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MICROBIAL ACTIVITY ALONG A SPECTRUM OF ANAEROBIC SWINE LAGOONS

(Aktivitas Mikroba pada Beberapa Lagun Anaerobik Pengolah Limbah Peternakan Babi)

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Abstract

Anaerobic lagoons treating swine waste rely upon balanced microbial activity from fermentation to methanogenesis. A balance of production and consumption of intermediate products, such as hydrogen and volatile fatty acids (VFAs), during the degradation of complex organic wastes is crucial for proper functioning of anaerobic lagoons. If activity is not balanced, odors can result from VFAs and ammonia accumulation. Additional odors may be due to H_2S produced by sulfate reducing bacteria (SRB). In this study, microbial activity was monitored in a spectrum of lagoons, ranging from well functioning to dysfunctioning, to determine what affects the functionality of these systems. Hydrogen concentrations were lower and achieved an apparent steady state earlier in lagoon microcosms with higher ratio methane/COD than those with lower ratio methane/COD as well as having higher MPN values of methanogens. Not surprising, SRB were more prevalent when sulfate input was higher, suggesting sulfate input contributes to odors. Phototrophic purple bacteria can reduce odor by consuming H_2S and VFA. Phototropic bacteria MPNs were high in lagoons with low conductivity, concentrations of COD, sulfate, ammonia and solids. Phototropic bacteria in the functional lagoons may not only help in controlling offensive odors by consuming H_2S but also in consuming excessive VFA thereby improving the lagoon's performance. Sulfate, COD, and solids concentrations are factors that can cause high variability of the microbial activity in anaerobic swine lagoons and furthermore determine the lagoon's functionality.

Keywords: anaerobic bacteria, methane, hydrogen, anaerobic lagoons, swine wastes

Abstrak

Laguna anaerobik pengolah limbah peternakan babi bergantung pada keseimbangan pada aktivitas mikroba dari fermentasi sampai metanogenesis. Keseimbangan produksi dan konsumsi produk intermediate, seperti hidrogen dan asam lemak selama perombakan limbah organik kompleks, merupakan kunci utama berfungsinya lagun anaerobik. Ketidakseimbangan aktivitas mikroba akan menyebabkan timbulnya bau yang berasal dari kelebihan produksi asam lemak dan asam lemak serta hidrogen sulfide yang dihasilkan dari aktivitas bakteri pereduksi sulfat. Pada penelitian ini aktivitas mikroba di beberapa lagun dari yang berfungsi baik sampai yang tidak berfungsi diteliti untuk mendeterminasi faktor-faktor yang mempengaruhi kinerja lagun anaerobic agar berfungsi baik. Konsentrasi hidrogen lebih rendah pada lagun dengan rasio metan/COD dan MPN bakteri penghasil metan yang tinggi, dibandingkan konsentrasi hidrogen pada lagun dengan rasio metan/COD

yang rendah. Bakteri pereduksi sulfat cukup tinggi pada lagun yang menerima beban masukan yang mengandung sulfat yang tinggi. Bakteri fototrofik ungu dapat mengkonsumsi hidrogen sulfide dan asam lemak. MPN bakteri fototrofik tinggi pada lagun dengan konduktivitas, kandungan COD, sulfat, ammonia dan padatan yang rendah. Selain mengontrol bau, bakteri fototrofik pada lagun yang berfungsi baik juga meningkatkan kinerja lagun. Kandungan sulfat, COD dan padatan merupakan faktor yang menyebabkan aktivitas bakteri sangat bervariasi di lagun anaerobik peternakan babi dan yang menentukan lagun berfungsi baik.

Kata kunci: mikroba, metan, hidrogen, lagun anaerobik, limbah peternakan babi

INTRODUCTION

Anaerobic lagoons are the most common method of storing and treating wastes from swine production facilities in the United States and Canada; however their performance may vary from site to site. Anaerobic lagoons are very effective at decomposing most kinds of organic matter; however, they frequently release large quantities of unpleasant odors that cause problem in the rural areas. Properly functioning lagoons rely on microbial activity and management practices such as solids separation prior into the lagoon, periodic solids removal and suitable organic loading rates that affect the microbial activity.

The degradation of complex substrates of swine waste to methane and carbon dioxide in anaerobic lagoons requires several trophic groups of microorganisms. Complex substrates such as polysaccharides, protein, and lipids are hydrolyzed by hydrolytic bacteria, subsequently fermented by fermentative bacteria to a variety of intermediate products, such as short-chain VFA (acetate, propionate and butyrate), alcohols, hydrogen and carbon dioxide. Syntrophic bacteria further convert propionate and butyrate to acetate, carbon dioxide and hydrogen (Schink, 1997; Conrad, 1999; McCarthy and Smith, 1986). Hydrogenotrophic methanogens finally convert hydrogen and carbon dioxide into methane, and acetoclastic methanogens convert acetate to methane (Zinder, 1993; Vogels *et al,* 1988). Hydrogen will also be consumed by other hydrogen consumers, such as homoacetogens (Schink, 1997; Conrad, 1999) and sulfate reducing bacteria (SRB) (Widdel, 1988). Excess VFA and hydrogen sulfide produced by SRB in anaerobic lagoons can also be consumed by other trophic groups such as phototrophic sulfur (PSB) and non-sulfur purple bacteria (PNSB) (Lotringen and Gerrish, 1978; Kobayashi *et al,* 1983; Do *et al,* 2003). The presence of phototrophic purple bacteria in anaerobic lagoons is indicated by the pink/purple hue of lagoon slurry.

