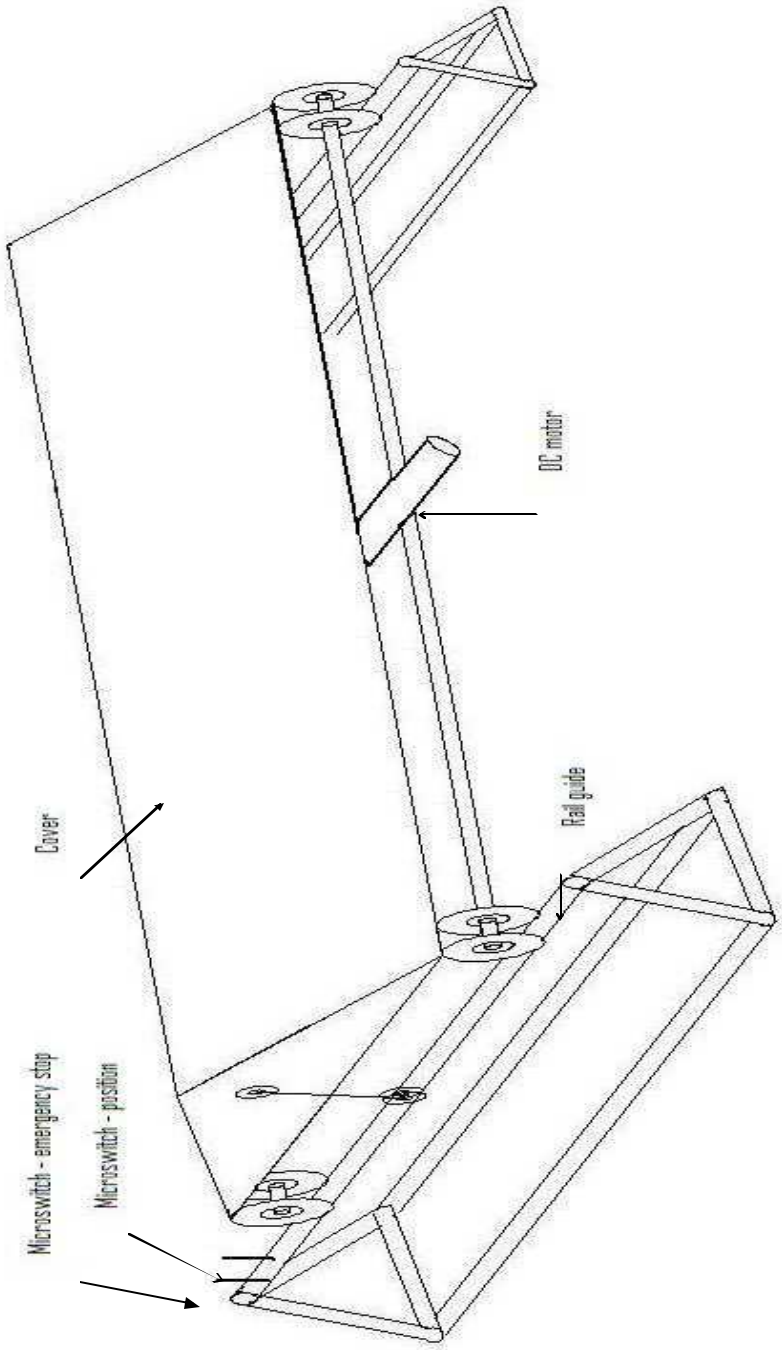

Figure C-2. Retractable cover, moves into position based on control from rain gauge.

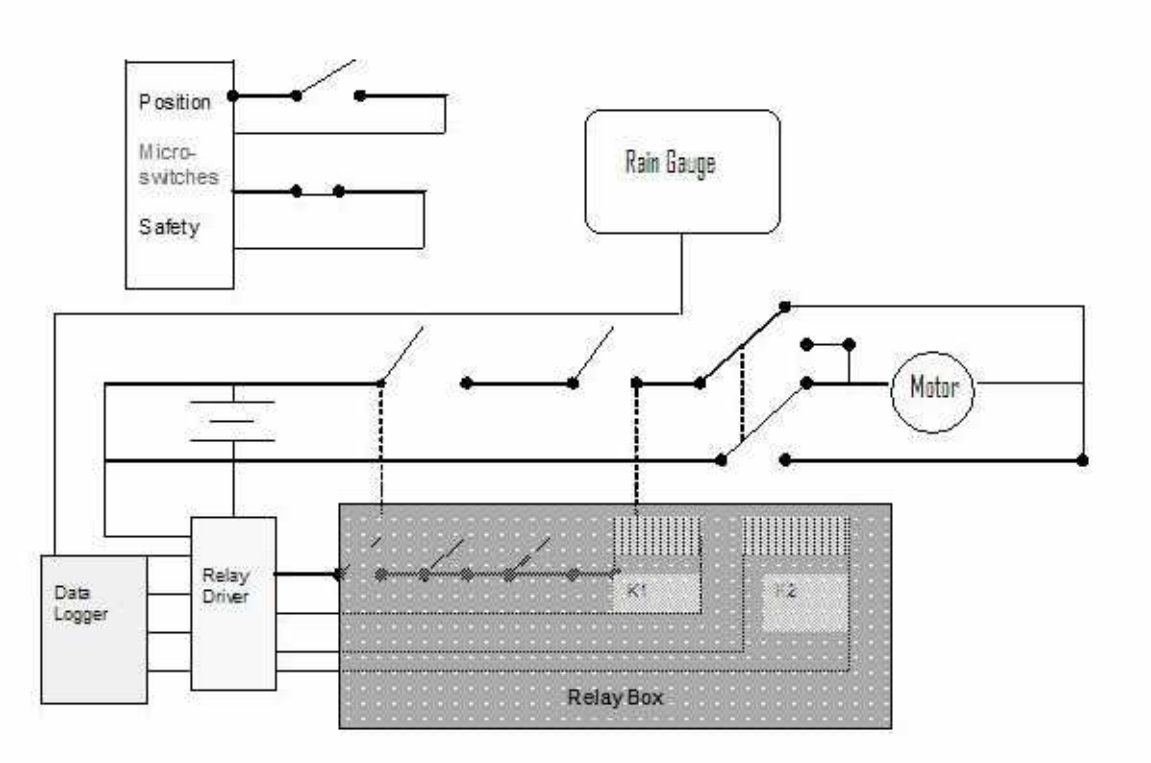

Figure C-3. Electronic schematic for automated rain shield- M=dc motor, K1=power relay, K2=direction relay, position micro switch = normally open, safety micro switch = normally closed.

2.2 ENVIRONMENTAL SAMPLING & MICROBIAL ASSAYS

Sampling and microbiological assays were conducted using the identical methods and materials described in Chapter 2, section 2.5.4.

3. RESULTS

3.1 ENVIRONMENTAL CONDITIONS

During the seasonal solar drying periods at the GVWWTP, moisture was the prevailing stress factor impacting fecal coliform inactivation. As shown in Figure C-5, the average ambient daily temperatures for the summer of 2005 experiment were approximately 5ºC greater than those for summer 2004 experiment. The relative humidity was approximately the same for both summers on Day 3 through Day 22. On Day 23 of the summer 2005 experiment, the monsoon rains commenced as indicated by the sharp rise in the average relative humidity. During the summer 2004 experiment, fecal coliform density levels reached Class A criteria prior to any rainfall events.

Figure C-6 indicates the average daily temperatures in the beds for summer 2004 and 2005. The figure clearly shows that: (1) the temperatures of the tilled beds were higher than the non-tilled (Control) bed for the 2005 summer experiment; (2) the temperatures of the biosolids during the summer of 2005 were greater than the bed temperatures for the summer of 2004; and (3) the biosolids in the covered and tilled bed for the summer 2005 had the highest average daily temperature of all the beds.

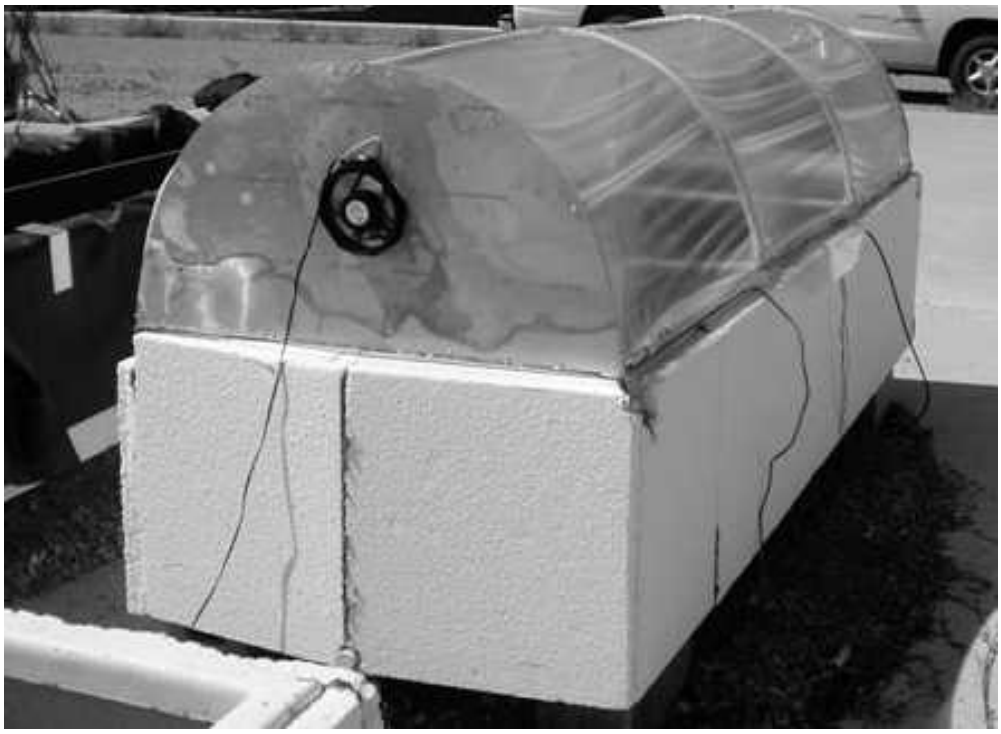

Figure C-4. Covered bed- polyethylene plastic over pvc frame, 1 watt dc powered fans regulated by temperature and relative humidity.

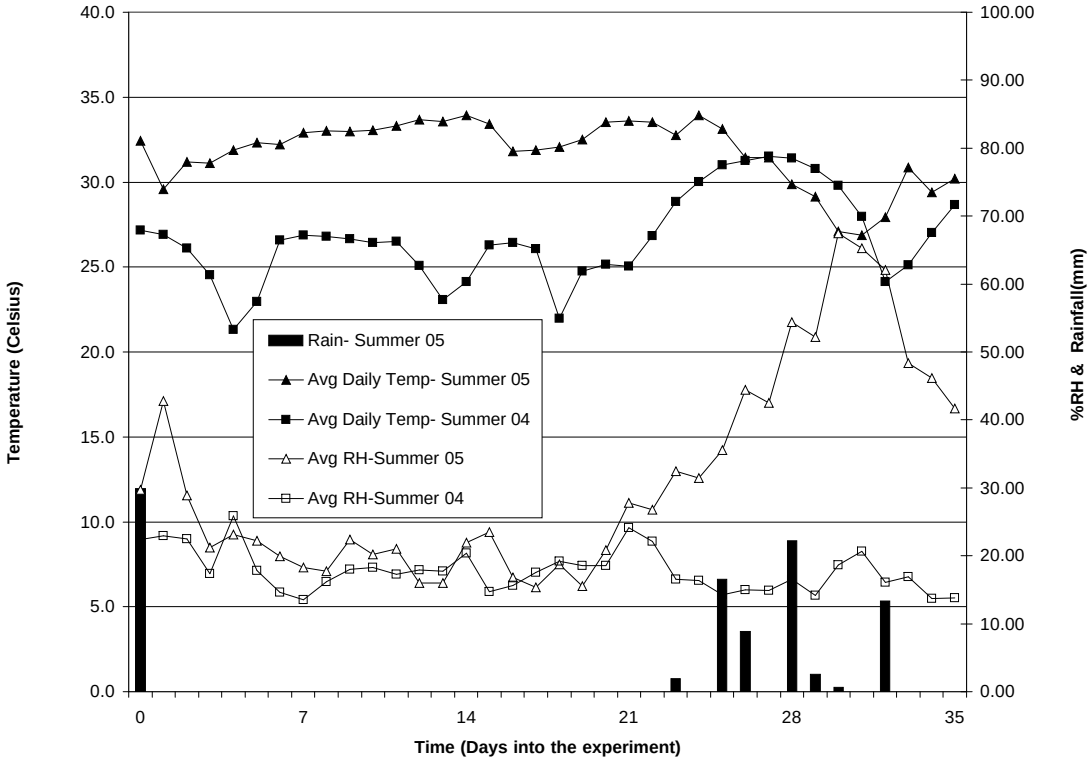

Figure C-5. Average daily temperature and relative humidity values for summers 2004 and 2005.

