REDUCTION OF SELECTED PATHOGENS IN ANAEROBIC DIGESTION

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ABSTRACT

Anaerobic digesters are becoming a popular waste treatment option in New York State. These systems generate energy for on-farm use and sale while providing significant odor reduction. Research has shown that mesophilic systems (T=100^{o}F) have the potential to reduce pathogens entering the environment. A plug flow digester was monitored for 14 months starting in May 2001. Samples taken from the digester influent and effluent were tested for the fecal coliform group of indicator organisms and *Mycobacterium avium subspecies paratuberculosis (Map)*. *M. avium paratuberculosis* is the microorganism responsible for Johne's disease in dairy cattle and other ruminants. Results show almost a 3-log reduction in fecal coliforms and slightly more than a 2-log reduction in *Map*. This paper describes an anaerobic digester and shows the comparative results of testing between a farm with a digester and a farm without a digester. Since both farms in this study compost and sell excess solids, analyses were done on the composted manure as well.

KEYWORDS. Anaerobic Digestion, Pathogen Reduction, *Mycobacterium Avium paratuberculosis,* Composting

INTRODUCTION

Daily spreading of manure, a long time continuing practice in animal agriculture in the United States will come under increasing pressure as environmental considerations prevent spreading during saturated conditions. The Natural Resource Conservation Service (NRCS) National Standard for Nutrient Management (590) prohibits spreading manure on saturated soils. Research has shown that manure, pathogens, and nutrients move quickly through preferential flow paths to tile lines when manure is spread during wet conditions (Goehring, 1999). Phosphorous Indexes that determine the timing and application of manure to be spread to meet phosphorus management requirements will discourage manure spreading when nutrients are prone to move off the field. Saturated conditions have been recognized in some of the Phosphorous Indexes developed so far. Therefore, for environmental reasons more manure will be stored and spread during the summer when the soils are drier and the chance for saturating rainfall events are less. This will limit the amount of pathogens washed downstream and will also provide nutrients to crops as they are growing when their uptake is the highest.

In New York State a low percentage of farms have enough storage to prevent them from spreading manure when the ground is saturated (Poe, 2001). Therefore,

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additional manure storage facilities will be built on farms. As manure is stored, byproducts from the partial decomposition of the stored manure produce malodorous gases. Farms that spread this manure during the warm season can cause severe odor events in their communities. Increased use of direct incorporation (mixing the manure with the soil as it is applied) and immediate incorporation (performing a tillage operation to immediately mix the manure with soil after manure is broadcast on the surface) will be used to reduce odors. Unfortunately these operations are very difficult to perform while a crop is growing.

Manure treatment methods to reduce odors are available. The only method that has the potential to control odors while providing an economic incentive to the farm over the long term is anaerobic digestion. The byproducts that can be produced with an anaerobic digestion manure treatment system include electricity, heat, solids, and maintained nutrient value. Dairy farms can produce about 1kw of power for every 7 cows (Wright, 2001). These benefits may provide a positive return per cow over the life of the digester (Wright and Inglis, 2001).

Since anaerobic digestion reduces the odors of the effluent enough to eliminate complaints, the nutrient benefits occur as the treated effluent is spread on growing crops. Pathogen reduction is important when doing this to reduce the chance of contaminating the crop. The solid byproducts can be sold as a soil amendment off site, composted and sold as a value added product, used on the farm as bedding or spread on fields. Pathogen reduction is important here by reducing the risk to the offsite user of importing pathogens and to protect the herd if the farm uses the solids as bedding. Each of these operations depends on reduced pathogens in the digested effluent. Applying pathogens in high numbers onto crops being grown for animal feed creates a fecal-oral pathway that has the potential to provide an increased disease presence in the herd. Using separated solids as bedding under the animals also has the potential to increase disease if pathogens are not reduced. Selling the separated solids offsite with pathogens present may present a liability issue.

