C. 2. c. EFFLUENT VERSUS ABSORPTION AREA WATER QUALITY DURING THE RESEARCH STUDY

Hydrogen ion concentrations (pH), EC values, and chloride ion concentrations remained consistent between effluent and subsystems samples each year. Effluent nitrate-N concentrations were lower and ammonium-N concentrations higher than those concentrations found within either subsystem in the absorption area. This was due to nitrification that occurs under the aerobic conditions in the subsystems (Reneau *et al.*, 1989). The phosphate-P concentration was also higher in the pump chamber effluent than in the subsystems, as expected.

Nitrate-N increased by as much as 182 times, ammonium-N decreased by as much as 20 times, and phosphate-P decreased by as much as 157 times from the effluent determinations to those determined from subsystems 1 and 2. The pump chamber operates under anaerobic conditions and ammonium-N from the human waste accumulates. When the effluent from the anaerobic pump chamber is dosed into an aerobic subsystem, ammonium-N is transformed to nitrate-N through nitrification and phosphate-P is immobilized on soil colloids (Degen *et al.*, 1991).

Although the effluent was of overall weak strength, all the quality parameters were at greatest values/concentrations in 1991, the third and last year of the research study, however, subsystem values/concentrations varied little during the study period. Both subsystems appeared to handle the effluent load with consistent performance, regardless of effluent quality.

C. 3. SUMMARY

Background water quality concentrations/values up-gradient from subsystems 1 and 2 (within the absorption area) were greater for most parameters than concentrations/values down-gradient from the subsystems. Effluent

concentrations/values fluctuated during 1989 and 1990 and then rose for all parameters in 1991, the third and last year of the research study. Most likely this was due to the system maturing. Both subsystems seemed to renovate the effluent well and appeared to handle the effluent load with consistent performance, regardless of the effluent quality. The effluent was weak and did not test or tax the subsystems as much as expected.

D. MICROBIAL TRACER STUDIES IN ALTERNATIVE OSWTDS

D. 1. BACKGROUND COLIFORM AND BACTERIOPHAGE COUNTS

Background coliform and bacteriophage counts were performed at the research site in both summer 1989 and winter 1990, prior to addition of the tracer organisms to the infiltrator subsystems. No phages were detected and coliforms were only detected sporadically. Usually coliform counts were zero, however, when detected, counts always ranged between one and 50 colony-forming units (CFU) per 100 mL. Detection mostly occurred at the shallow depth (45.7 cm) in subsystem 1 in the winter, however, these coliforms were not antibiotic resistant and therefore, in no way interfered with the tracer *E. coli*.

D. 2. TRACER STUDIES

Each subsystem contained four sampling wells at a shallow depth of 45.7 cm and four deep wells at 213.4 cm, or a total of eight wells per subsystem. Results were tabulated to indicate how many of these wells tested positive for the tracers and how much of the tracer was recovered. Bacterial and viral tracer counts from subsystem 1 during the summer and winter are presented in Tables 11 and 12, respectively. Summer and winter results for subsystem 2 are presented in Tables 13 and 14, respectively.

Table 11. Bacterial and viral tracers enumerated for Subsystem 1 with a design loading rate of 5.1 Lpd/m^2 (actual loading rate 2.4 Lpd/m^2) in the Blairton silt loam during the summer 1989*.

Time (hr)	Soil Depth (cm)	Total Number of Wells Tested	Bacteria** (CFU/50 mL) 1 2 3 4	Viruses*** (PFU/50 mL) 1 2 3 4 5 6
24	45.7	4	2 1 0 1	2 2 0 0 0 0
	213.4	4	2 1 0 1	3 1 0 0 0 0
48	45.7	4	3 1 0 0	4 0 0 0 0 0
	213.4	4	0 2 0 2	4 0 0 0 0 0
72	45.7	4	2 1 1 0	4 0 0 0 0 0
	213.4	4	0 2 0 2	4 0 0 0 0 0

*Bacterial and viral tracers applied at 1.4 x 10^8 CFU/m^2 and 1.9 x 10^5 PFU/m^2, respectively.

**Each well is represented under one of the following categories, based on bacterial counts enumerated from the water sample taken from the well during the particular tracer study sampling time:

1. 0 2. 1-50 3. 51-100 4. >100.

***Each well is represented under one of the following categories, based on viral counts enumerated from the water sample taken from the well during the particular tracer study sampling time:

1. 0 2. 1-50 3. 51-100 4. 10^2-10^3 5. 10^3-10^4 6. >10^4.

Table 12. Bacterial and viral tracers enumerated for Subsystem 1 with a design loading rate of 5.1 Lpd/m^2 (actual loading rate 2.4 Lpd/m^2) in the Blairton silt loam during the winter 1990*.

Time (hr)	Soil Depth (cm)	Total Number of Wells Tested	Bacteria** (CFU/50 mL) 1	2	3	4	Viruses*** (PFU/50 mL) 1	2	3	4	5	6
24	45.7	4	1	1	0	2	4	0	0	0	0	0
	213.4	4	1	2	0	1	4	0	0	0	0	0
168	45.7	4	1	1	1	1	4	0	0	0	0	0
	213.4	4	2	2	0	0	4	0	0	0	0	0
192	45.7	4	0	3	1	0	3	0	0	0	0	1
	213.4	4	1	2	1	0	3	1	0	0	0	0
288	45.7	4	0	1	0	3	2	1	0	0	0	1
	213.4	4	0	0	2	2	4	0	0	0	0	0
816	45.7	4	1	1	0	2	3	0	0	0	0	1
	213.4	4	4	0	0	0	4	0	0	0	0	0

*Bacterial and viral tracers applied at 3.7 x 10^8 CFU/m^2 and 1.9 x 10^8 PFU/m^2, respectively.