The average daily temperatures of the biosolids beds are shown in Figure C-7 and C-8 in relation to the moisture content of the beds over time. The temperature in the covered and tilled bed was consistently higher than in the other beds and peaked on Day 10 of the experiment. As the mass of biosolids in the uncovered beds began to dry, their temperatures also rose above ambient air temperature and began to reach levels similar to the covered & tilled bed. When rainfall began on Day 23, all temperature levels in the biosolids dropped, however, the temperatures in the covered and tilled bed (3a) and retractable-covered bed (4a), continued to remain above that of the "uncovered" beds.

3.2 Microbial Results

The inactivation rates for fecal coliforms versus the different treatments are listed in Table C- 6. Initial enteric virus levels for the summer of 2004 were 2.7 PFU/4 g dry weight for the aerobically-digested biosolids. For all other experiments, the enteric virus and helminth ova levels were determined to be < 1 PFU/4 g dry weight of biosolids and < 1 organism/4g dry weight of biosolids.

The inactivation rates for the Control beds (non-tilled) during the summer 2004 and summer 2005 experiments were relatively the same, 0.17 $days^{-1}$, and 0.19 $days^{-1}$, respectively. Tilling during the winter 2005 solar drying experiments did increase fecal coliform reduction by more than 7.5 times that of the untilled bed (Open and Tilled vs. Control). Similarly, comparing inactivation in the two covered beds for the winter of 2005, tilling did increase the die-off rate of fecal coliform by 2.7 times (Covered & Tilled bed versus the Covered and Untilled bed, Winter 2005). Tilling, however, during the winter 2006 solar drying experiment, did not demonstrate any significant increase in fecal coliform inactivation.

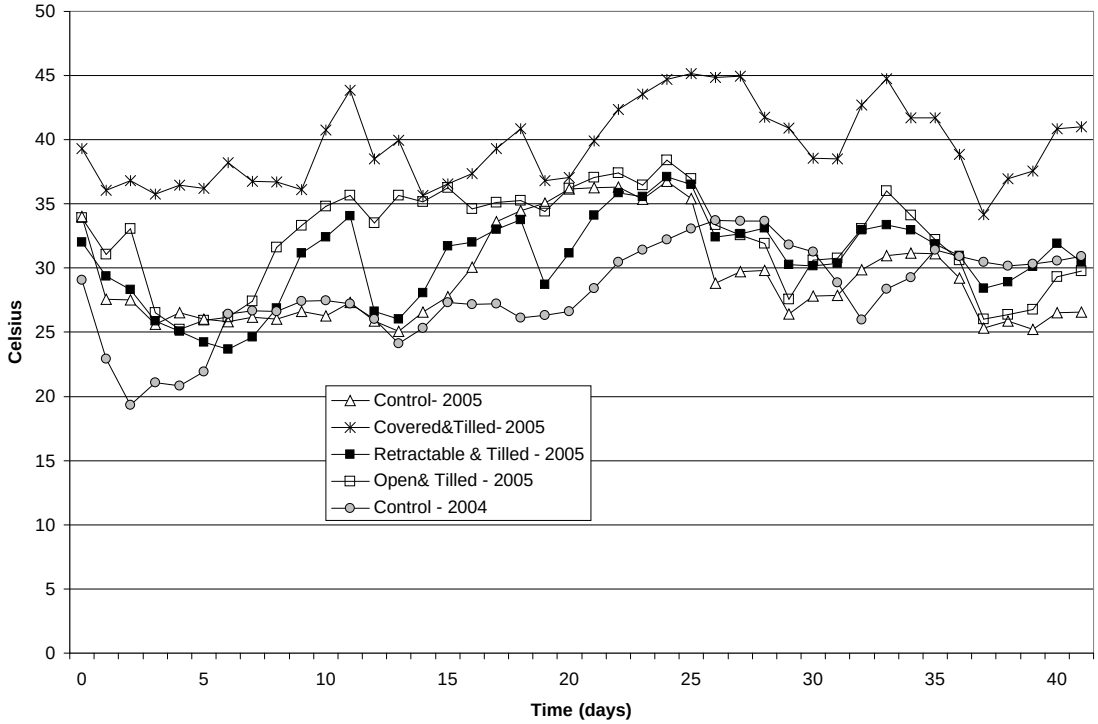

Figure C-6 Average daily temperatures of biosolids, summer 2004 and summer 2005.

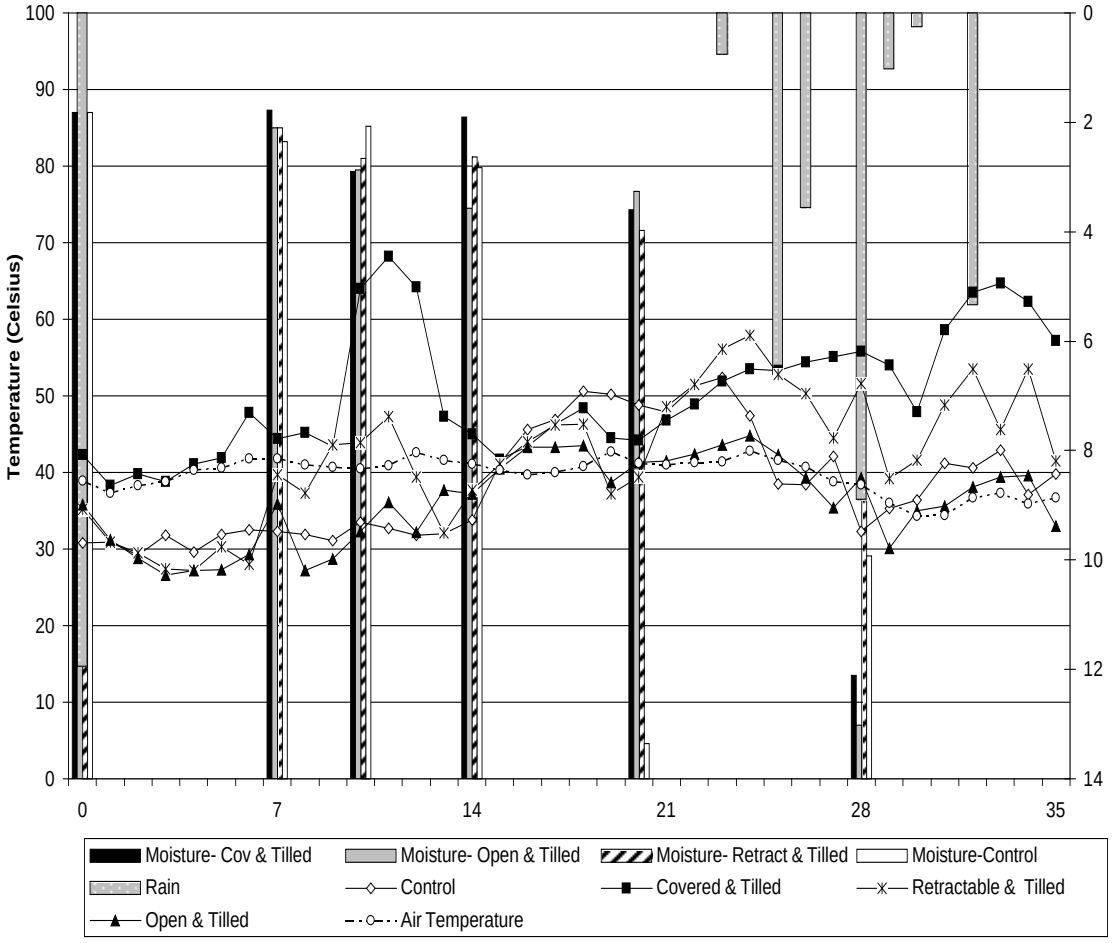

Figure C-7. Maximum Daily Ambient Air and Biosolids' Temperature, Summer 2005.

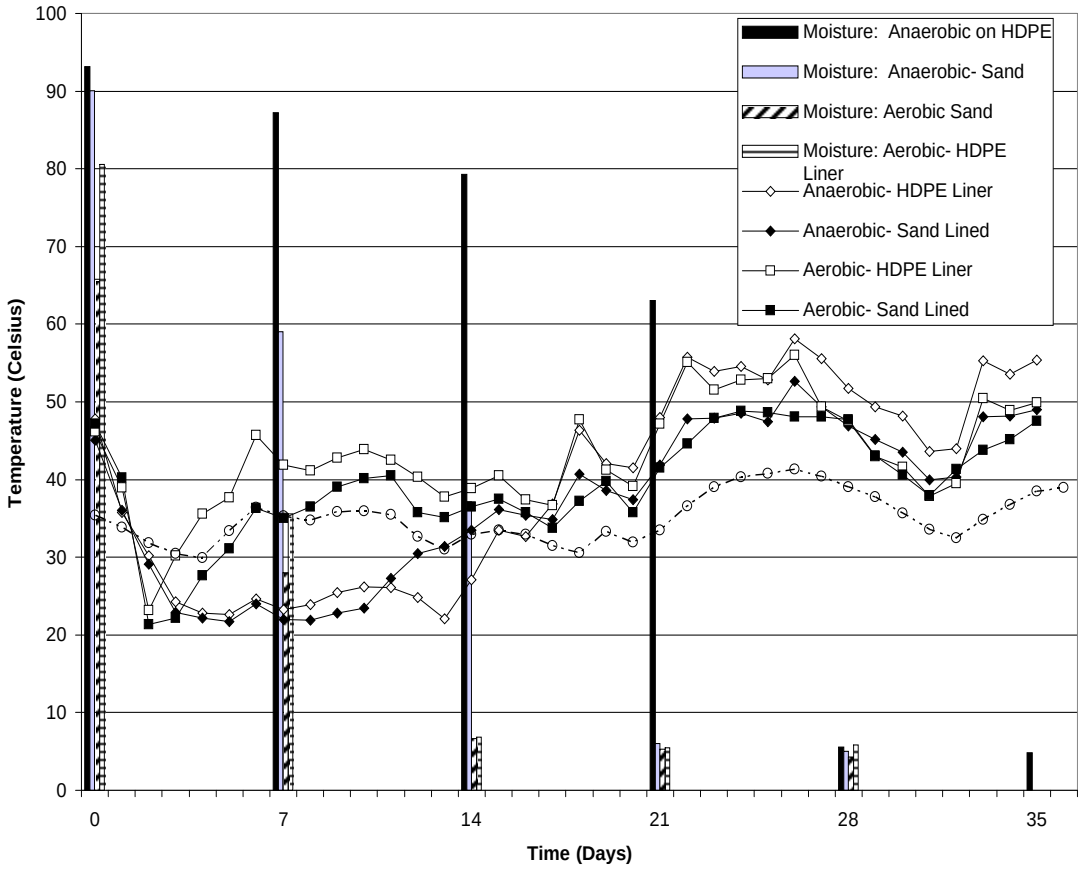

Figure C-8. Daily ambient air and biosolids' temperatures, summer 2004.