Reducing the pathogens present in the effluent from digesters is an important characteristic of the process. The objectives of this paper are to show reductions in an indicator organism (Fecal Coliform) and the reductions of *Map* as an obligate pathogen, representing bacteria and protozoa that are fairly difficult to kill. *Map* was chosen because this intestinal mycobacterial infection is of economic concern to the dairy cattle industry. An estimated 20 to 40% of herds in the United States are infected. There is also the proposed but as yet unproven association with Crohn's Disease in humans (Stabel, 1998). *Map* persists in cattle manure slurry held at 5°C for up to 252 days and for 98 days in slurry held at 15°C (Jorgensen, 1977). Depending on conditions, *Map* can survive in water for 9-14 months (Collins, 2001).

METHODS

Farm Description

The layout for Farm A is shown in Figure 1. Farm A is a 550-cow dairy in central New York State that works 2,200 acres of land. The farm started operation in 1993 and

installed an anaerobic digester in 1998 to reduce odors, improve water quality and community relations while producing electricity and compost for sale off farm. The anaerobic digester was designed for a 20-day retention time for1,000 cow capacity. Therefore, with the current herd capacity the actual retention time for the digester is closer to 36 days.

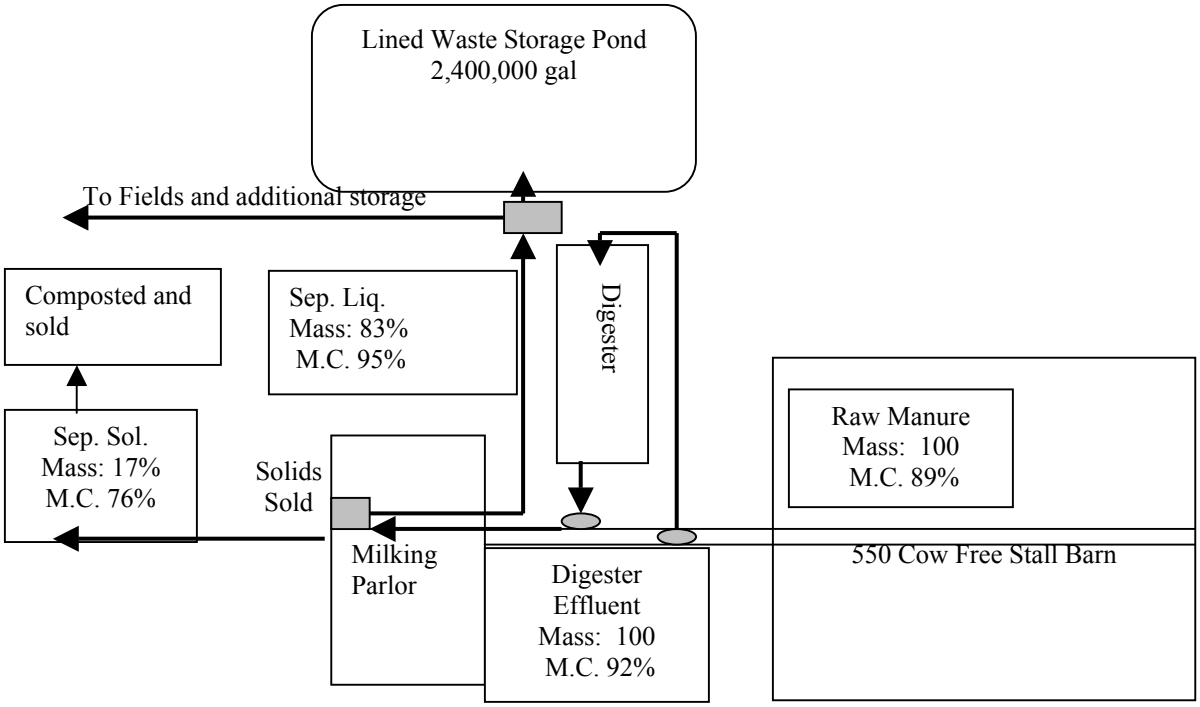

Figure 1. Farm A anaerobic digestion schematic

Figure 2 shows the layout of Farm P. This farm is a 900-cow dairy in central New York State that utilizes a manure separator as part of their manure management system. The separated liquids in this system are stored in a long-term anaerobic lagoon. The solids are conveyed to a forced air static pile composting system and are used for bedding in the freestall buildings. After a year the farm started accumulating enough excess solids that they compost in turned windrows for sale off farm.

