A.4 Pathogen Emissions Report

Evaluation of Agriclean Technology at the BR Harris Farm for Pathogens
Project OPEN Science Team for Pathogens

Alternative Technology: anaerobic digester followed by solids separation

Location: Bobby Ray Harris Farm, Pitt County, northwest of Greenville, NC

Period of Operation:
The evaluation dates are:

1st field experiment: 01/17/2005 (liquid/solid waste stream only)
2nd field experiment: 01/24/2005 (air only)
3rd field experiment: 01/31/2005 (air only)

Technology Supplier: Phil Lusk, David Palmer, Scott Pogue (Agriclean, LLC, 605-224-4334, 615-238-4477)

NCSU Representative PI: Leonard Bull (919-515-6838); Lynn Worley-Davis (919-515-6852)

Statement of Task:
- Measurement of microbial indicator and pathogen concentrations at key points throughout the waste treatment stream of the technology
- Measurement of airborne microbial indicator and pathogen concentrations at selected sites on the farm in close proximity to the treatment system and at the upper and lower property boundaries
- Measurement of microbial indicator and pathogen concentrations within soils from sites where treated waste water is applied, as well as background soil where spray irrigation does not occur (did not spray irrigate during sample periods)
- Microbial measurements were made during one session corresponding to a cold season.
- Microbial parameters measured for the waste stream: fecal indicators (fecal coliforms, *E. coli*, enterococci, spores of *Clostridium perfringens*, and total coliphages) and the bacterial pathogen *Salmonella*
- Microbial parameters measured in the air samples: total bacteria, total fungi, bacterial endotoxins, fecal indicators (fecal coliforms, *E. coli*, spores of *Clostridium perfringens*, and total coliphages) and the bacterial pathogen *Salmonella*
- Environmental conditions measured at sample points as air samples were collected: temperature, wind direction and speed, relative humidity, solar irradiance

Measurement of Pathogens:
Treatment Technology
The Bobby Ray Harris farm on which the Agriclean alternative technology is built is a finishing farm with twelve swine houses. The source of microbial contaminants on the farm is the fecal matter from the barns. At capacity, there are 960 animals per barn for a total of 11,520 animals on the farm site, with an average weight of 50 to 250 pounds (pigs are scheduled within one week of each other, all in-all out). Each of the houses utilizes tunnel ventilation and a pit-recharge system for removing the fecal matter from the barns. The pit recharge system utilizes 40,000 gallons of water per house with each house being flushed twice per week (5 houses are flushed each day (AgriJet technology)). For part of this project, there was an alternative AgriJet flush system that was to be retrofit into a portion of the houses; however, installation in all 12 houses had not been completed at the time of the evaluation. Due to time constraints, there was only a single cool season evaluation performed for this technology. For the waste treatment stream, the barns are flushed into an underground equalization tank from which the wastewater is moved into a mesophilic anaerobic digester. A portion of the mesophilic digester effluent is re-circulated through a heat exchanger to help heat in the influent from the houses. Under proper operating conditions, the 250,000 gallon digester will operate at 95^{o}F. Methane can be harvested from the digester and used as a heat source for the incoming waste stream from the houses. From the

digester, the treated waste moves to an equalization tank designed to buffer system flows and then into a solids fan separator unit. At the time of this evaluation, there were no resulting solids separated from the treatment system. The treated liquid from the solids separator is then returned to a storage lagoon where it is used to flush the houses.

Microbiological Samples

Single grab samples were collected from points within the waste treatment streams to assess the microbial concentrations associated with the technology. Microbial concentrations were quantitatively determined in the waste stream for fecal indicators (fecal coliforms, *E. coli*, enterococci, spores of *Clostridium perfringens*, and total coliphages) and the bacterial pathogen, *Salmonella*. Microbiological assays were performed according to protocols outlined in the Quality Assurance Project Plan (QAPP) prepared by the Pathogens group of the OPEN team. Briefly, fecal coliform, *E. coli*, and enterococci bacteria were assayed using commercial, quantitative, biochemically-based (defined substrate) microbial culture assay systems and other microbial indicators were assayed using standard quantitative microbial assay methods. *Salmonella* was assayed using an accepted most-probable number culture assay method based on published literature.

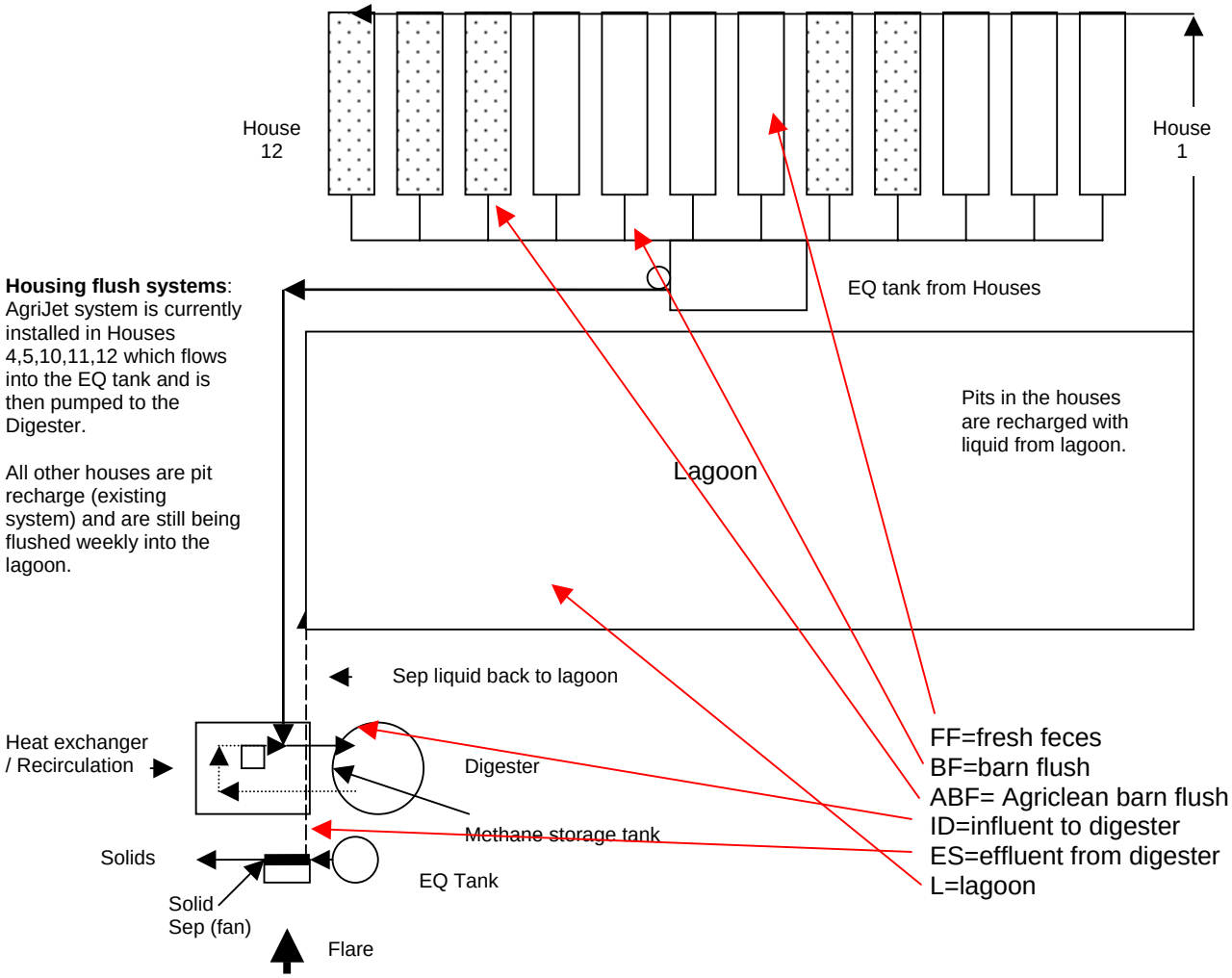
Air samples for microbial analysis were collected at sites throughout the farm. Airborne microbial concentrations were measured for total bacteria, total fungi, spores of *C. perfringens*, fecal coliforms, *E. coli*, and *Salmonella*. Microbiological air sampling was performed using AGI-30 all-glass impingers with sampling at 12.5 LPM for 30 minutes per sample. Each microorganism was analyzed by culture methods described in the QAPP document from the OPEN team. In addition to culturable airborne microorganisms, airborne endotoxins were collected using personal SKC air samplers at approximately 4 LPM for 4 hours. Samples were analyzed by the *Limulus amebocyte lysate* (LAL) test. Environmental conditions, including temperature, relative humidity (RH), wind velocity, and solar irradiation, were measured and recorded at specific locations and times when microbial air samples were collected. These microbial measurements took place according to the following schedule:

Table 21.1. Pathogen Measurement Schedule and Sample Locations at BR Harris Farm

Date Samples Collected	Air Samples Analyzed	Waste Stream Samples Analyzed	Environmental Samples Analyzed
1/17/2004	--	FF, BF, ABF, ID, ES, L	--
8/2/2004	UB, LB, B, L, T	--	--
8/9/2004	UB, LB, B, L, T	--	--

UB=upper boundary; LB=lower boundary; B=barn; L=lagoon; T=technology; FF=fresh feces; BF=barn flush; ABF=Agriclean Barn Flush; ID= influent to digester; ES= effluent from digester; L= lagoon

Figure 21.1. Microbial Waste Stream Measurements Taken at BR Harris Farm



Results:

Waste Stream Samples

Concentrations of microbial indicators and *Salmonella* were measured in the waste stream of two surrogate farms and of the BR Harris farm with the Agriclean technology. At each farm, the microbial "source strength" was measured directly in fresh fecal samples taken from the barns where the animals are housed (Table 21.2). Microbial concentrations in fresh feces at the surrogate farms showed some variations at different sampling times. Concentrations were higher and less variable for fecal coliforms, *E. coli* and enterococci than they were for *C. perfringens*, coliphages and *Salmonella*. Microbial concentrations were statistically lower in the fresh feces at the BR Harris farm than at the surrogate farms (Mann-Whitney U-test; p=0.0311). *Salmonella* concentrations in fresh feces were generally low for all three of the farms tested.

Table 21.2. Pathogen "Source Strength" in Fresh Swine Feces for the Surrogate Farms and the BR Harris Farm with the Agriclean Technology

Site	Date	Fecal Coliform (cfu/g)	*E. coli* (cfu/g)	Enterococci (cfu/g)	*Cl. perfringens* (cfu/g)	Coliphage (pfu/g)	*Salmonella* (cfu/g)
Surrogate 1	9/10/2002	1.4E+07	1.0E+07	3.6E+05	7.9E+04	2.5E+07	4.6E+01
	1/7/2003	8.1E+05	1.7E+05	1.6E+05	4.5E+01	< 4.5E+04	< 3.0E+01
Surrogate 2	10/1/2002	2.9E+05	1.2E+05	5.5E+05	2.3E+02	1.8E+03	< 3.0E-01
	1/28/2003	1.5E+06	2.4E+05	3.0E+05	5.4E+05	3.7E+05	2.1E-01
	5/13/2003	2.4E+06	3.8E+05	5.3E+05	4.5E+03	1.8E+04	3.6E-01
	7/28/2003	3.9E+06	2.9E+06	2.9E+05	3.5E+06	1.8E+06	1.1E+02
Agriclean	1/17/2005	5.2E+04	3.0E+04	1.0E+03	3.3E+02	1.8E+02	3.0E-01

In order to determine treatment efficacy of the surrogate sites and of the BR Harris farm with the Agriclean technology, $\log_{10}$ microbial reductions were computed for each of the treatment systems (Table 21.3). Reductions for the liquid waste streams were computed using the barn flush for each farm as the influent to the treatment systems and using the lagoon liquid microbial concentrations for the surrogate farms and the treated liquid from the digester at the BR Harris farm with the Agriclean technology. Microbial concentrations in the barn flush were used because these give a more representative estimate of the microbial concentrations of the influent to the treatment system than the microbial concentrations in fresh fecal matter. The barn flush represents a greater portion of the animals in the house and provides a more homogenous and time-integrated mixture of microbes. Additionally, these concentrations account for changes in microbial quality caused by any microbial degradation that may occur within the houses before the swine wastes enter the treatment system.

Due to time constraints, the BR Harris farm was evaluated only once during the cold season. This treatment system gave somewhat lower $\log_{10}$ reductions for all of the microbial indicators, as well as for *Salmonella*, during this evaluation period compared to the reductions achieved on the surrogate farms. There are only small differences between the influent and effluent microbial concentrations and this is reflected in the low, and sometimes even negative, values for calculated $\log_{10}$ microbial reductions at the surrogate farm and BR Harris farm sites. There were no statistically significant differences in the $\log_{10}$ reductions for the alternative technology as compared to the surrogate farms (Mann-Whitney U-test, p=0.8715).