3.2 EFFECT OF THE COVERED STRUCTURE

The covered structure increased the inactivation rate of fecal coliform by 36% (Figure C-9) over all other treatments during the summer solar drying season of 2005, but had no significant effect during the winter seasons, Table C-4. The lower performance during the winter was due mainly to the lower ambient air and bed temperatures as well as the accumulation of moisture by condensation inside the cover. 3.4 Effects of tilling

Tilling during the summer season had no significant impact on the inactivation of fecal coliforms when comparing fecal coliform inactivation rates for the retractable roof & tilled bed (k_d=0.18 $days^{-1}$ and 0.19 $days^{-1}$, respectively) and the open & tilled bed versus the results for the Control bed (k_d = 0.18 $days^{-1}$ and 0.19 $days^{-1}$, respectively), summer 2005 experiment. Similarly, tilling versus non-tilling treatments during the winter of 2006 experiment had no advantage on fecal coliform die-off. However during the winter of 2005, tilling treatments in the Open & Tilled bed were more than seven (7) times higher than the Control (untreated) bed.

The inactivation rates were relatively low in the winter time due to the cooler temperatures and the higher relative humidity. Figure C-11 indicates that fecal coliform levels do decline over time. The average bed temperature ranged from 12.6ºC to 21.7ºC during the last 15 days, while the moisture content of the treated biosolids approached 7% on Day 50 for the tilled beds and 23% moisture content in the Control bed on this same day.

Table C-4 Fecal coliform inactivation rates.

Summer Experiments	Treatment	k_d observed
2004	Control (HDPE Liner)	0.17
2004	Control (Sand)	0.17
2005	Control	0.19
2005	Covered & Tilled	0.24
2005	Retractable Roof & Tilled	0.18
2005	Open & Tilled	0.17
Winter Experiments	**Treatment**	**k_d observed**
2005	Control	0.007
2005	Covered & Un-Tilled	0.016
2005	Covered & Tilled	0.043
2005	Open & Tilled	0.054
2006	Control	0.099
2006	Covered & Tilled	0.072
2006	Retractable Roof & Tilled	0.074
2006	Open & Tilled	0.083

3.3 EFFECTS OF THE AUTOMATED SHIELD

During the summer solar drying season of 2005, rainfall occurred after Day 23, and although fecal coliform in the Retractable Cover bed did regrow, the regrowth levels remained near or below Class A criteria in the bed with the automated rain shield, Figure C-10. Fecal coliforms in the Open and Tilled bed, located to the east of the shielded bed, were influenced by the rain as regrowth levels extended beyond the original N_0 values measured at the beginning of the experiment. During the winter solar drying experiment of 2006, only a very limited amount of rainfall occurred and it came towards the end of the experimental run. The last sampling was performed on Day 50, while the last rainfall event was recorded on Day 48. Fecal coliform levels for the shielded bed were slightly greater than those levels for the unshielded bed (Figure C-11). The results are inconclusive as to whether the shield mitigates for the effect of rainfall during cold wet periods. In any case, utilizing sand as a liner, aids in the drainage of biosolids after rainfall.

Salmonella influent levels in the aerobically-digested biosolids were very low and did not increase beyond Class A levels during the summer or winter field experiments. Assayed values for *Salmonella* are shown in Tables C-5 and C-6.

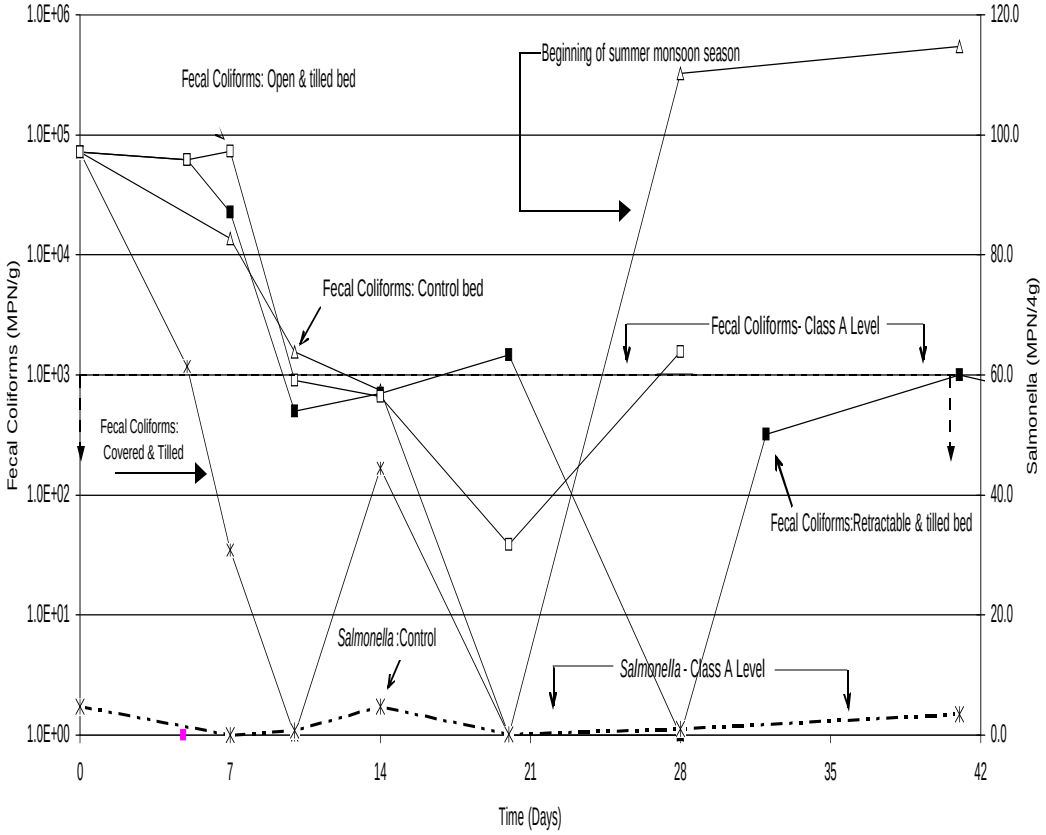

Figure C-9. Fecal coliform and Salmonella die-off patterns for summer 2005; all Salmonella levels were at or below Class A criteria, < 3 MPN/4g.

4. CONCLUSIONS

Open solar drying in the semi-arid region of Green Valley, Arizona is effective in producing Class A microbial quality biosolids during warm dry seasons. Class A criteria can be achieved within a time frame of 14-28 days without additional treatment of tilling or a covered structure if biosolids are placed at a maximum depth of 20 cm and no rainfall occurs.

The main physical factors that impact the inactivation of fecal coliform in biosolids in open solar drying beds are temperature, moisture and the interaction of these two parameters (Zaleski, 2005). Higher temperatures and lower moisture content will increase the inactivation of enteric bacterial pathogens and pathogen indicators. From these studies it is concluded that at an average daily temperature in the range of 25ºC to 35ºC, fecal coliform inactivation is significantly increased as the moisture level in the biosolids declines towards 5% moisture content (g H_2O/g Total Mass). Once the biosolids exceed this temperature range, temperature becomes the governing factor, increasing inactivation regardless of moisture content. This suggests tilling treatments alone do not increase pathogen inactivation, but that a minimum threshold temperature must be attained in the biosolids in order to significantly increase pathogen inactivation. According to results obtained by Choi *et al*. (2005) high average daily temperatures (of biosolids) from 40-57ºC were correlated with rapid fecal coliform die-off in open solar drying beds.

The reason that the Covered and Tilled bed did not produce significant results in the colder wetter climates is the result of a combination of consistently low temperatures and high moisture content of the biosolids throughout the winter experiments. Evaporation

inside the beds was limited due to the high “indoor” relative humidity and moisture condensation. Figure C-5 and C-7 indicate that significantly higher temperatures were consistently present from Day 0 to Day 15 in the Covered & Tilled bed as compared to all other beds.

5. RECOMMENDATIONS

In semi-arid regions of moderate ambient air temperatures, tilling treatments in open solar drying beds are beneficial during cold rainy seasons to reduce the moisture content of the biosolids and thereby increase onsite storage capacity or prime the biosolids for disinfection during anticipated warm dry seasons. The optimal manipulation of biosolids during warm solar drying seasons is achieved by utilizing a covered bed and tilling the biosolids to achieve rapid pathogen inactivation. Further studies need to be conducted to investigate the importance of tilling consistency (depth, frequency, tiller revolutions per minute, etc.) and the interaction between tilling and high ambient air temperatures. In semi-arid regions of moderate ambient air temperatures, tilling treatments are only recommended during cold rainy seasons. Optimal manipulation of biosolids during the solar drying process is achieved by utilizing a covered bed in the warmer seasons and tilling the biosolids to achieve rapid pathogen inactivation.

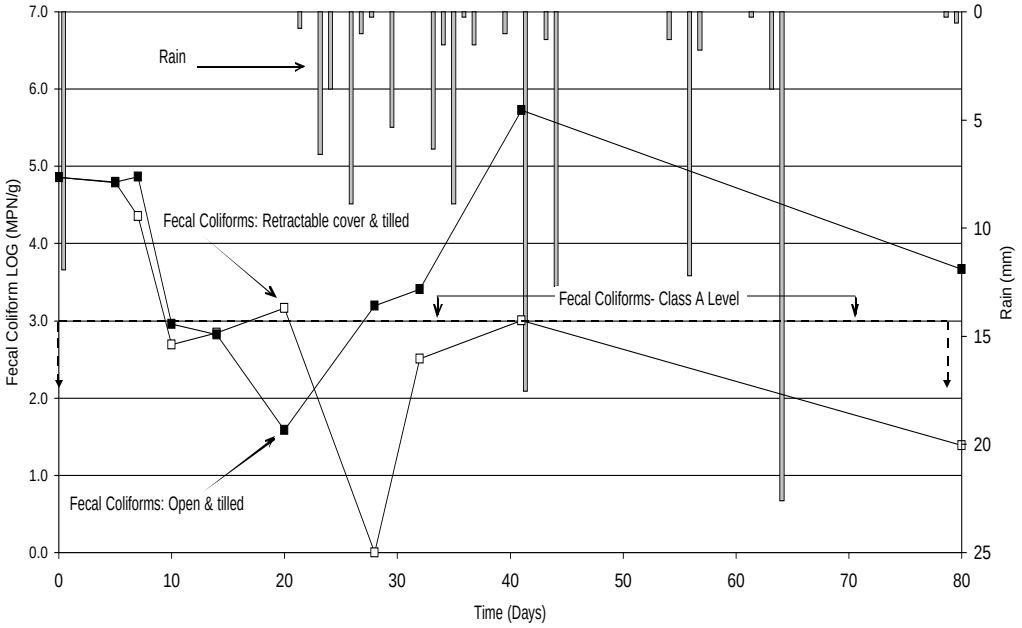

Figure C-10. Effects of automated shield on fecal coliform regrowth after rainfall, summer 2005.

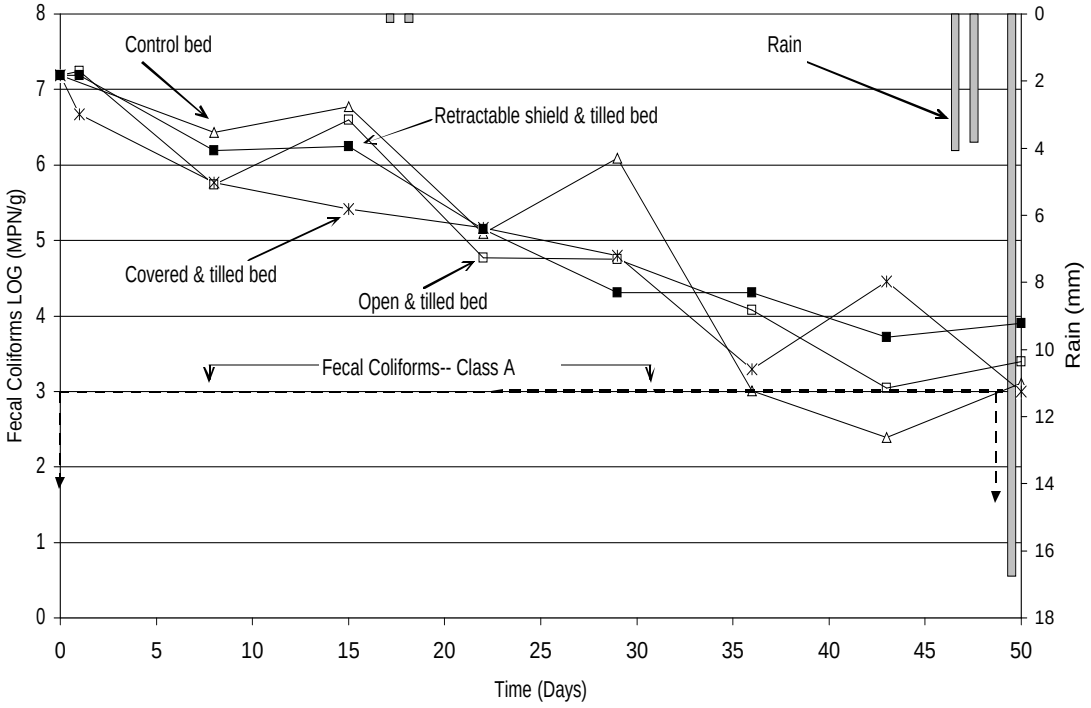

Figure C-11. Fecal coliform density levels during winter 2006 solar drying season.