Functionality of lagoons relies on the cooperative interaction of those trophic groups of microorganisms from fermentative bacteria to methanogenic bacteria. Other hydrogen consumers and possibly phototrophic bacteria may help balance the metabolic reaction of production and consumption of intermediates especially hydrogen and VFA. Hydrogen consumers appear to be important to maintain level of hydrogen concentrations low enough to support VFA oxidation by syntrophic bacteria. Hydrogen partial pressure exceeding 10-4 atm can inhibit VFA oxidation, resulting in VFA accumulations (Mormille, *et al,* 1996; Conrad *et al,* 1985). VFA accumulations cause the pH drop and subsequently inhibit the methanogens, further disrupt the treatment systems. Excessive VFA and also H2S produced by SRB can cause problematic odors. Therefore, the presence of phototrophic bacteria in the lagoons may help improve the functionality of lagoons by consuming odor compounds such as VFA and H2S (Kobayashi *et al,* 1983; Do *et al,* 2003).

In order to maintain functionality of anaerobic lagoons, controlling factors that have negative impacts on microbial activity in anaerobic lagoons is very crucial. These include

temperature fluctuation (Zinder, 1993), organic overloading and build up of toxic materials such as metals (Zahn *et al,* 2001; Kong *et al,* 1994), ammonia (Hansen et al, 1998), and high salts (Speece, 1996). The use of disinfectants to clean the barn and the use of antibiotics in feed and to treat ill animal can also affect microbial activity in lagoons (Hilpert *et al,* 1984; Poels *et al,* 1984).

Anaerobic lagoon considered functioning is usually odorless, has high organic removal (> 70%) and shows pink/purple hue of lagoon slurry indicating the presence of phototrophic purple bacteria (Miner *et al,* 2000). Microbial activity that affects the functionality of lagoons may vary from lagoon to lagoon due to other factors that affect microbial activity in lagoons.

Therefore, the objective of this study was to examine the microbial activity in a spectrum of lagoons, ranging from well functioning (effective treatment, minimal odor, and exhibiting a photosynthetic bloom) to dysfunctioning (organic overloading and odorous) and to determine the factors that affect the functionality of these systems.

EXPERIMENTAL METHODS

Lagoon Description and Sample Collection

Six lagoons with different management system ranged from non purple, light purple, purple and pink purple were selected for this study. The characteristic of lagoons operational and appearances is presented in Table 1. Groundwater containing approximately 37

Table 1. Lagoon appearances

Lagoon	Depth (m)	Volume (m^3)	Capacity	Management systems	Physical Conditions (Color, odor)
1	1 – 5.5	13055	2000 farrowing pigs	Recharge pit system, recycle, solid removal	Purple, odorless
2	0.3 – 2.5	1807	250 (sows, nursery, farrowing, and finishing pigs)	Direct flush with slug loadings and excess food, no recycle, no solid removal, over feeding	Brown/Black, odorous
3	1 – 2.7	4248	100 (farrowing and finishing pigs)	Direct flush through seepage (no slug loadings) with excess food, no recycle, no solid removal	Pink/purple, odorless
4	2.1 – 5.8	114835	6500 nursery pigs	Recharge pit system, solid removal	Green/ purple, odorless
5	1.2 – 4	112447	500 sows, 3000 farrowing pigs	Recharge pit system, solid removal	Light purple, odorless Pink/purple, odorless
6	1 – 2.8	56239	150 (sows, nursery, farrowing, and finishing pigs)	Recharge pit system, recycle, solid removal	

mg/L sulfate is used to flush waste for lagoons 2 and 3. Sludge accumulations, which reduce the lagoon's volume is apparent in lagoon 2 due to direct waste flush (slug loading) and no solids removal. Sampling was conducted at least on total five sampling locations. Depth profile samples, surface, middle, bottom and sediment were collected at each location. Samples for microcosm studies were collected at middle location. Surface, middle and bottom slurries were collected by using Water Sampler (Cole Parmer, Vernon Hills, IL). Sediment was collected by using an Ekman Dredge.

Field Measurements.

Temperature, pH, dissolved oxygen, ORP (Oxidation Reduction Potential) and conductivity, were measured at the surface, middle and the bottom depths of all sampling locations by using a Water Analyzer (Cole Parmer, Vernon Hills, IL) equipped with DO, pH , ORP, conductivity probes and thermo sensor. All probes were calibrated with standard solutions.

Chemical Characterization of Lagoon Slurry

Total solids (TS) and volatile solids (VS) were measured by using standard methods (APHA, 1992). Slurry was centrifuged at 10,000 G for 10 min and filtered with 0.2 μM nylon syringe filter (Whatman, Inc, Clifton, NJ) prior to soluble COD, ammonia-N, VFA and salts analyses.Chemical Oxygen Demand (COD) was measured by using Method 8000 (Hach, Loveland, CO). Ammonia-N in the slurry was analyzed by using Method 10031 (Hach, Loveland, CO). Sulfate was measured by using an ion chromatography (IC) (Model DX, Dionex, Sunnyvale, CA) equipped with an AS4A column and a AG4A guard column.

