Effect of Stratification on the Profile of an Anaerobic Swine Waste Treatment Lagoon in Kentucky

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Abstract. An understanding of the nature of wastes in an anaerobic swine lagoon is essential in the design and operation of alternative collection, treatment, and disposal facilities for environmental quality management such as odor control, nutrient and pathogen reduction. In this study, the characterization of an anaerobic swine waste treatment lagoon (0.40 ha) from a farrowing operation (~2000 sows) was carried out to examine the dynamics of the system due to stratification and seasonal variability. Swine waste samples were taken from an anaerobic swine lagoon at different depths (0, 50, and 250 cm) with a pulley system equipped with a special sampler that allows for sampling exclusively at certain depth. The sampling process was carried out from spring to fall season. The pH and temperature were monitored and recorded continuously from the epilimnion (top) and hypolimnion (bottom) layers of the lagoon. The samples were then analyzed for their mineral contents by using Inductively Coupled Plasma (ICP), Total Nitrogen, and Total Organic Carbon analyzers. Microbial dynamics were monitored by DNA extraction and Denaturing Gradient Gel Electrophoresis (DGGE). Results showed that nutrient (C, N, P, S) concentrations varied according to stratified lagoon layers and season. For example, total organic carbon concentrations range from 1800 mg/L (top) to 5400 mg/L (bottom) for late spring, and from 1100 mg/L (top) to 3600 mg/L (bottom) for the middle of summer. Trace minerals such as Al, Ca, Fe, K, Na, and Mg, on the other hand, appeared to be affected more by stratification than seasonal variability. The reason for the decrease in nutrient concentrations in summer time may be due to increase microbial activities which required more essential nutrients (i.e., C, N, P, S) rather than trace minerals for growth during active season. DGGE analysis also showed that microbial community structure appeared to be affected by the stratification and seasonal variability. There were distinct banding patterns for samples obtained from the epilimnion and hypolimnion. Based on these data, it is important to consider the effect of stratification and seasonal variability of waste loading from traditional anaerobic swine lagoon when designing and operating an alternative anaerobic digester.

Keywords. DGGE, ICP, stratification, anaerobic lagoon, swine.

Introduction

Anaerobic lagoons are used for the storage and treatment of high strength organic wastewater from concentrated animal feeding operations (CAFOs). Typically, an anaerobic lagoon is a deep earthen pond with appropriate soil-lined basins with inlet and outlet piping. Anaerobic lagoons have been known to have depths of up to 9 meters to conserve heat energy and to maintain anaerobic conditions (Metcalf and Eddy, 1991). The influent wastes from CAFOs settle to the bottom and the partially clarified effluent is usually discharged to another treatment process for further treatment. Normally, these lagoons are anaerobic throughout their depth, except for an extremely shallow surface zone. Stabilization is brought about by a combination of precipitation and the anaerobic processes that convert organic wastes to gaseous end products (i.e., carbon dioxide, methane, and volatile organic compounds), organic acids, and cell tissues.
There have been many studies involving the evaluation of anaerobic lagoon performance for treatment of wastes from CAFOs in the United States and around the world (Fulhage et al., 1978; Fulhage, 1980; Payne et al., 1980; Westerman et al., 1990; Biscudo, 1996; Costa et al., 1995; Georgakakis, 1996). Most anaerobic lagoons in the United States are single cell treatment, while systems in other countries consist of other treatment trains such as solid separators and multiple lagoons connected in series. The content and/or the effluent from these lagoons are usually land-applied, and, in some cases, discharged to surface water. However, the direct discharge of wastes to surface water without further treatment practice is no longer allowed in the U.S. Although these anaerobic lagoon systems are able to reduce organic matters, nutrients, and other heavy metals, their efficiency is dependent on seasonal variability and other environmental conditions. This is very critical when considering alternative treatment systems for these wastes. As more modern and different livestock operation practices are changing, the demands and requirements of the efficacy of these lagoons are also changing.