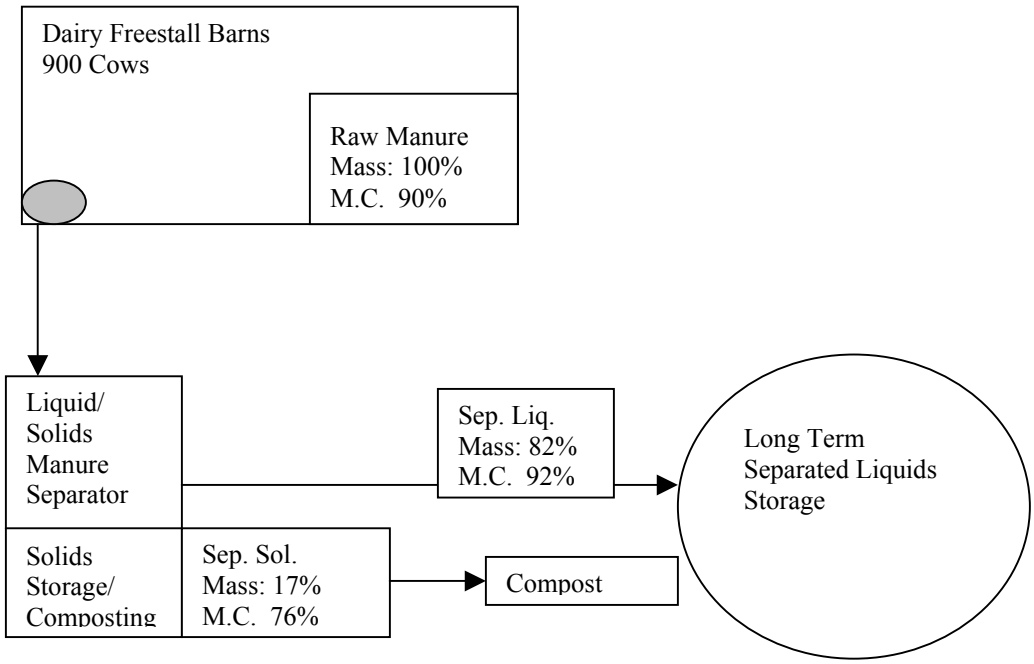

Figure 2. Farm P Separated solids treatment only

Sampling Procedure

On Farm A samples were taken from 5/2001 until 6/2002 as grab samples from the pipeline entering the digester (raw manure), the flow from the end of the digester (digester effluent), from the outflow pipe directly from the separator (separated liquids), from three depths and three locations in the waste storage facility (stored liquid), the solids taken from the end of the screw press separator (separated solids), and composted solids after windrow composting (compost).

On farm P samples were taken from the inlet of the roller press separator (raw manure), from the outflow of the separator (separated liquids), from the solids deposited in the compost building (separated solids), and from solids after composting (compost).

On farm A, samples were taken on 7/2/01, 7/9/01, 7/16/01, 8/6/01, 8/13/01, and 8/20/01 to relate the inflow and outflow of the 36-day retention time of the digester and to check the actual reduction of pathogens over its retention time. Also four sets (raw manure, digester outflow, separated liquids and separated solids) of samples were pulled over a 4-hour period and a composite sample created from each individual pull (total of 20

samples), this was performed to get an idea of variability of each grab sample. Two and one half gallons were collected in a 5-gallon bucket and were thoroughly mixed before being placed in two plastic containers not less than 4oz in size. The containers were labeled, sealed and placed in a cooler with ice.

