

Report as of FY2009 for 2006SD74B: " Microbial Indices of Soils and Water Associated with Vegetated Treatment Areas (VTAs) from Five Animal Feeding Operations (AFOs) in South Dakota"

Publications

- Other Publications:
 - ◆ Bleakley, B.H. 2007. Microbial indices of soil and water. South Dakota Water Resources Institute Water News. January 2007, volume 3, number 1.

Report Follows

Report as of FY2009 for 2006SD74B: " Microbial Indices of Soils and Water Associated with Vegetated Treatment Areas (VTAs) from Five Animal Feeding Operations (AFOs) in South Dakota"

Publications

Project 2006SD74B has resulted in no reported publications as of FY2009.

Report Follows

Microbial indices of soils and water associated with vegetated treatment areas (VTAs) from five animal feeding operations (AFOs) in South Dakota

Basic Information

Title: Microbial indices of soils and water associated with vegetated treatment areas (VTAs) from five animal feeding operations (AFOs) in South Dakota

Project Number:

Start Date: 3/1/2007

End Date: 2/28/2009; (with extension for an additional year to 6/30/2009)

Funding Source: 104B

Congressional District: First

Research Category: Water Quality

Focus Category: Water Quality; surface water; wastewater.

Descriptors: None

Principal Investigators: Bruce Bleakley; Todd Trooien

Introduction: *Write a brief overall introduction to your progress/completion report.*

Nutrient and sediment loads from animal feeding operations (AFOs) can reduce the quality of surface waters and groundwaters. Basin technologies can help alleviate some of these problems, but they can be costly, and cause odor problems. The potential for development and implementation of alternative non-basin technologies interests a variety of stakeholder groups. An EPA funded grant, "Evaluating the Performance of Vegetated Treatment Areas," (Dr. Todd Trooien, P.I.), seeks to evaluate the technical and financial feasibility of vegetated treatment areas (VTAs) as a non-basin alternative for reducing nutrient and sediment loads from AFOs having less than 1,000 animal units. For this EPA grant, each of five AFOs in different areas of South Dakota have had or will have VTAs established. Performance of each VTA is being measured by sampling inflows and outflows from vegetated areas. The samples are being analyzed for nutrients (N and P), salts, sediment, and numbers of fecal coliform bacteria. Data from these measurements will allow calculation of water and salt balances, loss or gain of nutrients, removal of sediment, and fecal coliform numbers. Data will be entered in a basin model to simulate basin performance, that will be compared to measured VTA performance. Samples have been obtained from 2005 to 2009, with the Howard, SD site receiving the most attention since it has been operational the longest; some of the other sites (like the one near Claire City in Roberts County, and the one in Minnehaha County) are only becoming operational in 2009. For the EPA project, only numbers of fecal coliform bacteria are being measured. Other aspects of the microbiology of the inflow and outflow areas associated with the VTAs are not addressed, and are the focus of this 104b proposal.

Information Transfer Program: Some of the information presented here was also presented at a Stakeholders Meeting on the SDSU campus in Spring of 2010.

Data from this project will continue to be presented at such stakeholder meetings; and at field days at one or more of the VTA sites of the study.

Problem:

Nutrient and sediment loads from animal feeding operations (AFOs) can negatively impact the quality of surface waters and groundwaters. One accepted way to reduce nutrient and sediment loads from AFOs is by use of basin technologies, which are effective but can be costly, and lead to air quality problems due to unpleasant odors. The potential for development and implementation of alternative non-basin technologies interests a variety of groups, including the South Dakota Cattlemen's Association, South Dakota Farm Bureau, South Dakota Association of Conservation Districts, South Dakota State University, South Dakota Department of Agriculture, Natural Resources Conservation Service, South Dakota DENR, and cattle producers. The Iowa Cattlemen's Association (ICA) is also interested.

An EPA funded grant, "Evaluating the Performance of Vegetated Treatment Areas," (Dr. Todd Trooien, P.I.), has been underway since 2005 to the present. Its goal is evaluation of the technical and financial feasibility of vegetated treatment areas (VTAs) as a non-basin alternative for reducing nutrient and sediment loads from AFOs having less than 1,000 animal units. Each of five AFOs in different areas of South Dakota have had or will have VTAs established. Performance of each VTA will be measured by sampling inflows and outflows from vegetated areas. The samples are being analyzed for nutrients (N and P), salts, sediment, and numbers of fecal coliform bacteria. Data from these measurements will allow calculation of water and salt balances, loss or gain of nutrients, removal of sediment, and fecal coliform numbers. Data will be entered in a basin model to simulate basin performance, that will be compared to measured VTA performance.