Thus the objective of this study is to examine the effect of stratification and seasonal variability on the profile of an anaerobic swine lagoon as a case study for designing alternative remediation options.

Material and Methods

Chemical Analyses of swine samples

Chemical elemental concentrations in swine wastes are dependent on the size of animals and dietary intake (Mulligan and Hesler, 1972; Sutton et al., 1974). The chemical elemental composition of an anaerobic swine lagoon is also dependent on the concentrations of swine manure flushed into the lagoon and biotic processes in the lagoon (Booram and Smith, 1974). Chemical concentrations in a lagoon are affected by environmental processes. Weather conditions and biological processes give rise to wide variations in chemical elemental composition in a swine lagoon as observed in this study.

Swine waste samples were taken from an anaerobic swine lagoon (0.4 ha, squared configuration with a slope of 3) at different depths (top, 50 cm from the top, and bottom of the lagoon) with a pulley system equipped with a sampler that allows for sampling at certain depth. The single-cell lagoon received swine manure from a farrowing operation of about 2000 sows with a pull-plug setup. The depths of the lagoon varied from 2 to 3 meters at the center due to drawdown for land application. However, the depth of the swine lagoon during our sampling period remained at about 2.5 meters. Swine samples were taken in late May, early June, and late September from the center of the swine lagoon in Kentucky with the pulley system. However, molecular analyses were only carried out for May and September samples. Chemical analyses were carried out for June and September samples. These samples were analyzed for pH, chemical elements using Inductively Coupled Plasma (ICP) spectrometer, total nitrogen, and total carbon. DNA extractions were also carried out on these samples for microbial study. Chemical and biochemical analyses were performed according to Standard Methods. Temperatures of the lagoon were monitored by using two HOBO water temperature probes. One was floated on the surface of the lagoon while the other was weighted with lead sinkers and sunk to the bottom of the lagoon. Both probes were moored to an anchor at the edge of the lagoon for retrieval.

Molecular Analysis

DNA was extracted from swine lagoon samples (0.5 ml) in duplicate for each stratum on each sampling day using the Q-Biogene FastDNA® Spin Kit for soil (Q-Biogene, Irvine, CA) according to manufacturer’s specifications. Bacterial community 16S rDNA (2 µl) from the 1:10 dilution was amplified with the bacterial specific primer set 341F-GC/907R, using a previously described PCR protocol (Casamayor et al., 2000) in a PTC-200 DNA thermal cycler (MJ Research, Las Vegas, NV). The GC designation on the 341F primer represents a 40 bp GC rich region on the 5’ end of the primer necessary to prevent complete denaturation of the DNA strands during electrophoresis. Sequences were amplified using HotStarTaq® MasterMix Kit (Qiagen Inc, Valencia, CA), with 800 nM each primer. The above PCR protocol was modified, adding an additional heating phase (95°C for 15 mins) at the beginning of the reaction, as required when using the HotStarTaq® MasterMix.

Denaturing gradient gel electrophoresis (DGGE) was used to separate and characterize 16S rDNA by using a gradient of denaturants (100% denaturant solution consisting of a combination of 40% [vol/vol] formamide and 7 M urea) in a polyacrylamide gel (37.5:1) to separate DNA fragments according to melting behavior (i.e. sequence, melting domains). GelBond PAG Film (Cambrex BioSciences Rockland, MA) was used during pouring of the DGGE gels to allow for easier manipulation of the polyacrylamide gel after electrophoresis. 5 µl of PCR product was electrophoresed through a 30-60 % denaturing gradient according to Nübel et al. (Nübel et al., 1997) for 4 h at 200 V in a Bio-Rad DCode universal mutation detection (Bio-
Rad Laboratories, Hercules, CA). The DGGE gels were stained with the Bio-Rad Silver Stain kit according to the manufacturer’s specifications, and the images were captured using an Epson Perfection 4990 Photo Scanner (Epson, Long Beach, CA).