Table 21.3. Log_{10} Microbial Reductions in the Waste Streams at the Surrogate Farms and at the BR Harris Farm with the Agriclean Technology

Site	Date	Fecal Coliforms	*E. coli*	Enterococci	*Cl. perfringens*	Coliphage	*Salmonella*
Surrogate 1	9/10/2002	2.0	2.1	2.0	1.3	2.0	1.4
	1/7/2003	1.5	1.5	1.1	0.4	1.1	2.4
Surrogate 2	10/1/2002	1.5	1.6	0.9	1.3	0.7	> 0.2
	1/28/2003	0.7	0.7	0.3	-0.3	0.7	-0.8
	5/13/2003	1.6	1.2	0.9	1.4	1.0	0.3
	7/28/2003	2.0	2.1	2.0	1.3	2.0	1.4
Agriclean	1/17/2005	1.5	1.5	1.5	0.1	1.1	1.7

* Negative Log_{10} Reduction values correspond to increases in microbial concentrations within the treatment systems

In order to better understand the overall microbial reduction for this waste management system, it is important to know the microbial concentrations in the final effluent, or treated wastewater from the system. These microbial concentrations are shown in Table 21.4. The microbial concentrations in the final treated liquids were statistically similar to those in the treated wastewaters at the surrogate farm sites (Mann-Whitney U-test, p=0.7328). There were no solids from the separation system available during the evaluation period.

Table 21.4. Microbial Concentrations in Final Treated Liquids at the Surrogate Farms and at the BR Harris Farm with the Agriclean Technology

Site	Date	Fecal Coliforms (cfu/100mL)	*E. coli* (cfu/100 mL)	Enterococci (cfu/100mL)	*Cl. perfringens* (cfu/100mL)	Coliphage (pfu/100 mL)	*Salmonella* (cfu/100mL)
Surrogate 1	9/10/2002	2.2E+05	1.1E+05	2.0E+04	1.3E+05	4.5E+04	4.6E+02
	1/7/2003	2.6E+05	1.6E+05	4.1E+05	4.9E+04	3.1E+05	4.3E+02
Surrogate 2	10/1/2002	1.3E+05	9.7E+04	2.7E+04	7.0E+04	4.6E+04	4.6E+02
	1/28/2003	1.6E+05	1.1E+05	4.4E+05	9.2E+05	3.6E+05	4.6E+02
	5/13/2003	2.0E+04	1.0E+04	2.8E+04	2.4E+05	3.2E+04	1.5E+01
	7/28/2003	4.9E+04	1.9E+04	1.1E+04	2.3E+06	2.0E+04	3.6E+00
Agriclean	1/17/2005	2.0E+05	1.2E+05	7.4E+05	4.9E+04	3.3E+04	9.2E+00

Environmental Samples

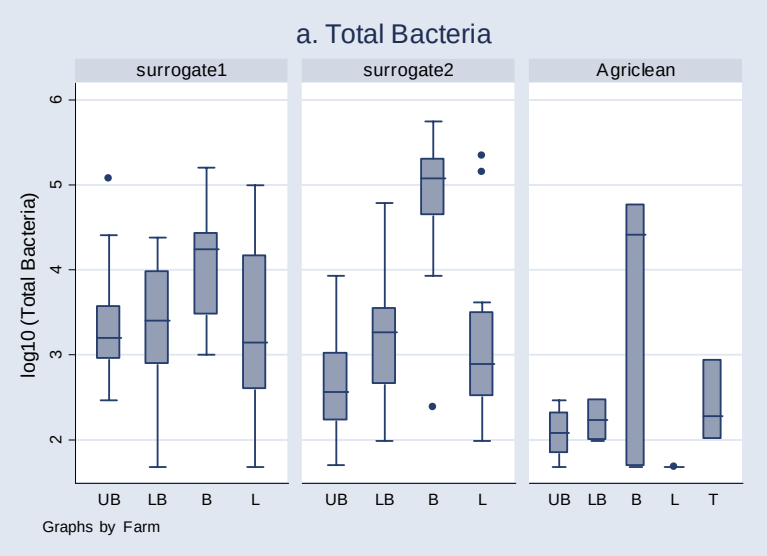
No environmental groundwater samples, soil or vegetation samples from land application sites of waste treatment solid or liquid residuals (byproducts), or vectors (flies) associated with this site were collected during the course of this evaluation (cold season evaluation only). It is hoped that the opportunity to evaluate the full technology and its possible environmental microbial (pathogen) impacts would come at some future time.

On-farm Air Samples

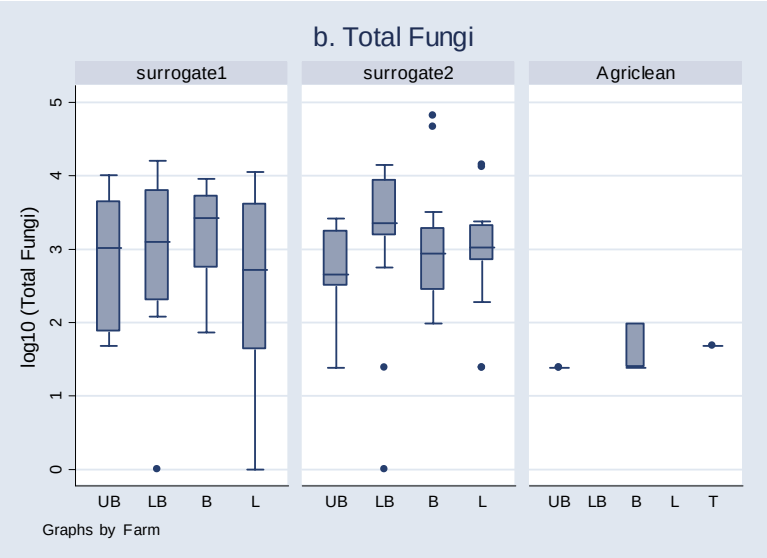
Bacteria and Fungi in Air. Concentrations of total bacteria and total fungi were measured in air on the surrogate farms and on the BR Harris farm with the Agriclean technology (Figures 21.2A and 21.2B). The results for total bacteria concentrations at the BR Harris farm were statistically lower than the concentrations on the surrogate farms (Mann-Whitney U-test; p=0.0005) (Figure 12.2A). The concentrations of total bacteria were generally in the range of 2 to 4 log_{10} per cubic meter at all three of these test sites. Bacterial concentrations at the surrogate sites were lowest at the upper (upwind) boundary, higher at the lower (downwind) boundary and highest near exhaust fans and barns. For the BR Harris farm, the highest levels of total bacteria in air were at the barns and the lowest concentrations were observed at the lagoon. Overall, these results indicate

increases in airborne bacteria on the farms compared to upwind boundary levels. Microbial increases at lower boundaries were higher on conventional farms than on the alternative technology (BR Harris) farm.

Figure 21.2. Concentrations (CFU/M^3) of airborne bacteria and fungi at the surrogate farms and the BR Harris Farm with the Agriclean technology (UB: upper boundary; LB: lower boundary; B: exhaust fan or near barn; L: lagoon; T: technology)



As shown in Figure 12.2(B), the levels of fungi in air tended to be similar on the surrogate farms and statistically lower at the BR Harris farm (Mann-Whitney U-test; p=0.0006). The highest airborne fungi concentrations were at certain sites on the conventional farms, such as near barns (Surrogate 1) and at the lower boundary (Surrogate 2). Concentrations were generally in the range of 2 to 4 $\log_{10}$ per cubic meter for the surrogate farms and in the range of 0 to 2 $\log_{10}$ per cubic meter for the BR Harris farm. Airborne fungi concentrations were very low at the lower boundary and at the lagoon for the BR Harris farm and were highest at the barns.



Fecal Indicator Bacteria in Air. Air samples were analyzed for fecal indicator organisms and for the pathogen, *Salmonella*. Because many of the results for these samples were below the lower level of detection for the assays, the percentage of positive samples based on the total number of samples collected was computed and these percentages are summarized in Tables 21.5 to 21.7. There were no air samples positive for *Salmonella* at any of these farms sites. Both of the surrogate farms had positive air samples at the upper boundary, suggesting that there may be airborne fecal impacts from other adjacent sources. The frequencies of samples positive for fecal indicator microbes in air were generally lowest for upper boundaries and highest for sample sites near waste sources, such as exhaust fans or near barns, lagoons, or the technology. The frequencies at which air samples were positive for fecal indicator microbes were slightly higher on the surrogate farms (38 of 416 samples or 9%) as compared to the BR Harris farm (3 of 100 samples or 3%). However, these frequencies of positive samples were not significantly different (Fisher's Exact Test; $p = 0.2277$) and microbial concentrations in positive microbial air samples were similar for the BR Harris farm with the Agriclean technology when compared to the surrogate farms (median concentrations of 210 and 69 CFU/M^3, respectively, Mann-Whitney U-test, $p = 0.6523$). These results indicate that there are environmental impacts associated with the BR Harris farm and each of the surrogate farms.

Table 21.5. The percentage of positive samples of *Clostridium perfringens* spores measured at different sampling sites on the Surrogate Farm1, Surrogate Farm 2, and BR Harris Farm

Site	Surrogate Farm 1	Surrogate Farm 2	BR Harris Farm
Upper boundary	0	0	0
Lower boundary	0	29%	0
Exhaust fans or near barn	50%	56%	0
Lagoon	13%	13%	0
Technology	n/a	n/a	0

[1] not applicable

Table 21.6. The percentage of positive samples of total coliphage measured at different sampling sites on the Surrogate Farm1, Surrogate Farm 2, and BR Harris Farm

Site	Surrogate Farm 1	Surrogate Farm 2	BR Harris Farm
Upper boundary	0	13%	0
Lower boundary	0	21%	25%
Exhaust fans or near barn	13%	33%	50%
Lagoon	0	13%	0
Technology	n/a	n/a	0

[1] not applicable

Table 21.7. The percentage of positive samples of fecal coliform bacteria (*E. coli*) measured at different sampling sites on the Surrogate Farm1, Surrogate Farm 2, and BR Harris Farm

Site	Surrogate Farm 1	Surrogate Farm 2	BR Harris Farm
Upper boundary	6% (0)	0	0
Lower boundary	0	0	0
Exhaust fans or near barn	13% (0)	0	0
Lagoon	13% (0)	0	0
Technology	n/a	n/a	0

[1] not applicable

The levels of endotoxins measured at the two surrogate farms and the BR Harris farm with the Agriclean technology are summarized in Table 21.8. The concentrations of endotoxins varied a great deal on a daily basis at the farm sites. High levels of endotoxins (mean 985 EU/m^3) were detected at the barn sample for the BR Harris farm and were similar to those at the surrogate

farm 2 (BR Harris and surrogate 2 each use tunnel ventilation). In some cases, the concentrations of endotoxins at the lower boundary were higher than (surrogate 2) or similar to (surrogate 1 farm and BR Harris farm with the Agriclean technology) those at the upper boundary, which suggests that endotoxins released from the swine barns were reaching the lower boundary of at least some farms (surrogate 2 and BR Harris)..

Table 21.8. The levels of endotoxin from airborne dust at sampling sites

Location	Sites	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Summary	
									Mean	SD
		Concentration (EU/m^3)								
Surrogate 1	Upper Boundary	20	5	107	49	n/d[1]	n/d	n/d	45	45
	Upper Wind	9	8	47	217	n/d	n/d	n/d	70	100
	Near Barn 1	70	62	358	481	n/d	n/d	n/d	243	210
	Near Barn 2	217	48	510	510	n/d	n/d	n/d	321	229
	Lagoon	160	14	108	23	n/d	n/d	n/d	76	70
	Lower Boundary	5	6	121	47	n/d	n/d	n/d	45	54
Surrogate 2	Upper Boundary	1	1	15	31	6	21	2	11	12
	Exhaust fan 1	28	312	2940	290	1861	288	55	825	1126
	Exhaust fan 2	225	n/d	2869	84	n/d	n/d	n/d	1059	1569
	Lagoon	3	2	68	26	13	10	21	20	23
	Lower Boundary	3	3	97	26	23	30	4	26	33
BR Harris Farm	Upper Boundary	4	6	n/d	n/d	n/d	n/d	n/d	5	1
	Barn	1922	48	n/d	n/d	n/d	n/d	n/d	985	1325
	Lagoon	26	3	n/d	n/d	n/d	n/d	n/d	15	17
	Technology	22	3	n/d	n/d	n/d	n/d	n/d	12	14
	Lower Boundary	14	10	n/d	n/d	n/d	n/d	n/d	12	3

[1] not done; [2] below limit of detection

Environmental conditions were recorded simultaneously at the points on the farms where air samples were collected, with these values summarized in Table 21.9. Temperatures were somewhat variable for the different sample days for each of the surrogate farms, as would be expected due to the varied seasons of sample collection. Temperatures were lower for the BR Harris farm due to the single season (cold season) evaluation. Mean relative humidity, mean wind velocity, and mean solar irradiation were similar for each of the farms.