**Each well is represented under one of the following categories, based on bacterial counts enumerated from the water sample taken from the well during the particular tracer study sampling time:

1. 0 2. 1-50 3. 51-100 4. >100.

***Each well is represented under one of the following categories, based on viral counts enumerated from the water sample taken from the well during the particular tracer study sampling time:

1. 0 2. 1-50 3. 51-100 4. 10^2-10^3 5. 10^3-10^4 6. >10^4.

Table 13. Bacterial and viral tracers enumerated for Subsystem 2 with a design loading rate of 10.2 Lpd/m^2 (actual loading rate 4.9 Lpd/m^2) in the Blairton silt loam during the summer 1989*.

Time (hr)	Soil Depth (cm)	Total Number of Wells Tested	Bacteria** (CFU/50 mL) 1	2	3	4	Viruses*** (PFU/50 mL) 1	2	3	4	5	6
24	45.7	4	1	2	1	0	2	2	0	0	0	0
	213.4	4	2	2	0	0	3	1	0	0	0	0
48	45.7	4	2	1	0	1	3	1	0	0	0	0
	213.4	4	2	0	1	1	3	1	0	0	0	0
72	45.7	4	2	1	0	1	4	0	0	0	0	0
	213.4	4	1	3	0	0	4	0	0	0	0	0

*Bacterial and viral tracers applied at 1.4 x 10^8 CFU/m^2 and 1.9 x 10^5 PFU/m^2, respectively.

**Each well is represented under one of the following categories, based on bacterial counts enumerated from the water sample taken from the well during the particular tracer study sampling time:

1. 0 2. 1-50 3. 51-100 4. >100.

***Each well is represented under one of the following categories, based on viral counts enumerated from the water sample taken from the well during the particular tracer study sampling time:

1. 0 2. 1-50 3. 51-100 4. 10^2-10^3 5. 10^3-10^4 6. >10^4.

Table 14. Bacterial and viral tracers enumerated for Subsystem 2 with a design loading rate of 10.2 Lpd/m^2 (actual loading rate 4.9 Lpd/m2) in the Blairton silt loam during the winter 1990*.

Time (hr)	Soil Depth (cm)	Total Number of Wells Tested	Bacteria** (CFU/50 mL)				Viruses*** (PFU/50 mL)					
			1	2	3	4	1	2	3	4	5	6
24	45.7	4	0	0	0	4	3	1	0	0	0	0
	213.4	4	2	2	0	0	4	0	0	0	0	0
168	45.7	4	0	1	1	2	4	0	0	0	0	0
	213.4	4	1	3	0	0	4	0	0	0	0	0
192	45.7	4	0	1	1	2	2	1	0	0	0	1
	213.4	4	1	3	0	0	3	1	0	0	0	0
288	45.7	4	0	0	0	4	2	0	0	0	0	2
	213.4	4	0	3	0	1	4	0	0	0	0	0
816	45.7	4	2	0	0	2	2	0	0	0	0	2
	213.4	4	4	0	0	0	4	0	0	0	0	0

*Bacterial and viral tracers applied at 3.7 x 10^8 CFU/m^2 and 1.9 x 10^8 PFU/m^2, respectively.

**Each well is represented under one of the following categories, based on bacterial counts enumerated from the water sample taken from the well during the particular tracer study sampling time:

1. 0 2. 1-50 3. 51-100 4. >100.

***Each well is represented under one of the following categories, based on viral counts enumerated from the water sample taken from the well during the particular tracer study sampling time:

1. 0 2. 1-50 3. 51-100 4. 10^2-10^3 5. 10^3-10^4 6. >10^4 CFU.

D. 2. a. SUBSYSTEM 1

D. 2. a. 1. SUMMER BACTERIAL STUDY

Bacterial tracers were applied at 1.4 x 10^8 CFU/m^2. At 24 h after tracer addition both depths had two negative wells; one well was positive with 1-50 CFU and one well with >100 CFU (Table 11). By 48 h, the depths began to show differing results with only one of the shallow wells positive, with 1-50 Two deep wells had 1-50 CFU and two had >100 CFU. At 72 h results were unchanged except another shallow well tested positive, with 51-100 CFU.

In retrospect, sampling was not carried out long enough to ensure that all wells tested negative for the tracer *E. coli*. Since only a small change was noted from 48 to 72 h, there is no way to estimate how long it took subsystem 1 to empty of viable tracer. As a coliform tracer is used to indicate coliform activity in wastewater disposed through OSWTDS, these results indicate that effluent coliform loads probably experience large amounts of die-off, soil filtration, and other types of movement inhibition within the first 72 h after dosing to the soil infiltrator.

D. 2. a. 2. SUMMER VIRAL STUDY

Viral tracers were applied at 1.9 x 10^5 PFU/m^2. At 24 h only two shallow wells and one deep well indicated phage, all with counts between 1-50 PFU (Table 11). At 48 and 72 h all four shallow and all four deep wells tested negative for the presence of the bacteriophage tracer. These are very favorable results indicating that soil and subsystem characteristics enable effluent viral loads to be greatly reduced within 48 h of entry into the soil infiltrators. Reduction of viral particles within the OSWTDS provides less

possibility of virus contained in the disposed wastewater traveling through the soil to join and contaminate groundwater that may be used for human consumption.

D. 2. a. 3. WINTER BACTERIAL STUDY

Bacterial tracers were applied at 3.7 x 10^8 CFU/m^2. Three of four shallow wells tested positive for tracer presence at 24, 168, and 816 h (Table 12). At 192 and 288 h, all four shallow wells tested positive. Counts fluctuated across all enumeration categories. At 192 h, it appeared that counts were declining with three wells positive for 1-50 CFU and one positive for 51-100 CFU, however, by 288 h, only one well tested positive with 1-50 CFU, while three wells tested with >100 CFU. By 816 h, counts began once again to decline slightly with only three wells positive, one with 1-50 CFU, and two with >100 CFU.