Microcosm Studies

Microcosm studies were conducted to determine hydrogen concentrations and methane production in the lagoon slurry and sediments. Microcosms were prepared in an anaerobic chamber containing 10 % H_2:90 % N_2. Autoclaved 160-mL serum bottles were filled with 100 mL of slurry or 50 mL of slurry plus 50 g sediment and 0.1 mL of resazurin (10 g/L). The bottles were sealed with butyl rubber stoppers (Bellco Glass, Inc, Vineland, NJ) and aluminum seals. The headspace of each microcosm was exchanged with oxygen free N_2 gas. Slurry collected at least 4 days prior to the experiment was autoclaved three days consecutively for 30 min at 121 °C in order to prepare negative controls. The microcosms were incubated at 25 and 35°C on shaker tables (Cole Parmer, Vernon Hills, IL) with rotation set at 52 rpm. Pressure, along with hydrogen and methane concentrations was measured over period of times for 8 days. The headspace pressure was determined by using a PX26-100GV (0-100 psig) series pressure transducer (Omega, Stamford, CT) with a 26-gauge disposable needle to penetrate the stopper of the microcosms. Headspace CH_4 was measured by using a GC equipped with FID (Model Varian 3400, Walnut Creek, CA). A stainless steel 80/100 Porapak Q (6 ft by 1/8 in) packed column (Supelco, Bellefonte, PA) was used to resolve methane. The MDL of CH4 was 13 μM. Headspace H2 concentrations were measured by using a gas analyzer (Model 3000, Molecular Analytical, Sparks, MD). The hydrogen concentrations in the slurry samples were calculated by using the Ostwald coefficient for each temperature and with the assumption that the solvent was pure water.

Microbial Population

Methanogens, fatty acid oxidizing bacteria (FAOB), sulfate-reducing bacteria (SRB) and phtotrophic bacteria were enumerated by using the most probable number (MPN assay) method. A mineral basal medium and carbon source for each type microbial community was used with resazurin added as a redox indicator as described in Mormille *et al*, (1996).

DNA Extraction, PCR Amplification and DGGE

Nucleic acid extraction was performed by using UltraCleanTM soil DNA isolation

kits (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's instructions. DNA was amplified by using universal bacterial primers 27F and 1392R. Polymerase chain reaction (PCR) was used to amplify extracted DNA prior to denaturant gradient gel electrophoresis (DGGE) analysis as described in Leung and Topp, 2001. Denaturant gradient gel electrophoresis (DGGE) was performed with a DCode™ 16 cm x 16 cm 10% polyacrylamine gel (BioRad, Hercules, CA, USA) maintained at 60 °C in 7 l of Tris-acetate-EDTA (TAE) buffer (20 mM Tris-acetate, 0.5 M EDTA, pH 8.0). Gels were stained with concentrated SYBR Green I (Molecular Probes, Eugene, OR, USA). Images were captured using an Alpha DigiDoc. DGGE enables the separation of DNA fragments with the same length but different base pair sequences. The method was first utilized to examine microbial community profiles by Muyzer (1993).

RESULTS AND DISCUSSIONS

Physical and Chemical Characteristics of Lagoons

Six different anaerobic lagoons were thoroughly characterized from late spring to early fall. Lagoon 2 is the only non-purple (brown/black) lagoon with black scummy slurry. Lagoon 1 displayed the bright purple slurry, while lagoons 4 and 5 displayed a little lighter purple slurry. Lagoons 3 and 6 displayed bright pink purple colored slurry.

Temperature ranged from 22 to 28 °C during sampling. Despite the physical appearance differences, dissolved oxygen (DO) levels in the lagoon slurry for all lagoons were not significantly different. DO levels ranged from 0.0 to 0.1 mg/L at the surface slurry and were 0.0 for all lagoon slurry below the surface and the bottom. All lagoons were highly anaerobic. To support most of anaerobic microbial activity

Table 2. Chemical characteristics of lagoons

Lagoons (Visual Observation)	pH	ORP (mV) Bottom → Surface	Conductivity (mS/cm)
1 (purple)	7.35 ± 0.27^{a}	-310 → -137	3.93 ± 0.19^{a}
2 (brown/black)	7.14 ± 0.13^{b}	-430 → -237	5.39 ± 0.46^{b}
3 (pink/purple)	7.66 ± 0.21^{a}	-350 → -170	2.64 ± 0.14^{c}
4 (green/purple)	7.67 ± 0.19^{a}	-317 → +38	2.26 ± 0.26^{c}
5 (light purple)	7.56 ± 0.30^{a}	-278 → -112	4.99 ± 0.29^{b}
6 (pink/purple)	7.56 ± 0.36^{a}	-277 → -6	3.60 ± 0.25^{a}

Table 3. Chemical characteristics of lagoons (continued)