Table 21.9. Summary of environmental conditions during microbial air sampling at the Surrogate Farm1, Surrogate Farm 2, and BR Harris Farm

(a) Temperature (°C)

Farm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean ± SD
Surrogate 1	27±1°C	23±5 °C	1±1 °C	-2 ±1°C	n/a[1]	n/a	n/a	13±14°C
Surrogate 2	31±3°C	30±2°C	8±3°C	19±3°C	25±1°C	32±2°C	33±3°C	25±9°C
BR Harris Farm	4±4°C	6±2°C	n/a	n/a	n/a	n/a	n/a	5±3°C

(b) Relative Humidity (%)

Farm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean ± SD
Surrogate 1	82±3%	52±17%	28±3%	33±7%	n/a	n/a	n/a	49±23%
Surrogate 2	46±8%	61±6%	22±5%	80±12%	28±2%	63±5%	58±5%	51±20%
BR Harris Farm	28±13%	53±8%	n/a	n/a	n/a	n/a	n/a	41±17%

(c) Average wind velocity (m/sec)

Farm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean ± SD
Surrogate 1	2.2±0.6	1.0±0.8	3.0±1.4	1.2±0.4	n/a	n/a	n/a	1.9±1.2
Surrogate 2	0.6±0.3	1.2±0.3	2.2±0.8	3.7±2.6	2.1±0.8	1.5±0.7	1.7±1.1	1.9±1.0
BR Harris Farm	1.0±0.5	4.1±1.4	n/a	n/a	n/a	n/a	n/a	2.6±1.9

(d) Solar irradiation (mW/cm^2)

Farm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean ± SD
Surrogate 1	2.4±1.4	7.1±1.3	3.8±0.8	4.3±0.7	n/a	n/a	n/a	4.4±2.0
Surrogate 2	5.0±1.8	5.4±2.3	5.0±0.5	3.4±2.6	10.3±1.3	6.7±3.7	11.5±0.8	6.8±3.0
BR Harris Farm	5.2±0.4	5.6±0.5	n/a	n/a	n/a	n/a	n/a	5.4±0.5

[1] not applicable

Summary Analysis:

Based on the current information from a single cold season evaluation, the Agriclean system at the BR Harris farm cannot be judged environmentally superior to the current technology because there were statistically similar $\log_{10}$ reductions and there were high concentrations of microbial indicators and pathogens remaining in the resulting waste stream final residuals. Levels of airborne bacteria and fungi were lower at the BR Harris farm when compared to the surrogate farms; however, frequencies, levels and concentrations of some airborne fecal indicator organisms also were statistically similar for the surrogate farms and the BR Harris farm with the Agriclean technology.

Evaluation of Sustainable NC Technology at the Stokes Farm for Pathogens
Project OPEN Science Team for Pathogens

Alternative Technology: Closed-loop liquid treatment and solids composting

Location: Redhill Farm, Greene County, near Ayden, NC

Period of Operation:
The evaluation dates are:

1st field experiment: 03/21/2005 (air only)
2nd field experiment: 03/28/2005 (liquid/solid waste stream only)
3rd field experiment: 07/18/2005 (air and liquid/solid waste stream)
4th field experiment: 07/25/2005 (air only)
5th field experiment: 08/01/2005 (composter liquid/solid waste stream and air)

Project Investigators: Don Lloyd (Environmental Technologies, LLC; 919-922-5399)

NCSU Representative PI: Kurt Creamer (919-515-4092)

Statement of Task:
- Measurement of microbial indicator and pathogen concentrations at key points throughout the waste treatment stream of the technology
- Measurement of airborne microbial indicator and pathogen concentrations at selected sites on the farm in close proximity to the treatment system and at the upper and lower property boundaries
- Measurement of microbial indicator and pathogen concentrations within soils from sites where treated waste water is applied, as well as background soil where spray irrigation does not occur (did not spray irrigate during sample periods)
- Microbial measurements were made during two sessions corresponding to a warm and cold season.
- Microbial parameters measured for the waste stream: fecal indicators (fecal coliforms, *E. coli*, enterococci, spores of *Clostridium perfringens*, and total coliphages) and the bacterial pathogen *Salmonella*
- Microbial parameters measured in the air samples: total bacteria, total fungi, fecal indicators (fecal coliforms, *E. coli*, spores of *Clostridium perfringens*, and total coliphages) and the bacterial pathogen *Salmonella*
- Environmental conditions measured at sample points as air samples were collected: temperature, wind direction and speed, relative humidity, solar irradiance

Measurement of Pathogens:
Treatment Technology
The Sustainable NC alternative technology is located on the Redhill farm. The Redhill site is a finishing facility that consists of 3 animal barns capable of housing 1224 animals per house for a total capacity of 3672 animals on the farm. The source of microbial contamination on the farm is the animals in the houses. The fecal wastes from the barns are removed using a flush system consisting of two flush tanks per house with a capacity of 450 gallons each. The houses are flushed 4 to 5 times daily for a total volume of wastewater of 4500 to 5400 gallons per house per day. The average weight of the pigs at the facility is 150 pounds and the barns are naturally ventilated. The alternative waste management system utilizes an equalization tank to stabilize flows from the houses, after which a solids separator removes the solids from the wastes. The solids are removed using a trailer to convey them to the composter constructed at a separate site in close proximity to the Redhill farm. A sanitizer (TCM – trichlormelamine) and polymer are injected into the liquid portion of the waste stream, flocculated using a static mixer, and solids are further removed through two settling tanks in series. The settled solids and polymer is removed to the composter for further treatment. A portion of the liquid waste stream is returned to the flush

tanks for use in removing the wastes from the barns and the other portion is further treated through a Norweco aeration and filtration system to produce drinking water for the pigs. The Norweco treated water is mixed with well water to dilute the dissolved salts to achieve potable drinking water for the pigs. One-ppm chlorine is maintained in the water for the pigs through the use of an automatic residual chlorine monitor.

Microbiological Samples

Single grab samples were collected from points within the waste treatment streams to assess the microbial concentrations associated with the technology. Microbial concentrations were quantitatively determined in the waste stream for fecal indicators (fecal coliforms, *E. coli*, enterococci, spores of *Clostridium perfringens*, and total coliphages) and the bacterial pathogen, *Salmonella*. Microbiological assays were performed according to protocols outlined in the Quality Assurance Project Plan (QAPP) prepared by the Pathogens group of the OPEN team. Briefly, fecal coliform, *E. coli*, and enterococci bacteria were assayed using commercial, quantitative, biochemically-based (defined substrate) microbial culture assay systems and other microbial indicators were assayed using standard quantitative microbial assay methods. *Salmonella* was assayed using an accepted most-probable number culture assay method based on published literature.

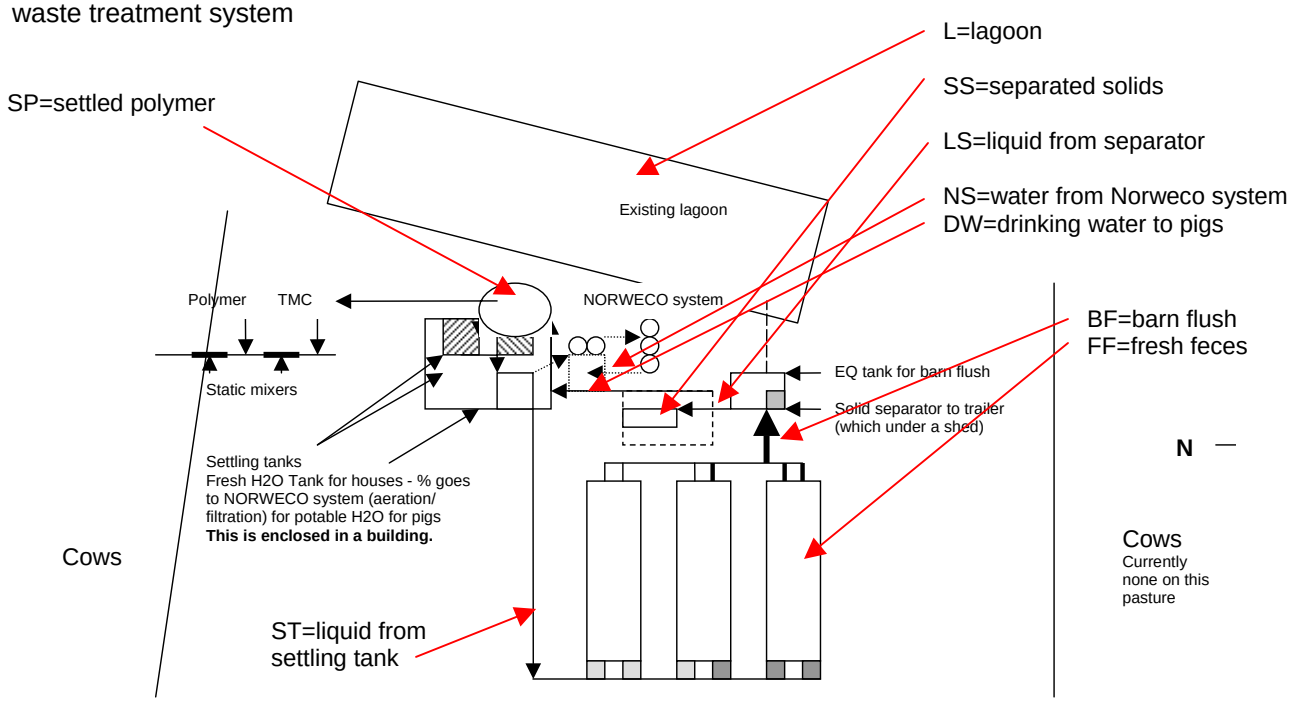
Air samples for microbial analysis were collected at sites throughout the farm. Airborne microbial concentrations were measured for total bacteria, total fungi, spores of *C. perfringens*, fecal coliforms, *E. coli*, and *Salmonella*. Microbiological air sampling was performed using AGI-30 all-glass impingers with sampling at 12.5 LPM for 30 minutes per sample. Each microorganism was analyzed by culture methods described in the QAPP document from the OPEN team. Environmental conditions, including temperature, relative humidity (RH), wind velocity, and solar irradiation, were measured and recorded at specific locations and times when microbial air samples were collected. These microbial measurements took place according to the following schedule:

Table 22.1. Pathogen Measurement Schedule and Sample Locations at Stokes Farm

Date Samples Collected	Air Samples Analyzed	Waste Stream Samples Analyzed	Environmental Samples Analyzed
3/21/2005	UB, LB, L, B, T	--	--
3/28/2005	--	FF, SS, SP, BF, LS, ST, NS, DW, L	--
7/18/2005	UB, LB, L, B, T	FF, SS, SP, BF, LS, ST, NS, DW, L	--
7/25/2005	UB, LB, L, B, T	--	--
8/1/2005 (composter)	UB, LB, T	IN, AM, EF, CT	--

UB=upper boundary; LB=lower boundary; L=lagoon B=barn; T=technology; FF=fresh feces; SS=solids from separator; SP=settled polymer; BF=barn flush; LS=liquids from separator; ST=liquids from settling tank; NS=liquid from Norweco system; DW=drinking water to houses; L=lagoon; IN=influent to composter; AM= compost amendment (cotton offal); EF=effluent from composter; CT=tea from composter

Figure 22.1. Microbial Waste Stream Measurements Taken at the Stokes Farm with the SNC waste treatment system



Results:

Waste Stream Samples

Concentrations of microbial indicators and *Salmonella* were measured in the waste stream of two surrogate farms and of the Redhill farm with the Sustainable NC (SNC) technology. At each farm, the microbial "source strength" was measured directly in fresh fecal samples taken from the barns where the animals are housed (Table 22.2). Microbial concentrations in fresh feces at the surrogate farms showed some variations at different sampling times. Concentrations were higher and less variable for fecal coliforms and *E. coli* than they were for enterococci, *C. perfringens*, coliphages and *Salmonella*. Microbial concentrations were statistically similar in the fresh feces at the Redhill farm on 3/28/05 and 7/18/05 and at the surrogate farms (Mann-Whitney U-test; p=0.8583). Microbial concentrations were statistically lower in the fecal matter at the Redhill farm that was subjected to composting on 8/1/05 as compared to the microbial concentrations in the fresh feces at the surrogate farms (Mann-Whitney U-test; p=0.0195). When all of the data for the Redhill farm is combined, there are no statistically significant differences between the fecal matter at this farm compared to the surrogate farm sites (Mann-Whitney U-test; p=0.1805). *Salmonella* concentrations in fresh feces were generally low for all three of the farms tested.

Table 22.2. Pathogen "Source Strength" in Fresh Swine Feces for the Surrogate Farms and the Stokes Farm with the Sustainable NC Technology

Site	Date	Fecal Coliform (cfu/g)	*E. coli* (cfu/g)	Enterococci (cfu/g)	*Cl. perfringens* (cfu/g)	Coliphage (pfu/g)	*Salmonella* (cfu/g)
Surrogate 1	9/10/2002	1.4E+07	1.0E+07	3.6E+05	7.9E+04	2.5E+07	4.6E+01
	1/7/2003	8.1E+05	1.7E+05	1.6E+05	4.5E+01	< 4.5E+04	< 3.0E+01
Surrogate 2	10/1/2002	2.9E+05	1.2E+05	5.5E+05	2.3E+02	1.8E+03	< 3.0E-01
	1/28/2003	1.5E+06	2.4E+05	3.0E+05	5.4E+05	3.7E+05	2.1E-01
	5/13/2003	2.4E+06	3.8E+05	5.3E+05	4.5E+03	1.8E+04	3.6E-01
	7/28/2003	3.9E+06	2.9E+06	2.9E+05	3.5E+06	1.8E+06	1.1E+02
SNC	3/28/2005	2.6E+06	1.8E+06	2.0E+05	2.3E+05	3.0E+06	7.4E-01
	7/18/2005	2.0E+06	7.5E+05	4.8E+03	4.9E+01	3.3E+04	3.0E-01
SNC composter	8/1/2005	2.0E+02	2.0E+02	3.1E+03	4.6E+03	6.0E+02	3.0E-01

In order to determine treatment efficacy of the surrogate sites and of the Redhill farm with the SNC technology, $\log_{10}$ microbial reductions were computed for each of the treatment systems (Table 22.3). Reductions for the liquid waste streams were computed using the barn flush for each farm as the influent to the treatment systems and using as final residuals the lagoon liquid microbial concentrations for the surrogate farms and the combined treated liquid and solid residuals at the Redhill farm with the SNC technology. Microbial concentrations in the barn flush were used because these give a more representative estimate of the microbial concentrations of the influent to the treatment system than the microbial concentrations in fresh fecal matter. The barn flush represents a greater portion of the animals in the house and provides a more homogenous and time-integrated mixture of microbes. Additionally, these concentrations account for changes in microbial quality caused by any microbial degradation that may occur within the houses before the swine wastes enter the treatment system.

