

MICROBIAL CHARACTERIZATION OF THE TREATMENT ZONE IN SWINE WASTE  
LAGOONS

and

SWINE LAGOON SLUDGE DEFINITION AND MEASUREMENT

Combined Reports for  
North Carolina Pork Council Project 06-006 and 06-007

John J. Classen  
Mark Rice  
Leonard Bull  
Amy Grunden  
Anthony Devine

March 24 2009

## **Key Findings**

Measurements of sludge depth and samples from the sludge layer were taken at several locations in each of 10 to 15 lagoons. Two measurements of depth to sludge were made and recorded. The first measurement, the sludge/liquid boundary, was determined using the sonar method. The second measurement, the layer of dense sludge capable of supporting a resistance of approximately 0.1 pounds per square inch, was determined using a weighted disk. The depth of the dense sludge that supported the weighted disk was, on average, 2.6 feet below the sludge/liquid boundary as determined by the sonar method. A new sampling device was developed to enable samples to be taken from the sludge/liquid boundary and every six inches below that to the bottom of each lagoon. Samples were analyzed for total and volatile solids, UV absorbance, and microbial identification based on variations in the 16S rDNA genes identified using the Terminal Restriction Fragment Length Polymorphism (TRFLP) method.

Based on these analyses, we have identified the interface layer between the dense sludge and the sludge/liquid boundary as the area of greatest microbial activity. This interface is a region in the lagoon where sludge material from the lagoon depths can mix with the lagoon liquid layer, which allows microbial communities access to the nutrients found in sludge material. Regulations in North Carolina specifically address the volume of sludge found in the lagoon system (NPDES Permit NCA200000 from NCDWQ website) and currently recommend a measurement technique that includes the interface layer. The results of this study suggest current management practices to remove sludge based on these regulations may disrupt the active microbial communities found at the point where liquid and solid layers of the lagoon systems meet.

## **Final Report, March 2009**

As suggested at the time of award, the principal investigators of this project have cooperated with the principal investigators of the project, Microbial Characterization of the Treatment Zone in Swine Waste Lagoons (06-006), adding Dr. Amy Grunden and Anthony Devine to the team that addressed the following combined objectives:

- Develop an operational definition of sludge based on measurements of various components of solids of a lagoon, especially volatile and fixed suspended solids as well as microbial characterization of the liquid / sludge interface.
- Develop and evaluate an ultraviolet spectrophotometric method of sludge measurement.
- Compare methods of determining the top of the sludge layer, including the weighted disk, sludge gun, sonar and UV spectrophotometry, and identify strengths and weaknesses of each method.
- Define the active treatment zone on top of the inactive sludge layer based upon microbial counts and identifications in an anaerobic swine waste lagoon.
- Correlate microbial counts and identifications to concentrations of various solids, especially volatile and fixed suspended solids.

The original work plan called for operation of large columns to accurately model lagoon behavior in a system that was easier to obtain samples from precise depths than could be obtained from full size operational lagoons. Doubts that these columns would develop the same

physical and biological characteristics as lagoons led the team to develop a sampling device that could take suitable samples from actual lagoons. Requirements of the sampler are listed: Samples must be taken from specific depths without contamination as the device is raised. Samples must be collected so that disturbance of nearby lagoon material is minimized. The device must be able to take samples within two inches of the bottom of a lagoon.

Several designs were built by the Research Shop in the Biological and Agricultural Engineering Department and tested at the Lake Wheeler Road Field Laboratory Swine Education Unit lagoon. After several iterations of modifications and testing, an acceptable sampler was put in use in August 2007. The sample chamber is based on a 3.5 inch diameter PVC tube and is 2.5 inches in height. Another PVC tube that is slightly larger fits over the inner tube to seal the chamber. The device will collect a lagoon sample (approximately 300 mL) from any depth within plus or minus the height of the sample chamber.

We determined that taking samples from over the side of small boat on waste lagoons is not advisable. Not only is the boat unstable but keeping the sampler vertical to take a sample from the desired depth is difficult. We modified a 10 ft jon boat by cutting a 7.5 inch hole in the center, wide enough to pass the sampler through. Secured to the inside is a stand pipe as tall as the gunwales of the boat, well sealed to prevent leaks.