Lagoons (Visual Observation)	COD (mg/L	$N\text{-}NH_3$ (mg/L)	SO_4 (mg/L)	TSa (mg/L)	VSa (mg/L)
1 (purple)	228 ± 54^{a}	na	6 ± 2^{a}	1811 ± 96^{a}	617 ± 44^{a}
2 (brown/black)	995 ± 213^{b}	na	26 ± 23^{b}	3578 ± 20^{b}	2410 ± 20^{b}
3 (pink/purple)	802 ± 209^{bc}	230 ± 4^{a}	11 ± 8^{bc}	1686 ± 351^{a}	837 ± 154^{c}
4 (green/purple)	294 ± 76^{a}	155 ± 22^{b}	8 ± 3^{a}	2002 ± 167^{c}	740 ± 7^{c}
5 (light/purple)	595 ± 162^{c}	555 ± 45^{c}	22 ± 18^{bc}	2614 ± 168^{d}	880 ± 353^{ac}
6 (pink/purple)	651 ± 212^{c}	132 ± 64^{b}	$19 \pm 13^{b}c$	2208 ± 202^{c}	1135 ± 164^{c}

Values (mean ± standard deviations) with different letters are significantly different ($P<0.01$). a Values were from lagoon surface and middle slurries.

such as sulfate reduction and methanogenesis, highly anaerobic conditions with ORP at least -200 mV is required (Zinder *et al*, 1993; Widdel, 1988). ORP values for bottom slurry of all lagoons ranged from -430 for lagoon 2 to -277 mV for lagoon 6, which were highly reduced conditions (Table 2). While the ORP values of surface lagoon slurry for all lagoons ranged from +38 for lagoon 4 to -170 mV for lagoon 3, except for lagoon 2 (brown/black). As shown in table 2, purple lagoons had greater (positive) ORPs at the surface than the brown/black lagoon. These findings are in agreement with previous study that at the surface, purple lagoons had a lesser reducing environment than did brown/black lagoons (Chen *et al*, 2003).

The average pH values for lagoons ranged from 7.14 to 7.67, with the lagoon 2 had the lowest pH (Table 2). ANOVA test showed that the pH of lagoon 2 slurry was significantly different with pH of all other lagoon slurries ($P<0.01$).

The pH of five other lagoons was not significantly different. The pH values of all lagoons appear to be near or within the range for the growth of common microbes present in the lagoons especially methanogens (Zinder *et al*, 1988), and phototrophic bacteria (Do *et al*, 2003).

Conductivity, a measure of the level of salts, total and volatile solids were significantly high in lagoon 2 (brown/black) (Table 2). Overall purple lagoons had lower conductivity values than the brown/black lagoon. Conductivity values for the purple lagoons had a range of 2.26 to 4.99 mS/cm. The brown/black lagoon had the average value of approximately 5.39 mS/cm.

The average total and volatile solids in lagoon 2 were 3578 and 2410 mg/L respectively. These values were high and significantly different from the total and volatile solids of all other lagoons (Table 3). Organic loading rate in anaerobic lagoons is measured as volatile solids loading rate (VSLR). The VSLR for all lagoons studied were not measured. However, based on the total and volatile solids measured in the lagoon slurries and the volume of the lagoon, it can be deduced that the VSLR of lagoon 2 was high. Lagoon 2 is a small and shallow lagoon with black scummy slurry. Solids accumulation was also apparent due to no application of solids removal.

The average of Chemical Oxygen Demand (COD) concentrations, which measured as soluble COD concentration, for all lagoons were below 650 mg/L except for lagoon 2 (brown/black) and 3 (pink purple) (Table 3).

Lagoon 2 had the highest COD concentration, although it was not significantly different from COD concentration of lagoon 3. Lagoons 1 and 4 had the lowest average COD concentrations i.e., below 300 mg/L. Overall, purple lagoons had lower COD concentrations.

Ammonia is one of the odor compounds to be concerned in anaerobic lagoons. Ammonia was not measured for lagoon 1 and lagoon 2. The ammonia levels for all purple lagoons measured ranged from 132 to 555 mg/L. These values are much lower than ammonia concentration that can be toxic to anaerobic bacteria. Ammonia concentration above 1500 mg/L can have adverse effect to anaerobic bacteria (Hansen *et al*, 1998).

Sulfate concentration in anaerobic lagoons also is being concerned because it can be reduced by sulfate reducing bacteria (SRB) to hydrogen sulfide, one of the major odor compounds produced in anaerobic lagoons. Most of purple lagoons had low sulfate concentrations, especially for lagoons 1 and 4, which had average sulfate concentrations of 6 and 8 mg/L respectively. On the other hand lagoon 2, the only non-purple (brown/black) lagoon studied had the highest average sulfate concentrations althoug h it was not significantly different with that of lagoons 5 and 6 (purple lagoons) at $P<0.01$. Lagoon 2 received the waste flushed by groundwater containing 35 mg/L sulfate from the swine barn. High influent sulfate concentration in lagoon 2 and with no solids removal application contributes high sulfate concentration in the lagoon slurry. High sulfate concentration in the lagoon slurry can result in high production of hydrogen sulfide.

Regmi (2004) reported that air hydrogen sulfide concentration in this lagoon reached as high as 18 mg/L.