During the first two evaluation periods, the composter system was not operational and microbial reductions were computed based on no further treatment of the separated waste solids from the system. The technology provider and NCSU representative provided flow estimates that allowed us to make computations for the microbial reductions achieved by the system. These microbial reductions are based on 16% of the overall flow through the system removed as solids by the solids separator, 17% of the flow removed as solids by the flocculation and settling, 56% of the flow returned as liquid to the flush tanks of the barns, and 11% of the flow treated through the Norweco system for producing potable drinking water for the pigs. This treatment system gave relatively low $\log_{10}$ reductions for all of the microbial indicators, as well as for *Salmonella*, during these two evaluation periods compared to the reductions achieved on the surrogate farms. The comparatively low microbial reductions in the alternative technology were because of the higher microbial concentrations in the solids from the separator and from the settling tanks. There are only small differences between the influent and effluent microbial concentrations and this is reflected in the low, and sometimes even negative, values for calculated $\log_{10}$ microbial reductions at the surrogate farm sites and at the BR Harris farm with the SNC technology. The alternative SNC technology actually performed worse than the surrogate farms as evidenced by statistically lower $\log_{10}$ reductions in the waste stream (Mann-Whitney U-test, $p=0.0033$).

The composter was operational and was evaluated on 8/1/2005. Microbial $\log_{10}$ reductions were computed using the separated solids from the SNC technology as the influent material and the composter effluent liquids and solids. There were statistically higher $\log_{10}$ reductions achieved by the composter alone as compared to the reductions at the surrogate farm sites (Mann-Whitney U-test; $p=0.0051$).

Table 22.3. Log_{10} Microbial Reductions in the Waste Streams at the Surrogate Farms and at the Stokes Farm with the Sustainable NC Technology

Site	Date	Fecal Coliforms	*E. coli*	Enterococci	*Cl. perfringens*	Coliphage	*Salmonella*
Surrogate 1	9/10/2002	2.0	2.1	2.0	1.3	2.0	1.4
	1/7/2003	1.5	1.5	1.1	0.4	1.1	2.4
Surrogate 2	10/1/2002	1.5	1.6	0.9	1.3	0.7	> 0.2
	1/28/2003	0.7	0.7	0.3	-0.3*	0.7	-0.8
	5/13/2003	1.6	1.2	0.9	1.4	1.0	0.3
	7/28/2003	2.0	2.1	2.0	1.3	2.0	1.4
SNC	3/28/2005	0.3	0.7	1.0	0.5	0.4	> 0.1
	7/18/2005	0.3	> 0.4	-0.2	> 0.1	> 1.6	> 0.0
SNC composter	8/1/2005	2.1	2.3	2.6	3.0	2.9	0.8

* Negative Log_{10} Reduction values correspond to increases in microbial concentrations within the treatment systems

Table 22.3 demonstrates the microbial reductions achieved by the SNC technology without and with the subsequent composter for treatment of the separated solids. In order to understand the overall efficacy of the SNC system, it is important to compute the combined reductions that would be expected by the SNC technology for treatment of waste liquids and the composter for treatment of the separated waste solids. The microbial reductions are summarized in Table 22.4. As expected, greater overall microbial reductions are achieved by the complete system for solids and liquid treatment. These microbial reductions for the overall system are statistically similar to the reductions achieved by the conventional systems on the surrogate farm sites (Mann-Whitney U-test; p=0.7750).

Table 22.4. Log_{10} Microbial Reductions in the Waste Streams at the Surrogate Farms and at the Stokes Farm with the Combined Sustainable NC Technology and Composter

Site	Date	Fecal Coliforms	*E. coli*	Enterococci	*Cl. perfringens*	Coliphage	*Salmonella*
Surrogate 1	9/10/2002	2.0	2.1	2.0	1.3	2.0	1.4
	1/7/2003	1.5	1.5	1.1	0.4	1.1	2.4
Surrogate 2	10/1/2002	1.5	1.6	0.9	1.3	0.7	> 0.2
	1/28/2003	0.7	0.7	0.3	-0.3*	0.7	-0.8
	5/13/2003	1.6	1.2	0.9	1.4	1.0	0.3
	7/28/2003	2.0	2.1	2.0	1.3	2.0	1.4
SNC liquid + composter	--	1.2	1.3	1.8	1.4	1.3	> 0.3
	--	1.1	> 1.2	0.9	> 1.1	> 2.1	> 0.3

* Negative Log_{10} Reduction values correspond to increases in microbial concentrations within the treatment systems

These values for microbial reductions are somewhat misleading because the calculations take into account the portion of the waste from the settling tanks (56% of the total flow) that is re-circulated to flush the barns and that never leave the system. In order to better understand the efficacy of this combined treatment system, it is important to consider the microbial concentrations in the final treated waste residuals resulting from treatment (Table 22.5). The residuals from the system are the resulting liquid from the Norweco system (Table 22.5 – liquid) that is diluted as potable drinking water for the pigs (Table 22.5 – drinking water) and the resulting liquid and solids from the compost unit. During the first evaluation, the microbial concentrations were still quite high in the treated Norweco liquids (1.7E+03 to 4.6E+05 cfu of pfu/100 mL; except

Salmonella which was below detection limits). For the second evaluation, these microbial concentrations were considerably lower (below detection limits to 1.3E+02 cfu/100 mL). The microbial concentrations in the treated liquids from the Norweco system are statistically lower than the microbial concentrations in the liquids at the surrogate farms with conventional treatment (Mann-Whitney U-test; p=0.0008). The drinking water reflects these microbial concentrations, with detectable levels of indicators in drinking water during the initial evaluation period and generally below detection limits during the second evaluation period. Of particular note and concern are the viral indicators (coliphage) during the initial period with 680 pfu/100 mL that was going to the pigs for potable drinking water.

There are two waste residuals from the compost unit: a solid residual and a compost "tea" material. The technology providers noted during the evaluations that the liquid "tea" material had been previously used from other similar systems as a nutrient source for plants. This may not be an acceptable practice for raw food plants (e.g., produce) without further treatment of this material. This is because of the high microbial concentrations that we measured during this evaluation, including measurable concentrations of the frank pathogen, *Salmonella* (1.5E+01 to 3.6E+05 cfu or pfu/100mL). However, these microbial concentrations are statistically lower than the residual materials from the surrogate farms with conventional treatment and those liquids are land applied at agronomic rates (Mann-Whitney U-test; p=0.0423).

For the solid residual from the compost unit, the microbial concentrations are reported in Table 22.5 as concentrations per 100 grams wet weight. These microbial concentrations in the composted solid materials are statistically lower compared with the microbial concentrations in the treated residuals from the surrogate farms (Mann-Whitney U-test; p=0.0269). It should also be noted that this material meets Class A biosolids standards for fecal coliform bacteria (<1000 fecal coliform bacteria per gram) and for *Salmonella* (<3 *Salmonella* per 4 grams). However, further studies would be needed to demonstrate whether the material meets the total culturable virus (<1 total per 4 grams) and viable helminth ova standards (<1 viable helminth (*Ascaris*) ova per 4 grams). Overall microbial concentrations from this combined system (treated liquid from the SNC technology and treated liquid and solids from the compost unit) were statistically lower that those microbial concentrations in treated residual materials from the surrogate farm site (Mann-Whitney U-test; p<0.0001).

Table 22.5. Microbial Concentrations in Final Treated Residuals at the Surrogate Farms and at the Stokes Farm with the Sustainable NC Technology

Site	Date	Material	Fecal Coliforms (cfu/100mL)	*E. coli* (cfu/100 mL)	Enterococci (cfu/100mL)	*Cl. perfringens* (cfu/100mL)	Coliphage (pfu/100 mL)	*Salmonella* (cfu/100mL)
Surrogate 1	9/10/02	liquid	2.2E+05	1.1E+05	2.0E+04	1.3E+05	4.5E+04	4.6E+02
	1/7/03	liquid	2.6E+05	1.6E+05	4.1E+05	4.9E+04	3.1E+05	4.3E+02
Surrogate 2	10/1/02	liquid	1.3E+05	9.7E+04	2.7E+04	7.0E+04	4.6E+04	4.6E+02
	1/28/03	liquid	1.6E+05	1.1E+05	4.4E+05	9.2E+05	3.6E+05	4.6E+02
	5/13/03	liquid	2.0E+04	1.0E+04	2.8E+04	2.4E+05	3.2E+04	1.5E+01
	7/28/03	liquid	4.9E+04	1.9E+04	1.1E+04	2.3E+06	2.0E+04	3.6E+00
SNC	3/28/05	liquid	1.4E+04	1.2E+04	3.7E+03	1.7E+03	4.6E+05	<3.0E+01
		drinking water	5.6E+01	3.7E+01	5.1E+00	2.0E+01	6.8E+02	<3.0E+01
	7/18/05	liquid	1.3E+02	<1.0E+01	2.0E+01	<1.8E+01	<4.5E+01	<3.0E+01
		drinking water	1.9E+01	<1.0E+01	1.5E+01	<1.8E+01	<4.5E+01	<3.0E+01
SNC composter	8/1/05	liquid	1.5E+04	3.5E+03	3.6E+05	4.5E+01	5.0E+01	1.5E+01
		solids	6.8E+04	6.1E+03	1.7E+04	2.3E+03	5.0E+03	2.0E+01

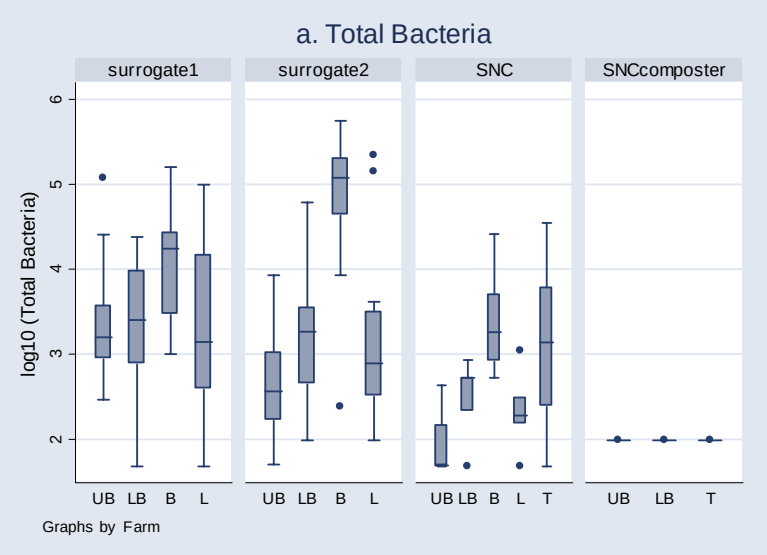
Environmental Samples

No environmental groundwater samples, soil or vegetation samples from land application sites of waste treatment solid or liquid residuals (byproducts), or vectors (flies) associated with this site were collected during the course of this evaluation. We attempted to collect vectors (flies) at this technology site on both 3/28/2005 and 7/18/2005, but none were caught. This presumably was due to low numbers of flies at this site during those evaluation periods. We were not able to collect houseflies at the SNC compost site on 8/1/05; however, we did note houseflies around the compost unit. We suggested to the technology providers that the piled composted material should be covered to avoid any potential contamination issues as a result of houseflies at the site. It is hoped that the opportunity to evaluate the full technology and its possible environmental microbial (pathogen) impacts would come at some future time.

On-farm Air Samples

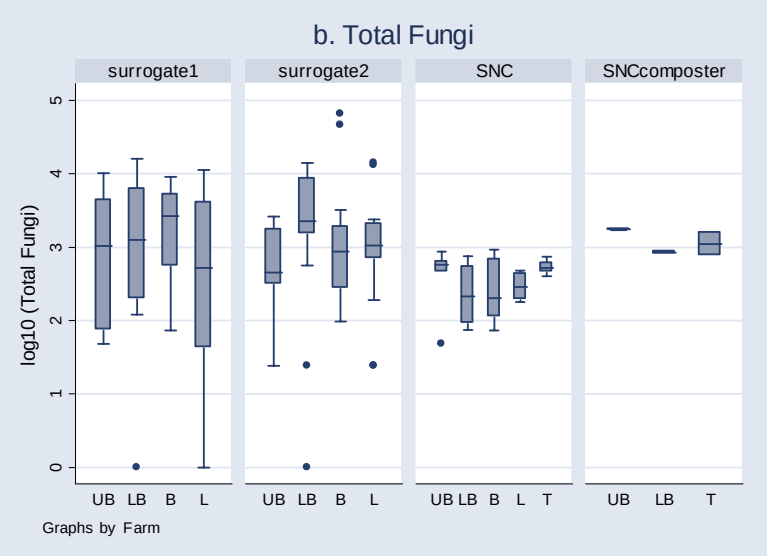
Bacteria and Fungi in Air. Concentrations of total bacteria and total fungi were measured in air on the surrogate farms and on the Redhill farm with the SNC Technology (Figures 22.2A and 22.2B). Total bacteria concentrations at the Redhill farm were statistically lower than the concentrations at the surrogate farms (Figure 12.2A)(Mann-Whitney U-test, $p<0.0001$). The concentrations of total bacteria were generally in the range of 2 to 6 $\log_{10}$ per cubic meter at all three of these test sites. Bacterial concentrations at the surrogate sites were lowest at the upper (upwind) boundary, higher at the lower (downwind) boundary and highest near exhaust fans and barns. For the Redhill farm, the highest levels of total bacteria in air were at barns followed closely by the technology samples. Overall, these results indicate increases in airborne bacteria on the farms compared to upwind boundary levels. Microbial increases at lower boundaries were higher on conventional farms than on the alternative technology (Redhill) farm on the basis of $\log_{10}$ concentrations at upper boundaries, especially when the composter was operational at the alternative technology site.