The depth of sludge for each lagoon was determined consistent with North Carolina State University Extension recommendations (Westerman et al., 2008). An additional measurement was taken to monitor any changes in the depth of dense sludge. The total depth of each lagoon was determined by measurement with a calibrated PCV pole. Two measurements of depth to sludge were made and recorded. The first measurement, the sludge/liquid boundary, was determined using the sonar method. The second measurement, the layer of dense sludge capable of supporting a resistance of approximately 0.1 pounds per square inch, was determined using a weighted disk (Fulhage et al., 2005). The depth of the dense sludge that supported the weighted disk was, on average, 2.6 feet below the sludge/liquid boundary as determined by the sonar method.

Samples collected were analyzed by both the microbial analysis team and the sludge determination team. Samples for sludge determination were taken at the top of the sludge layer as determined by sonar analysis of a lagoon and every six inches below that to the lagoon bottom. This process was repeated at several locations within a lagoon as well as at several different lagoons. Some lagoons were sampled at two different times during the overall sampling period.

A new method was developed by the microbial analysis team to provide rapid identification of microorganisms in the hog lagoon systems; the method is based on sequence variations in the 16S rDNA genes identified using the Terminal Restriction Fragment Length Polymorphism (TRFLP) method coupled with our newly developed the *In Silico* analysis system.

Based on the analyses using this system we have identified the interface layer between the dense sludge and the sludge/liquid boundary as the area of greatest microbial activity. The results of this analysis have been described in the article ("Determining the Microbial Community of Hog

Waste Lagoon Systems Using Terminal Restriction Fragment Length Polymorphism Analysis”, authored by Devine, Anthony, Ellis, Joseph, Bull, Leonard, Rice, Mark and Grunden, Amy) which is being submitted to *Water Research* in April 2009. Specifically in this study, TRFLP analysis in combination with *In Silico*© analysis was used to examine microbial communities from three swine waste lagoon systems in Harnett County, North Carolina. TRFLP was chosen over more traditional methods in an effort to provide the most extensive identification of organisms present in the lagoons. *In Silico*© was able to detect significant numbers of fragment patterns, which ranged from 273-1,124, depending on the sampling location. The patterns were matched to distinct microbial classes by the *In Silico*© software. Fragment utilization by the *In Silico*© software was high, between 79-100%. *In Silico*© was able to detect patterns in the lagoon systems belonging to *Proteobacteria*, Low GC microorganisms, microorganisms in the CFB group, among other phylogenetically distinct groups. The majority of the patterns detected were identified as either unclassified, meaning they had no deposited phylogenetic information, or multiple, meaning that one pattern belonged to multiple organisms that are phylogenetically different.

For the study, the interface location of each lagoon was chosen for analysis due to the presence of both sludge material from lower depths of the lagoon and liquid from upper portions of the lagoon. This interface is a region in the lagoon where sludge material from the lagoon depths can mix with the lagoon liquid layer, which allows microbial communities access to the nutrients found in sludge material (Figure 1). Since regulations in North Carolina specifically address the volume of sludge found in the lagoon system (NPDES Permit NCA200000 from NCDWQ website) and currently recommend a measurement technique that includes the interface layer, it was considered important to present the interface data to show the active microbial communities found at the point where liquid and solid layers of the lagoon systems meet.

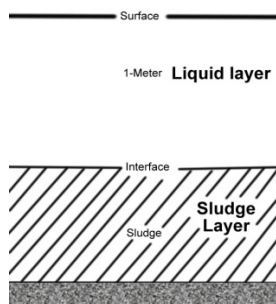


Figure 1

The microbial community present at the interface plays a significant role in the degradation of the sludge material in the lagoon. Presented in Figure 2 is an example of the data we were able to obtain to characterize the microbial communities in hog waste lagoon samples. In this particular case, TRFLP analysis was able to identify patterns belonging to 16 distinct phyla, 19 classes, 44 orders, 67 families and 93 genera from lagoon sludge-liquid interface samples from a lagoon in Harnett County NC.