Bacterial tracer presence in the deep wells seemed to occur in waves with two wells at 1-50 CFU and one well at >100 CFU at 24 h. At 168 h counts declined to only two wells positive for 1-50 CFU. By 288 h, counts were again high with two wells positive at 51-100 CFU and two positive with >100 CFU, but by 816 h, all four wells were negative.

Once again, not all wells were negative by the completion of sampling. Collection of samples continued for over one month during the winter study and therefore, it is more worrisome that this subsystem had not cleared all of the dosed tracers. However, it must be noted that the tracer sampling schedule was modified for this site in the winter study due to the tracer culture being added to the system through the septic tank rather than the pump chamber.

D. 2. a. 4. WINTER VIRAL STUDY

Viral tracers were applied at 1.9 x 10^8 PFU/m^2. The tracer virus did not appear in subsystem 1 until 192 h, and then only in one shallow well as >10^4 PFU and one deep well as 1-50 PFU (Table 12). By 288 h all deep wells were negative while two shallow wells remained positive (one with 1-50 PFU and one maintaining counts of >10^4 PFU). One shallow well still remained positive with >10^4 PFU, even at 816 h.

D. 2. b. SUBSYSTEM 2

D. 2. b. 1. SUMMER BACTERIAL STUDY

At the shallow depth the number of positive wells decreased from 24 to 48 h and then stayed constant, however, counts were higher at 48 and 72 h with one well at >100 CFU and the other with 1-50 CFU (Table 13).

Counts increased in the deep wells from 24 to 48 h and then decreased again by 72 h. Once again, the sampling schedule did not permit a finding of how long it actually took to clear the subsystem of the tracer *E. coli*.

D. 2. b. 2. SUMMER VIRAL STUDY

The viral tracer was detected at 24 and 48 h, with all positive wells at 1-50 plaque-forming unit (PFU) per 100 mL, and was found to decrease with time as all wells tested negative for the presence of the bacteriophage tracer by 72 h (Table 13).

D. 2. b. 3. WINTER BACTERIAL STUDY

At 24 h, all shallow wells tested >100 CFU (Table 14). These numbers declined slightly at 168 and 192 h to only two samples positive at >100 CFU, one well with 51-

100 CFU, and one well with 1-50 CFU. By 288 h, all four wells were once again positive with >100 CFU. Two wells continued to test positive at >100 CFU at 816 h, while the other two wells were negative.

Two deep wells were positive with 1-50 CFU at 24 h, while three wells were positive with 1-50 CFU at 168 and 192 h. At 288 h, three wells still tested positive at 1-50 CFU, while the fourth well test at >100 CFU. By 816 h, however, all wells were negative for the tracer *E. coli*.

D. 2. b. 4. WINTER VIRAL STUDY

The tracer phage was detected at 24 h with 1-50 PFU in one shallow well (Table 14). While at 168 h all shallow wells were negative, by 192 h two samples were again positive, one as 1-50 PFU and one as $>10^4$ PFU. While two samples were negative at both 288 and 816 h, the other two wells tested at $>10^4$ PFU. Detection occurred in only one deep well at 192 h with a count of 1-50 PFU.

Once again, sampling did not occur long enough to determine the amount of time it took to clear the subsystem of tracer organisms. As tracers were detected shallowly at the end of the study, it would appear that these organisms might continue to migrate with time to the deeper wells.

D. 2. c. SUBSYSTEM COMPARISON AND OVERVIEW

A sample was taken from each of the four shallow and four deep wells per subsystem during each of the days samples were collected. The summer sampling study yielded 24 samples per subsystem for each tracer (Tables 11 and 13). The winter sampling period extended over five different days, yielding 40 samples per subsystem for each tracer (Tables 12 and 14).

D. 2. c. 1. SUMMER BACTERIAL TRACER STUDY

In subsystem 1, over the three day summer sampling, nine wells tested negative, eight wells tested 1-50 CFU, one well tested 51-100 CFU, and six tested at >100 CFU (Table 11). Of the fifteen wells positive for tracer *E. coli*, five were shallow and ten were deep wells.

In subsystem 2 over the summer sampling period, ten wells tested negative, nine tested 1-50 CFU, two wells tested 51-100 CFU, and three tested at >100 CFU (Table 13). Of the 14 wells positive, seven were shallow and seven were deep wells.

Overall, these subsystems performed very similarly with the only notable exception regarding the >100 CFU observations. Of the six >100 CFU wells in subsystem 1, five were deep wells while only one of the three >100 CFU wells in subsystem 2 was a deep well.

Subsystem 2 (design loading rate 10.2 Lpd/m^2, actual loading rate 4.9 Lpd/m^2) received two times more effluent per dose than subsystem 1 (design loading rate 5.1 Lpd/m^2, actual loading rate 2.4 Lpd/m^2). The higher observations began earlier in subsystem 1 at both depths and dropped off more slowly than in subsystem 2 (Tables 11 and 13). The bacteria, due to their relatively large size, are predominantly filtered from the system while viral particles predominantly adsorb to the soil particles. These methods of restricting microbial transport help to purify wastewater. Moisture data reflected that subsystem 1 was wetter in the absorption field and subsystem 2 was wetter below the field (Table 7). Subsystem 2 must have been shedding effluent and, most likely tracers, downslope and away from the absorption area.