Table C-5. Salmonella levels (MPN/4 g dry weight biosolids), summer solar drying field experiments.

Summer 2004				
Day	2a- Aerobic Biosolids- HDPE liner		2c- Aerobic Biosolids Sand Liner	
0	1.3		1.3	
7	0.62		1.05	
14	0		0.22	
21	0		0.15	
28	0.15		0.34	
35	0		0	
42	0		0	
Summer 2005	Bed			
Days	Control	Covered & Tilled	Retractable & Tilled	Open & Tilled
0	4.82	4.82	4.82	4.82
7	0.00	0.58	0.00	1.80
10	0.81	0.00	0.00	0.00
14	4.75	0.00	0.37	0.00
20	0.13	0.00	0.95	0.16
28	1.07	0.21	0.00	0.13
41	3.41	0.00	0.00	0.15

Table C-6. Salmonella levels (MPN/4 g dry weight), winter solar drying experiments

Winter 2005	Bed			
Day	Control	Covered & Untilled	Covered & Tilled	Open & Tilled
0	0	0	0	0
1	NS	0.91	5.69	2.1
3	NS	1.8	0	0
8	1.76	0	0	0
15	0	0.73	0	0
22	0.91	0.86	0.86	3.1
29	0	0.84	0	0.22
36	0.88	0	0.25	0
43	0	0	0	0.495
50	0	0	0	0.495
55	0	0	0	0
NS= not sampled				

Winter 2006	Bed			
Day	Control	Covered & Tilled	Retractable & Tilled	Open & Tilled
0	1.00	0.93	0.78	0.00
8	1.00	0.00	0.59	0.32
15	1.82	0.25	0.28	0.73
22	0.65	0.23	0.22	0.51
29	0.32	0.17	0.50	0.16
36	0.27	0.16	0.49	0.15
43	0.26	0.00	0.30	0.14
50	0.00	0.00	0.15	0.00

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APPENDIX D: AMMONIA VOLATILIZATION IN SOLAR DRYING BEDS

ABSTRACT

Samples of biosolids during winter and summer solar drying experiments were analyzed for their ammonium (NH_4^+) and nitrate (NO_3^-) ion content. The nitrate-N for each sample was measured at or below detectable levels. 34 - 92% of ammonia-N (NH_3-N) was lost through volatilization during the summer solar drying experiments. During the wet winter season, anaerobically-digested tilled biosolids lost 35.5% NH_3-N and aerobically digested tilled biosolids lost 63.6% NH_3-N. Untilled biosolids did not experience a net loss of ammonia during the wet winter season.

Macronutrients, especially nitrogen and phosphorous, are valuable ingredients making biosolids a relatively inexpensive source of fertilizer. Therefore recovery of N-loss is of importance. Recommendations to avoid the loss of nitrogen during solar drying include drying biosolids in a closed system, capturing ammonia gas by scrubbing it with sulfuric acid and recovering the ammonium sulfate as a fertilizer.

Keywords: ammonia, ammonium ion, biosolids, nitrogen, solar drying

1. INTRODUCTION

Biosolids may be applied onto agricultural farms across the United States to help reduce our dependence on oil-based commercial fertilizers. In addition to the nutrients, wet (slurry) biosolids (< 10% total solids) can be an important intrinsic resource of water in arid regions. During anaerobic digestion, ammonification produces ammonia which can in some cases account for up to 50% of the total N found in biosolids. Therefore, investigating ammonia volatilization is important since the conservation of ammonia in land applied biosolids and manures can improve nutrient management and maximize utilization of available nitrogen.

There are two forms of reduced nitrogen ammonia (NH_3) and the ammonium ion (NH_4^+). Ammonia is extremely soluble in water and toxic to bacteria, while the ammonium ion is nontoxic and is used by bacteria as a source of N. Volatilization occurs when free ammonia is present. At pH levels above 10.5 to 11.5, the majority of the ammonia-nitrogen present in solution is in the form of free ammonia gas (NH_3). As temperature increases, more ammonia is converted to free ammonia gas because of the temperature dependence of the acid dissociation constant. At a pH level of 7, under standard conditions (i.e., temperature is 25°C and pressure is 1 atm), 0.56% of ammonia present is in the form of free ammonia. When the temperature increases to 60°C, a temperature commonly found in aerobic landfills, the percentage of free ammonia present at pH 7 increases to 4.90% (Berge *et al.*, 2005).

Ammonium is known to sorb onto various inorganic and organic compounds which may be important in the case of biosolids due to their high organic content. The amount

of ammonium sorbed on some organics has been reported to exceed the mass found in the bulk liquid. Sorption of ammonium to the waste allows for temporary storage of ammonium prior to its nitrification or volatilization, and may also result in the slow dissolution of ammonium over time (Berge *et al.*, 2005).

Many studies have focused on the ammonia losses from manures and biosolids during storage, treatment and land application. In developing a mathematical model for NH_3 volatilization, Soogard *et al.*, 2002, determined that the significant variables affecting ammonia loss rates were soil water content, air temperature, wind speed, slurry type, dry matter content of slurry, total ammoniacal nitrogen content of the slurry, application method and rate, slurry incorporation and measurement technique. NH_3 emission from manure slurry that has been land applied is primarily dependent on the physical processes controlling movement of the slurry within the soil, interaction of slurry liquid with soil cation exchange capacity (CEC) (Sommer *et al.*, 2003).

Analogous to investigations related to manure, studies measuring the emissions of ammonia from biosolids that have been land applied indicate the important emission factors are related to the physical and chemical properties of the biosolids (e. g. pH, moisture content and stabilization methods), the application method during and after land application (e.g. slinging versus land injection, time prior to tilling, depth of tilling), the physical (clay particles fix NH_4+ and is readily exchanged and held on the cation-exchange complex) and chemical properties of the soil , and the atmospheric environment such as vapor pressure deficit, wind and air temperature (Soogard, 2002). Ammonia emissions from land applied biosolids were evaluated in a series of laboratory and outdoor experiments by Matlaga

(2005). The laboratory experiments ranged from testing inside a chamber, to measuring three treatments (biosolids alone, surface application of biosolids, and incorporation of biosolids into the soil. The results showed that surface applied biosolids lost 21.5% of the applied NH_4-N lost as ammonia within 26.4 hours, while biosolids incorporated into soil resulted in minimal losses of 2.9% of the applied NH_4-N (Matlaga, 2005). Harmel *et al* (1997), provide pertinent information about ammonia volatilization from land applied biosolids in the Southwestern United States. Quemada, performed laboratory studies on soil- surface biosolids treatments. Among the treatments were surface-applied biosolids compared to biosolids incorporated into soil. Pertinent results indicated that biosolids integrated with soil reduced ammonia loss by 15% compared to the losses experienced by surface applied biosolids.

A complaint of an alleged ammonia related illness, prompted measurements of ammonia emissions from the land applied biosolids to be recorded near the home of the purported victim. The literature reported a maximum emission rate of 18.7 ug/m^2/sec for the first 12 hours following application of biosolids and by the fifth day, an emission rate of 4.2 ug/m^2/sec, while after 30 days, a rate of only 0.4 ug/m^2/sec was recorded (Chrostowski, 2002). In terms of risk assessment, these values are very low as the American Industrial Hygiene Association reports that the ammonia threshold exposure limit is 250 ug/L (Nicoletti and Taylor, 2005).

Another critical issue to sustaining biosolids disposal by land application concerns the reduction of detrimental social and environmental impacts. Residential encroachment into traditional agricultural land-use areas has been cause for addressing odor issues and the potential regulation of air quality near treatment plants (Lim *et al.*, 2003). Ammonia

gas (NH_3) generated by the decomposition of organic matter in wastewater. Levels of ammonia in wastewater influent typically range from 10 mg kg-1 to 45 mg kg-1 (Metcalf and Eddy, 2003). Ammonia may be generated throughout the anaerobic wastewater treatment processes, thereby concentrating levels of ammonia and the ammonium ion (NH_4^+-N) in wastewater residuals or biosolids; typical ammonia levels in treated effluent are between 0.1-1 mg kg-1. (Metcalf and Eddy, 2003). Ammonium-N as cited in the literature varies greatly depending on the method of stabilization, e.g., Christie *et al.* (2001) reports 7200 mg kg^{-1} for alkaline stabilized biosolids, a range between 700 mg kg-1 to approximately 2726 mg kg^{-1} (He *et al.*, 2003 & Tercero *et al.*, 2005) for cake biosolids, and 42700 mgkg-1 found in liquid biosolids (Gilmour *et al.*, 2003). Zeng *et al.* (2005) reports a difference between anaerobically and aerobically-digested biosolids, 824 mg kg-1 for anaerobic biosolids and 241 mg kg-1 for aerobic biosolids, respectively.

Field studies by Gilmour (2005) have shown that plant-available nitrogen (PAN) is reduced when biosolids are surface-applied increasing ammonia volatilization. Laboratory studies (from Gilmour, 2005) further indicate that organic N from the same biosolids incubated in different soils mineralizes at different rates. Other important factors in ammonia volatilization include soil temperature, soil type, and soil moisture content and microbial activity (Agehara and Warncke, 2005). Their influence on the rate of decomposition, net N mineralization and PAN and the C to N ratio, organic N, and total N content in biosolids was related to biosolids C to N ratio, organic N, and total N.

Ammonium nitrogen is very soluble in an aqueous solution. But during the solar drying process, the NH_4^+ ion is deprotonated, forming the very volatile NH_3 gas that can be lost with the evaporation of moisture. Conversely, ammonia ions can be oxidized (via

nitrification) to form nitrate (NO_3^-) ions. Key factors affecting volatilization of ammonium from biosolids should be analogous to those described by He *et al.*, 2003 when integrated biosolids into soil. These factors are moisture, temperature, pH, and microbial activity.

Solar drying of biosolids is a cost-effective method of dewatering the material for volume reduction (Metcalf and Eddy, 2003) and transportation management. More recently solar drying of biosolids has been used as an economical means for pathogen reduction (Choi *et al.*, 2005 and Hamoud *et al.*, 2006, unpublished data). The disadvantages of open solar drying can include maintaining compliance with vector attraction requirements, abating odor nuisances, loss of NH_3-N and moisture from the biosolids during the drying process. This represents the loss of a valuable fertilizer resource or a potential loss in energy. The purpose of this study was to quantify the ammonia losses from biosolids during the conversion process from Class B biosolids to a Class A material using open solar drying.

2. METHODS AND MATERIALS

Biosolids' samples of approximately 50 g were collected at various times during the length of the drying process. Key variables associated with the biosolids include the method of stabilization, aerobic vs. anaerobic, the tilling treatment received, i.e. either tilling or no tilling, and the season in which the biosolids were exposed to solar drying, winter vs. summer. The moisture content of the samples was determined by air drying in an oven (Lindberg) over 24 hours at a temperature of 104ºC.

The concentrations of NH^{+}_{4}-N and NO^{-}_{3}-N in the biosolids were determined by extracting biosolids samples (as is) with 0.1 NH_2SO_4 solution. The colorimetric analysis was performed with the Hach kit by using a spectrophotometer (Hitachi model U-2000).