Methane and Hydrogen

Methane and hydrogen concentrations were measured in microcosm of lagoon slurry to determine microbial activity in anaerobic swine lagoons. Lagoons with high methane concentrations i.e., 50 to 86 mM had low hydrogen concentrations that ranged from 0.016 to 0.029 μM. On the other hand, lagoons with low methane concentrations i.e., 12.12 to 20.75 mM had much higher hydrogenconcentrations (4.17 to 7.95 μM) (Figure 1). Lagoon 2 (brown/black) had the highest methane concentrations, however when normalized with COD, there were significant differences of the ratio of methane/COD for bottom slurry and sediment samples between lagoon 2 and lagoon 1 (purple) (Figure 2).Overall, lagoon 2 (brown/black) still had higher values of ratio of methane/COD than other four purple lagoons. Lagoon 1 had the highest ratio of methane/COD for bottom slurry i.e., 0.23 mmol/mg, on the other hand lagoon 6 had the lowest ratio of methane/COD for sediment sample i.e., 0.02 mmol/mg (Figure 2).

Hydrogen is a critical intermediate in anaerobic processes to allow methanogesis to occur. Hydrogen concentration above 80.7 nM can inhibited acetogenic dehydrogenations of organic acids in landfill environments.

This leads to accumulation of organic acids, which in turn decreases the pH to a level inhibitory to methanogenic bacteria and methanogenesis (Mormille *et al*, 1996). Hydrogen concentrations were higher than 80.7 nm, and the methane generations were low in microcosm lagoon slurries of lagoon 4, 5, and 6; however, there was no indication of volatile fatty acids (VFA) accumulation in these lagoons. The pH of lagoon slurry for these lagoons was all above 7.

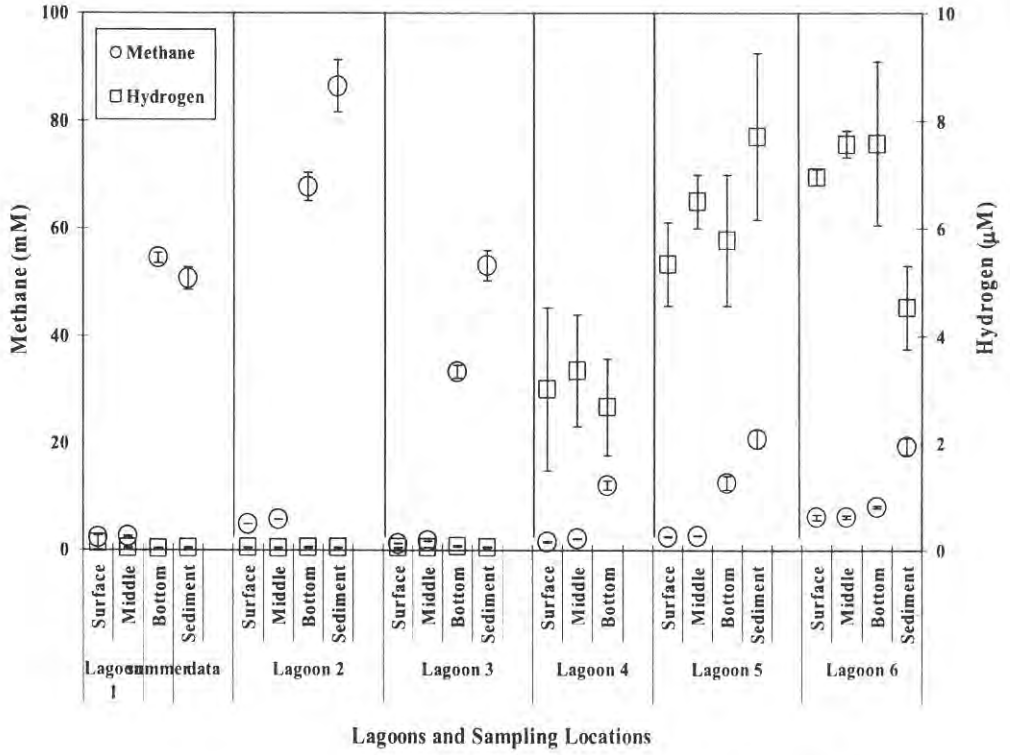

Figure 1. Methane and hydrogen concentrations in microcosm lagoon slurries and sediment at 35 °C