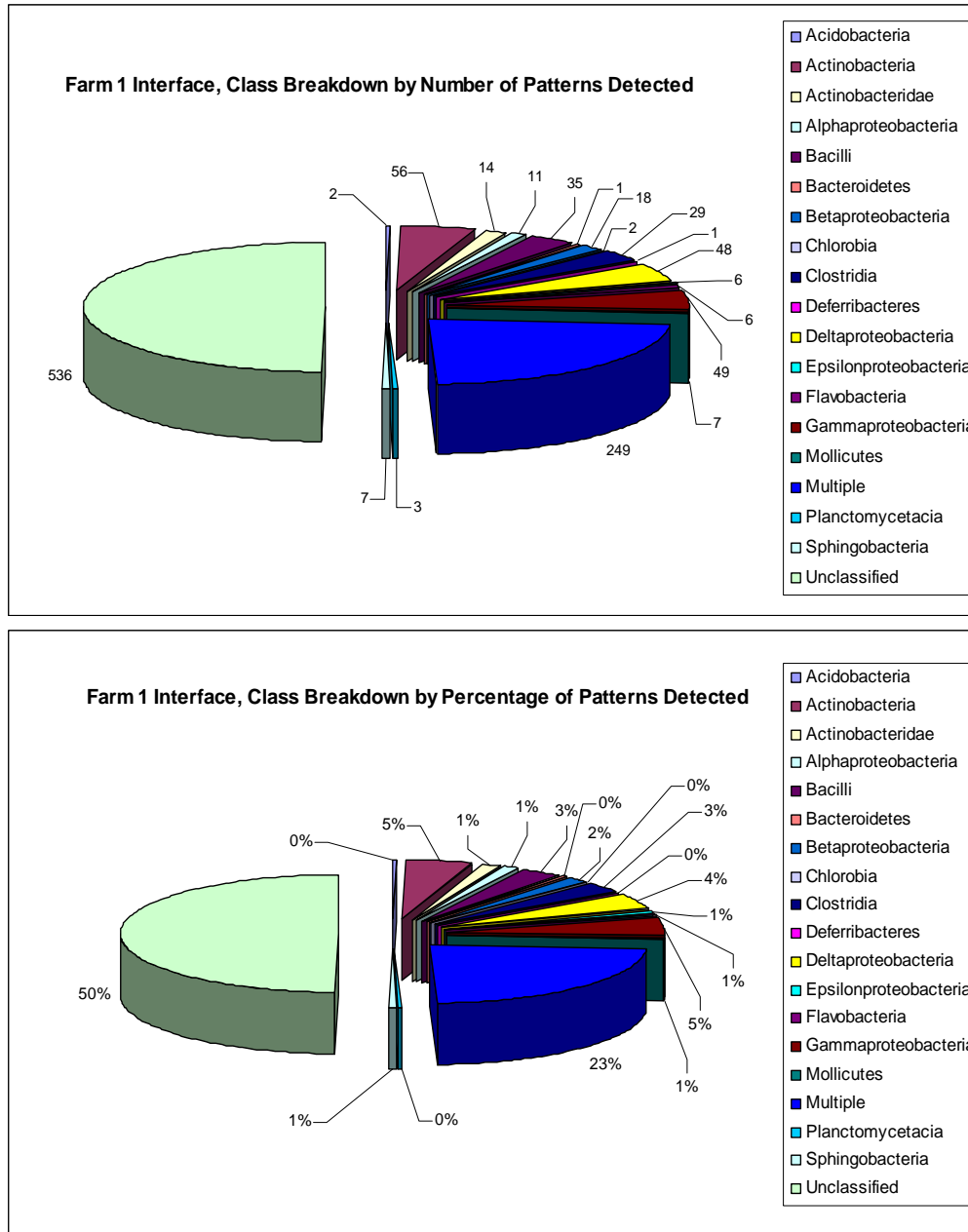


Figure 2

The TRFLP analysis method pioneered for this hog lagoon project has also been used in collaboration with Dr. Vivek Fellner in the NCSU Department of Animal Sciences to assess changes in cow rumen microflora when various treatments are added to the animal diets. This study has recently been submitted to the Journal of Dairy Science (M. C. Johnson\*, A. A. Devine<sup>†</sup>, J. C. Ellis<sup>‡</sup>, A. M. Grunden<sup>†</sup> and V. Fellner, Effects of antibiotics and oil on microbial profiles in mixed cultures of ruminal microorganisms. J. of Dairy Science. Submitted). This work has also attracted the interest of private industry. The firm DSM Nutritional Inc. has contributed \$80,000 over two and a half years to expand this project to evaluate the physical/chemical properties and microbial communities of twenty lagoons, ten of which are

control lagoons and ten that are receiving a treatment additive developed by DSM Nutritionals Inc. For this project microbial populations and chemical compositions have been determined for the 10 control and treatment lagoons over the course of one year (sampled over 4 quarter periods). We have completed the analyses of quarters 1 through 3 and have established baseline microbial communities for the twenty lagoons and are now in the process of analyzing the quarter 4 data and determining the effects of seasonal changes and the presence of DSM lagoon additive on the microbial populations and the chemical properties of the treated lagoons.