Compost Sampling Procedure

On farm A, solids are separated daily from the digester and hauled to the compost site and stacked in 100-foot windrows to be turned on a weekly basis with a windrow turner. On farm P, piles of excess organic material were placed in windrows to be turned with a bucket loader monthly. Two paired samples were taken from each farm 5/13 and18/02. Eight sub-samples were collected from each side of the windrow, put into clean or plastic lined bucket and mixed well. Care was taken to get sub-samples from all sections of the pile especially the center of the pile. Four composite samples were taken from each farm and quart samples were delivered to the *Map* lab for analysis.

Test Methods

Fecal Coliform was determined in an Environmental Protection Agency certified lab using Standard Methods 18 9221C (Greenberg, 1992).

Map testing was performed at the New York Animal Health Diagnostic Laboratory at Cornell (NYAHDL). United States Department of Agriculture approves the NYAHDL for *Map* culture annually by meeting proficiency testing standards defined by the National Veterinary Services Laboratory, Ames Iowa. Because *Map* has a tendency to clump and because there is no standardized method for culturing *Map*, the procedure used for testing is described below.

The fecal/manure culture for *Map* was carried out at the NYAHDL using the double incubation and centrifugation technique with solid Herrold's Egg Yolk Medium (HEYM) (Shin, 1989, Whitlock, 1990). Briefly, 2 gr. from each mixed composite fecal or manure sample was mixed with 35 ml of sterile distilled water. The suspension was allowed to stand for 30 min. at room temperature. Five ml was transferred from the upper portion of the supernatant and mixed with 25 ml of half-strength brain heart infusion broth (BHI) containing 0.9% hexadecypyrimidinium chloride (HPC). The fecal sample was incubated overnight at 37°C. The sample was harvested by centrifugation at 3,000-x g for 20 min. The pellet was resuspended in 1 ml of half-strength BHI containing vancomycin, amphotericin B, nalidixic acid (5 mg/ml of each) and incubated overnight at 37^{o}C. Finally, 0.15 ml of the solution was transferred to each of 3 tubes of HEYM with mycobactin J and antibiotics. Tubes were incubated for 10 weeks and examined weekly for typical colonies after the initial 4 weeks of incubation. *Map* was confirmed by typical colony morphology, typical growth rate, mycobactin dependence and acid fast staining. If required, IS900 PCR and subculture to HEYM tubes, with and without mycobactin J, provided additional confirmation. Fecal culture results in the dataset were reported in total colony forming units (TCFU) per gram. Total colonies on the three tubes for each sample were counted and multiplied by 7-7.5 to give a semiquatitative result of cfu/gram. Dilutions of 1:10 and 1:100 were done on the pelleted samples from the raw manure to provide an end point dilution estimate for those samples with > 300 cfu (too numerous to count or TNTC results). Again, the method is considered semiquantitative

because *Map* has a tendency to clump; each colony forming unit may represent a clump of organisms or a single organism.

RESULTS

Table 1 is a listing of the overall means from all testing results. As shown in Table 1, there is a significant reduction in organisms tested as the manure goes through some manure system components. We have no explanation for the rise in Fecal Coliform from raw manure to separated liquid on Farm P. The rise in Fecal Coliform from digested separated liquid to storage on farm A is likely from the addition of raw milk and wastewater that is introduced into the storage. The reduction in Fecal Coliform and *Map* in this process is significant. This reduction can help justify using the effluent on growing crops, for bedding, and for sale. Some organisms persist despite the reduction. Care should be taken when using this material for bedding or when feeding crops fertilized during the growing season.

Table 1. Pathogen results for two dairy manure treatment systems

	Farm A		Farm P	
	Fecal Coliform CFU/Gram	*Map* CFU/Gram	Fecal Coliform CFU/Gram	*Map* CFU/Gram
Raw Manure	3,836,400	20,640	1,525,700	20,990
Digested Effluent	3,400	136	N/A	N/A
Separated Liquids	1,700	77	2,085,000	1,200
Storage	7,700	6	87,200	880
Separated Solids	620	20	1,126,400	670
Compost	130	0	12,100	0

Charts 1 and 2 show the variation in the levels of organisms from farm P over time. The raw Fecal Coliform had a standard deviation of 3,300,000. The raw manure for *Map* had a standard deviation of 21,000.