NO^{-}_{3}-N: samples were extracted with water (1:2 ratio) and analyzed by using Ion Chromatography: Dionex, (model ICS-1000). Total N(excluding ammonia-N) was determined by oven drying samples @ 104^{o}C, followed by analysis using Carlo Erba N-C-S elemental analyzer (Model NA 1500).

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3. RESULTS

Figures D-1 through D-7 indicate the concentration of ammonium-N, water, and solids for an initial 1000 kg (metric ton) of biosolids drying over time, t. Values are based on the initial %TS and the %TS measured over time during the various solar drying field experiments. The absolute value of NH^{+}_{4}-N is shown numerically on each graph. It is assumed that the total amount of solids remains constant over time, while water is subject to loss via evaporation. Nitrogen is lost as the ammonium-ion is converted to NH_3 gas and the gas is volatized to the atmosphere. The loss of nitrogen is expected to be linear with time; deviations are likely due to spatial variation in sampling (depth and area). The graphs also include the changing concentration of NH^{+}_{4}-N per total mass of biosolids over time.

Application of a relatively dry cake of anaerobically-digested biosolids indicates that NH_4-N is in the range of 0.40-0.45% of the total mass of biosolids applied (Figure D-2). A dried cake of aerobically-digested biosolids contains roughly 0.25% NH_4-N per total mass of product applied (Figure D-3).

Figure D-1 indicates that non-tilled mesophilic anaerobically-digested biosolids produced at the Ina Road WWTP in Tucson, Arizona and dried at the GVWWTP facility lost 92% of its original concentration of ammonia during the summer 2004 drying season. This loss is 2.7 times greater than the ammonia lost in the tilled mesophilic anaerobically-digested biosolids generated in Los Angeles County Sanitary District (LACSD) and solar dried in open fields at Quartzsite, Arizona. The reasons for this difference in volatilization rates may be due to the chemical composition of the biosolids and/or the method of generation. The biosolids produced at the Ina Road WWTP were initially 6% TS while the biosolids produced at LACSD were approximately 27% TS. Both biosolids contained a polymer additive and both were centrifuged prior to release for transportation, however the latter biosolids may have less free water available for evaporation, meaning a greater percentage of water is chemically bound to the organic matter (Cheng *et al.*, 2002).

Tilling during the winter months increased the amount of ammonia lost in both the anaerobically and aerobically-digested biosolids as shown in graphs D-4 vs. D-5 and D-7 vs. D-6 respectively. Despite moderately cool temperatures and rainfall during these winter periods, tilling promoted ammonia-N loss. Between 40% and 58% was lost during both of these solar drying experiments (average air temperature was 12.6 º C and 14.1º C and rainfall was 44.5 mm and 31.75 mm for Quartzsite and GVWWTP, respectively).

Table D-1 details the level of net NH^{+}_{4}-N and water loss from the different solar drying experiments, as well as the concentration of NO^{-}_{3}-N levels and average organic N levels throughout the drying process.

Table D-1. Net losses of Ammonium and Water and Existing Nitrate levels in Biosolids during Solar Drying Field Experiments

Type of Biosolids/ Origin	Season	Biosolids' Treatment	Net NH_4-N loss (%)	Net Water Loss (%)	Nitrate Levels (ug/g)	Average Organic Nitrogen (%)
Anaerobic/ Ina WWTP	Summer	None	92.4	93.9	BDL	1.64
Anaerobic/ LACSD	Summer	Tilled	34.3	77.1	BDL	NE*
Aerobic/ GVWWTP	Summer	None	52.3	92.7	0.6	2.22
Anaerobic/ LACSD	Winter	Tilled	35.5	40.0	<0.5 – 8.1	3.78
Anaerobic/ LACSD	Winter	None	0.0	-1.8	<0.5-1.95	3.89
Aerobic/ GV WWTP	Winter	Tilled	63.6	57.9	BDL	6.29
Aerobic/ GVWWTP	Winter	None	0.0	2.6	BDL	3.78

LACSD= Los Angeles County Sanitary District biosolids shipped to Quartzsite for drying; GVWWTP= Green Valley Wastewater Treatment Plant, Green Valley, Arizona
BDL = below the detection limit of 0.5 mg/kg NO^-_3
*NE- not evaluated

4. CONCLUSIONS

The majority of N loss in biosolids in open solar drying beds is due to the volatilization of NH_3 which is enhanced by moisture evaporation from the biosolids during the drying process. As shown in Table D-1, very little NH_3 is converted to NO^-_3, therefore N loss must be occurring by volatilization. In general tilling of biosolids enhances the loss of N due to increased evaporation rates of moisture from the biosolids regardless of the drying season (summer vs. winter). However, because of the variability in data and final results, field studies need to be repeated to gain more reliable trends concerning ammonia loss in relation to type of biosolids and induced treatments (i.e. tilling and a covered bed), replication of samples would prove useful.

Most U.S. states require that the application of biosolids to agricultural land be done at agronomical rates dependent on N crop needs plus expected mineralization of organic N because most of the N in biosolids is organic (Shober *et al.*, 2003). A review of typical agronomic rates that biosolids are applied and the cost of synthetic commercial fertilizer illustrate the benefits of preventing ammonia loss from biosolids during the open solar drying process.

Data in Table D-2 refers to a typical land application rate (Shober *et al.*, 2005). Utilizing a land application rate of 5 Mg (dry weight of biosolids)ha^{-1} for data in Table D-2, which represents an average application rate value for the crops of corn, soy bean and alfalfa. These analytic chemical results show that moisture loss from biosolids during open solar drying leads to a proportional loss in nitrogen (NH_4-N) content in the biosolids. This loss can be measured in terms of energy units or fertilizer costs. For example, if synthetic based ammonia sulfate is applied to a 240 ha cotton farm to

Table D-2. Ammonium-N losses associated with solar drying of biosolids.

Application Rate = 5 Mg dry wt. Biosolids/ha[a]	Initial %TS[1]	Absolute value of NH_4-N at the start [2] (kg/ 1000 kg)	Total NH_4-N Applied via biosolids @ start of drying[3] (kg/ha)	Projected Absolute loss due to solar drying[4] (kg)	Dollar Value of NH_3-loss /ha (based on $0.22[5]/kg)	Total dollar loss on 420 ha[6] farm ($)
Anaerobic/ Ina WWTP, Summer	5.4	2.9	500	469	$103.18	43,336
Anaerobic/ LACSD, Summer	27	2.13	39.44	13.52	$2.97	1,247
Aerobic/ GVWWTP, Summer	15	0.88	29.33	15.3	$3.37	1,415
Anaerobic/ LACSD, Winter	26	3.1	59.62	21.2	$4.66	1,957
Aerobic/ GVWWTP, Winter	13	1.1	42.3	26.92	$5.92	2,486

a. Typical application rate, Shober *et al.*, 2003

1 Data from lab notebook

2 Initial data point from Graphs D1-D6

3 Value is based on 5000 kg/TS * Absolute value of NH4-N

4 Corresponding % loss from appropriate graphs, shown in figures D1-D7

5 Cost of ammonia sulfate, USDA National Agriculture Statistics Service, July 2001

6 Typical size of a cotton farm in U.S., USDA, Economic Research Service, July 2001

augment the N loss from solar dried land applied biosolids, then the total dollar loss to the farmer per year would be in the range of $1,247 to $43,336, see Table D-2.

In addition to the inability to fully utilize the N in the generated biosolids, solar drying has the adverse effect of evaporating the moisture content of the initially generated biosolids. Water can be a critical source of moisture to crops in arid climates and wet slurry sludges can provide a portion of this needed resource to crops in these dry regions as well as sustain dryland cropping. In addition, incorporation of biosolids into soil can improve land use and management practices to sequester soil organic carbon. The high organic matter in biosolids may help improve the hydrological properties of the soil (Rostagno and Sosebee, 2001). This is the goal for afforestation - enhance activity of soil macrofauna, for example termites (Parker and Laha, 2005).

While solar drying of biosolids can be an effective method for dewatering and disinfection, it does promote the loss of N by ammonia volatilization and moisture reduction in the biosolids. While these are disadvantages inherent to the solar drying process, there are ways to mitigate for the loss of these resources and still achieve a high quality of biosolids for the purpose of agronomic benefit when land applying.

5. RECOMMENDATIONS

Biosolids are land applied for disposal reasons but are desired by the agricultural industry due to their agronomic value of N. Therefore recovering volatilized ammonia during the solar drying process is a prudent and feasible endeavor by either chemical means or structural methods. It has been shown that ammonia volatilization in chicken manure can be managed by the addition of chemical compounds such as $FESO_4$, alum or

$CaCO_3$. (Pierzynski and Gehl, 2005). Similarly, it has also been demonstrated that acidification of biosolids to a pH of 5 prevents ammonia loss from manure samples and in a preliminary study, the same was found for fresh biosolids during sample drying (Cogger *et al.*, 2001). A second proposed method of management is to design the drying bed as an enclosed structure, similar to a greenhouse building. Complete enclosure allows for the collection of NH_3 gas inside of the greenhouse structure while abating odors. The collected gas can be scrubbed with sulfuric acid, H_2SO_4, or hydrochloric acid, HCl and the by-product, ammonia sulfate, and ammonium chloride respectively can be recovered and marketed as a fertilizer (Agrawal, 2004).. Field studies can be set up to scrub the air inside a covered bed by using an exhaust fan to draw the inside air and moisture into a narrowed ducted outlet. The air can then be condensed and the moisture collected in a glass sampling bottle.

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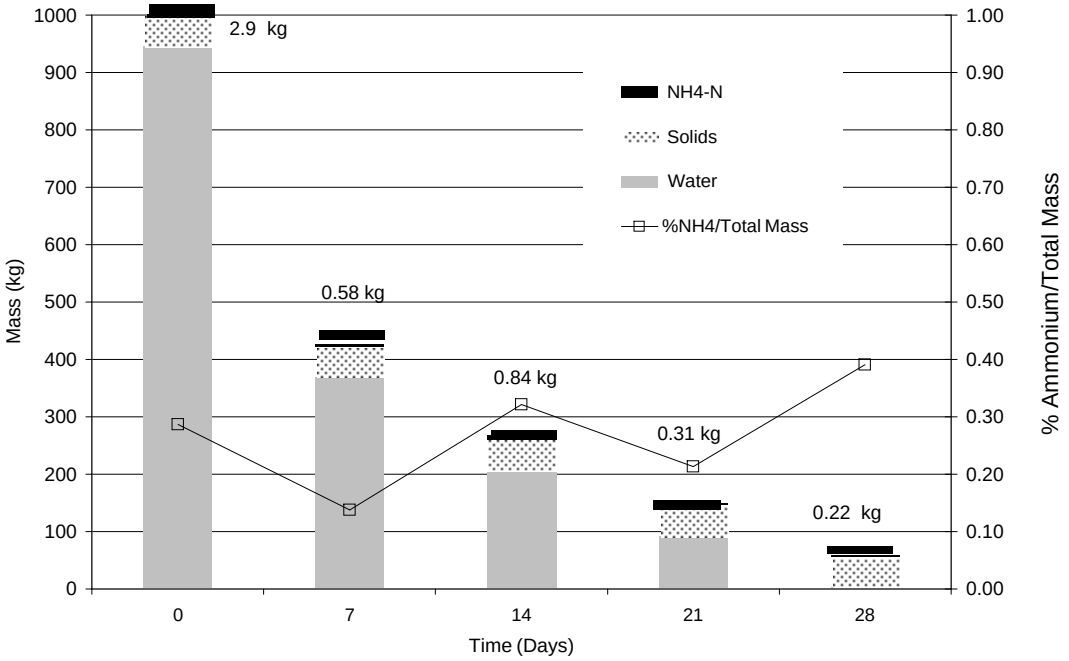

Figure D-1. Composition of anaerobic mesophilically-digested untilled biosolids (kg/1000 kg of biosolids), solar dried in Green Valley, Arizona, summer 2004. Values on graphs refer to mass NH4+-N in kg.