For the EPA project, counts of *E. coli* before and after each VTA are the only measure being taken of bacterial effects on water quality in pre- and post-VTA areas. Other microbial measures affecting water quality would also be valuable, such as detecting presence or absence of toxigenic *E. coli*, such as *E. coli* O157:H7; and numbers of non-toxigenic *E. coli* in pre- and post-VTA areas at each site, to better assess whether water quality in post VTA areas is better (has lower numbers of these bacteria) than in pre-VTA areas. Also, since microbial activity can influence physical and chemical parameters of soil and water, such as whether aerobic or anaerobic processes are occurring, other measures of microbial activity would be valuable for both pre-VTA and post-VTA areas at each site, to further assess water quality in these areas. The 104b project described here is providing a more detailed and broader understanding of some microbiological issues relating to this waste management system.

Research Objectives:

For the EPA project described above, counts of *E. coli* before and after each VTA are the only measure being taken of bacterial effects on water quality in pre- and post-VTA areas. Other microbial measures affecting water quality would also be valuable, such as detecting presence or absence of toxigenic *E. coli*, such as *E. coli* O157:H7; and total *E. coli* other than *E. coli* O157:H7 in pre- and post-VTA areas at each site, to better

assess whether water quality in post VTA areas is better (has lower numbers of these bacteria) than in pre-VTA areas; or if the presence of *E. coli* diminishes across the landscape as one moves further away from the feedlot across the VTA. Also, since microbial activity can influence physical and chemical parameters of soil and water, such as whether aerobic or anaerobic processes are occurring, other measures of microbial activity would be valuable for both pre-VTA and post-VTA areas at each site, to further assess water quality in these areas.

Differences in the microbiology of soils in the inflow and outflow areas associated with VTAs is being assayed by measuring the following microbial indices: (a) Soil respiration; (b) oxidation/reduction potential; (c) heterotrophic microbial activity; (d) soil bacterial diversity; (e) numbers of total culturable *E. coli* in water ; and (f) presence or absence of culturable pathogenic *E. coli* O157:H7. Data for these microbial indices will be added to the data sets from the EPA project, to get a better idea of the number and activity of microbes in soils associated with inflow and outflow areas.

Methodology:

Soil respiration was initially measured in the field with a portable soil respirometer. The assay is now being done on samples in the lab. Both plant root respiration and microbial respiration can contribute to the values obtained in the field (Alef, 1995a; Beck, 1996). Oxidation/reduction potential of wet soils are being assayed with a portable meter fitted with an oxidation/reduction electrode (Zausig, 1995). Heterotrophic microbial activity is being evaluated by assaying ability of soil samples to hydrolyze fluorescein diacetate (Alef, 1995b). Soil bacterial diversity is being assayed in two ways: (1) by use of Biolog EcoPlates that assay the ability of a soil microbial community to utilize different carbon sources (Insam and Goberna, 2004) ; and (2) by molecular methods, using polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) (Hastings, 1999; Baker and Harayama, 2004). We are evaluating the numbers of *E. coli* of humans and animals in both inflow and outflow water. Water samples have been analyzed by counting coliforms on mFc agar, then picking these colonies over onto Chromagar plates that are more specific in identifying colonies of *E. coli*. A serologic test is being used to determine if water samples contain *E. coli* O157:H7.

Principal Findings

i) Assessing the redox state of the soil and/or water in inflow versus outflow areas: Because of dry conditions throughout the state for most of 2006 including at the Howard site, redox potential was not measured at Howard or elsewhere for that year. At the end of 2007, initial readings were taken at Howard in the fall when standing water was present at several of the sampling areas for this site. Using a portable redox electrode, redox values were found to be negative at most of the water saturated sites, with negative values ranging from -120 millivolts to -250 millivolts. These negative readings indicate that the sample sites were largely or entirely anaerobic, so that processes such as fermentation, sulfate reduction, and denitrification were likely occurring. In June of 2008, soon after major rainfall events, redox electrode readings were again taken in Howard when the soil was moist but not water saturated. Readings at all sample sites were positive, ranging from 124 to 280 millivolts, indicating that the soils were largely

aerobic and conducive to aerobic microbial processes and plant root metabolism. Engineering changes occurred in the layout of the drainage pipes at the Howard site between 2007 and 2008 to further spread flow of water from the feedlot over the landscape, and may be promoting more positive redox values/aerobic conditions over the entire landscape, even during wet periods.

Some problems in taking the redox measurements in situ have included breakage of the delicate and expensive redox electrodes in heavy clay soils/clay pans that are often hard to detect in the field. Also, it has been more difficult to access and take measurements during wet periods in the field (the only times when redox measurements are meaningful in the field) than we initially expected. We are now making redox measurements on soil samples brought back to the lab, where measurements can be made more easily.