DGGE fingerprint analysis was performed using the Fingerprint II software program (Bio-Rad Laboratories) using the basic, Clustering Analysis, Comparative Quantification and Polymorphism Analysis and Dimensioning Techniques modules. The gel images were imported into the software and analyzed according to manufacturer’s specifications, with UPGMA analyses being performed based upon the banding patterns present in each gel lane. The strength of the clusters obtained from the UPGMA analysis was based on cophenetic correlations, which are an estimate of the faithfulness of a grouping within a dendrogram, with a score of 100 indicating that a grouping is extremely well supported. The software package performed band matching between the fingerprints, allowing for the principle component analysis (PCA) and multidimensional scaling (MDS) analysis.

Results and Discussion

Chemical Analyses

Swine samples were collected at various depths from a swine lagoon in Kentucky during late spring (June) through early fall (September). The samples were analyzed for their chemical contents. The results showed that primary nutrient (C, N, P, S) concentrations varied according to stratified lagoon layers and seasonal changes. The nitrogen and carbon concentrations range from 916 (at the top of the lagoon) to 1200 mg/L (at the bottom of the lagoon, 250 cm) and 1800 (top) to 5400 mg/L (bottom), respectively, during June sampling (Table 1). Sulfur and phosphorous concentrations exhibited a similar trend. The concentrations for S and P range from 36 (top) to 460 mg/L (bottom) and 180 (top) to 1940 mg/L (bottom), respectively, for June. However, the results obtained during the month of September showed that the concentrations were lower for all nutrients. Nevertheless, the effect of stratification remained the same (i.e., lower concentrations at the top and higher concentrations at the bottom of the lagoon) (Table 1). The concentrations of chemical oxygen demand (COD), the measurement of organic matter content, also exhibited the same trend with values ranging from 1414 to 8023 mg/L in June and 165 to 806 mg/L in September (Table 1). These results were similar to values obtained from other studies (Bicudo et al., 1999; DeSutter et al., 2005).

Table 1. Nutrient concentrations in swine lagoon in June and September*.

<table>
<thead>
<tr>
<th>Month</th>
<th>Layer</th>
<th>N (mg/L)</th>
<th>C (mg/L)</th>
<th>S (mg/L)</th>
<th>P (mg/L)</th>
<th>COD (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>Top</td>
<td>916 (20)</td>
<td>1800 (50)</td>
<td>36 (5)</td>
<td>180 (13)</td>
<td>1412 (100)</td>
</tr>
<tr>
<td></td>
<td>50 cm</td>
<td>1100 (30)</td>
<td>2000 (43)</td>
<td>38 (3)</td>
<td>195 (18)</td>
<td>1555 (210)</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>1200 (23)</td>
<td>5400 (48)</td>
<td>460 (9)</td>
<td>1940 (29)</td>
<td>8023 (241)</td>
</tr>
<tr>
<td>September</td>
<td>Top</td>
<td>620 (120)</td>
<td>1100 (21)</td>
<td>19 (4)</td>
<td>119 (20)</td>
<td>165 (42)</td>
</tr>
<tr>
<td></td>
<td>50 cm</td>
<td>600 (26)</td>
<td>1500 (34)</td>
<td>38 (4)</td>
<td>187 (31)</td>
<td>205 (35)</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>800 (24)</td>
<td>3600 (61)</td>
<td>299 (21)</td>
<td>1289 (41)</td>
<td>806 (68)</td>
</tr>
</tbody>
</table>

* ( ) denotes standard errors of two samples.

The results also showed that concentrations of trace minerals such as Al, Ca, Fe, K, Na, Mg, and others exhibited the same fluctuation as the nutrients in the swine lagoon according to stratified processes and seasonal variability (Table 2). However, the concentrations of these trace minerals appeared to be affected more by stratification than seasonal variability. For example, the concentrations of iron range from 10.7 (top) to 350 mg/L (bottom) and 4.8 (top) to 232 mg/L (bottom) in June and September, respectively (Table 2). The reason for the decrease in nutrient concentrations in September may be due to increased microbial activities which required more essential nutrients. This is evident by the increase in the microbial cell counts (Table 3). Based on the 16s real time polymerase chain reaction (RT-PCR) results, the microbial concentrations were observed to be higher in September than in June samples. The microbial concentrations range from 2.65 x 10^8 (top) to 3.98 x 10^8 cells/ml (bottom) in June and 2.76 x 10^9 (top) to 1.32 x 10^9 cells/ml (bottom) in September (Table 3).