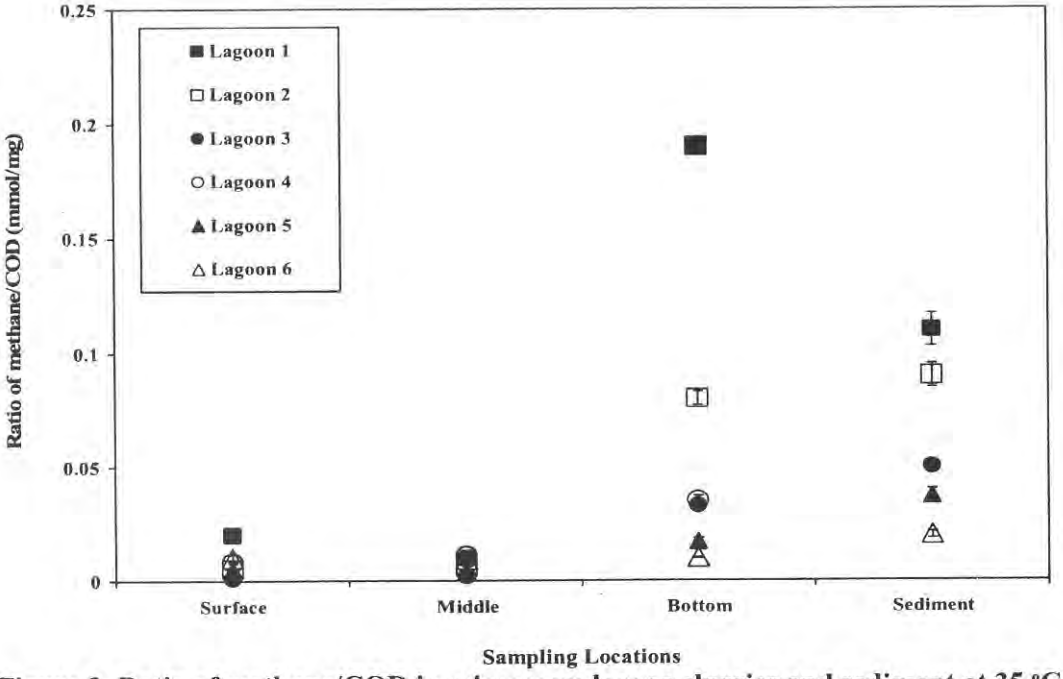

Figure 2. Ratio of methane/COD in microcosm lagoon slurries and sediment at 35 °C

Microbial Population

The most probable number (MPN) analysis was used to quantify four different trophic groups of bacteria (methanogens, sulfate-reducing bacteria, fatty acid oxidizing bacteria and phototrophic bacteria) found in six different swine lagoons (Table 4). The MPN results did not indicate any obvious disruption to the anaerobic treatment process. Although different lagoons had higher concentrations of anaerobic microorganisms than others, the overall distribution of trophic groups were somewhat similar in all of the study lagoons. In general, sulfate-reducing bacteria were most numerically prevalent in all lagoons, whereas methanogens generally had the lowest numbers. Fatty acid oxidizing bacteria were in general slightly more prevalent than methanogens. Phototrophs varied the most among lagoons in comparison to the other groups. Phototrophic purple bacteria measured as phototrophic non sulfur bacteria (PNSB) were not quantified for lagoons 1 and 2 slurries. The presence of phototrophic purple bacteria is indicated by purple hue of lagoon slurry. Visually there was indication of phototrophic purple bacteria present in lagoon 1 (purple lagoon) but not in lagoon 2 (brown/black lagoon).

DGGE

Denaturant gradient gel electrophoresis was used to compare overall community structure in the lagoons. In DGGE, sequences with different base pair compositions are separated in the gel due to their different melting temperatures in an increasing formamide/urea gradient.

Table 4. Microbial populations in lagoons

Lagoon (visual observation)	Depth	Methanogens	SRB	FAOB	Phototrophs
1 (purple)	Surface (cells/mL)	2.4×10^3	2.4×10^5	2.3×10^4	NA
	Middle (cells /mL)	4.6×10^3	4.6×10^6	2.4×10^4	
	Bottom (cells /mL)	4.6×10^4	2.4×10^6	2.4×10^4	
	Sediment (cells /g)	1.1×10^5	1.5×10^6	4.6×10^5	
2 (brown/black)	Surface (cells /mL)	2.4×10^5	2.4×10^7	2.4×10^5	
	Middle (cells /mL)	2.1×10^4	2.4×10^7	1.1×10^5	NA
	Bottom (cells /mL)	2.4×10^5	2.1×10^7	4.6×10^5	
	Sediment (cells /g)	1.1×10^6	2.4×10^7	1.1×10^6	
3 (pink/purple)	Surface (cells /mL)	4.6×10^2	4.6×10^6	2.4×10^3	4.6×10^4
	Middle (cells /mL)	1.1×10^3	2.4×10^6	1.1×10^4	4.6×10^4
	Bottom (cells /mL)	2.4×10^3	2.4×10^6	2.4×10^3	4.6×10^4
	Sediment (cells /g)	4.6×10^4	1.1×10^7	2.4×10^4	1.1×10^5
4 (green/purple)	Surface (cells /mL)	1.1×10^4	4.6×10^6	1.1×10^4	4.6×10^6
	Middle (cells /mL)	1.1×10^4	4.6×10^7	4.6×10^4	4.6×10^6
	Bottom (cells /mL)	2.4×10^7	2.4×10^7	2.4×10^5	4.6×10^6
5 (brown/purple)	Surface (cells /mL)	2.4×10^3	1.1×10^5	1.1×10^4	2.6×10^3
	Middle (cells /mL)	2.4×10^3	2.4×10^5	1.1×10^3	2.6×10^3
	Bottom (cells /mL)	2.4×10^4	2.4×10^5	1.1×10^4	1.7×10^4
	Sediment (cells /g)	2.4×10^4	4.6×10^5	1.1×10^4	4.6×10^3
6 (pink/purple)	Surface (cells /mL)	2.4×10^3	2.4×10^6	2.4×10^4	1.1×10^5
	Middle (cells /mL)	4.6×10^4	2.4×10^7	4.6×10^3	4.6×10^5
	Bottom (cells /mL)	2.4×10^4	2.4×10^6	2.4×10^4	2.4×10^6
	Sediment (cells /g)	4.6×10^5	4.6×10^7	4.6×10^4	4.6×10^5

NA: not applied

Figure 3. The profile of band pattern of DGGE analysis of lagoon middle slurries

DGGE provides a visual picture to compare prevalent sequences between samples. While it is often difficult to identify specific sequences with DGGE, DGGE does provide a method for comparing diversity on a broader scale. DGGE results showed strong different DNA bands pattern in each lagoon (Figure 3). These results showed considerable diversity between all six lagoon bacterial communities. Lagoon 2, the most odiferous lagoon, appears the most divergent in population from the other lagoons sampled. Lagoons 1, 4 and 5 shared several predominant bands. These results indicate that while there are overall similarities in the community structure, different populations dominate each lagoon.