The hypothesis being tested is that redox values will be more negative in areas having largest amounts of organic load from the AFOs; where microbial respiration will have depleted oxygen gas concentration and led to anaerobiosis. By relying more heavily on redox measurements made in the lab, we will be able to generate more data on more soil samples from the field sites, and better evaluate this hypothesis. About half the soil samples so far obtained have had redox measured in the lab; the remaining samples will be completed in 2010.

ii) Assessing the activity of microorganisms in soil and/or water in inflow versus outflow areas by use of a portable soil respiration monitor (for drier soils only): Carbon dioxide measurements were made at the Howard site in pre VTA and post VTA areas from late summer to fall of 2006. Preliminary analysis of respiration data (not yet statistically analyzed) suggests that there were not significant differences in CO₂ production between pre VTA and post VTA areas on the dates sampled, possibly because the respiration of grass roots was so dominant and equivalent in both areas. It is clear from data obtained to date that soil temperature is a major determinant of soil respiration, more so than moisture. Soil temperatures around 21° C gave respiration values (g CO₂/m²/hour) ranging from 2 to 6; while soil temperatures around 8° C gave values that were three to eight times lower.

During 2007 and 2008, soil respiration readings at Howard were taken in summer and fall, (after the soil had dried enough to be assayed). Values were no higher than 2006, especially in the summer. How soil respiration values correlate to FDA hydrolysis measures, and to soil temperature and moisture, needs to be examined. We have established a standard assay for soil respiration of samples brought back to the lab (where the contribution of living plant roots can be eliminated; so that measured respiration will be only microbial).

We are testing the hypothesis that respiration will be higher in inflow areas compared to outflow areas, due to greater organic matter load stimulating microbial respiration in the inflow areas than in the outflow areas. Data obtained to date from the Howard site do not clearly support this, but work over the next year including data from Howard and the other VTA study sites will help decide whether the hypothesis is supported. About half the soil samples so far obtained have had soil respiration measured in the lab; the remaining samples will be completed in 2010.

iii) Assessing the heterotrophic activity of microorganisms in soil and/or water in inflow versus outflow areas by use of a spectrophotometric assay of fluorescein diacetate (FDA) hydrolysis in inflow vs. outflow soil and/or water: Soil samples from pre VTA and post VTA areas of the Howard site collected from 2006, 2007, 2008, and 2009 have been analyzed for FDA activity, (with some samples yet to be processed); but data have not yet been statistically analyzed. It is clear that different sites at Howard have different FDA hydrolysis values over time, and there are differences between sites. How these values correlate to values of soil respiration, temperature, and soil moisture needs to be examined.

We hypothesize that FDA hydrolysis will be greater in inflow areas than outflow areas, since higher amounts of organic matter in inflow areas should stimulate more microbial activity than in outflow areas. Data obtained to date do not clearly support this, but data analysis completed in 2010 will help decide whether this hypothesis is supported.

iv) Assessing soil bacterial diversity in inflow versus outflow soil and water by means of denaturing gradient gel electrophoresis (DGGE) to compare number of gel bands obtained from samples from inflow versus outflow soil and water, employing 16S ribosomal DNA primers and polymerase chain reaction (PCR) methodology: This is a molecular method that will be able to assess both the culturable and non-culturable bacteria in soil samples. Soil samples from pre VTA and post VTA areas of the VTA sites will be analyzed using DGGE.

An initial DGGE gel running samples from the Sturgis, SD site was shown in last year's progress report. Work on samples from the Brandon, SD and Howard, SD sites has since been completed, and is shown in Fig. 1 and Fig 2.

At the Brandon, SD site, samples from different parts of the VTA landscape were labeled by their orientation to pipes (P1, P2, and P3) emerging from the large earthen berm separating the VTA from the feedlot, and by the smaller berms (B1, B2, B3, and B4) in the VTA that water flows across after emerging from the pipes. As can be seen in Fig. 1, there are some differences in presence or absence of bands, and in band intensity, between samples from different sites. Similarly, for the Howard site (Fig. 2), differences in presence or absence of bands and in band intensity are evident in samples from the same site in different years, and between sites in the same year. Each band likely represents one specific DNA sequence of a numerically dominant prokaryote in the soil (including both culturable and non-culturable prokaryotic groups). Detailed analysis of this and other DGGE gels from these sites will be completed in 2010.