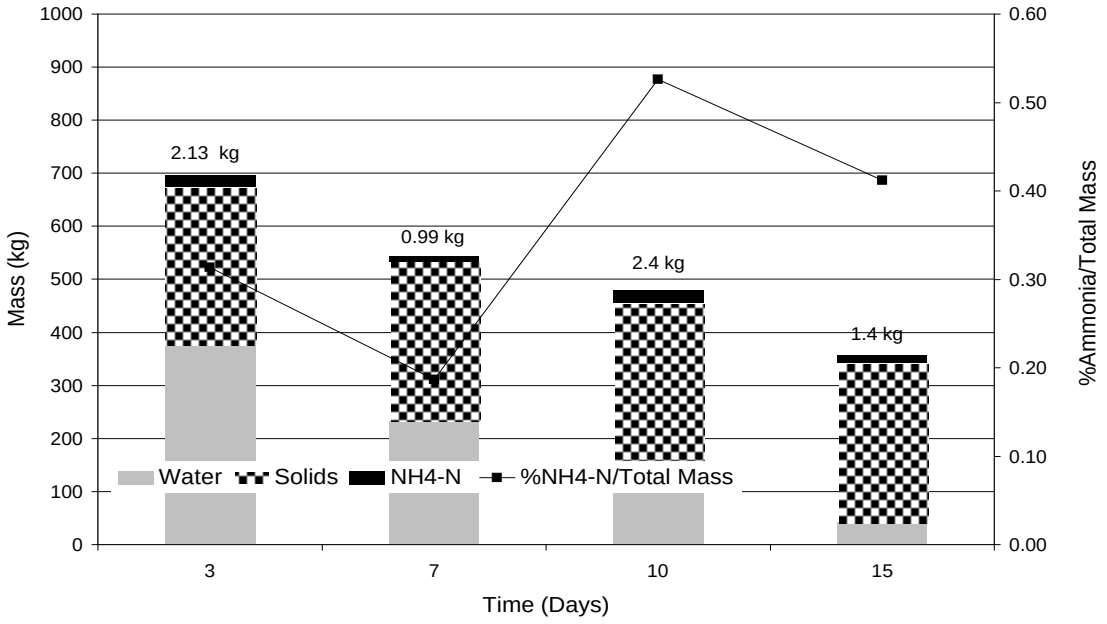

Figure D-2. Composition of anaerobic mesophilically-digested and tilled biosolids, (kg/1000 kg of biosolids), solar dried in Quartzsite, Arizona, summer 2004.

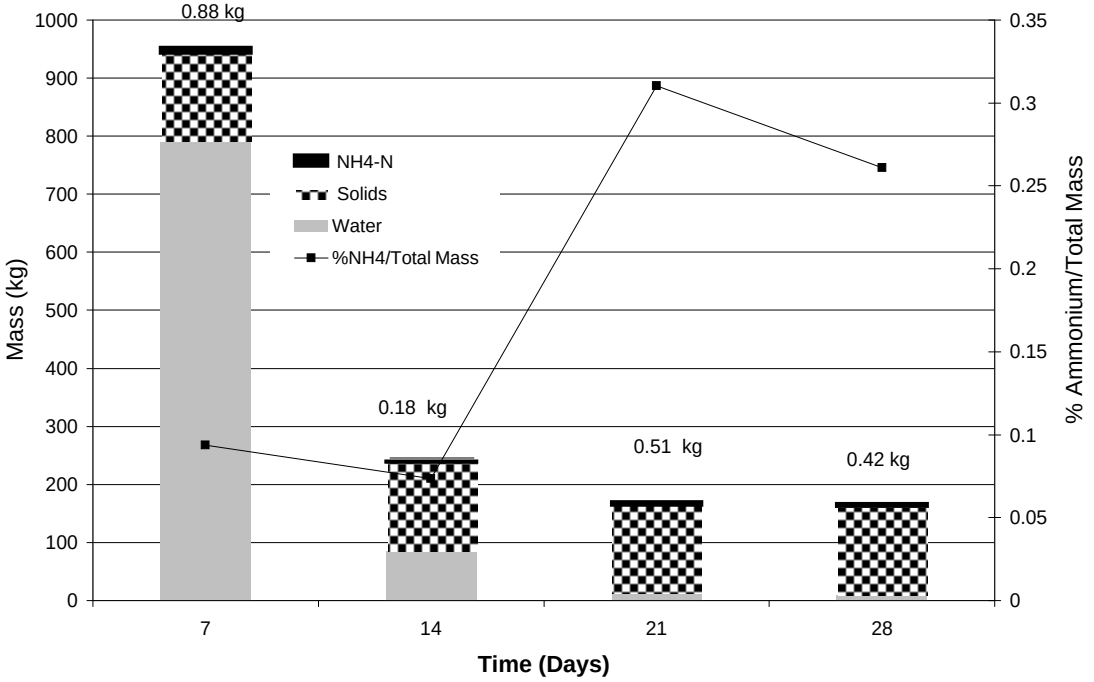

Figure D-3. Composition of aerobically-digested, composition in kg/1000 kg of biosolids, untilled biosolids, solar dried in Green Valley, AZ, summer 2004.

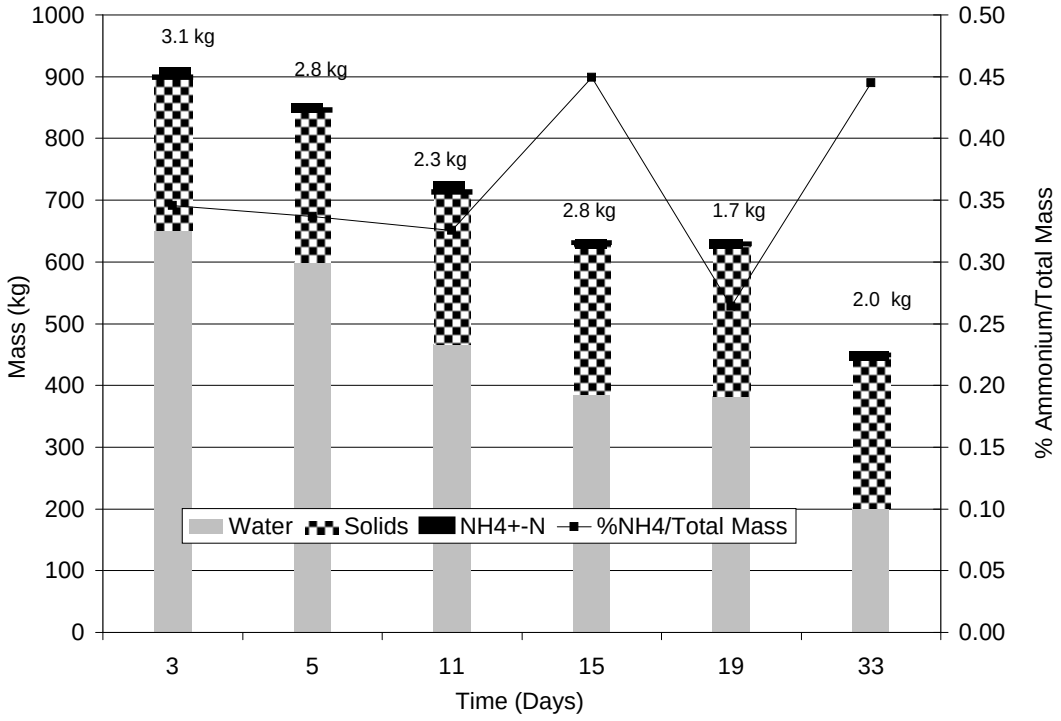

Figure D-4. Composition of anaerobically-digested, tilled biosolids (kg/ 1000 kg of biosolids), solar dried in Quartzsite, Arizona, winter 2005.

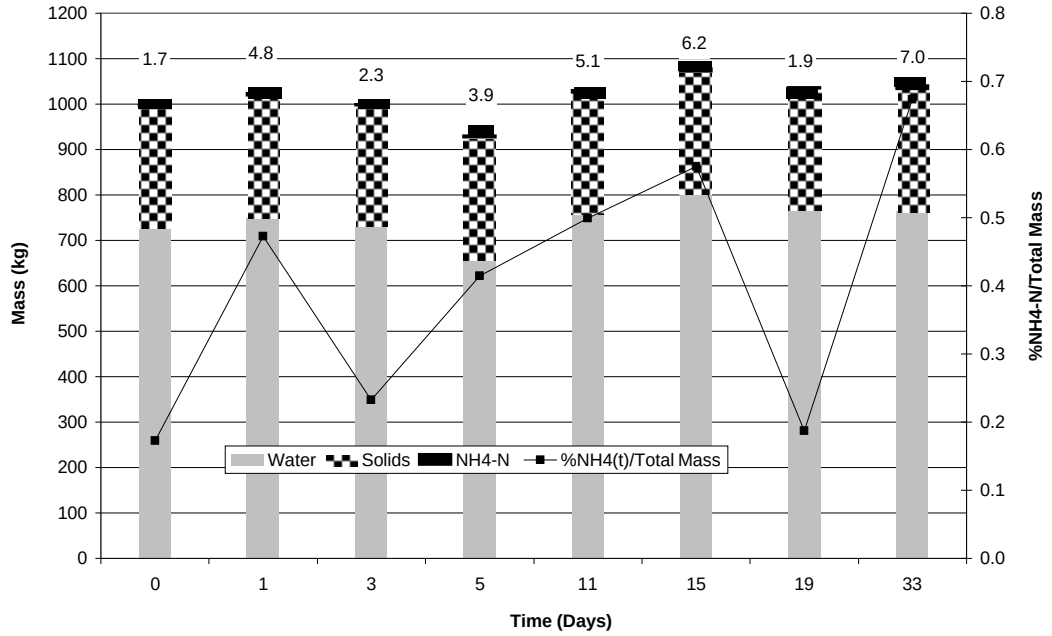

Figure D-5. Composition of anaerobically-digested, untilled biosolids, (kg/1000 kg of biosolids), solar dried in Quartzsite, Arizona, winter 2005.

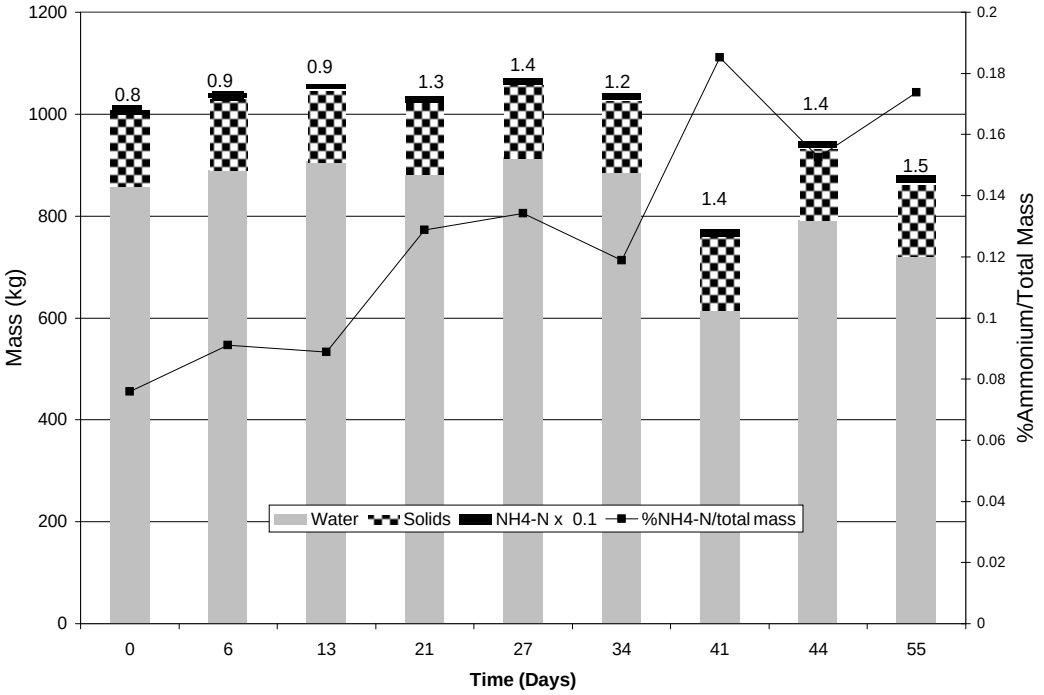

Figure D-6 . Composition of aerobically-digested, untilled biosolids (kg/1000 kg of biosolids),, solar dried at GVWWTP, winter 2005.