Sample analysis for the sludge analysis team consisted of laboratory determination of total solids, volatile solids, dissolved solids and suspended solids. Total solids and volatile solids were determined by conventional EPA methods in the Environmental Analysis Laboratory in the Biological and Agricultural Engineering Department at North Carolina State University. Because these samples had such high solids content and the sample size was limited due to the sampling, suspended solids measurement by vacuum filtration was not feasible and an alternative approach developed in the laboratory was used. The approach is based on the assumption that dissolved solids will migrate to equal concentration between liquid and solid phases when centrifuged and that suspended solids will be captured 100% in the solid phase. When the liquid portion after the centrifuge operation is dried, dissolved solids remain and are measured gravimetrically in the same way as total solids. Suspended solids are then determined by subtracting the dissolved solids concentration from the total solids concentration.

Sludge samples were then analyzed by UV spectroscopy by measuring absorbance at wavelengths between 200 and 1,100 nm at 1 nm intervals and related to total solids concentration and volatile solids concentration. An example of the absorbance of one of the lagoons is shown in Figure 3. The differences in absorbance at both higher and lower wavelengths and the difference in both total solids and volatile solids suggests a useful relationship exists that can be used to more precisely define the interface at which the maximum microbial activity occurs. Preliminary analyses are complete and more detailed analysis of the relationships is underway.

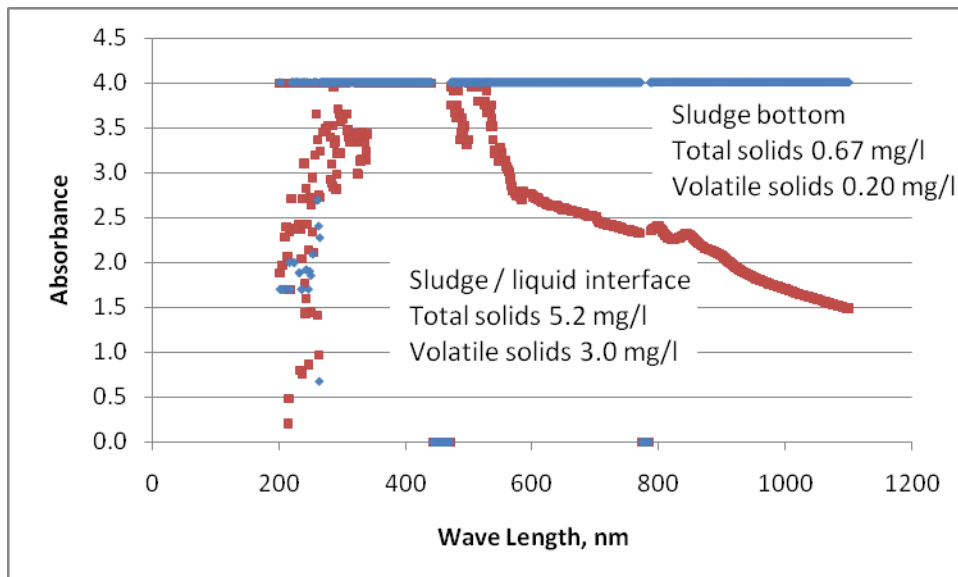


Figure 3. Example of UV response at one location of a swine lagoon

## References

Fulhage, C. D., A. Schmidt, and L. Lory. 2005. Long-Term Sludge and Nutrient Accumulation in Swine Lagoons: Case Study. Proceedings of the 2005 Animal Waste Management Symposium; The Development of Alternative Technologies of the Processing and Use of Animal Waste. Volume 1: 416-423. October 5-7, 2005, Sheraton Imperial Hotel, Research Triangle Park, NC.

Westerman, Philip W., Karl A. Shaffer, J. Mark Rice. 2008. Sludge Survey Methods for Anaerobic Lagoons, North Carolina Cooperative Extension Publication AG639, 2008.