As wells at both depths in both subsystems were still positive at the end of the collection period, it is concluded that sampling did not occur long enough to observe complete tracer removal. Overall counts were very low at both 24 and 48 h and were

decreasing by the 72 h sampling so it can only be assumed that it took some additional days for all the absorption field wells to clear the tracer organisms.

D. 2. c. 2. WINTER BACTERIAL TRACER STUDY

In subsystem 1 over the winter sampling, eleven wells tested negative, thirteen tested 1-50 CFU, five tested 51-100 CFU, and eleven tested >100 CFU (Table 12). Of the 29 wells positive for tracer *E. coli*, 17 were shallow and 12 were deep wells.

In subsystem 2 over the winter sampling, ten wells tested negative, thirteen tested 1-50 CFU, two tested 51-100 CFU, and 15 tested >100 CFU (Table 14). Of the 30 wells positive, 18 were shallow and 12 were deep wells.

As in the summer study, both subsystems performed very similarly in the winter. The number of positive wells, however, was approximately twice that recorded in the summer.

In both subsystems there appeared to be a pattern of increasing and then decreasing counts at 45.7 cm. At 213.4 cm there appeared to be a gradual increase in counts in subsystem 1 until the 288 h sampling. In subsystem 2 at 213.4 cm, a small increase was seen at 288 h and both subsystems declined in observed counts by 816 h.

Soil matric potentials showed both subsystems at both depths in the absorption field to have been wetter in the summer season than in the winter (Tables 7 and 8) but twice as many wells were positive. Possibly a more unsaturated condition during the winter allowed the tracers to stay within the absorption system rather than move out in a saturated flow which may somewhat reflect the summer results.

Although ponding was observed most often in subsystem 1 during the three year research study, this was attributed to a fragic layer in the soil underlying subsystem 1

(Table 2). As both subsystems yielded such similar summer and winter results, no one characteristic of either subsystem seems to have affected its performance.

During the winter season, the microbial tracers were added to the septic tank (pump chamber was not accessible) and monitoring occurred over the course of one month. It is suspected that many of the tracer organisms either attached to the accumulated organic matter within the septic tank and did not move to the pump chamber with the liquid phase or that the organisms died off during the time inside the septic tank. By addition to the septic tank, tracer entry to the pump chamber may have occurred at low levels over days, thus requiring observations over a much longer time frame than that utilized during the summer monitoring.

The increasing and decreasing numbers found at 45.7 cm may have been representative of the actual movement of tracers from the septic tank to the pump chamber. By 288 h, it appeared that observations had peaked and a subsequent decline at 816 h was evident. A sludge sample from the septic tank was not collected so it could not be determined how many of the microbial tracer organisms were dispersed to the absorption field.

At the end of the sampling period, all deep wells in both subsystems had tested negative for the tracer and the number of positive shallow wells was decreasing; counts in those wells were also decreasing. It would be difficult to estimate when all of the shallow wells would finally test negative for the tracer though, overall, the data appeared to indicate that, within one month, tracer numbers were definitely declining.

D. 2. c. 3. SUMMER VIRAL TRACER STUDY

During the summer virus study, only small numbers of phage were found in both subsystems. Few phage particles were even detected. In the summer phage study in

subsystem 1, 21 wells were negative and three tested with 1-50 PFU (Table 11). Of the three positive wells, two were shallow and one was a deep well. No phage particles were detected after 24 h, however, they were detected at both depths.

In subsystem 2, 19 wells were negative and five were positive with 1-50 PFU (Table 13). Of the five positive wells, three were shallow and two were deep wells. Phages were detected at both depths at 24 and 48 h, but at low levels of 1-50 PFU. There were no viral particles detected at 72 h in either system.

Although bacteria are larger than viruses and thought to be filtered easier than viral particles, the results indicate that bacteria during the summer study moved further and in larger numbers in the two subsystems than the virus particles. Viruses are charged particles that are predominantly retained in a soil system by adsorption to charged soil particles. In both subsystems, adsorption may have prevented viral particles from moving with the liquid phase as well as an inability to elute viruses off of soil particles in collected water samples which would have reduced both phage transport and detection.

It is also conceivable that there are root channels and other crevices which may be large enough to enable microbial movement without filtration or adsorption occurring, but this would make it easier for viral particles to travel that same route due to their small size. Expectations would then be to find viral particles with the tracer bacteria, however this was not the case.

D. 2. c. 4. WINTER VIRAL TRACER STUDY

During the winter study the viral recoveries were all quite low, with only slight peaks in viral numbers occurring at 192 and 288 h in both subsystems (Tables 12 and 14). In the winter viral study in subsystem 1, 35 wells were negative, two were positive with

1-50 PFU, and three were positive with $>10^4$ PFU. Of the five positive wells, four were shallow and one was a deep well.

In subsystem 2, 32 wells were negative, three were positive with 1-50 PFU, and five were positive with $>10^4$ PFU. Of the eight positive wells, seven were shallow and one was a deep well.

Peak observations occurred in shallow wells in both subsystems within the same time periods—192, 288, and 816 h. These observations yielded counts of $>10^4$ PFU. Throughout the tracer studies, both subsystems performed very similarly and did so even with this very different trend in the overall tracer study results.

The tracer addition to the septic tank rather than the pump chamber affected the time it took to record tracer observations for both subsystems. Most likely die-off or adsorption of tracers occurred in the septic tank, thereby decreasing numbers of organisms that even were dosed into the absorption field. It appears that it took between 168 and 192 h to disperse the organisms from tank to chamber to field.