Different microbial activity as well as chemical characteristics of lagoons was observed in our studied anaerobic swine lagoons, which affect their functionality. Functionality of lagoons was usually identified based on the waste stabilization and the odor produced. In this study the ratio of methane generation/COD was used as a parameter of waste stabilization. Lagoon 1 (odorless, purple) had the highest waste stabilization based on the methane/COD ratio. Though Lagoon 2 (black) had higher methane/COD ratio; however, odor intensity was obviously high in this lagoon. Air hydrogen sulfide and total hydrocarbon concentrations in lagoon 2 reported reached as high as 18 mg/L and 121 mg/L respectively (Regmi, 2004). Despite high methane generation, lagoon 2 still contained high COD and Volatile Solids concentrations indicating high organic loading rate. Lagoon 2 also had the highest sulfate concentration which might stimulate the growth of sulfate reducing-bacteria thereby increasing the production of hydrogen sulfide that caused odor. Odor intensity and volatile organic compounds (VOCs) were highly correlated with high organic loading rate (Zahn *et al*, 2001). In other study reported that organic loading in lagoon 1 (considered as functional lagoon) from 24.7 to 56.5 g of VS/m^3/day, while in lagoon 2 (considered as non-functional lagoon) had organic loading of 100.7 to 188.9 g of VS/m^3/day (Henny *et al*, 2006). Maximum loading rate recommended in the USA ranges from 61.8 -79.5 g of VS/m3/day respectively (ASAE, 1998), and lagoon 2 as the non-functional lagoon exceeded these recommendations by approximately double. Organic loading appears to be a major determinant of whether a photosynthetic bloom occurred in an anaerobic swine lagoon (Do *et al*, 2003). The presence of phototrophic bacteria was not observed in the spring and summer in lagoons that had average total solids of 3500 mg/L and average COD concentrations of 1300 mg/L (Chen *et al*, 2003). High organic loadings and solids concentrations inhibit the growth of purple photosynthetic bacteria in lagoon 2. Purple lagoons, indicating the sign of the presence of purple photosynthetic bacteria, often are considered as functional lagoons due to low odor. Interesting results were perceived in this study. Although purple lagoons had low odor; however, not all purple lagoons had high methane generation indicating low organic removal.

Lagoons studied here have different management systems which appear to cause the variability in lagoons microbial activity. Lagoon management systems such as recharge pit system control the organic loading rate to avoid slug loading that can cause organic overloading and solids accumulation. Annual solids removal application also can reduce the solids buildup to maintain the treatment volume of lagoon. Lagoons receiving waste from recharge pit system with application of solids removal had better performance than lagoons receiving direct flush of waste or slug loading with no application of solids removal. Lagoons receiving waste from the barn containing one type of pigs such as lagoons 1 and 4 also seemed to function better (moderate to high ratio of methane/COD, high bacterial counts, and low chemical characteristics) than lagoons receiving waste from the barn containing more types of pigs. Using water containing low sulfate concentration and low chemical characteristics recycled effluent in preference to groundwater containing high sulfate concentration to flush the waste from the

barn also can reduce sulfate concentration in lagoons. In addition, different type of diet and antibiotics used for promoting swine growth contributes different swine manure compositions (waste constituents) that can affect the biochemical origins, composition and accumulation of odorous compounds (Miller and Varel, 2003). Certainly the management systems of lagoon affect microbial activity by providing environmental conditions to support degradation of organic waste by anaerobic bacteria and further control odor produced in lagoons.

CONCLUSIONS

This study elucidates microbial activity differences as well as chemical differences in anaerobic lagoons. Production of methane and hydrogen which was measured to determine microbial activity in lagoons varied from lagoon to lagoon. Concentration of methane and hydrogen is inversely correlated. This finding suggests that elevated hydrogen concentration can be an indicator of low waste stabilization in anaerobic lagoons. The study also indicates that COD, sulfate and solids concentrations are factors that can cause high variability of the microbial activity as well as elevate requisite microbial community in anaerobic swine lagoons. Overall, our study suggests that COD, sulfate, and solids concentrations of lagoons can be important factors that affect the functionality of lagoons. Controlling these factors is a key for the functionality of lagoons. The variability of microbial activity in anaerobic lagoons strongly associated with lagoon management systems. This study suggested that in addition to good management system, uniform waste constituents might improve the performance of anaerobic lagoon treatment systems. Lagoon management systems such as the use of recharge pit systems, water recycling and solids removal can possibly help maintain functionality by controlling organic overloading (COD), reducing sulfate and solids concentrations, thereby maintaining the requisite microbial community for waste treatment.

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REFERENCES

APHA, 1992. *Standard Methods*. 18th edition.

ASAE, 1998. *Design of Anaerobic Lagoons For Animal Waste Management*, ASAE EP 403.3. ASAE Standards 1998. American Society of Agricultural Engineers. 2950 Niles Road, St. Joseph, MI, p. 656-659.