Figure 22.2. Concentrations (CFU/M^3) of airborne bacteria and fungi at the surrogate farms and the Redhill farm with the Sustainable NC technology (UB: upper boundary; LB: lower boundary; B: exhaust fan or near barn; L: lagoon; T: technology)



As shown in Figure 12.2(b), the levels of fungi in air were statistically higher on the surrogate farms as compared to the Redhill farm with the SNC technology. The highest airborne fungi concentrations were at certain sites on the conventional farms, such as near barns (Surrogate 1) and at the lower boundary (Surrogate 2). The highest fungi concentrations associated with the

SNC technology were at the compost unit. Concentrations were generally in the range of 2 to 4 $\log_{10}$ per cubic meter. In general, airborne fungi concentrations were lower at the upper (upwind) boundary of surrogate farms, higher at the lower (downwind) boundary and highest near exhaust fans and barns. Overall, airborne fungi concentrations were somewhat lower on the alternative technology (Redhill) farm as compared to the surrogate farms.



Fecal Indicator Bacteria in Air. Air samples were analyzed for fecal indicator organisms and for the pathogen, *Salmonella*. Because many of the results for these samples were below the lower level of detection for the assays, the percentage of positive samples based on the total number of samples collected was computed and these percentages are summarized in Tables 22.6 to 22.8. There were no positive air samples at any of the sites for the frank pathogen, *Salmonella*. Both of the surrogate farms had positive air samples at the upper boundary, suggesting that there may be airborne fecal impacts from other adjacent sources. The frequencies of samples positive for fecal indicator microbes in air were generally lowest for upper boundaries and highest for sample sites near waste sources, such as exhaust fans or near barns, lagoons, or the technology. The frequencies at which air samples were positive for fecal indicator microbes were higher on the surrogate farms (38 of 416 samples or 9%) as compared to the Redhill farm (0 of 120 samples or 0%). These frequencies of positive samples were significantly lower (Fischer's Exact Test; $p<0.0001$) for the Redhill farm as compared to the frequencies of positives at the surrogate farm sites. These results indicate that there are fewer environmental impacts from airborne fecal microbes associated with the Redhill farm than at the surrogate farms.

Table 22.6. The percentage of positive samples of *Clostridium perfringens* spores measured at different sampling sites on the Surrogate Farm1, Surrogate Farm 2, and Redhill farm with the SNC Technology

Site	Surrogate Farm 1	Surrogate Farm 2	SNC
Upper boundary	0	0	0
Lower boundary	0	29%	0
Exhaust fans or near barn	50%	56%	0
Lagoon	13%	13%	0
Technology	n/a	n/a	0
Technology 2 (compost)	n/a	n/a	0

[1] not applicable

Table 22.7. The percentage of positive samples of total coliphage measured at different sampling sites on the Surrogate Farm1, Surrogate Farm 2, and Redhill farm with the SNC Technology

Site	Surrogate Farm 1	Surrogate Farm 2	SNC
Upper boundary	0	13%	0
Lower boundary	0	21%	0
Exhaust fans or near barn	13%	33%	0
Lagoon	0	13%	0
Technology	n/a	n/a	0
Technology 2 (compost)	n/a	n/a	0

[1] not applicable

Table 22.8. The percentage of positive samples of fecal coliform bacteria (*E. coli*) measured at different sampling sites on the Surrogate Farm1, Surrogate Farm 2, and Redhill farm with the SNC Technology

Site	Surrogate Farm 1	Surrogate Farm 2	SNC
Upper boundary	6% (0)	0	0
Lower boundary	0	0	0
Exhaust fans or near barn	13% (0)	0	0
Lagoon	13% (0)	0	0
Technology	n/a	n/a	0
Technology 2 (compost)	n/a	n/a	0

[1] not applicable

Environmental conditions were recorded simultaneously at the points on the farms where air samples were collected, with these values summarized in Table 22.9. Temperatures were somewhat variable for the different sample days for each of the farm sites, as would be expected due to the varied seasons of sample collection. Mean relative humidity, mean wind velocity, and mean solar irradiation were similar for each of the farms tested.

Table 22.9. Summary of environmental conditions during microbial air sampling at the Surrogate Farm1, Surrogate Farm 2, and Stokes Farm with the SNC Technology

(a) Temperature (°C)

Farm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean ± SD
Surrogate 1	27±1°C	23±5 °C	1±1 °C	-2 ±1°C	n/a[1]	n/a	n/a	13±14°C
Surrogate 2	31±3°C	30±2°C	8±3°C	19±3°C	25±1°C	32±2°C	33±3°C	25±9°C
SNC	18±2	35±3 °C	33±3 °C	26±1 °C	n/a	n/a	n/a	28±7°C

(b) Relative Humidity (%)

Farm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean ± SD
Surrogate 1	82±3%	52±17%	28±3%	33±7%	n/a	n/a	n/a	49±23%
Surrogate 2	46±8%	61±6%	22±5%	80±12%	28±2%	63±5%	58±5%	51±20%
SNC	31±5%	60±7%	60±8%	81±3%	n/a	n/a	n/a	55±18%

(c) Average wind velocity (m/sec)

Farm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean ± SD
Surrogate 1	2.2±0.6	1.0±0.8	3.0±1.4	1.2±0.4	n/a	n/a	n/a	1.9±1.2
Surrogate 2	0.6±0.3	1.2±0.3	2.2±0.8	3.7±2.6	2.1±0.8	1.5±0.7	1.7±1.1	1.9±1.0
SNC	0.8±0.5	1.6±1.0	2.0±1.1	1.5±0.7	n/a	n/a	n/a	1.5±1.0

(d) Solar irradiation (mW/cm^2)

Farm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean ± SD
Surrogate 1	2.4±1.4	7.1±1.3	3.8±0.8	4.3±0.7	n/a	n/a	n/a	4.4±2.0
Surrogate 2	5.0±1.8	5.4±2.3	5.0±0.5	3.4±2.6	10.3±1.3	6.7±3.7	11.5± 0.8	6.8±3.0
SNC	8.1±1.7	9.8±3.0	9.7±1.2	3.3±0.8	n/a	n/a	n/a	8.2±3.0

[1] not applicable

Summary Analysis:

Overall, the SNC technology built at the Redhill farm site can potentially be viewed as an environmentally superior technology even though it does not show extensive microbial $\log_{10}$ reductions in the waste stream. This is because a significant portion of the flow (56%) is contained within the system. The resulting microbial concentrations in the final treated residual material (drinking water to pigs and composted solids and liquids) are statistically lower than the residual materials from the surrogate farm sites with the conventional treatment technologies. These treated materials include the water that is treated through the Norweco system for potable use by the pigs on the farm, and the liquid and solid material from the compost unit. Although the microbial concentrations in the compost liquid "tea" are somewhat high, they are statistically lower than the treated residual liquids from the surrogate farms with conventional treatment that are generally land applied in much greater volumes for agronomic purposes to crops at farm sites. Additionally, the residual solid material from the compost unit had microbial concentrations that are statistically lower that the residuals from the conventional technology sites (Mann-Whitney U-test; p=0.0052), and further go on to meet Class A biosolids standards for fecal coliform bacteria and *Salmonella* (analyses were not performed to determine if they meet Class A biosolids standards for total culturable viruses and helminth ova). It should further be noted that the volumes of residual materials from the compost unit are less than the volumes of treated liquids on farm sites utilizing conventional technology. Therefore, not only are the microbial concentrations lower at the SNC technology site, but the microbial loads (concentration X volume) to the environmental will be lower than at farm sites with the conventional technologies.

Furthermore, there were statistically lower airborne microbial indicator (total bacteria and fungi) concentrations at the Redhill farm with the SNC technology as compared to concentrations in air at the surrogate farm sites with conventional technologies. There were no airborne fecal indicators (fecal coliform bacteria, *E. coli*, spores of *Cl. perfringens*, and coliphage) or *Salmonella* detected in any of the samples collected to the Redhill farm site, as compared to 9% frequency at the surrogate farm sites. Finally, there were no houseflies collected at the Redhill site during each of the sampling periods; this presumably was because there were low concentrations of houseflies associated with the technology at this site.

Antimicrobial Resistance of Bacteria collected from Surrogate Farms and Farms with Alternative Waste Management Systems

(Note: This report is a revision of information presented in the 2005 Phase 2 Report for Technology Determinations, Appendix A8, Pathogen Emissions Report)

This section of the report summarizes the essential study findings on the performance of the alternative swine waste technologies for presence and reductions of antimicrobial resistance for *E. coli* and *Salmonella* relative to the antimicrobial resistance for bacteria isolated from the surrogate farms and relative to each other. Antimicrobial resistance analyses were done on bacteria isolates from eighteen swine farms: two surrogate farms and sixteen farms or sites with alternative technologies. The alternative technology farms include:

- Goshen Ridge farm with the Super Soils technology for treatment of liquid wastes,
- Hickory Grove site with the Super Soils composting technology for the waste solids from the Goshen Ridge farm,
- Grinnells laboratory on the NCSU campus with the ReCycle belt system,
- Lake Wheeler Research farm with the ReCycle gasifier,
- Harrells farm with the ISSUES technology,
- Carrolls farm with the ISSUES technology,
- Vestal farm with the ISSUES technology,
- Agriclean farm,
- Corbett farm #1 (BEST) technology,
- Corbett farms #3/4 technology,
- Murphy-Brown farm # 93 (Ekokan) technology,
- ORBIT/HSAD (High Solids Anaerobic Digester) technology,
- Corbett Farm #2 (ReCip) Technology,
- Sequencing Batch Reactor (SBR) Technology,
- Howard Farm (Constructed Wetlands) technology, and
- Barham farm with a covered ambient temperature digester, biological nitrification of digester liquid effluent and recycle of the nitrified effluent for fertilization of greenhouse tomatoes.

Both *E. coli* and *Salmonella* were chosen for antibiotic testing because they are common in the intestinal tracts of swine and it is possible for each of them to cause disease outbreaks in animals and humans. *Salmonella* is clearly a frank pathogen. Multiple antibiotic resistance is also important for *E. coli*, although it is not always a frank pathogen. However, some strains of *E. coli* are human pathogens and antibiotic resistance can be associated with genetic material on plasmids that can be transferred from one bacterium to another. Strains of *E. coli* harbored by animals that are human pathogens include *E. coli* O157:H7 harboring Shiga-like toxin genes and various enterotoxigenic strains harboring genes for heat stable and/or heat labile toxins. It is possible that the widespread antibiotic resistance seen in the *E. coli* isolates, including the non-pathogenic strains, can be transferred to other frank pathogens, such as pathogenic strains of *E. coli*, *Salmonella* and *Campylobacter*.

An important consideration in judging if alternative technologies were superior to conventional technologies on the basis of reducing antimicrobially resistant bacteria was to determine whether or not alternative treatments resulted in greater extents of reduction of these bacteria in treated waste residuals based on lower occurrence of antimicrobial resistance traits in *E. coli* and *Salmonella* bacteria isolates after treatment than before treatment as compared to the conventional technology.

For antimicrobial resistance testing, a micro-dilution method was used as described in detail in the QAPP document for this project. Bacterial isolates were tested for their resistance to nine different antibiotics: streptomycin (STR), chlortetracycline (CTET), tetracycline (TET), trimethoprim (TMP), sulfamethoxazole (SMX), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), and ampicillin (AMP). Briefly, bacteria were isolated from waste stream, air,

and environmental samples from the farms. Such isolation was a subsequent step following enumeration of the bacteria for evaluation of remaining microbial concentrations in treatment residuals and environmental media potentially impacted the systems and determination of microbial reductions by treatment processes. The bacterial isolates were purified by three successive pure colony isolation steps and resulting isolates were subjected to biochemical confirmation of their identity (i.e. *E. coli* or *Salmonella*). Provided the isolates confirmed as the bacteria of interest, they were then diluted to a proper concentration for testing by analysis on antimicrobial micro-titer plates. The micro-titer plates have varying concentrations of the antibiotics of interest pre-distributed to wells on the plates and equal amounts of bacteria are inoculated to each of the wells. Following a 24-hour incubation, wells are scored as positive (antimicrobially resistant) or negative (antimicrobially sensitive) for bacterial growth.

From this information, a minimum inhibitory concentration (MIC) for each antibiotic can be calculated. This MIC corresponds to the least amount of each antibiotic that is required to inhibit growth of the bacterial isolate. Depending on the MIC, the *E. coli* and *Salmonella* isolates were classified into susceptible or resistant categories based on the breakpoints for humans set by the National Committee for Clinical Laboratory Standards (NCCLS). This classification was used instead of any corresponding MICs for animals because of the interest in human public health impacts associated with antimicrobial resistant bacteria from these farms.

An additional use for antimicrobial resistance patterning is for microbial source tracking, where it is possible compare the numbers and types of antimicrobials detected in the waste stream samples on the farms to those from environmental samples (i.e. air, ground and surface waters, and soils and vegetation from land application sites on the farms of treated residual material). Unfortunately, we were unable to isolate a sufficient number of *E. coli* and *Salmonella* from environmental samples to effectively make these comparisons for microbial source tracking analysis. Where environmental isolates were collected, they are noted in the report.