D. 3. SUMMARY

Each bacterial and viral tracer was detected by 24 h except in subsystem 1 during the winter study. Summer sampling was not carried out over a long enough period of time to have all wells test negative for bacterial tracers. The summer viral tracer was detected only at 24 h in subsystem 1 and only through 48 h in subsystem 2. By the end of the extended winter sampling, all deep wells (213.4 cm) tested negative for both tracer organisms. From starting cultures on the order of 10^8 organisms, relatively few organisms were detected at either depth in either subsystem in either seasonal study.

E. LABORATORY DENITRIFICATION STUDIES FROM SUBSYSTEM SAMPLES

For denitrification to occur, aerobic conditions first are needed to permit oxidation of ammonia to nitrate. Anaerobic conditions are then needed to produce nitrous oxide through microbial denitrification. The laboratory experiments performed simulated field conditions and were an indication of the denitrification potential microbial population within each subsystem. This potential is dependent on the presence of a denitrifying population and an adequate source of nitrate-N and energy.

Effluent quality determinations yielded ammonium-N concentrations ranging between 7.42 and 31.00 mg/L over the three year research study (Table 10). Up-gradient, absorption area (subsystems 1 and 2), and down-gradient sampling showed ammonium-N concentrations approximately 3 mg/L up-gradient and within the absorption area (subsystems 1 and 2) and decreasing to less than 0.4 mg/L down-gradient (Tables 9 and 10).

Nitrate-N concentrations ranged from 0.07 to 0.87 mg/L in effluent samples over the study period. Up-gradient, within absorption area (subsystems 1 and 2), and down-gradient concentrations ranged between 0.12 and 13.04 mg/L. Down-gradient concentrations for the most part were greatly reduced.

It would appear from this water quality data that nitrification was occurring within the absorption area (subsystems 1 and 2), greatly reducing high ammonium-N concentrations (from the effluent) within the first 30.5 cm of soil in the absorption area (Table 10). Even greater reductions in ammonium-N concentrations were apparent at the 45.7 cm depth. Alternately, nitrate-N concentrations drastically increased from effluent to absorption area (subsystems 1 and 2). In 1989, the first year of the research, nitrate-N concentrations at the 30.5 cm depth were 6.02 and 12.80 mg/L, respectively for subsystems 1 and 2. The following year, the concentrations were 4.80 and 8.5 mg/L for

subsystems 1 and 2, respectively. Samples from down-gradient of the absorption area (subsystems 1 and 2) indicate a significant loss of nitrate-N, to less than 1.2 mg/L in all areas except for a high concentration of 9.65 mg/L 762.0 cm down-gradient of subsystem 2 at the 30.5 cm depth (Table 9).

E. 1. NITROUS OXIDE PRODUCTION FROM UNAMENDED SOIL SAMPLES

Nitrous oxide emissions from each subsystem are presented in Table 15. These soil samples were collected from subsystems 1 and 2 and studied, unamended, in the laboratory.

E. 1. a. SUBSYSTEM 1

Summer emissions from the 0-15.2 cm sample were more than six times greater than the winter emissions at that same depth. In the 15.2-30.5 depth, a slight significant difference occurred with the winter product slightly greater than the summer product.

During the summer study, the 0-15.2 cm depth produced two and one-half times more nitrous oxide than the 15.2-30.5 cm depth. These quantities were significantly different. In the winter study, a reversal occurred; emissions from the 15.2-30.5 depth were more than two and one-half times greater than the emissions from the 0-15.2 cm depth.

E. 1. b. SUBSYSTEM 2

Although not significantly different, the 0-15.2 cm depth produced quantitatively higher concentrations of nitrous oxide in the winter than in the summer. In the 15.2-30.5 cm depth, quantitatively more nitrous oxide was produced in the summer than in the winter. The products, however, were not significantly different.

The 0-15.2 cm depth produced about one and one-half times more nitrous oxide than the 15.2-30.5 cm depth in the summer. In the winter, the 0-15.2 cm depth continued to produce greater emissions with more than two times as much nitrous oxide as was produced in the 15.2-30.5 cm depth. In both seasons, the 0-15.2 cm emissions differed significantly from the 15.2-30.5 cm product.

E. 1. c. SUBSYSTEM COMPARISON

Samples from the 0-15.2 cm depth performed identically in the summer in both subsystems, however, subsystem 2 produced eight times more nitrous oxide in the winter than subsystem 1. The 15.2-30.5 cm samples from subsystem 2 performed quantitatively better than the samples from subsystem 1 in both summer and winter, although there was little significant difference in the emission concentrations.

Samples from the 0-15.2 cm depth produced quantitatively more nitrous oxide in subsystem 1 in the summer and in subsystem 2 in the summer and winter. Nitrous oxide yields in subsystem 1 during the winter were greater in the 15.2-30.5 cm depth. Overall subsystem 1 in the winter yielded quantitatively the smallest amount of nitrous oxide in the unamended soil study.

Subsystem 1 showed significant differences between emissions produced during the 0-15.2 cm depth in the summer and winter with quantitatively more nitrous oxide produced in the summer (Table 15). No significant difference between the seasonal emissions was noted for the samples from 15.2-30.5 cm.

Subsystem 2 quantitatively produced as much or more nitrous oxide at both depths over both seasons as subsystem 1. Although values between seasons at both depths were not significantly different between summer and winter, quantitatively higher

Table 15. N_2O-N emissions from soil samples collected during the summer 1989 and the winter 1990 from Blairton silt loam.

Subsystem	Design Loading Rate (Lpd/m^2)	Actual Loading Rate (Lpd/m^2)	Soil Depth (cm)	Summer 1989 Winter 1990* ($ug\ N_2O\text{-}N/g$ dry soil)	
1	5.1	2.4	0-15.2	0.064A	0.010C
			15.2-30.5	0.026C	0.028BC
2	10.2	4.9	0-15.2	0.063A	0.081A
			15.2-30.5	0.044BC	0.038BC

*Means in the same column with the same letter are not significantly different using Duncan's multiple range test ($p<0.05$).

emissions were produced in the winter at the shallow depth and in the summer at the greater depth.