Chen T., Schulte D. D. Koelsch R. K. and Parkhurst A. M., 2003. Characteristics of Phototrophic and Non-Phototrophic Lagoons for Swine Manure. Transaction of the ASAE 46(4): 1285-1292.

Conrad R., 1999. Contribution of Hydrogen to Methane Production and Control of Hydrogen Concentrations in Methanogenic Soils and Sediments. *Fems Microbiology Ecology*, 28: 193-202.

Conrad R., Phelps T. J. and Zeikus J. G., 1985. Gas Metabolism Evidence in Support of the Juxtaposition of Hydrogen-Producing and Methanogenic Bacteria in Sewage Sludge and Lake Sediments. *Appl. Environ. Microbiol.*, 50: 595-601.

Do Y.S., Schmidt M. T., Zhan J. A., Boyd E. S., de la Mora A. and Dispirito A. A., 2003. Role of Rhodobacter sp. Strain PS9, A Purple Non-Sulfur Photosynthetic Bacterium Isolated From Anaerobic Swine Waste Lagoon, in Odor Remediation. *Appl. Environ. Microbiol.*, 69(3): 1710-1720

Hansen K. H., Angelidaki I. and Ahring B. K., 1998. Anaerobic Digestion of Swine Manure: Inhibition by Ammonia. *Wat Res.* 32(1): 5 – 12.

Henny C., M. Mormille and J. G. Burken, 2006. *Performance Characteristics and Microbial Activity of Anaerobic Swine Lagoons, Prosiding Pengelolaan Sumberdaya Perairan Darat Secara Terpadu di Indonesia, Seminar Nasional Limnologi 2006*. Jakarta. 5 September.

Hilpert R., Winter J. and Kandler O., 1984. Agricultural Feed Additives and Disinfectants as Inhibitory Factors in Anaerobic Digestion. *Agricultural Wastes*. 10: 103-116

Kobayashi H. A., Stenstrom M. and. Mah R. A., 1983. Use of Photosynthetic Bacteria for Hydrogen Sulfide Rremoval from Anaerobic Waste Ttreatment Effluent. *Wat. Res.*, 17(5): 579-587.

Kong I. C., Hubbard J. S. and Jones W. J., 1994, Metal-induced Iinhibition of Anaerobic Metabolism of Volatile Fatty Acids and Hydrogen, Appl. Microbiol. Biotecnol. 42: 396-402.

Leung, K. and Topp E., 2001. Bacterial Community Dynamics in Liquid Swine Manure during Storage: Molecular Analysis using DGGE/PCR of 16S rDNA. *Fems Microbiology Ecology*, 38(2-3): 169-177.

Lotringen J. M. V. and Gerrish J. B., 1978. H_2S *Removal by Purple Sulfur Bacteria in Swine Waste Lagoons, In Proceeding of the 32nd Industrial Waste Conference*, Purdue University: 440-448.

Mc Carthy P. L. and Smith D. P., 1986. Anaerobic Wastewater Treatment. *Environ Sci Technol.*, 20: 1200-1206.

Miller D. N. and Varel V. H., 2003, Swine Manure Composition Affects the Biochemical Origins, Composition, and Accumulation of Odorous Compounds, *J. Anim.* Sci. 81: 2131-2138.

Miner J. R., F. J. Humenik, and M. R. Overcash, 2000. *Managing Livestock Wastes to Preserve Environmental Quality*. Iowa State University Press. Ames.

Muyzer, G. E. C. D. W. A. G. U., 1993. Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaxtion- Amplified Genes Coding for 16S rRNA. *Appl. & Env. Microbiol.*, 59(3): 695-700.

Poels J., Van Assche P. and Verstraete W., 1984. Effects of Disinfectants and Antibiotics on The Anaerobic Digestion of Piggery Waste, *Agric. Wastes*, 9: 239-247

Regmi S., 2004. *Odor Evaluation and Control at An Anaerobic Swine Lagoon*, Thesis, University of Missouri-Rolla.

Schink B., 1997. Energetics of Syntrophic Cooperation in Methanogenic Degradation. *Microbiol. & Molec. Biol. Rev.*, 61(2): 262-280.

Speece R. E., 1996. *Anaerobic Biotechnology for Industrial Wastewaters*. Archae Press Nashville, Tennessee.

Vogels G. D., Keltjens J. T. and C. van Der Drift, 1988. *Biochemistry of Methane Production*, In Zehnder, A. J. B (ed), Biology of Anaerobic Microorganisms. John Wiley & Sons, New York: 469-586.

Widdel F., 1988. *Microbiology and Ecology of Sulfate- and Sulfur-Reducing Bacteria*, In A. J. B. Zehnder (ed), Biology of Anaerobic Microorganisms. John Wiley & Sons, New York: 469-586.

Zahn J. A., Hatfield J. L., Laird D. A., Hart T. T., Do Y. S. and DiSiprito A. A., 2001. Functional Classification of Swine Manure Management Systems Based on Effluent and Gas Emission Characteristics. *J. Environ. Qual.*, 30: 635-647.

Zinder S. H., 1993. *Physiological Ecology of Methanogens*, In Ferry J. G (ed), Methanogens: Ecology, Physiology, Biochemistry and Genetics. Chapman & Hall, New York: 26 – 206.