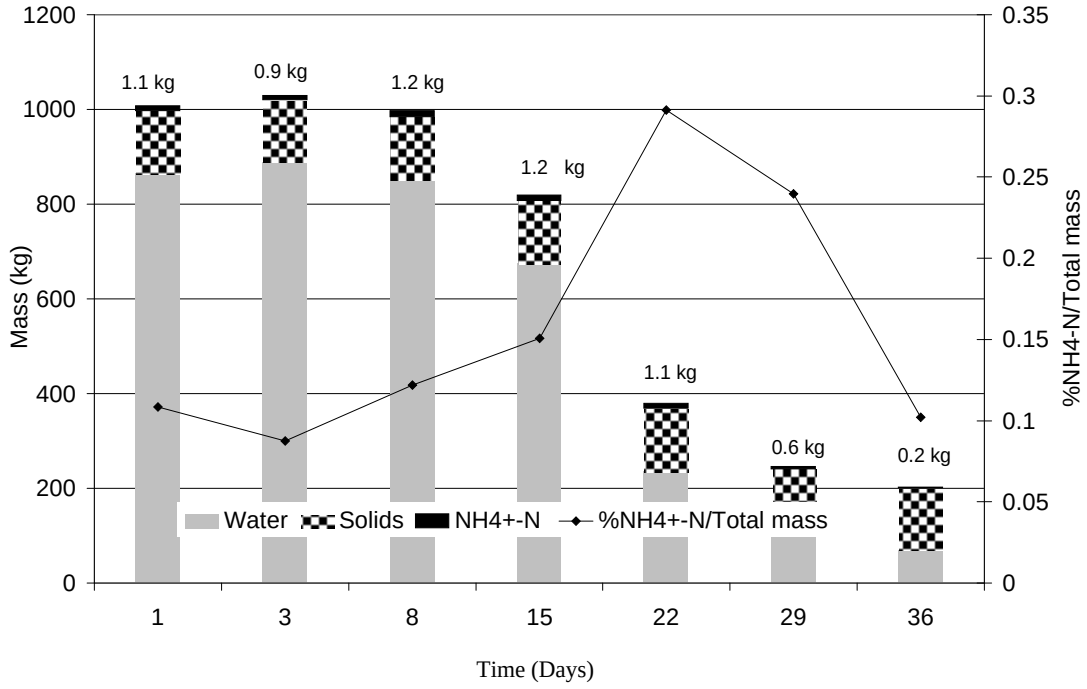

Figure D-7. Composition of aerobically-digested tilled biosolids, (kg/1000 kg of biosolids), solar dried at GVWWTP, Arizona, winter 2005.

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APPENDIX E: SHORT STUDY- BIOTIC EFFECTS ON PATHOGEN REDUCTION IN BIOSOLIDS

ABSTRACT

A preliminary controlled laboratory study was performed to investigate the inherent biotic effects in biosolids on the survivability of fecal coliforms. Two 1.0 kg samples of solar dried, aerobically-digested biosolids from the Green Valley WWTP were collected. One set of material, 1.0 kg, was sterilized by autoclaving it in a Nalgene® container for one hour at 131ºC and 1.5 atm. This procedure was repeated three times to ensure sterility. 10.0 g of sterilized biosolids were placed into 84 separate Nalgene® sterilized jars (5.0 cm (height) x 3.0 cm (diameter)). Each jar was amended with a specified amount of sterile water and then inoculated with 1.0 ml or less of *Escherichia coli* ATCC 15592 (Department of Soils, Water and Environmental Science Department, University of Arizona). The Nalgene® jars were placed in an oven located in the walk-in refrigerator at the Microbiology Building at the University of Arizona; the oven temperature was set at 38ºC, 1 day prior to the start of the experiment. The 3-tube MPN process was utilized to enumerate and estimate the inactivation rate of fecal coliforms over a 20-day period at the moisture levels of 10%, 35%, 65%, and 80% (g/g). These inactivation results (see Figure E-1) were compared to those for the non-sterilized biosolids that were heated to the same temperature, see Appendix B. The inactivation rates for all moisture levels with the exception of the 65% moisture level were greater than the inactivation rates for the non-sterilized biosolids. However paired T-test analysis indicates that only the inactivation rates at the 10% and 80% moisture levels are statistically significant at $p = 0.05$. These results indicate that there is a biotic factor which affects fecal coliform inactivation in biosolids. Future studies need to be performed with additional replication and different type of biosolids to ensure that this

trend is consistent at various temperature levels and with anaerobically-digested biosolids. It is also noted that the standard deviation among the inactivation rates for the sterile biosolids is less than the standard deviation for the inactivation rates of the non-sterilized biosolids, 0.16 vs. 0.81, respectively). This indicates that there exists a large amount of variability within the context of microbial competition.

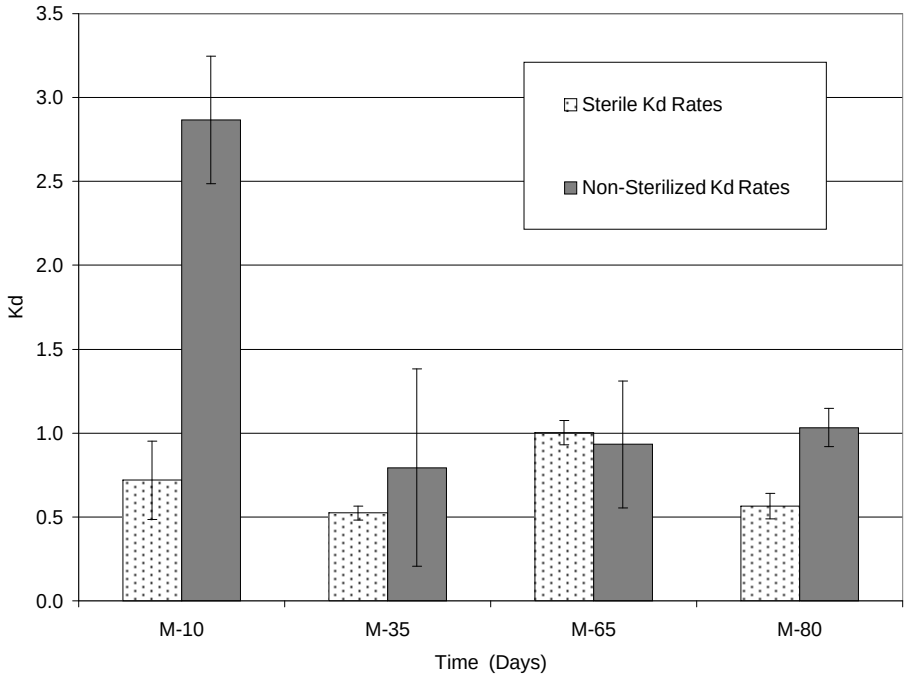

Figure E-1. Comparison of inactivation rates for fecal coliforms in sterilized and non-sterilized aerobically-digested biosolids, where kd = log (Nt/No) (day-1).

Table E- 1. Inactivation rates and T- test results in sterile versus non-sterile biosolids

Moisture Level	10%		35%		65%		80%	
Biosolids	Sterile	Non-Sterile	Sterile	Non-Sterile	Sterile	Non-Sterile	Sterile	Non-Sterile
k_d	0.58 0.59 0.99	3.3 2.7 2.6	0.57 0.50 0.50	1.47 0.41 0.50	0.96 0.96 1.09	1.1 1.2 0.5	0.48 0.63 0.58	1.1 0.9 1.1
p value	p = 0.022		p = 0.48		p = 0.81		p = 0.045	

APPENDIX F: PROTOCOLS FOR MICROBIOLOGICAL ASSAYS

F-1. FECAL COLIFORM ISOLATION FROM BIOSOLIDS

PROCEDURE:

1. Add 10 ml of biosolids to 90 ml of buffered peptone water and place on a shaker for 10 minutes. This is the 10^{-1} dilution.

2. Make 10 fold dilutions by adding 1 ml of the 10^{-1} sample to a test tube containing 9 ml of buffered peptone water. Serial dilute to 10^{-8} or 10^{-9}

3. Add 1 ml of each dilution to tubes containing 10 ml of Lauryl Tryptose Broth (LTB) and durham tubes. Prepare 3 or 5 replicates for each dilution depending on MPN method.

4. Incubate samples in a circulating water bath at 35°C for 24-48 hours.

5. Score +/- for gas production. There will be air present in the durham tube and the broth will be turbid. Bubbles may also rise in the tube when shaken. These tubes are positive for coliforms.

6. Add 0.1 ml from each positive LTB tube to tubes containing 10 ml of EC broth and a durham tube. Incubate in a circulating water bath at 44.5C for 24-48 hours.

7. Score tubes +/- for gas production. These tubes are positive for fecal coliforms.

8. Refer to the MPN table to determine MPN/gram for fecal coliforms

MATERIALS and MEDIA:

Buffered Peptone
Laurel Tryptose Broth
EC Broth

F-2. PROTOCOL FOR *SALMONELLA* SPP. ASSAY, 3 MPN-METHOD

PROCEDURE:

1. Add 10g for biosolids sample to each one of four bottles containing 10ml of sterile buffered peptone water and shake or stir for at least 10 minutes. Each bottle may be labeled as A, B, C, and D.
2. Add 10 ml of the mixture from bottle D to each one of three empty sterile test tubes. Take 1ml of the mixture of the same bottle D and add to each one of three test tubes containing 9ml of sterile buffered peptone water. Vortex dilution. Discard this bottle after taking the samples. In this step it can be observed that bottles are inoculated with 10g of biosolids, three test tubes with 1g of biosolids, and three test tubes with 0.1g of biosolids. A triplicate dilution and nine solutions are created.
3. Incubate bottles A, B, and C and all test tubes at 35^{o}C for 24 hours in a water bath.
4. Without mixing the dilutions, take 0.1ml of each bottle and test tubes and add it to tubes containing 10ml of Rappaport-Vassiliadis R10 broth. Vortex and incubate at 42^{o}C for 24 hours in a water bath. RV media preparation includes addition of novobiocin, compound that inhibits gram-positive bacteria and increases *Salmonella* population.
5. Take one droplet with an inoculation loop and strike it in Hektoen Agar plates. Incubate at 37 ^{o}C for 24 hours. *Salmonella* colonies grow on Hektoen as separate black and round dots with a colorless halo. Results of possible *Salmonella* colonies should be recorded.
6. Take two typical and separate suspected *Salmonella* colonies and strike them separately in the same Trypticasein Soy Agar (TSA) plate. TSA plates may be half

divided for each colony. Incubate for 37^{o}C for 24 hours. In this step, pure colonies are grown in plates.

7. Using a straight needle, pick the center of a well-isolated colony from TSA plates.
8. Inoculate by stabbing to the base of the butt and streaking the slant of LIA and TSI tubes. TSA plates need to be saved for a later confirmation step.
9. Cap the tubes loosely to ensure aerobic conditions. Incubate at 35^{o}C for 18 to 24 hours.
10. For TSI tubes, read for acid production in base of slants which will turn yellow. Also inspect for alkaline slants which will turn in red. Hydrogen sulfide reactions may be present.
11. For LIA, examine at 18 to 24 hours and 40 to 48 hours for alkaline production in the base of the slant (purple color). Blackening at the apex of the slants will reveal hydrogen sulfide productions.
12. Record tubes as positives for *Salmonella* spp. if TSI and LIA conditions of *Salmonella* presence are observed. Otherwise, confirmation of results should be made using a latex *Salmonella* test kit.
13. Place one drop of each the reacting and control reagents on a testing card.
14. Transfer the center of a well-isolated colony on the TSA plate to the reacting reagent drop in the testing card using a sterile inoculating loop. Rotate the inoculating loop around the test circle for about 10 to 15 seconds until the circle is entirely covered.
15. Transfer the center of another well-isolated colony on the TSA plate to the control reagent drop in the testing card using a sterile inoculating loop. Rotate the inoculating

loop around the test circle for about 10 to 15 seconds until the circle is entirely covered.