Sources of Bacterial Isolates on Farms

Bacterial isolates used for determining antimicrobial resistance were collected from throughout the waste streams on each of the test farms. These locations are summarized in Tables 18.1 to 18.18. For each of the farm sites, an attempt was made to collect equivalent numbers of isolates from each of the sample locations on the farms. This was possible for *E. coli* due to the sizeable number of isolated colonies. However, it was not always possible for *Salmonella* because few if any of these bacteria were isolated from some sites and samples.

Surrogate farm 1 was a commercial swine farm that used a conventional waste treatment system consisting of an anaerobic lagoon and sprayfield. This system flushed wastes from the barns into a conventional anaerobic lagoon system. Nineteen *E. coli* and twenty Salmonella isolates were tested from Farm 1 and their sources are summarized in Table 18.1.

Table 18.1 Sources of *E. coli* and *Salmonella* isolates from Surrogate Farm 1

Source	# of *E. coli* isolates (n=19)	# of *Salmonella* isolates (n=19)
Fresh Feces	6	3
Influent to Lagoon	6	8
Lagoon	7	8

Surrogate farm 2 was a commercial swine farm that used a conventional waste treatment system consisting of an anaerobic lagoon and sprayfield in which the waste from the barn was flushed into an anaerobic lagoon. Thirty-one *E. coli* isolates and 33 *Salmonella* isolates were collected and analyzed. Isolates were also collected and analyzed from environmental samples of soils and vegetation to which lagoon liquid had been land applied in accordance with the waste

management plan for the farm. The locations from which bacteria were isolated on the surrogate farm 2 are summarized in Table 18.2.

Table 18.2 Sources of *E. coli* and *Salmonella* isolates from Surrogate Farm 2

Source	# of *E. coli* isolates (n=31)	# of *Salmonella* isolates (n=33)
Fresh Feces	8	10
Influent to Lagoon	6	7
Lagoon	9	13
Environmental	8	3

The Barham Farm used an alternative waste treatment system employing an in-ground, covered, ambient temperature, anaerobic digester and biofilters for nitrification of digester effluent that was first stored briefly in a lagoon. Treated effluent from the system was used in a drip irrigation system with additional micronutrients provided as supplemental material for growing greenhouse tomatoes. Forty-eight *E. coli* isolates and thirty-six *Salmonella* isolates were tested from the Barham Farm and the locations are summarized in Table 18.3.

Table 18.3 Sources of *E. coli* and *Salmonella* isolates from the Barham Farm with the Covered Anaerobic Digester, Biofilters, and Irrigation for Greenhouse Tomatoes

Source	# of *E. coli* Isolates (n=48)	# of *Salmonella* isolates (n=36)
Fresh Feces	7	3
Barn Flush	15	11
Digester Influent	5	10
Digester Effluent	8	10
First Biofilter	5	-
Second Biofilter	6	-
Greenhouse Leached Effluent	2	2

The Goshen Ridge Farm with the Super Soils technology used an alternative waste treatment system consisting of initial separation of waste solids from waste liquid, followed by further treatment of the separated liquid by a series of biological processes and a chemical precipitation process for phosphorous. Seventeen *E. coli* isolates and 20 *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of these isolates are shown in Table 18.4.

Table 18.4 Sources of *E. coli* and *Salmonella* isolates from the Goshen Ridge Farm with the Super Soils Technology for Treatment on Waste Liquids

Source	# of *E. coli* isolates (n=17)	# of *Salmonella* isolates (n=17)
Fresh Feces	3	4
Solid Separator	2	4
Barn Flush	3	4
Influent to Denitrification Tank	3	0
Homogenation Tank	2	5
Post-Phosphorus Solids	2	0
Effluent from settling tank (solids/nitrogen removed; no phosphorous removal)	2	0

The Grinnell's laboratory system (ReCycle Belt Conveyor System) was designed to separate the liquid and solid wastes, and was not specifically aimed at waste treatment for pathogen reduction.

This technology was linked with the ReCycle gasifier system at the Lake Wheeler Research farm. This was a pilot-scale facility and the final fate of the separated liquid (urine) from the system was not specified. Six *E. coli* isolates were collected and analyzed for antimicrobial resistance, and the sources of these isolates are shown in Table 18.5.

Table 18.5 Sources of *E. coli* isolates from the ReCycle Belt System at the Grinnells Laboratory

Source	# of *E. coli* isolates (n=6)
Feces from belt	5
Urine	1

The ReCycle gasifier technology was the gasification system utilized for treatment of separated solids and was linked with the ReCycle belt system (Grinnells laboratory) for removal and separation of the liquids and solids from the barns. Five *E. coli* isolates were collected and analyzed for antimicrobial resistance, and the source of these isolates is shown in Table 18.6.

Table 18.6 Sources of *E. coli* isolates from the ReCycle Gasifier at the Lake Wheeler Research Farm

Source	# of *E. coli* isolates (n=5)
Influent Solids	5

The Carrolls farm used a combined in-ground anaerobic digester with an aerobic blanket, combined with a BioKinetic aeration process for nitrification-denitrification of the swine wastes from the barns. Twenty *E. coli* and sixteen *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of theses isolates are shown in Table 18.7.

Table 18.7 Sources of *E. coli* and *Salmonella* isolates from the Carrolls Farm

Source	# of *E. coli* isolates (n=20)	# of *Salmonella* isolates (n=16)
Fresh Feces	3	5
Flies	2	2
Barn Flush	3	2
Lagoon 1	3	3
Aeration basin	3	1
Spray irrigated soil/vegetation	2	1
Aerated Spray/abs effluent	4	2

The Harrells farm used an in-ground digester with a permeable cover and aerobic blanket combined with a BioKinetic aeration process for nitrification-denitrification of the swine wastes from the barns. Thirty-seven *E. coli* and nine *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of theses isolates are shown in Table 18.8.

Table 18.8 Sources of *E. coli* and *Salmonella* isolates from the Harrells Farm

Source	# of *E. coli* isolates (n=37)	# of *Salmonella* isolates (n=9)
Fresh Feces	5	1
Influent to digester	5	1
Barn Flush	5	1
Influent to polishing reservoir	5	1
Polishing reservoir	6	2
Clarifier Solids	4	3
Spray irrigated soil/vegetation	3	-
Background Soil	4	

The Vestal farm used a mesophilic anaerobic digester with methane recovery and power generation, followed by an aerobic digester and water reuse system. Nineteen *E. coli* and seventeen *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of theses isolates are shown in Table 18.9.

Table 18.9. Sources of *E. coli* and *Salmonella* isolates from the Vestal Farm

Source	# of *E. coli* isolates (n=19)	# of *Salmonella* isolates (n=17)
Fresh Feces	1	-
Barn Flush	3	3
Clarifier Solids	1	1
Influent to water reuse	1	2
Clarifier Liquids	2	3
Effluent from water reuse	2	2
Effluent from mesophilic digester	3	2
Digested solids	2	-
Settling basin	4	4

The Super Soils composting technology was a centralized waste solids composting and processing facility linked to the Super Soils system at the Goshen Ridge farm site. Waste solids were processed for value-added products that were to be for use and sale off site. Seventeen *E. coli* and ten *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of these isolates are shown in Table 18.10.

Table 18.10 Sources of *E. coli* and *Salmonella* isolates from the SS Composting Farm

Source	# of *E. coli* isolates (n=17)	# of *Salmonella* isolates (n=10)
Wood shavings	2	1
Flies	4	3
Semi composted (15 days)	2	2
Effluent solids	5	4
Influent solids	4	-

The Agriclean system utilizes an anaerobic digester followed by solids separation using a fan separator unit. The treated liquid from the solids separator is then returned to a storage lagoon where it is used to flush the houses. Eight *E. coli* and seventeen *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of these isolates are shown in Table 18.11.

Table 18.11 Sources of *E. coli* and *Salmonella* isolates from Agriclean Farm

Source	# of *E. coli* isolates (n=8)	# of *Salmonella* isolates (n=4)
Fresh Feces	2	-
Barn Flush	1	1
Effluent from storage tank	2	2
Lagoon	1	1
Influent to Digester	2	-

The Corbett Farm #1 with the BEST technology is primarily for solids separation and utilizes a fan separator. This technology is not intended to treat swine waste, but instead to separate the solid and liquid components. The separated solids are to be further treated and processed through a centralized biomass energy recovery system, consisting of a gasification and combustion unit, which was not evaluated by the OPEN team. This solids separation technology does not treat the liquid portion of the waste. The technology providers have suggested this solids separation technology can be combined with some other technology that can further treat the liquid wastes. Twenty *E. coli* and eleven *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of these isolates are shown in Table 18.12.

Table 18.12 Sources of *E. coli* and *Salmonella* isolates from Corbett Farm # 1 (BEST) Farm

Source	# of *E. coli* isolates (n=20)	# of *Salmonella* isolates (n=11)
Fresh Feces	4	1
Barn Flush	4	-
Effluent from storage tank	4	6
Pond	4	1
Solids from Separator	4	3

The Corbett Farms #3/4 with the BEST technology focuses primarily on solids separation utilizing a screen and screw press system. This technology does not include processes for treating swine waste. It consists only of processes for separating the waste components so that the separated solids can be subjected to processing through a centralized biomass energy recovery system, consisting of a gasification and combustion unit. This technology does not treat the liquid portion of the waste and the technology providers have suggested this technology be combined with some technology that can further treat the liquid wastes. Seventeen *E. coli* and nine *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of these isolates are shown in Table 18.13.

Table 18.13 Sources of *E. coli* and *Salmonella* isolates from Best 3/4 Farm

Source	# of *E. coli* isolates (n=17)	# of *Salmonella* isolates (n=9)
Fresh Feces	1	-
Barn Flush	3	2
Effluent from storage tank	2	2
Pond	4	1
Solids from Separator	3	2
Influent to pond	2	2
Spray irrigated soil/vegetation	2	-

The Ekokan technology utilizes solids separation followed by an up-flow biofilter technology to treat the liquid portion of waste. This system is designed to treat only the liquid portion of the waste from the farm and the separated solids are held in a conventional anaerobic lagoon on the

farm site. Nineteen *E. coli* and twenty *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of these isolates are shown in Table 18.14.

Table 18.14 Sources of *E. coli* and *Salmonella* isolates from Ekokan Farm

Source	# of *E. coli* isolates (n=19)	# of *Salmonella* isolates (n=20)
Fresh Feces	1	-
Barn Flush	2	1
Effluent	1	3
Polishing reservoir	3	2
Solids from Separator	2	2
Equalization tank	1	2
Backwash	2	4
Biosolids Reservoir	3	2
Liquid from separator	2	1
Lagoon	2	3

The ORBIT/HSAD (High Solids Anaerobic Digester) technology is designed as a central processing facility for anaerobic digestion of swine waste solids from several farms in one geographical area. Ten *E. coli* and nine *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of these isolates are shown in Table 18.15.

Table 18.15 Sources of *E. coli* and *Salmonella* isolates from Orbit Farm

Source	# of *E. coli* isolates (n=10)	# of *Salmonella* isolates (n=9)
Influent to digester	5	9
Flies	5	0

The RECIP (Solids separation/reciprocating water technology system) system is designed only to treat the liquid portion of the waste stream separated by an initial solids separation process. Separated solids are transferred to an existing lagoon system on the farm. Thirty-one *E. coli* and eighteen *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of these isolates are shown in Table 18.16.

Table 18.16 Sources of *E. coli* and *Salmonella* isolates from ReCip Farm

Source	# of *E. coli* isolates (n=31)	# of *Salmonella* isolates (n=18)
Fresh Feces	1	-
Barn Flush	2	-
Lagoon for separated solids	4	3
Storage pond	2	5
Sludge from Separator	3	4
Spray irrigated soil/vegetation	7	-
Dosing Tank	2	2
Day Holding Tank	4	4
House flies	6	-

The Sequencing Batch Reactor (SBR) technology is a batch reactor that utilizes aerobic processes to treated the combined fecal wastes from the barns (liquids and solids). Sixteen *E. coli* and seven *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of these isolates are shown in Table 18.17.

Table 18.17 Sources of *E. coli* and *Salmonella* isolates from SBR Farm

Source	# of *E. coli* isolates (n=16)	# of *Salmonella* isolates (n=7)
Fresh Feces	2	-
Barn Flush	3	2
Equalization tank effluent	4	1
SBR effluent	2	1
SBR bacteria/activated sludge	3	2
Lagoon 1	1	1
Lagoon 2	1	-

The Constructed Wetlands technology at the Howard farm utilizes a solids separator to remove the solid portion of the waste after which, the liquids are treated through two parallel wetlands cells. As operated, the solids are land applied without further treatment. Forty-two *E. coli* and thirty-eight *Salmonella* isolates were collected and analyzed for antimicrobial resistance, and the sources of these isolates are shown in Table 18.18.

Table 18.18 Sources of *E. coli* and *Salmonella* isolates from Constructed Wetland Farm

Source	# of *E. coli* isolates (n=42)	# of *Salmonella* isolates (n=38)
Fresh Feces	3	-
Barn Flush	3	8
Storage pond	1	3
Soil applied with solids	1	2
Soil and vegetation	4	5
Barn exhaust fan	10	-
Inner wetland influent	4	5
Outer wetland effluent	5	4
Outer wetland influent	4	3
Solids Separator	4	8
Inner wetland effluent	3	-

Antimicrobial Resistance of *E. coli* Isolates from the Farms

E. coli bacteria were isolated from eighteen test farms and subjected to antimicrobial resistance testing. Results from these analyses are summarized in Table 18.19.

