Performance Characteristic and Microbial Activity of Anaerobic Swine Lagoons

Cynthia Henny[1], Melanie R. Mormile[2], Joel G. Burken[2]

[1] Research Center for Limnology, LIPI, Cibinong, 16911

[2] Environmental Research Center, University of Missouri-Rolla, MO 65409, USA

Key words: Anaerobic Metabolism, Methanogenesis, Anaerobic lagoons, Swine Wastes

Abstract

Anaerobic lagoons are routinely used to effectively treat swine waste. However such lagoons can fail and often generate offensive odors. To function properly, anaerobic lagoons rely upon system management such as proper organic loading, and solids removal, and balanced anaerobic microbial activity from fermentation to methanogenesis. Disturbances to this balance may result in elevated hydrogen and a buildup of volatile fatty acids that can inhibit methanogens, ultimately disrupting treatment. Excessive volatile fatty acids cause problematic odors, as does H_2S produced by sulfate-reducing bacteria (SRB). Performance characteristics and microbial activity in a functional (actively methanogenic, non-odoriferous) and non-functional lagoon (lacking gas production, malodorous) was studied to determine organic carbon removal efficiency as methane production and levels of hydrogen concentrations as possible indicators of metabolic health. Factors that might negatively impact methane production were also investigated. In addition, a microcosm study conducted at four different temperatures: 4, 10, 25 and 35^{o}C was performed to determine the impact that temperature had on methane production

and hydrogen concentrations. The methane production rates were positively correlated to temperature for both lagoons. Surprisingly, the number of methanogens was higher in the non-functional lagoon. Both lagoons established apparent low steady state concentrations of hydrogen as well as near neutral pH values. Organic overloading that might negatively impact the methane production was apparent in the non-functional lagoon; albeit, the ratio of methane/SCOD for both lagoons was not significantly different. However, high sulfate concentration in the waste resulted in high SRB numbers in the non-functional lagoon, suggesting that excessive production of H_2S by SRB might create an odor problem in the non-functional lagoon. Purple sulfur bacteria, as indicated by a purple colored layer in anaerobic lagoons, can reduce odor by consuming H_2S. Moreover, the non-functional lagoon displayed no signs that purple sulfur bacteria were present, suggesting that purple sulfur bacteria may have an important role in odor reduction.

INTRODUCTION

Anaerobic lagoons commonly treat swine wastes in the United States and are effective at decomposing many kinds of organic matter [1, 2, 3]. These lagoons rely on microbial activity and management practices such as solids separation prior to treatment, periodic solids removal and suitable organic loading rates to help maintain functionality [1-7]. Lagoons, however, can frequently generate considerable odors due to hydrogen sulfide and volatile organic compounds [4, 8-10].

Animal waste consists of complex organic compounds including carbohydrates, protein, and fats. Anaerobic degradation of animal waste is carried out by a series of reactions catalyzed

by anaerobic microorganisms ranging from fermentative to methanogenic bacteria (Figure 1) [6, 7, 11]. These bacteria rely on synergistic relationships to produce metabolic products/intermediates resulting in balanced anaerobic metabolic reactions. During the initial metabolic step, the fermentative bacteria convert biodegradable organic matter to organic acids such as volatile fatty acids (VFA), and hydrogen. This is followed by the activity of fatty acid oxidizing bacteria (FAOB) that convert VFA such as propionate and butyrate into acetate and hydrogen that are transient intermediates along with carbon dioxide [7, 11-13]. Hydrogen and carbon dioxide in turn can also be converted to acetate by homoacetogens [12, 13]. In the final metabolic steps, acetate can be converted to methane by acetoclastic methanogens, while hydrogen and carbon dioxide can be converted to methane by hydrogenotrophic methanogens [7, 11-15]. However, if sufficient sulfate is present, sulfate-reducing bacteria (SRB) will utilize these intermediates and generate hydrogen sulfide and carbon dioxide [16, 17]. A balance of metabolic reactions from fermentation to methanogenesis is critical for swine lagoons to function properly. For example, disturbances to this balance may result in elevated hydrogen and a buildup of VFA. Hydrogen partial pressures exceeding 10^{-4} atm (80.7 nM dissolved hydrogen) inhibits the oxidation of fatty acids resulting in elevated concentrations of VFA [6, 7, 11, 18, 19]. These elevated acids lower pH and can inhibit methanogens, further disrupting treatment. Moreover, excessive VFA cause problematic odors, as can the H_2S produced by SRB.