We hypothesize that bacterial diversity will be greater in inflow versus outflow areas, due to greater amounts and types of organic matter available in the inflow areas; and to more likely frequent periodic aerobic/anaerobic transitions in the inflow areas, versus the outflow areas.

v) Assessing soil bacterial diversity in inflow versus outflow soil and water by means of carbon source utilization profiles of soil microbial communities in the inflow and outflow areas using Biolog EcoPlates: Soil samples from pre VTA and post VTA areas of the Howard and Sturgis sites have been/are being analyzed using Biolog plates, but have not yet been statistically analyzed. We will contact Dr. Mike Lehman of the USDA NCAUR lab in Brookings for aid in the statistical analysis, since he has had extensive background and experience in analyzing Biolog results for environmental samples.

We hypothesize that bacterial diversity will be greater in inflow versus outflow areas, due to greater amounts and types of organic matter available in the inflow areas; and to more likely frequent periodic aerobic/anaerobic transitions in the inflow areas, versus the outflow areas.

vi) Evaluating the numbers of specific fecal coliforms and/or potential pathogens of humans and animals in both inflow and outflow soil and water by use of several agar media that are selective and differential for specific bacterial types: Water samples from the Howard site have been analyzed by counting coliforms on Mfc agar, with numbers ranging from 10^4 to 10^5 CFU coliforms/ml in the pre-VTA and post-VTA samples. Picking these colonies over onto Chromagar plates has shown for 2006, 2007, and 2008 samples that most (in excess of 50%) but not all of the initial isolated colonies were *E. coli* in the pre-VTA and post-VTA areas. In 2006 water samples from the river in the area behind the post-VTA area, fecal coliforms were found in lower numbers (10^2 CFU coliforms/ml) than in pre and post VTA areas, with from 20% to 70% of those in the river verified as *E. coli*. For 2006 samples we detected presence of *E. coli* O157:H7 in pre-VTA and post-VTA water samples, but not in river samples situated after the post-VTA area. This indicates that the VTA is effectively removing *E. coli* strains of the greatest health concern before they reach the river.

For Howard in 2007, with water samples from the river in the area behind the post-VTA area, fecal coliforms were found in lower numbers than in pre-VTA and post VTA areas, but for some sampling dates the numbers in the river were only one log less than in the post VTA area. For 2007 samples we detected presence of *E. coli* O157:H7 in pre-VTA and post-VTA water samples, but not in river samples situated after the post-VTA area, again indicating that the VTA is effectively removing *E. coli* strains of the greatest health concern before they reach the river. However, there were times where cattle were grazing beside the river, so that numbers of *E. coli* in the river cannot be totally tied to the VTA but may instead originate from these grazing cattle near the river bank. Results of samples for 2008 from the Howard and Sturgis sites are shown in Table 1. The fecal coliform numbers at the Howard site were similar to counts from previous years in the VTA areas preceding the river. Counts from the river were very low or zero, indicating that the VTA is succeeding in helping to reduce the fecal coliform load entering the river.

In 2009, we concentrated on sampling from the creek that is close to the Howard VTA site, for fecal coliform counts. Based on previous years of data, we knew that surface flow of water carrying sediment in the VTA would likely have high fecal coliform counts. We wanted to see if fecal coliform counts in the nearby creek were similar or lower than in the VTA. The data (Table 1) strongly suggest that the creek is well protected from elevated fecal numbers. Most of the fecal coliform counts of the creek water column (taken under the bridge that runs beside a gravel road) were at or near zero. The water column was almost always clear, with little or no suspended solids visible to the unaided eye. When the bottom sediment was sampled, fecal coliform counts were higher than for the water column, but still not extremely high (Table 1).

Significance

Management issues that could be impacted by results of the study include management of undesirable odors affiliated with the AFOs; extent of anaerobic versus aerobic microbial processes in inflow versus outflow areas; ability of the VTAs to filter out specific pathogenic bacteria such as *Escherichia coli* O157:H7 ; amount of CO₂ gas produced from soil in pre-VTA versus post-VTA areas; and overall heterotrophic microbial activity and microbial community diversity in soil and water in pre-VTA versus post-VTA areas as a measure of the ability of the VTA in removing organic compounds from the AFO inflow. Results could influence future management decisions for AFOs making use of VTAs; and afford information to better understand how to manage microbial populations in the soils affiliated with the VTAs to achieve desirable air and water quality in these areas.

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Publication Citations

Bleakley, B.H. 2007. Microbial indices of soil and water. *South Dakota Water Resources Institute Water News*. January 2007, volume 3, number 1.