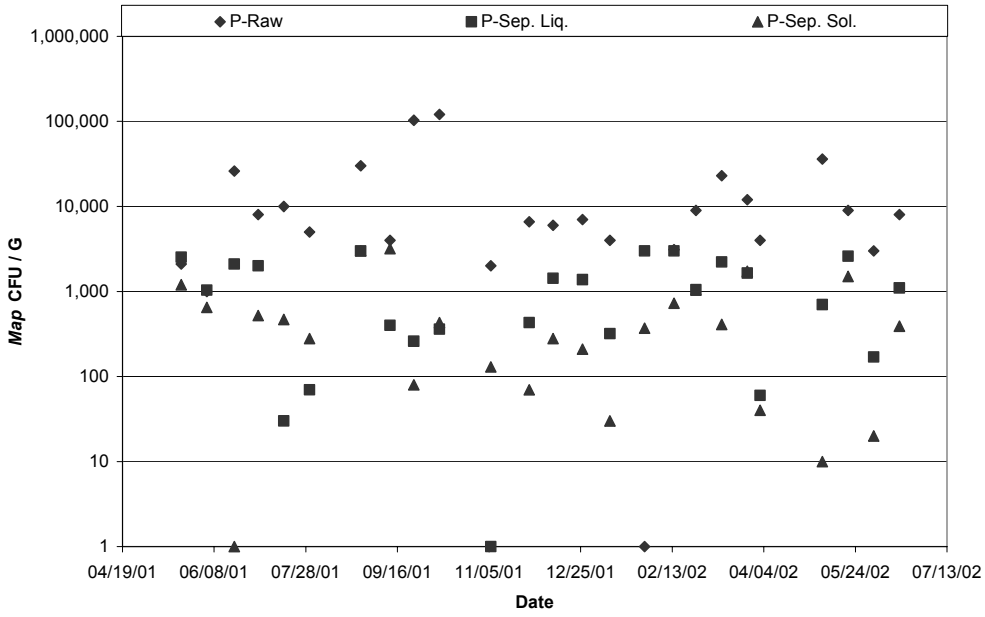

Chart 1. Farm P *Map* versus Time.

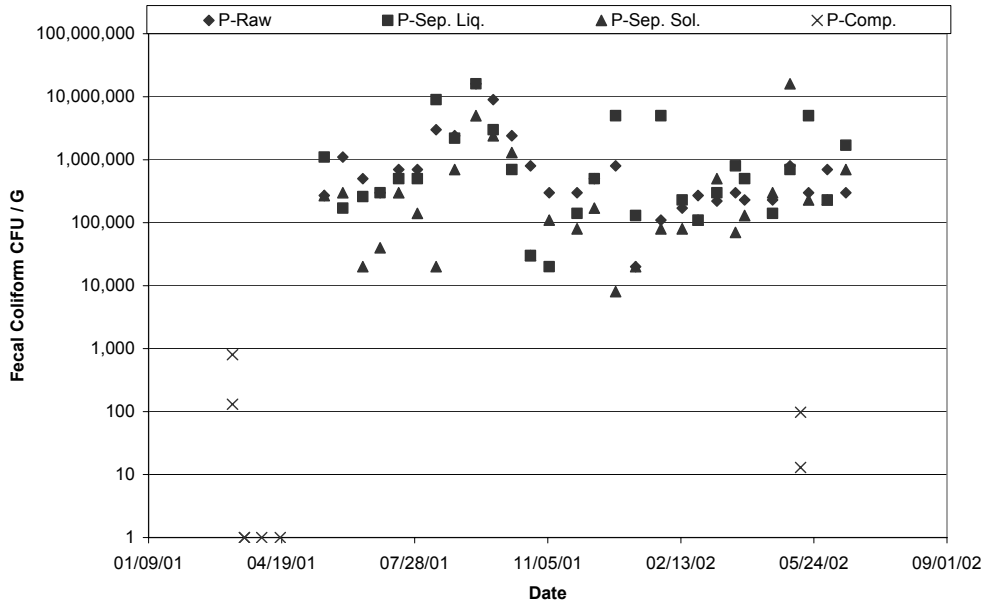

Chart 2. Farm P Fecal Coliform versus Time.