Water quality data indicate that subsystem 2 had greater concentrations of nitrate-N in 1989 and 1990 than did subsystem 1. In 1989, subsystem 2 averaged 12.8 mg/L nitrate-N as compared to subsystem 1 which averaged 6.02 mg/L. In 1990, subsystem 2 averaged 8.50 mg/L and subsystem 1 averaged 4.80 mg/L. These data represent water samples drawn from 30.5 cm deep sampling wells. The greater quantity of nitrate-N available in subsystem 2 as compared to subsystem 1 may have influenced the denitrification potential of subsystem 2. Subsystem 2, dosed with twice the effluent loading of subsystem 1, stayed drier in both seasons and seems to have provided a soil environment influenced by aerobic conditions for nitrification and anaerobic conditions for denitrification to occur. Especially significant is that in winter, the nitrous oxide production was eight times higher at the shallow depth for subsystem 2 (0.081 ug) as compared to subsystem 1 (0.010 ug).

E. 2. NITROUS OXIDE PRODUCTION IN UNAMENDED AND AMENDED SOIL SAMPLES

Soil samples taken from the soil infiltrator LPD were studied unamended, as discussed previously, and amended with a glucose solution to further evaluate denitrification potential. Emission concentrations from unamended and amended soil samples representing each subsystem are presented in Table 16.

Table 16. N_2O-N emissions from Subsystem 1 (design loading rate 5.1 Lpd/m^2, actual loading rate 2.4 Lpd/m^2) and 2 (design loading rate 10.2 Lpd/m^2, actual loading rate 4.9 Lpd/m^2) samples collected and amended during the summer 1989 and the winter 1990 from Blairton silt loam.

Subsystem	Soil Depth (cm)	Treatment	Mean N_2O-N* (ug N_2O-N/g dry soil)	
			Summer	Winter
1	0-15.2	Glucose	0.08A	0.03A
		No Amendment	0.06A	0.01A
	15.2-30.5	Glucose	0.05A	0.05A
		No Amendment	0.03A	0.03A
2	0-15.2	Glucose	0.07A	0.14A
		No Amendment	0.06A	0.08A
	15.2-30.5	Glucose	0.06A	0.05A
		No Amendment	0.04A	0.04A

*Means in the same column within the same depth followed by the same letter are not significantly different using Duncan's multiple range test ($p<0.05$).

E. 2. a. SUBSYSTEM 1

E. 2. a. 1. SUMMER DENITRIFICATION STUDY

The 0-15.2 cm soil sample yielded higher concentrations of nitrous oxide under both unamended and glucose-amended status than the 15.2-30.5 cm samples. The addition of glucose to the samples did increase nitrous oxide emissions at both depths.

E. 2. b. 2. WINTER DENITRIFICATION STUDY

The 15.2-30.5 cm soil samples yielded greater emissions of nitrous oxide than the 0-15.2 cm samples. The glucose amendment did stimulate greater emissions at both depths. Although water quality data indicated average nitrate-N concentrations in subsystem 1 during 1989 and 1990 to have been 6.02 and 4.80 mg/L, respectively, little denitrification occurred in subsystem 1 at the 0-15.2 cm depth in the winter study. The addition of glucose did increase nitrous oxide production, however, by about the same margin, 0.02 ug, as in the other study situations.

E. 2. b. SUBSYSTEM 2

E. 2. b. 1. SUMMER DENITRIFICATION STUDY

The 0-15.2 cm depth produced slightly greater emissions of nitrous oxide than the 15.2-30.5 cm depth. The amendment of glucose to the soil samples did increase production.

E. 2. b. 2. WINTER DENITRIFICATION STUDY

The subsystem 2 0-15.2 cm soil samples performed much better during the winter than in the summer. The glucose amendment to the soil increased summer emissions by two times. The 15.2-30.5 cm depth produced less than one-half the emissions from 0-15.2 cm samples, however the glucose amendment did produce higher concentrations of nitrous oxide.

E. 2. c. SUBSYSTEM COMPARISON

E. 2. c. 1. DEPTH COMPARISON

During the summer study in subsystem 1, quantitatively more nitrous oxide was produced within the shallow sample while more nitrous oxide was produced in the winter study in the deeper sample (Table 16). In subsystem 2 the shallow depth produced quantitatively more emissions with both amendments in both seasons.

The 0-15.2 cm horizon most likely experienced the better balance between the anaerobic and aerobic states, allowing denitrifying bacterial populations to survive and prosper. Additional amendments of glucose allowed surviving populations to further prosper and produce nitrous oxide, as noted in subsystem 2 at the 0-15.2 cm depth in the winter (Table 16). Measurements taken from tensiometers indicate that subsystem 1 was wetter with depth and subsystem 2, drier with depth, in both seasons (Tables 7 and 8). This may indicate that over time, subsystem 1 may have been building up water that could not percolate down through the profile. Subsystem 2 appeared able to disperse the added liquid throughout the upper layers and actually hold less water at a depth of 45.7 cm than at a depth of 30.5 cm.

E. 2. c. 2. AMENDMENT COMPARISON

The statistical analysis for subsystems 1 and 2 indicated there was no significant difference between treatments of no amendment and glucose, however, the addition of an energy source did quantitatively increase nitrous oxide emissions in each subsystem, at each depth, and in each season. The addition of glucose to the 0-15.2 cm winter soil sample from subsystem 2 did greatly enhance nitrous oxide production (Table 16). This increase of 0.06 ug N_2O-N/g dry soil was produced with the addition of an energy source. Water quality data indicated that subsystem 2 contained more nitrate-N at a depth of 30.5 cm during the years the denitrification study was performed than subsystem 1. Perhaps the addition of a carbon source allowed more of the nitrate-N already present in the subsystem to be denitrified.