16. Rock the card counterclockwise motion for about one to two minutes and inspect for agglutination. A positive confirmation for *Salmonella* spp. is recorded if agglutination occurs in the test reagent (latex test) and does not occur on the control latex sample. If both latex do not present agglutination, the sample is recorded as negative. In the case that both latex agglutinate, the sample should be retested; otherwise results cannot be considered as either positive or negative samples.
17. Based on the number of positive tubes, determine the MPN of *Salmonella*/4g of dry weight biosolids.

MATERIALS AND MEDIA

- Buffered peptone water
- Rappaport Vassiliadis broth (RV)
- Novobiocin
- Hektoen Enteric Agar
- Tryptic Soy Agar (TSA)
- Lysine Iron Agar (LIA)
- Triple Sugar Iron (TSI)
- Oxoid Latex Test Kit for *Salmonella*
- Petri dishes
- Test tubes
- Straight and round-ended loops
- REFERENCE:

F-3. DETECTING AND ENUMERATING ASCARIS OVA IN SLUDGE

1. Dried or thicken samples: weigh about 150 g and place in about 250 mL phosphate-buffered water in a beaker and let soak overnight at 4ºC. Transfer to blender and blend at high speed for one min.
2. Liquid samples: Measure 500 mL or more (estimated to contain at least 25 g dry solids) of liquid sample. Place one half of sample in blender. Add about 100 mL phosphate-buffered water. Blend at high speed for one min, transfer to a beaker. Repeat for other half of sample.
3. Pour the homogenized sample into a 1000 mL tall beaker and using a wash bottle, thoroughly rinse blender container into a beaker. Add 1% 7X to reach 450 mL final volume.
4. Allow sample to settle four hr or overnight at 4ºC. Stir with a wooden applicator, as needed to ensure that material floating on the surface settles. Additional 1% 7X may be added, and the mixture stirred if necessary.
5. After settling, vacuum aspirate supernatant to just above the layer of solids. If is not settled, centrifuge the sample. Transfer sediment to blender and add phosphate-buffered water to 250 mL, blend again for one min at high speed.
6. Transfer to beaker, rinsing blender and add 1% 7X to reach 450 mL. Allow to settle for two hr at 4ºC, vacuum aspirate supernatant to just above the layer of solids. If is not settled, centrifuge the sample.
7. Add 150 mL 1% 7X and stir for five min on a magnetic stirrer.
8. Strain homogenized sample through a 20 or 50 mesh sieve place in a funnel over a tall beaker. Wash sample through sieve with a spray of 1% 7X from a spray bottle.
9. Add 1% 7X to 450 mL final volume and allow to settle for two hr at 4ºC.
10. Vacuum aspirate supernatant to just above layer of solids. Mix sediment and distribute equally to 50 mL graduated conical centrifuge tubes. Thoroughly wash any sediment from beaker into tubes using water from a wash bottle. Bring volume in tubes to 25 mL with water.
11. Centrifuge for 10 min at 1,000 G (2,120 RPM). Vacuum aspirate supernatant from each tube down to just above the level of sediment. (The packed sediment in each tube should not exceed 5 mL. If it exceeds this volume, add water and distribute the sediment evenly among additional tubes, repeat centrifugation, and vacuum aspirate supernatant).
12. Add 5 to 10 mL $MgSO_4$ solution (specific gravity 1.20) to each tube and mix for 15 to 20 seconds on a vortex mixer. (Use capped tubes to avoid splashing of mixture from the tube).
13. Add additional $MgSO_4$ solution (specific gravity 1.20) to each tube to bring volume to 25 mL. Centrifuge for five to ten min at 800 to 1,000 G(1,890-2120 RPM). Do not use brake.
14. Allow the centrifuge to coast to a stop without the break. Pour the top 25 to 35 mL of supernatant from each tube through a 400-mesh sieve supported in a funnel over a tall beaker. (The supernatant is our sample).
15. Using a water spray bottle, wash excessive flotation fluid and fine particles through sieve.

16. Rinse sediment collected on the sieve (this is your sample) into a 100 mL beaker by directing the stream of water from the wash bottle onto the upper surface of the sieve.
17. After thoroughly washing the sediment from the sieve, transfer the suspension to the required number of 15 mL centrifuge tubes, taking care to rinse the beaker into the tubes. Usually one beaker makes one tube.
18. Centrifuge the tubes for three min at 800 G (1890 RPM), and then discard the supernatant.
19. If more than one tube has been used for the sample, transfer the sediment to a single tube, fill with water, and repeat centrifugation.
20. Discard supernatant, add a drop of Lugol solution (Iodine/Potassium iodine solution) and add DI water to a final volume of 1 mL. Examine microscopically to enumerate the detected ova.

MATERIALS

1. 1.0 or 2.0 L graduate plastic beaker
2. Stir bars
3. Blender
4. 50 mesh sieve
5. 400 mesh sieve
6. 50 mL centrifuge tubes
7. 15 mL tubes

REAGENTS

1. 1% 7X: 1 L = 999 mL phosphate-buffered water, 1 mL 7X detergent, adjust pH to 7.2 ± 0.1 with 1N NaOH.
2. Magnesium Sulfate (sp. gr. 1.20: 1L = 215 g $MgSO_4$).
3. Phosphate-buffered water: 1L = 34.0 g KH_2PO_4, pH adjusted to 7.2 ± 0.5 with 1N NaOH.

REFERENCE

EPA/625/R-92/013: Environmental Regulations and Technology, Control of Pathogens and Vector Attraction in Sewage Sludge.

F-4. PROPAGATION OF *E. COLI*.

Note: *E. coli* 15597 should be transferred to a new Petri dish at least once every 4 weeks.

E. coli 25922 should be transferred to a new Petri dish every 2 weeks.

1. Transfer 1 colony of *E. coli* from a Petri dish into a 125 ml flask containing 50 mL of TSB. Cover with sterile sponge.

2. Leave flask in walk-in incubator overnight at ~ 35ºC. Flask should either be in shaker or stirring on high level on stir plate.

3. Transfer portion of inoculated Tryptose Soy Broth (TSB) to desired final volume of autoclaved TSB. (Roughly 1 mL inoculated TSB for every 100 mL of uninoculated TSB).

4. Incubate flask in walk-in incubator at least 8 hrs. Flask should either be in shaker or stirring on high level on stir plate. (Note: TSB should be turbid after specified inoculation time).

5. Titer stock by plating on EMB agar plates, incubate at 35ºC and read after 24 hrs. Store stock in walk-in refrigerator for a maximum of 5 days.

APPENDIX G: STATISTICAL ANALYSIS FOR CONTROLLED STUDY

Table G-1. Raw microbial data, solar drying in Green Valley, Arizona.

Green Valley WWTP, summer 2004					
Day	DOY	Fecal Coliforms		%Total Solids	
		Aerobic HDPE	Aerobic Sand	Aerobic-HDPE	Aerobic-Sand
0		1.50E+05	1.50E+05	15.37	15.37
7		4.78E+05	2.92E+06	19.46	34.22
14		6.68E+04	3.19E+04	64.39	72.02
21		2.47E+01	1.90E+03	93.16	93.31
28		2.43E+01	2.43E+02	94.57	94.73

Green Valley WWTP, Solar drying data for Bed 2, summer 2005			
		Fecal Coliforms	%TS
Day of Experiment	DOY	AE-2	AE-2
0	174	7.2E+04	12.98
7	181	1.4E+04	16.77
10	184	1.56E+03	14.77
14	188	7.43E+02	20.19
20	194	1	95.4

Green Valley WWTP, Solar drying data for Beds 3, 4 & 5, summer 2005				
		Fecal Coliforms		
Day of Experiment	DOY	AE-3	AE-4	AE-5
0	174	7.20E+04	7.20E+04	7.20E+04
5	179	1.18E+03	6.19E+04	6.22E+04
7	181	3.48E+01	2.26E+04	7.33E+04
10	184	1.00E+00	4.95E+02	9.03E+02
14	188	1.67E+02	7.04E+02	6.58E+02
20	194	1.00E+00	1.47E+03	3.87E+01
28	202	1.00E+00	1.00E+00	1.56E+03

Table G-2. Raw Microbial data, solar drying in Wadi Hassan, Jordan.

Days	Solar drying microbial data from Wadi Hassan, summer 204					
	Fecal Coliforms (MPN/g)		*Salmonella* (MPN/4g)		% TS	
	Fecal Coliforms Sample- 1	Fecal Coliforms Sample- 2	*Salmonella* Sample- 1	*Salmonella* Sample- 2	1	2
0	2.60E+06	2.60E+06	1222	1222	3.6	3.6
3	1.97E+05	2.17E+05	376	174	11.7	10.6
7	1.45E+05	1.50E+05	11	11.5	15.9	15
10	1.30E+04	4.90E+04	5.3	6	17.5	19
14	1.70E+04	1.80E+04	1.1	0.7	55.5	51
16	2.40E+03	9.30E+02	0.3	0.16	96.5	97.3
20	2.40E+02	4.46E+02	0.12	0.12	96.5	96.4
23	9.10E+01	9.10E+01	0.12	0.12	98.7	98.1
Solar drying microbial data from Wadi Hassan, spring 2005						
Days	Fecal Coliforms (MPN/g)		*Salmonella* sp. (MPN/4g)		% TS	
	Fecal Coliforms Sample- 1	Fecal Coliforms Sample- 2	*Salmonella* Sample- 1	*Salmonella* Sample- 2	1	2
0	5.00E+04	2.40E+05	202.0	98.0	9	10
6	1.50E+05	1.00E+05	64.8	44.4	28	42
12	7.90E+01	7.91E+01	0.4	1.0	95	95
14	4.58E+02	9.89E+02	1.0	1.0	94	94
19	2.45E+01	2.44E+01	0.9	0.2	94	94
22	4.67E+01	4.63E+01	1.0	1.0	92	93

TableG-3. Statistical analysis- T-test for open vs. closed on Fecal Coliform Inactivation.

Two-Sample T-Test and Confidence Interval: Closed versus Open

Two-sample T- test for Closed vs Open

	N	Mean	StDev	SE Mean
Closed	2	0.0533	0.0250	0.018
Open	2	0.0793	0.0126	0.0089

Difference = μClosed - μOpen
Estimate for difference: -0.0261
95% CI for difference: (-0.1111, 0.0590)
T-Test of difference = 0 (vs not =): T-Value = -1.32 P-Value = 0.318 DF = 2
Both use Pooled StdDev = 0.0198

Table G-4. Two-way ANOVA: kd versus moisture and temperature

Factors: Moisture (4 levels) and Temperature (3 levels)
(SYSTAT® ,Version 8.0)

Analysis of Variance for kd

Source	DF	SS	MS	F	P
Moisture	3	6.2782	2.0927	33.59	0.000
Temp	2	8.4302	4.2151	67.65	0.000
Interaction	6	3.7646	0.6274	10.07	0.000
Error	24	1.4955	0.0623		
Total	35	19.9685			

Individual 95% CI

Moisture	Mean	---+---------+---------+---------+--------
10	1.443	(----*----)
35	0.428	(----*----)
65	0.562	(----*----)
80	0.467	(----*----)
		---+---------+---------+---------+--------
		0.350 0.700 1.050 1.400

Individual 95% CI

Temp	Mean	-----+---------+---------+---------+------
25	0.332	(---*----)
32	0.437	(---*----)
38	1.407	(---*---)
		-----+---------+---------+---------+------
		0.350 0.700 1.050 1.400

Table G-5. Statistical analysis- kd= f(moisture, temperature).

Non-linear Regression – Gauss Newton Method to Determine Best Fit Estimate (SYSTAT®, Version 8.0)

Dependent variable is k_d

Source	Sum-of-Squares	df	Mean-Square
Regression	34.875	3	11.625
Residual	4.016	33	0.122
Total	38.891	36	
Mean corrected	19.968	35	

Raw R-square (1-Residual/Total) = 0.897
Mean corrected R-square (1-Residual/Corrected) = 0.799
R(observed vs predicted) square = 0.808

Wald Confidence Interval

Parameter	Estimate	A.S.E.	Param/ASE	Lower < 95%>	Upper
K1	0.112	0.046	2.444	0.019	0.205
K2	-41.881	1.117	-37.481	-44.154	-39.608
K3	-0.536	0.190	-2.815	-0.923	-0.149