Report as of FY2007 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

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

Introduction:

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 2007. 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 tied to the Ag United meeting for the Vegetative Treatments Systems project (sponsored by EPA via DENR) held on December 7, 2006 at the Sioux Falls Ramkota Inn.

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 total fecal coliforms (including both non-toxigenic *E. coli* and other genera that are found in feces) 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 fecal coliforms (including both non-toxigenic *E. coli* and other genera that are found in feces) 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.

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 fecal coliforms (including non-pathogenic *E. coli* but including other culturable fecal coliforms as well); and (f) numbers 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 is being measured in the field with a portable soil respirometer. Both plant root respiration and microbial respiration can contribute to the values obtained (Alef, 1995a; Beck, 1996). Oxidation/reduction potential of wet soils will be assayed in the field 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 will be 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 specific fecal coliforms and/or potential pathogens of humans and animals in both inflow and outflow 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, then picking these colonies over onto Chromagar plates that are more specific in identifying colonies of *E. coli*. A serologic test was used to determine if water samples contained *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 has not yet been measured at Howard or elsewhere, but will be when moist soil conditions allow.

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.

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 suggests that there were not significant differences in CO_2 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^o C gave respiration values (g CO_2/m^2/hour) ranging from 2 to 6; while soil temperatures around 8^o C gave values that were three to eight times lower.

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.

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 late summer to fall of 2006 have been analyzed for FDA activity, but data have not yet been statistically analyzed. 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.

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 Howard site will be analyzed using DGGE. 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 site have been analyzed using Biolog plates, but have not yet been statistically analyzed. 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 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 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), with from 20% to 70% of these verified as *E. coli*. We have 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.

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 CO_2 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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