Charts 3 and 4 show the variation in the levels of organisms for farm A over time. The raw Fecal Coliform had a standard deviation of 5,000,000. The raw manure for *Map* had a standard deviation of 36,500. Both farms exhibited a wide range of values. This variation can be attributed to variation in actual organisms shedding, sampling and testing methods. There was not a significant difference in the results obtained when taking 4 samples over a 4-hour period and the composite sample. Apparently a composite sample of 4 samples varies as much as one grab sample for both Fecal Coliform and *Map*.

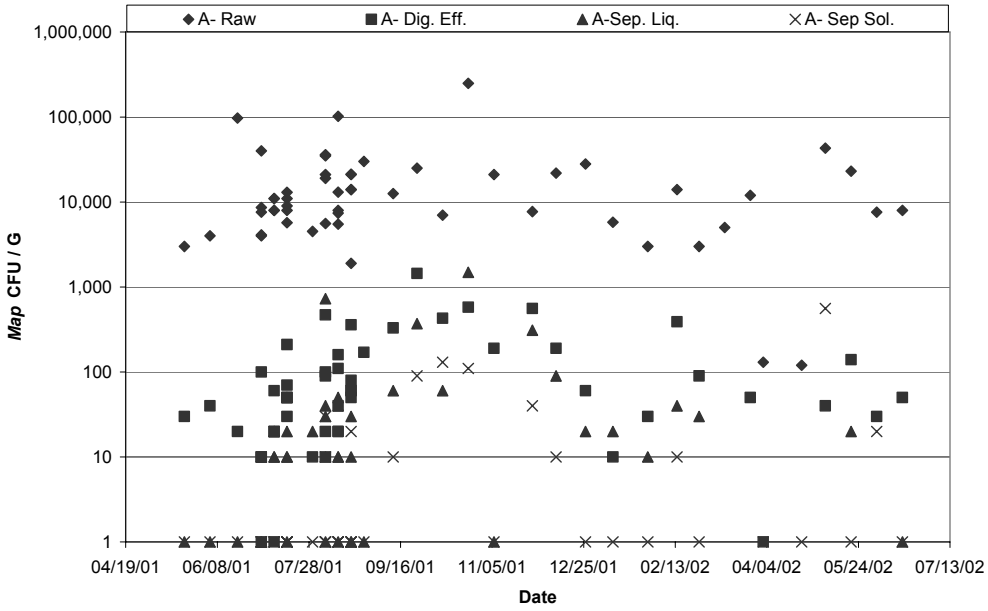

Chart 3. Farm A *Map* versus Time.