E. 2. c. 3. SEASONAL COMPARISON

At the 0-15.2 cm depth, subsystem 1 produced quantitatively greater emissions in the summer with both unamended and glucose-amended samples than in the winter study. At that same depth in subsystem 2, quantitatively greater emissions were produced during the winter as compared to the summer results for both unamended and amended samples. The 15.2-30.5 cm depth emissions were essentially the same regardless of amendment status or season in both subsystems (Table 16). Subsystem 1 yielded higher emissions in the summer study at 0-15.2 cm depth than in the winter while quantities produced at 15.2-30.5 cm depth stayed constant between the two seasons. Subsystem 2 yielded higher emissions in the winter study at 0-15.2 cm depth while quantities produced at 15.2-30.5 cm depth stayed almost constant between the two seasons. It would appear that a better population of denitrifiers existed higher in the soil profile year round.

E. 2. c. 4. OVERALL SUBSYSTEM COMPARISON

The addition of glucose did increase nitrous oxide emissions in both subsystems, at both depths, and in both seasons. Subsystem 1 failed to produce a greater quantity of nitrous oxide with the addition of glucose at both depths and in both seasons. The additional energy increased denitrification only in subsystem 2 at the shallowest depth in the winter. Even though it appears that subsystem 2 was drier with depth in both seasons; perhaps the fact that effluent was dosed to the original soil surface in cycles allowed for both aerobic and anaerobic conditions to exist within the first 15.2 cm thereby allowing sporadic nitrification to occur and a denitrifying microbial population to survive. Water quality data averaged over the years the denitrification study was performed indicated that greater concentrations of nitrate-N were present in subsystem 2 at the depth of 30.5 cm than in subsystem 1. The presence of the nitrogen source is one factor in denitrification potential in a soil environment. Results from subsystem 2 appear to indicate a greater potential for denitrification to occur than results from subsystem 1. It is quite possible that nitrate-N was indeed the limiting factor in these laboratory simulations.

E. 3. SUMMARY

Subsystem 2 quantitatively produced greater emissions of nitrous oxide than subsystem 1 in both seasons in the unamended study. Subsystem 1 in the winter produced the least nitrous oxide in the unamended study. The addition of glucose as a readily available carbon source quantitatively, but not significantly, increased nitrous oxide emissions over all samples. Glucose, suspected to be the limiting denitrification factor did not appear to be in the soil studied in these laboratory simulations. Both these

subsystems appear to be nitrate-limited, possibly due to the microbial populations present within the biological mat at the soil-effluent interface and within the first 30.5 cm of the soil profile utilizing any and all nitrate-N transformed from nitrification. Further studies and simulations would need to be performed to better determine whether nitrate-N is the overall limiting factor and to what extent it does limit denitrification in the subsystems installed in the Blairton silt loam.

V. SUMMARY AND CONCLUSIONS

A. LOADING RATES

The soil infiltrator LPD system placed in the Blairton silt loam (Stephens City, VA) was designed for a daily flow of 1135.6 Lpd based on current Commonwealth of Virginia Sewage Handling and Disposal Regulations (1982) for a two bedroom house. Monitoring flow rates over time showed an actual flow of 709.1 Lpd, approximately 62 percent of the designed daily flow rate. Every 1.98 d 851.7 L of effluent were dosed into the absorption area.

The absorption area consisted of two subsystems, numbered as 1 and 2, with design effluent loading rates of 5.1 and 10.2 Lpd/m^2, respectively. Subsystem 1, designed to approximate one-half the current Virginia regulation, had an actual loading rate of 2.4 Lpd/m^2 or approximately 47 percent of the design rate. Subsystem 2, designed to approximate the current Virginia regulation, had an actual loading rate of 4.9 Lpd/m^2 or 48 percent of the design flow.

With actual flow rates at less than 65 percent of the design flow, no risk of overloading occurred within either subsystem, however, the household use at this site was very low and did not typify that of a usual two-bedroom house and the associated potential occupancy of four persons. Some ponding was observed over the three year research study through soil access wells placed within the absorption field. However, there were never any indications of a subsystem actually failing by effluent ponding on the surface or backing up into the house plumbing. Overall, both subsystems appeared to function well under the designed/actual effluent loading rates.

B. SOIL MATRIC POTENTIALS

Soil matric potentials averaged over the periods that microbial tracer and denitrification studies were conducted. Both subsystems appeared to perform similarly during both these study periods.

C. WATER QUALITY

Background water quality parameters from up-gradient and down-gradient areas from both subsystems 1 and 2 were comparatively similar. Travel through the absorption area appears to have enhanced background water quality in the Blairton silt loam.

Water quality of pump tank effluent was averaged yearly over the three year research study. Concentrations/values fluctuated during years one and two, however, the highest concentration/value for each parameter occurred in the third and last year of the study. Most likely this occurred due to the general aging process of a septic system with the waste load building up inside the septic tank. The effluent generated from the household was of a weaker strength than expected, therefore, the subsystems were not tested as fully as they were designed to be on this site.

There were no notable differences between the overall performance of either subsystem 1 or 2 with regard to water quality. Each subsystem seemed to renovate the effluent to an acceptable quality and both subsystems appeared to handle the effluent load with consistent performance, regardless of the effluent quality, which was not consistent over the research period.