Table 18.19 Antibiotic Resistance (%) for *E. coli* Isolates from Eighteen Test Farms

Farm	No. of Isolates	% Antibiotic Resistance to:								
		STR	CTET	TET	TMP	SMX	CHL	CIP	GEN	AMP
Surrogate 1	19	21	100	100	0	32	21	0	0	32
Surrogate 2	31	19	100	100	0	29	29	0	0	16
Barham Farm	48	40	94	96	8	29	4	0	0	12
Goshen Ridge	17	29	100	100	0	12	24	0	0	18
Grinells	6	0	17	17	17	0	17	0	0	17
Gasifier	5	0	60	80	0	40	0	0	0	0
Carrolls	20	20	100	100	0	70	15	0	0	35
Harrells	37	16	86	89	11	30	16	0	0	32
Vestal	19	16	100	100	26	84	37	0	0	5
SS Composting	17	12	100	100	35	59	18	0	0	6
Agriclean	8	37	100	100	0	50	0	0	0	25
BEST #1	20	20	100	100	0	55	5	0	0	30
BEST #3/4	17	6	88	88	0	41	6	0	0	35
Ekokan	19	0	100	100	5	53	5	0	0	74
ORBIT	10	0	50	60	0	20	0	0	0	10
ReCip	31	3	90	90	3	71	42	0	0	45
SBR-Hunt	16	6	94	94	0	6	6	0	0	1
Wetlands	42	7	90	93	2	17	0	0	0	10

* Streptomycin (STR), Chlortetracycline (CTET), Tetracycline (TET), Trimethoprim (TMP), Sulfamethoxazole (SMX), Chloramphenicol (CHL), Ciprofloxacin (CIP), Gentamicin (GEN), Ampicillin (AMP)

E. coli isolates from all eighteen farms studied (surrogate farm 1, surrogate farm 2, Barham farm, Goshen Ridge Super Soils, Grinnells Laboratory, Carrolls farm, Harrells farm, Vestal farm, Hickory Grove site with the Super Soils Composting, Agriclean farm, Best #1 farm, Best #3/4 farm, Ekokan farm, Orbit farm, ReCip farm, SBR – Hunt farm, Howard farm with constructed wetland technology, and the Lake Wheeler Research farm gasifier) showed resistance to chlortetracycline and tetracycline. Isolates from fourteen farms showed resistance to streptomycin (all except Grinnells Laboratory, Lake Wheeler Research farm gasifier, Ekokan farm and Orbit farm) (Table 18.19). *E. coli* isolates from eight farms (Barham, Grinnells, Harrells, Vestal, Ekokan, ReCip, Wetlands, and Super Soils composting) showed resistance to trimethoprim. *E. coli* isolates from all farms except the Grinnells laboratory showed resistance to sulfamethoxazole. Resistance to chloramphenicol was found in all *E. coli* isolates from fourteen farms (except for isolates from the Lake Wheeler Research farm gasifier, Agriclean farm, Orbit farm and constructed wetlands). Similarly *E. coli* isolates from eighteen farms tested were resistant to ampicillin (all except Lake Wheeler Research farm gasifier). *E. coli* isolates from all eighteen farms showed no resistance to ciprofloxacin or gentamicin (Table 18.19).

It is noteworthy that streptomycin, chlortetracycline, tetracycline, sulfamethoxazole, and ampicillin are approved for swine use, but chloramphenicol is prohibited in the feed of the animals. *E. coli* resistance levels on surrogate farm 1 varied from 21% for chloramphenicol up to 100% for chlortetracycline and tetracycline. Resistance levels on surrogate farm 2 ranged from 16% for ampicillin to 100% for chlortetracycline and tetracycline (Table 18.19). Resistance levels on the Barham farm ranged from 4% for chloramphenicol to 96% for chlortetracycline. Resistance levels on the Goshen Ridge farm with the Super Soils technology ranged from 12% for sulfamethoxazole to 100% for both chlortetracycline and tetracycline. Resistance levels on the Grinnell farm were 17% for ampicillin, chloramphenicol, trimethoprim, chlortetracycline and tetracycline. Resistance levels on Carrolls farm ranged from 15% for chloramphenicol to 100%

for chlortetracycline and tetracycline. Resistance levels on the Harrells farm ranged from 11% for trimethoprim to 89% for tetracycline. Resistance levels on the Vestal farm ranged from 5% for ampicillin to 100% for both chlortetracycline and tetracycline. Resistance levels on the Super Soils composting farm ranged from 6% for the ampicillin to 100% for both chlortetracycline and tetracycline. Resistance levels on Agriclean farm ranged from 25% for ampicillin to 100% for both chlortetracycline and tetracycline. Resistance level on Corbett farm # 1 (BEST) ranged from 5% for chloramphenicol to 100% for both chlortetracycline and tetracycline. Resistance levels on Corbett farm #3/4 (BEST) ranged from 6% for streptomycin and chloramphenicol to 88% for both chlortetracycline and tetracycline. Resistance levels on Ekokan farm ranged from 5% for trimethoprim and chloramphenicol to 100% for both chlortetracycline and tetracycline. Resistance level on Orbit farm ranged from 10% for ampicillin to 60% for tetracycline. Resistance level on ReCip farm ranged from 3% for streptomycin and trimethoprim to 90% for both chlortetracycline and tetracycline. Resistance levels on SBR-Hunt farm ranged from 1% for ampicillin to 94% for both chlortetracycline and tetracycline. Resistance levels on constructed wetland farm ranged from 2% for trimethoprim to 93% for tetracycline. Finally, resistance levels for the Lake Wheeler Research farm gasifier ranged from 40% for sulfamethoxazole to 80% for tetracycline (Table 18.19). The presence of *E. coli* resistant to five different antimicrobials at the Goshen Ridge site is an interesting finding because antibiotics are not routinely used in the animal feed for growth promotion and for disease prevention.

Antimicrobial Resistance of *Salmonella* Isolates from the Farms

Salmonella bacteria were isolated from sixteen test farms and subjected to antimicrobial resistance testing. Results from these analyses are summarized in Table 18.20.

Table 18.20 Antibiotic Resistance (%) for *Salmonella* Isolates from sixteen Test Farms

Farm	No. of Isolates	% Antibiotic Resistance to:								
		STR	CTET	TET	TMP	SMX	CHL	CIP	GEN	AMP
Surrogate 1	20	45	90	85	0	35	30	0	0	25
Surrogate 2	33	55	94	94	3	88	18	0	0	21
Barham Farm	36	8	64	64	19	42	3	0	0	8
Goshen Ridge	20	50	100	100	0	45	25	0	0	30
Carrolls	18	6	12	12	6	19	12	0	0	19
Harrells	9	44	89	89	11	89	67	0	0	67
Vestal	17	12	88	88	0	47	24	0	0	71
SS Composting	10	60	80	80	10	70	10	0	20	30
Agriclean	4	25	100	100	0	75	75	0	0	50
BEST #1	11	73	100	100	0	45	9	0	0	0
BEST #3/4	9	33	89	89	0	67	33	0	0	22
Ekokan	20	100	100	100	0	55	5	0	0	5
ORBIT	9	56	89	89	0	78	0	0	0	0
ReCip	18	44	78	78	11	78	61	0	0	61
SBR-Hunt	7	14	100	86	0	29	0	0	0	0
Wetlands	38	29	95	97	39	53	50	0	0	55

* Streptomycin (STR), Chlortetracycline (CTET), Tetracycline (TET), Trimethoprim (TMP), Sulfamethoxazole (SMX), Chloramphenicol (CHL), Ciprofloxacin (CIP), Gentamicin (GEN), Ampicillin (AMP)

Salmonella isolates from all sixteen farms studied (surrogate farm 1, surrogate farm 2, Barham farm, Goshen Ridge Super Soils, Carrolls farm, Harrells farm, Vestal farm, Hickory Grove site with the Super Soils Composting, Agriclean farm, BEST #1 farm, BEST #3/4 farm, Ekokan farm, ORBIT farm, ReCip farm, SBR – Hunt farm, and Howard farm with constructed wetland technology) showed resistance to streptomycin, chlortetracycline, tetracycline, and sulfamethoxazole (Table 18.20). *Salmonella* isolates from seven farms (surrogate 2, Barham, Carrolls, Harrells, super soils composting, ReCip and Wetlands) also showed resistance to trimethoprim. *Salmonella* isolates from all sixteen farms showed no resistance to ciprofloxacin or

gentamicin with the exception of 20% isolates from Super Soils composting farm, which were resistant to gentamicin (Table 18.20).

Salmonella resistance levels on surrogate farm 1 varied from 25% for ampicillin up to 90% for chlortetracycline. Resistance levels on surrogate farm 2 ranged from 3% for trimethoprim to 94% for chlortetracycline and tetracycline (Table 18.20). Resistance levels on the Barham farm ranged from 3% for chloramphenicol to 64% for chlortetracycline and tetracycline. Resistance levels on the Goshen Ridge farm with the Super Soils technology ranged from 25% for chloramphenicol to 100% for chlortetracycline and tetracycline. Resistance levels on the Carrolls farm ranged from 6% for the streptomycin and trimethoprim to 19% for sulfamethoxazole and ampicillin. Resistance level on the Harrells farm ranged from 11% for trimethoprim to 89% for sulfamethoxazole, chlortetracycline and tetracycline. Resistance levels on the Vestal farm ranged from 12% for streptomycin to 88% for chlortetracycline and tetracycline. Resistance level on Agriclean farm ranged from 25% for streptomycin to 100% for chlortetracycline and tetracycline. Resistance level on Corbett Farm #1 (BEST) ranged from 9% for chloramphenicol to 100% for both chlortetracycline and tetracycline. Resistance level on Corbett farm #3/4 (BEST) ranged from 22% for ampicillin to 89% for both chlortetracycline and tetracycline. Resistance level on Ekokan farm ranged from 5% for ampicillin and chloramphenicol to 100% for streptomycin, chlortetracycline and tetracycline. Resistance level on Orbit farm ranged from 56% for streptomycin to 89% for chlortetracycline and tetracycline. Resistance level on ReCip farm ranged from 11% for trimethoprim to 78% for chlortetracycline and tetracycline. Resistance level on SBR-Hunt farm ranged from 14% for streptomycin to 100% for chlortetracycline. Resistance level on constructed wetlands ranged from 29% for streptomycin to 97% for tetracycline. Finally, resistance levels at the Hickory Grove site with the Super Soils composting technology ranged from 10% for trimethoprim and chloramphenicol to 80% for chlortetracycline and tetracycline (Table 18.20).

Multiple Antibiotic Resistance in *E. coli* Isolates from Test Farms

Resistance of bacteria to a single antibiotic is a concern to public health, but this is only a small part of the potential impact or risk. Multiple antibiotic resistance of bacteria is a growing concern for environmental impacts from confined animal feeding operations. Resistance to multiple antibiotics can result in the inability to successfully treat an infection or illness because there may be no antibiotics to which an infecting microbe is susceptible. To address this issue, we identified the number of *E. coli* isolates that were resistant to multiple antibiotics and the number of antibiotics to which each of the isolates were resistant. The results for multiple antibiotic resistance of *E. coli* isolates from these farms are summarized in Table 18.21.

Table 18.21 Multiple Antibiotic Resistance (%) of *E. coli* Isolates from the eighteen Test Farms

Farms	% of antibiotic each isolate is resistant to							
	0	1	2	3	4	5	6	7
Surrogate 1 (n=19)	-	5	26	37	32	-	-	-
Surrogate 2 (n=31)	-	-	55	19	23	3	-	-
Barham Farm (n=48)	2	4	31	42	15	4	2	-
Goshen Ridge (n=17)	-	-	71	-	29	-	-	-
Grinnells Laboratory (n=6)	67	-	17	17	-	-	-	-
Gasifier (n=5)	40	-	20	40	-	-	-	-
Carrolls farm (n=20)	-	-	20	30	40	10	-	-
Harrells farm (n=37)	11	3	30	27	16	8	5	-
Vestal farm (n=19)	-	-	5	42	26	26	-	-
SS Composting (n=17)	-	-	35	29	12	29	-	-
Agriclean (n=8)	-	-	37	25	25	12	-	-
BEST #1 (n=20)	-	-	45	10	30	-	15	
BEST #3/4 (n=17)	6	6	41	24	12	12	-	-
Ekokan (n=19)	-	-	5	58	32	5	-	-
ORBIT (n=10)	40	-	50	-	10	-	-	-
ReCip (n=31)	10	-	13	19	29	29	-	-
SBR-Hunt (n=16)	6	-	87	-	-	6	-	-
Wetlands (n=42)	7	2	62	21	7	-	-	-

As shown in Table 18.21, *E. coli* isolates from all eighteen farms were resistant to some of the test antibiotics. All farms had *E. coli* isolates that were multiply resistant (resistant to 2 or more antibiotics). On nine of the eighteen farms there were no *E. coli* isolates lacking resistance to one or more antibiotics; all isolates were resistant to one or more antibiotics. Of the other nine farms, absence of antibiotic resistance was found in 2, 6, 6, 7, 10, 11, 40, 40 and 67% of isolates. *E. coli* isolate resistance to only 1 antibiotic occurred in only 2, 3, 4, 5, and 6% isolates from wetlands, Harrells farm, Barham farm, surrogate farm 1, and BEST #3/4 farm, respectively. Eleven farms, including Surrogate farm 2 and ten alternative technology farms, had isolates that were resistant to as many as 5 different antibiotics; Barham farm, Harrell's farm and BEST #1 farm had isolates that were resistant to as many as 6 different antibiotics (Table 18.21). This demonstrates that multiple antibiotic resistance was widely present in *E. coli* from all of these farms and that the alternative waste management systems appeared to have similar occurrence rates for multiple antibiotic resistance as did the surrogate farms.