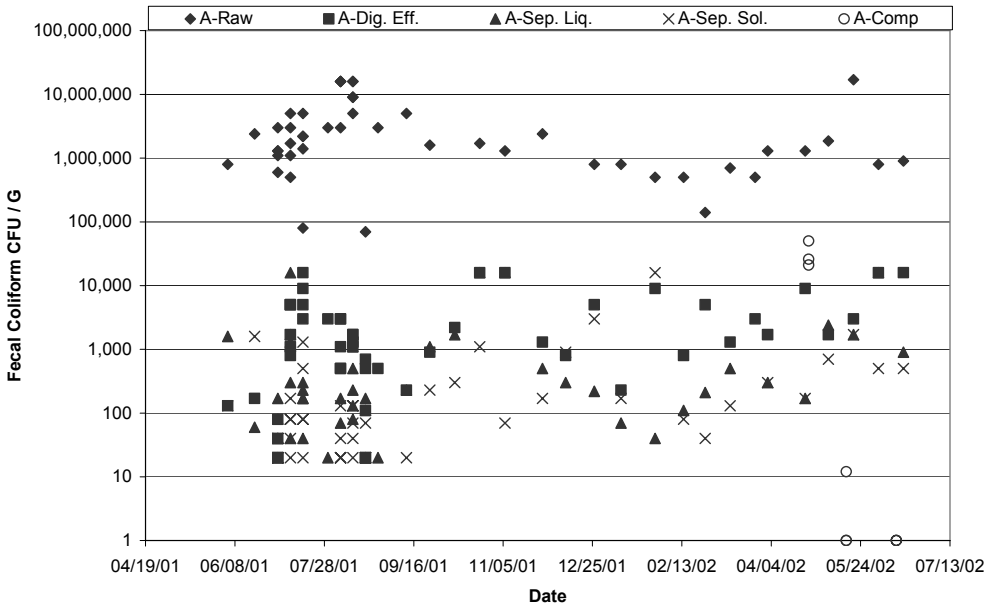

Chart 4. Farm A Fecal Coliform versus Time.

Two other digesters were also studied on a more limited basis. Farm F is a fixed film anaerobic digester with a retention time of 4 days. Farm D is a plug flow digester with a 20-day retention time. Although very few samples were taken on these farms the fecal Coliform reduction in each compares with the reduction in Farm A as shown in Chart 5.

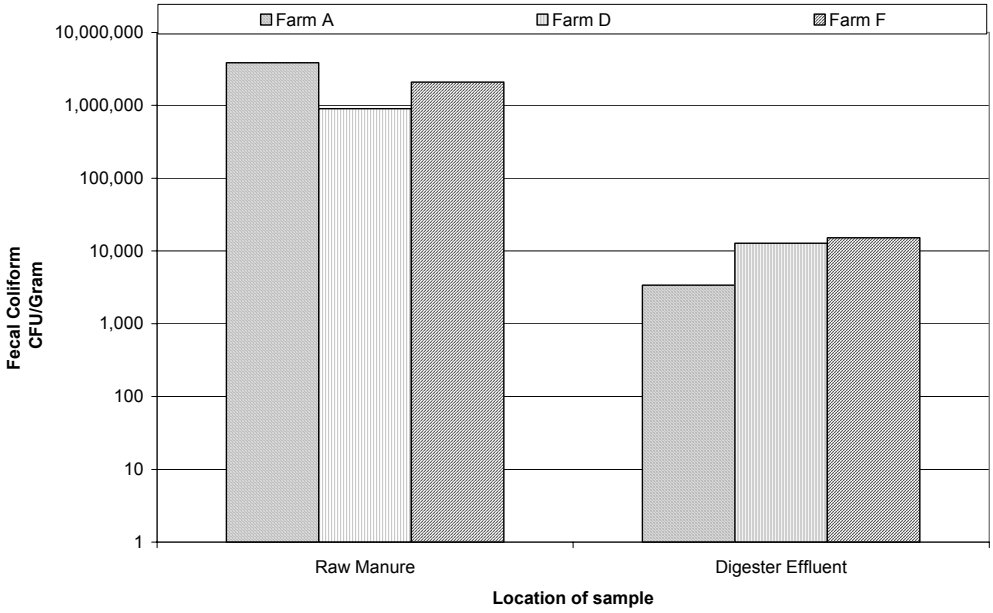

Chart 5. Comparison of three anaerobic digesters and their potential pathogen reduction.

CONCLUSIONS

Anaerobic digestion has the potential to significantly reduce the number of potential pathogens in the effluent. Composting can further reduce or eliminate pathogens. This is an important consideration as manure is applied to growing crops or used as bedding. When more emphasis is placed on reducing the potential for pathogen contamination from spreading manure, anaerobic digestion should be one of the techniques considered.

Additional ways to reduce the number of surviving pathogens even more include:

- Aeration to provide auto heating and an adverse environment,
- Anaerobic digestion at thermophillic temperatures
- Pasteurization of the manure utilizing waste heat from the electric generation process.

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