Table 2. Trace mineral concentrations in swine lagoon in June and September*.
Table 3. Microbial concentrations obtained from 16s RT-PCR.

<table>
<thead>
<tr>
<th>Month</th>
<th>Layer</th>
<th>Cells/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>Top</td>
<td>$2.65 \times 10^8$</td>
</tr>
<tr>
<td></td>
<td>50 cm</td>
<td>$2.13 \times 10^8$</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>$3.98 \times 10^8$</td>
</tr>
<tr>
<td>September</td>
<td>Top</td>
<td>$2.76 \times 10^9$</td>
</tr>
<tr>
<td></td>
<td>50 cm</td>
<td>$2.00 \times 10^9$</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>$1.32 \times 10^9$</td>
</tr>
</tbody>
</table>

**Molecular Analysis**

Swine samples were collected at various depths from a swine lagoon in Kentucky during late May and late September. DNA extractions and DGGE analysis were carried out on these samples. Fingerprint analyses of DGGE banding patterns (Fig. 1) revealed distinct swine bacterial community structures coinciding with different strata of the swine lagoon and different seasons (i.e., May for late Spring and September for early Fall). Fingerprint patterns were analyzed using UPGMA and the strength of the resultant branching patterns were evaluated using cophenetic correlations. Cophenetic correlations are an estimate of the faithfulness of a grouping within a dendrogram, with a score of 100 indicating that a grouping is extremely well supported. Definite shifts in the swine lagoon samples fingerprint patterns were observed (Fig. 1). The community fingerprints for the liquid swine samples from the epilimnion (top or T) layer of the swine lagoon were distinct from those from the hypolimnion (bottom or B) layer of the lagoon for both May and September samples. Furthermore, there were distinct banding patterns between May and September samples as well. However, we did not have sample from the middle depth of the swine lagoon for May. The top, bottom, and middle (50 cm from the surface of the lagoon) samples from September branched together and were distinct from those from May samples. This evidence is further supported by the PCA analysis of the DGGE gels from different sampling dates (Fig. 2). Figure 2 is the three-dimensional PCA output or the MDS. The May samples contained the highest number of distinctive bands than September samples, suggesting a more diverse bacterial community (Fig. 1). The difference may be due to the ideal conditions for microbial growth, proliferation, and diversity during the month of May as compared to early Fall (September). Within each sampling date, the top layers appear to have the highest distinctive bands than the bottom layers suggesting that the top layers contain more diverse microbial community. This may be due to the abundance of oxygen in the region near the top of the lagoon which is conducive to aerobic growth versus the anaerobic conditions of the bottom layer of the lagoon which may be conducive to a narrow range of microbial populations.
Figure 1. 16S rDNA DGGE bacterial community fingerprint analyses. A dendrogram representing the percent similarity of banding patterns based on UPGMA cluster analysis are shown to the left of the DGGE image. The numbers at the nodes of the dendrogram represent the cophenetic correlations, which is an estimate of the reproducibility of each sub-cluster. The DGGE image represents a 30 – 60% denaturant gradient with sample ID shown to the right of the DGGE image.
Conclusion

In conclusion, we found that stratification and seasonal changes do affect the biochemical profile of a swine lagoon. The microbial population dynamics and chemical constituents in the swine lagoon were observed to vary according to different stratified layers and different seasons. Thus, it is important to consider the effect of stratification and seasonal variability of waste loading from traditional anaerobic swine lagoon when designing and operating alternative treatment systems.

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References