Multiple Antibiotic Resistance in *Salmonella* Isolates from Test Farms

Of greater potential for public health risk to humans may be multiple antibiotic resistance in individual *Salmonella* isolates. This is because *Salmonella* bacteria are frank pathogens, capable of causing illness and disease outbreaks in both animals and humans. Multiple antibiotic resistance would make it more difficult to use antibiotic therapy treat patients with Salmonella infections. *Salmonella* isolates were found to be not only frequently antimicrobially resistant, but many were also resistant to multiple antibiotics. The numbers of antibiotics to which *Salmonella* isolates from these farms were resistant are summarized in Table 18.22.

Table 18.22. Multiple Antibiotic Resistance (%) of *Salmonella* Isolates from the sixteen Test Farms

Farms	% of antibiotics each isolate is resistant to							
	0	1	2	3	4	5	6	7
Surrogate 1 (n=20)	15	-	40	5	15	5	20	-
Surrogate 2 (n=33)	-	-	18	24	43	6	9	-
Barham Farm (n=36)	22	8	25	22	19	-	3	3
Goshen Ridge (n=20)	-	-	45	5	25	5	20	-
Carrolls farm (n=18)	78	-	6	-	6	-	11	-
Harrells farm (n=9)	11	-	-	-	22	44	22	-
Vestal farm (n=17)	18	-	-	47	12	18	6	-
SS Composting (n=10)	20	-	10	10	30	20	-	10
Agriclean (4)	-	-	25	-	25	25	25	-
Best 1 (11)	-	-	-	73	27	-	-	-
Best ¾ (9)	-	11	11	33	22	22	-	-
Ekokan (20)	-	-	-	45	50	-	5	-
Orbit (9)	-	11	22	11	56	-	-	-
ReCip (18)	22	-	-	-	17	22	39	-
SBR-Hunt (7)	-	14	57	14	14	-	-	-
Wetlands (38)	3	-	39	5	-	11	24	18

All farms had *Salmonella* that were resistant to multiple antibiotics, and the majority of *Salmonella* isolates from all sixteen farms had resistance to 2 or more of the 9 antibiotics for which resistance was tested. On eight farms all *Salmonella* isolates were antibiotic resistant, and on other eight farms the majority of *Salmonella* were antibiotic resistant. The exception was for only one farm, Carrols, where the majority of *Salmonella* (78%) were not resistant to antibiotics. Four farms, (Barham farm, BEST #3/4, ORBIT, and SBR-Hunt) had an isolate resistant to a single antibiotic. The remaining Barham Farm isolates and all other isolates that were antibiotic resistant had multiple resistance traits. Eleven farms had isolates resistant to six different antibiotics and three farms had isolates resistant to 7 different antibiotics. Overall, these results indicate high prevalence of multiply resistant *Salmonella* in fecal waste samples from both surrogate and alternative technology swine farms.

Statistical Comparisons of Antimicrobial Resistance Levels of *E. coli* and *Salmonella* in Raw and Treated Wastes of Surrogate and Alternative Technology Farms

Further analyses were conducted to determine if alternative technologies were environmentally superior to the surrogate technology on the basis of reducing the levels of antimicrobial resistance (AR) of *E. col*i and *Salmonella* isolates in treated swine waste residuals. The key criterion for comparisons of AR was the number of antimicrobially resistant traits harbored by bacterial isolates from raw and treated wastes of surrogate and alternative technology farms and the extent to which conventional or alternative treatment reduced the number of AR traits relative to the initial number such AR traits in the bacteria of the raw waste. Comparisons of numbers of AR traits in *E. coli* and *Salmonella* isolates from untreated swine wastes and treated residuals (solids or liquids) were made statistically using an unpaired, nonparametric t-test, the Mann-Whitney U-statistic. The results of these comparisons are summarized in Table 18.23

Table 18.23. Statistical Comparisons of Antimicrobial Resistance Levels of *E. coli* and *Salmonella* in Raw and Treated Wastes of Surrogate and Alternative Technology Farms

Bacteria (number of isolates)	Technology Type	Swine Waste Type	Mean No. (SD) AR Traits/Isolate	Comparison	P-value (Signifi-cance)
E. coli (102)	Alternative	Untreated	2.78 (1.4)	AR traits in Raw vs.	0.41
E. coli (93)		Treated	2.67 (1.4)	Treated Waste Isolates	(NS)*
E. coli (14)	Surrogate	Untreated	2.6 (0.74)	AR traits in Raw vs.	0.43
E. coli (14)		Treated	3.0 (1.0)	Treated Waste Isolates	(NS)
Salmonella (46)	Alternative	Untreated	2.56 (1.96)	AR traits in Raw vs.	0.0074
Salmonella (68)		Treated	3.61 (1.96)	Treated Waste Isolates	(VS)
Salmonella (13)	Surrogate	Untreated	3.5 (1.1)	AR traits in Raw vs.	0.90
Salmonella (26)		Treated	3.5 (1.9)	Treated Waste Isolates	(NS)

* NS = not significant; VS = Very significant; 5% level of significance or $P < 0.05$

As shown in Table 18.23, the levels of antibiotic resistance traits of *E. coli* isolates in untreated and treated wastes were not significantly different for either the alternative technologies or the surrogate technology. The numbers of antibiotic resistance traits in *E. coli* remained essentially unchanged after treatment from their numbers in the untreated waste. These results suggest that neither alternative nor conventional treatments reduced the carriage of antibiotic resistance traits by *E. coli* in swine wastes.

The results in Table 18.23 for *Salmonella* also indicate that the levels of antibiotic resistance traits of isolates in untreated and treated wastes were not significantly reduced by the alternative technologies or the surrogate technology. The numbers of antibiotic resistance traits in *Salmonella* after treatment by alternative technologies were actually significantly greater than their numbers in the raw waste. For the surrogate (conventional) technology farms, the numbers of antibiotic resistance traits in *Salmonella* isolates in treated waste residuals remained essentially unchanged from their numbers in the untreated waste. These results suggest that neither alternative nor conventional treatments reduced the carriage of antibiotic resistance traits by *Salmonella* in swine wastes.

Overall, the results of these analyses indicate that neither alternative nor conventional treatment systems significantly changed the levels of carriage of antibiotic resistance by *E. coli* and *Salmonella* in swine wastes. The majority of these bacteria continued to harbor multiple resistance to antibiotics. However, it is important to recall that the actual concentrations and total loads of these bacteria were appreciably reduced by swine waste treatment. Therefore, while the extent of carriage of antimicrobial resistance by *E. coli* and *Salmonella* was not reduced, the numbers of such bacteria were reduced to varying extents, depending upon the type of treatment and waste management system. Nevertheless, some *E. coli* and *Salmonella* harboring multiple antibiotic resistance remained in the treated waste residuals of the treatment technologies. Therefore, such waste residuals need to be properly managed to reduce human and animal exposure to such bacteria, some of which are known pathogens (all *Salmonella* and some strains of *E. coli*).

Antimicrobial resistance of E. coli and Salmonella from Environmental Samples of Soil, Vegetation and Flies

Further evidence for the environmental persistence and presence of multiple antimicrobial resistant *E. coli* and *Salmonella* was provided by the antibiotic resistance characteristics of the environmental isolates from soil, vegetation and flies. As shown by the results summarized in Table 18.24, multiply antimicrobial resistant *E. coli* and *Salmonella* were found in environmental isolates of these bacteria in samples from both alternative and surrogate (conventional) technology farms.

Table 18.24. Antimicrobial Resistance Properties of *E. coli* and *Salmonella* Isolates from Environmental Samples of Tested Swine Farms

Isolates	Farm	Medium	No. of resistance trails	Antibiotic
E. coli				
S2F1	**Surrogate 2**	Flies	4	STR,CTET,TET,SMX
S2F2		Flies	4	CTET,TET,SMX, AMP
S2F3		Flies	3	CTET,TET, AMP
S2F4		Flies	2	CTET,TET
S2F5		Flies	5	STR,CTET,TET,SMX,CHL
S2S2		Soil	2	CTET,TET
S2S3		Soil	2	CTET,TET
S2S5		Soil	2	CTET,TET
CF1	**Carroll - ISSUES**	Flies	4	STR,CTET,TET,AMP
CF2		Flies	4	STR,CTET,TET,SMX
SV1		Soil/Vegetation	4	CTET,TET,SMX, AMP
SV2		Soil/Vegetation	2	CTET,TET
SCF1	**Super Soils Composting**	Flies	5	STR,CTET,TET,TMP,SMX
SCF2		Flies	5	STR,CTET,TET,TMP,SMX
SCF4		Flies	3	CTET,TET,SMX
SCF6		Flies	2	CTET,TET
Salmonella				
S2S1	**Surrogate 2**	Soil	2	SMX, AMP
S2S2		Soil	2	SMX, AMP
S2S3		Soil	5	STR,CTET,TET,SMX,AMP
CF3	**Carroll - ISSUES**	Flies	0	
CF4		Flies	0	
CSV2		Soil/Vegetation	0	
SCF4	**Super Soils Composting**	Flies	0	
SCF5		Flies	3	CTET,TET,SMX
SCF6		Flies	0	

* Streptomycin (STR), Chlortetracycline (CTET), Tetracycline (TET), Trimethoprim (TMP), Sulfamethoxazole (SMX), Chloramphenicol (CHL), Ciprofloxacin (CIP), Gentamicin (GEN), Ampicillin (AMP)

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All *E. coli* isolates from flies or soil and vegetation of both alternative technology and surrogate farms were multiply antibiotic resistant, with some isolates harboring up to 5 resistance traits. Fewer *Salmonella* isolates than *E. coli* isolates were obtained from environmental samples. Of these, some but not all *Salmonella* isolates from soil or flies on surrogate or alternative technology farms harbored multiple antibiotic resistance. As for *E coli*, resistance to as many as 5 antibiotics was detected in some *Salmonella* isolates.

Overall Summary for Antimicrobial Resistance on Study Farms

Antibiotic resistance and multiple antibiotic resistance has been associated with bacteria from confined animal feeding operations due to the common practice of using these pharmaceuticals in the feed for therapeutic disease prevention, as well as sub-therapeutically for growth promotion of the animals. These antimicrobial resistance properties of bacteria present on the farm can have subsequent adverse public health impacts if people on farms are exposed to them or if

these bacteria are carried off the farms and people and other animals become exposed to them. These potential human and animal health impacts are a concern because some of the antibiotics used on the farms are also used to combat human infections. This widespread use of antibiotics, and the corresponding increases in antibiotic resistant bacteria, impact the medical and veterinary communities, making it difficult for physicians and veterinarians to treat human and animal bacterial disease cases and outbreaks with first-line antibiotics. Because of the lack of epidemiological data, it is still difficult to fully quantify health risks or the extent to which antibiotic use and resulting antibiotic resistant bacteria results in exposures that impact the health of human and animal populations.

Both *E. coli* and *Salmonella* isolates from the waste management systems on these farms and the environmental samples from surrogate farm 2 and some alternative technology farms tested were widely resistant to antibiotics. The two most common antibiotics for which the bacteria were resistant were chlortetracycline and tetracycline. There were two bacteria isolates resistant to gentamicin and only one isolate was resistant to ciprofloxacin. This resistance to ciprofloxacin is of particular concern because this antibiotic has been banned for use in animal feeds. There were also few bacteria isolates resistant to trimethoprim.

Multiple antibiotic resistance of bacteria isolated from these farms was widespread. For *E. coli*, all of the isolates were resistant to antibiotics, with all eighteen tested farms having isolates resistant to two or more antibiotics. Potentially of greater public health concern is the multiple antibiotic resistance in *Salmonella*. On all but one of the farms the majority of *Salmonella* isolates had multiple antibiotic resistance, and four farms had isolates resistant to 7 different antibiotics. The reasons for the persistence of these multiple antibiotic resistance traits in both *E. coli* and *Salmonella* is uncertain at this time. It could be due to the continued presence of the antibiotic in the waste material and the environmental samples or to the ability of the bacteria to maintain the antibiotic resistance gene and its expression in the environment, even if the antibiotic and its selective pressure were no longer present.

When comparing these test farms for the extent of antimicrobial resistance among *E. coli* and *Salmonella*, there appear to be no differences between the extent of the resistance on the surrogate farms with conventional waste management technology and on the farms with alternative waste management technologies A large proportion of the bacteria isolated from each of the farms were resistant to multiple antibiotics. Furthermore, the levels of multiple antibiotic resistance in *E. coli* and *Salmonella* were not reduced by the alternative or surrogate (conventional) swine waste treatment processes and management systems. Levels of multiple antibiotic resistance in *E. coli* and *Salmonella* isolates from treated wastes or from environmental media (flies, soil and vegetation), remained unchanged from the resistance levels found in isolates of these bacteria from untreated wastes. Based on these results, none of the alternative waste management systems can be judged environmentally superior to the conventional technology at the surrogate farms based on reduction of the levels of antimicrobial resistance in bacteria in treated swine waste residuals or environmental media. However, the concentrations of these bacteria and the total loads of these bacteria were often significantly reduced by alternative treatment processes and systems. On the basis of remaining concentrations or total numbers (loads) of antimicrobially resistant bacteria, many of these alternative technologies were superior to the surrogate (conventional) technology.