Student Support:

Number of students supported with section 104b: 2006-2010
One Ph.D. graduate student (summer 2006 and 2007 support)
Five undergraduate students (2007 to present)

Student Information Table

	Total	Major
Undergrad.	5	(2) Microbiology; (1) Biology; (2) Pre-pharmacy
Masters	0	
PhD.	1	Plant Science
Post-Doc.	0	
Total	6	

Notable Awards and Achievements:

Table 1: Fecal coliform counts: 2009

I) Howard, SD:

Date: 6/27/09: Location: Creek under bridge by road

Count: 0.0 CFU/ml

Date: 07/05/09: Location: Creek under bridge by road

Count: 0.0 CFU/ml

Date: 10/07/09: Location: Creek under bridge by road

Count: 0.0 CFU/ml

Date: 10/08/09: Location: Creek under bridge by road

Count: 0.0 CFU/ml

Date: 10/15/09: Location: Creek under bridge by road

Count: 2.6 CFU/ml

Date: 10/22/09: Location: Creek under bridge by road

Count: 0.0 CFU/ml (water only; no sediment)

1.04 X 10³ CFU/ml (water with bottom sediment); with 80% of isolates verified *E. coli* on Chromagar

Date: 10/29/09: Location: ¾ toward creek; near trees

Count: 1.106 X 10⁴ CFU/ml; with 100% of isolates verified as *E. coli*

Location: Creek under bridge by road:

8.0 X 10¹ CFU/ml; with 25% of isolates verified *E. coli* on Chromagar

II) Sturgis, SD:

Date: 6/28/09

Count: 0.0 CFU/ml in each chevron

Fig. 1: Brandon Gel (All samples collected 5-19-09)

Lane	Site
1	B1 P1
2	B1 P2
3	B1 P3
4	B2 P1
5	B2 P2
6	B2 P3
7	B3 P1
8	B3 P2
9	B3 P3
10	B4 P1
11	B4 P2
12	B4 P3

This gel was run with a 40% to 75% gradient at 70 V for 18 hours at 60 °C

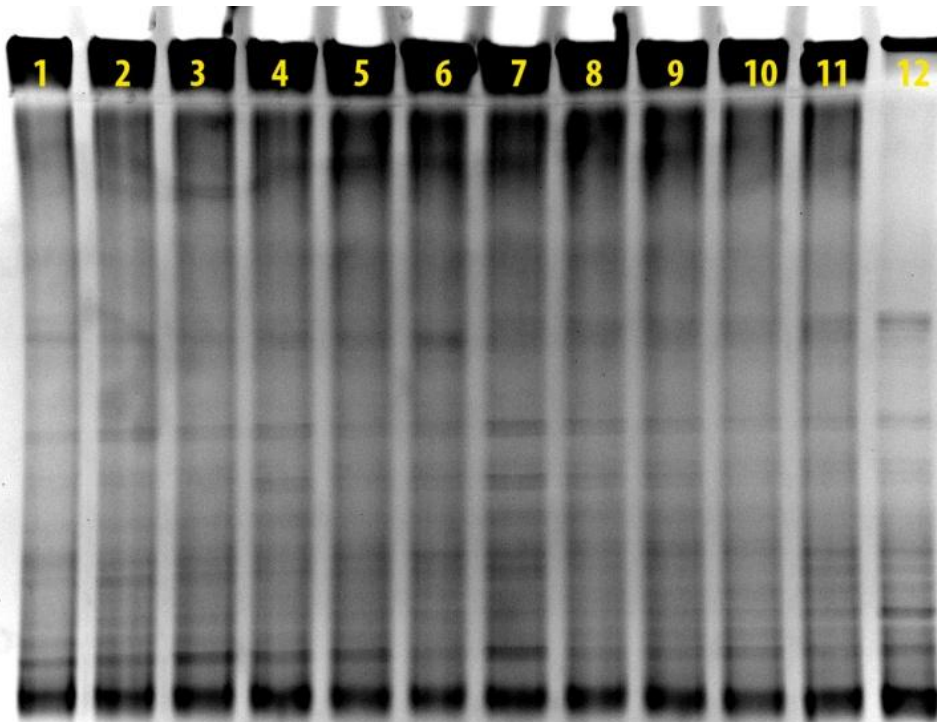


Fig. 2: Howard Gel (All samples collected during August of different years)

Lane	Site	Year Collected
1	1	2006
2	1	2007
3	1	2008
4	4	2006
5	4	2007
6	4	2008
7	7	2006
8	7	2007
9	7	2008
10	11	2006
11	11	2007
12	11	2008
13	12	2006
14	12	2007
15	12	2008
16	14	2006
17	14	2007
18	14	2008

This gel was run with a 40% to 75% gradient at 70 V for 18 hours at 60 °C