D. MICROBIAL TRACER STUDIES

D. 1. BACTERIAL TRACER

The summer bacterial tracer was recovered in greater numbers and from more deep wells in subsystem 1. During the winter sampling more observations of higher counts were recorded from subsystem 2, however, these were recovered mainly from the shallow wells. Tracer counts declined in both subsystems by 816 h during the winter.

Tracer study data indicated that the bacterial tracer not only moved into the shallow wells by 24 h but also to the deeper wells located at 213.4 cm in both subsystems and in both seasons by the first sampling period. Observations at each depth and each sampling further indicated that the tracer continued to survive and move through the profile for at least 72 hours in the summer season. Winter samplings carried out on a different time frame yielded tracer detection at shallow and deep wells at 816 h, with comparatively high counts from some wells.

Before sampling began each day, all wells were emptied of any standing water, therefore, each sample collected was soil water that moved into the well directly after the well was emptied. This ensured that the sample was indicative of soil water moving through the profile and the amount of bacterial tracer moving within it. The fact that deep wells showed tracer quantities 24 h after the dosing event indicated substantial water movement.

D. 2. VIRAL TRACER

The viral tracer was detected up to 48 h in shallow and deep wells in subsystem 2. In the winter sampling the tracer was detected sporadically in both shallow and deep wells in both subsystems.

In the summer viral tracer study in subsystem 1 no particles were detected after 24 h, however, they were detected at both depths prior to 24 h. In subsystem 2 phages were detected at both depths at 24 and 48 h. During the winter study viral recoveries were low although peaks were detected at 192, 288, and 816 h in both systems. Except for very low phage counts in each subsystem, no other virus was detected at 213.4 cm. Subsystem 2 had higher counts than subsystem 1 at 45.7 cm at 288 and 816 h.

The Blairton soil at the field site suffers from a high seasonal water table as well as low hydraulic conductivity. Finding these tracer organisms so deep in the profile so early after the original dosing demonstrated that enteric organisms are most likely traveling to those depths in that time frame as well. The potential for groundwater contamination with insufficiently treated effluent is certainly present, especially at locations like the experimental site, with a very shallow groundwater table.

Each family of viruses behaves differently within the soil matrix and may not represent how other families behave. Conclusions from a viral tracer study can only be made for the behavior of the particular viral strain utilized in the study, however, any research with viruses yields a bit more information on the complex nature of their interaction within the soil matrix.

E. DENITRIFICATION STUDIES

Aeration is a very important condition in the denitrification process as it allows for the ammonium-N dosed from the septic tank to be oxidized to nitrate-N which the denitrifiers then utilize to form nitrous oxide and nitrogen gas. Water quality data averaged over the years the denitrification study was performed indicated that greater concentrations of nitrate-N were present in subsystem 2 at the depth of 30.5 cm than in subsystem 1. Results from subsystem 2 showed a greater potential for denitrification to

occur in subsystem 1. Subsystem 2 quantitatively produced as much or more nitrous oxide at both depths over both seasons as subsystem 1. Especially notable was that, in winter, the nitrous oxide production was eight times higher at the shallow depth for subsystem 2 as compared to subsystem 1.

Although there was no significant difference between treatments of no amendment and glucose in all instances, the addition of an energy source did quantitatively increase nitrous oxide production in each subsystem, at each depth, and in each season. The highest quantity produced occurred in the 0-15.2 cm winter soil sample from subsystem 2, which increased in emissions by 0.06 ug N_2O-N/g dry soil with the addition of an energy source. Overall, subsystem 2 appeared to have greater denitrification potential whether or not amended with an energy source.

Glucose was predicted to be the limiting factor in field denitrification at the site, however, results did not support that prediction. Nitrate-N is possibly the limiting factor in these subsystems; further research would need to be performed to determine this possibility.

F. OVERALL SYSTEM PERFORMANCE

The design goal of low-pressure distribution of effluent is to utilize the entire absorption area by delivering effluent under pressure through pipes with varying hole diameters and hole spacing. The delivery should occur on a uniform schedule to promote both aerobic and anaerobic conditions within the treatment area. An anaerobic state would exist when the effluent is sprayed evenly over the trench bottom. As the effluent percolates into the soil, an aerobic state would begin to develop. The soil infiltrators enhance this design further by allowing effluent to be directly applied to the soil surface, instead of to the more impermeable subsurface layers. The Blairton silt loam has low

hydraulic conductivity and a shallow groundwater table. With the use of the infiltrators placed directly on the surface soil, enough permeable soil depth is available to theoretically renovate the effluent.

No physical signs indicated failure of either subsystem 1 or 2 during the research study. Both subsystems performed very well in renovating the chemical elements from the dosed effluent. Both subsystems appear to have handled the effluent load assigned. It must be noted that the effluent produced from the household was of a lesser volume and weaker strength than expected.

Subsystem 1 tended to be wetter within the absorption area that may be attributed to the discontinuous indurated layer identified beneath it. The reduced design loading rate most likely enabled this subsystem to function and to not become overloaded with effluent. Bacterial and viral tracer recoveries varied little between the subsystems, although tracer organisms did move through both subsystems. There were no significant differences in nitrous oxide emissions from either subsystem.

Overall, there appeared to be only slight differences in the performances of subsystems 1 and 2. Even though the design effluent loading for subsystem 2 was twice that for subsystem 1, the effluent was renovated to a high quality in both. The soil infiltrator LPD functioned very well as designed for the site and soil limitations. It produced good soil water quality both in the absorption area and in up-gradient and down-gradient areas from each of the two subsystems.

Further full-scale field research with soil infiltrator LPD systems is needed to determine the effects of greater actual loading rates and stronger effluents than were available at this site and potential performance limiting factors. The soil infiltrator LPD, as designed and maintained in this research, appears to be a potential alternative OSWTDS for use in marginal/unsuitable soils.

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