Other microorganisms that can be present in anaerobic lagoons are photosynthetic purple sulfur and non-sulfur bacteria (Figure 1) [20-22]. The presence of photosynthetic purple bacteria in anaerobic lagoons is indicated by pink or purple hues of lagoons. Pink to purple colored lagoons are thought to indicate that the lagoons are functioning well and generally, have less offensive odor than grayish or black lagoons lacking populations of purple bacteria.

Photosynthetic purple bacteria have been shown to consume ammonia, hydrogen sulfide, and the excess VFA that cause odor [20, 22].

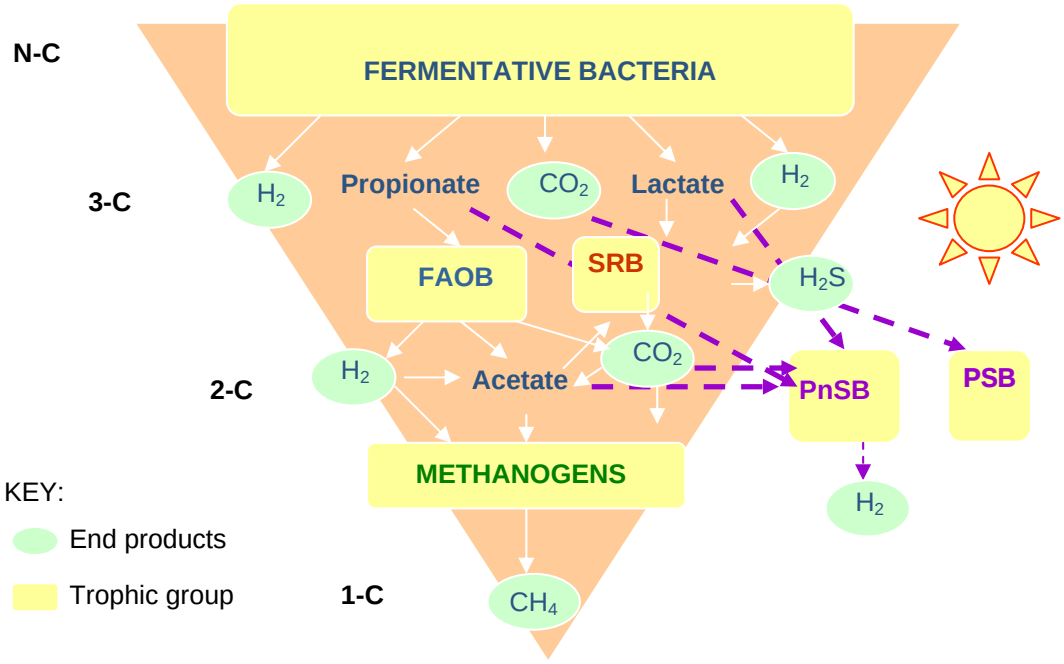


Figure 1. Sulfate reduction and methanogenesis in anaerobic processes

Lagoon management practices should provide suitable environmental conditions for the requisite microorganisms leading to the stabilization of swine wastes. Various factors that potentially can have negative impacts on the microbial population resulting in disturbances during the operation of anaerobic lagoons include organic overloading, temperature fluctuations, decreases in pH, salts buildup, ammonia accumulation, and the use of antibiotics and disinfectants. Organic overloading can result in intermediate buildup resulting in an imbalance to the anaerobic metabolic reactions in the lagoons to lagoon failure [4, 6, 7, 11, 14]. Additionally, organic overloading can inhibit the purple photosynthetic bacteria in the lagoon. Organic solids loading of more than 3 g [dry weight]/ L is inhibitory towards the growth of photosynthetic bacteria [22]. Low temperature and pH values can reduce the rate of

methanogenesis and can lead to the subsequent accumulation of VFA in anaerobic lagoon systems [15, 23]. Among potential negative impacts, salt and ammonia accumulation can be toxic to anaerobic bacteria in the lagoon [4, 6, 24, 25]. The application of disinfectants to clean the animal facilities and antibiotics used in the feed [26, 27] as well as to treat ill animals, can potentially disturb the anaerobic processes in the lagoons. Antibiotics and disinfectants have potential to reduce the number of requisite bacteria to a point where they can no longer recover, resulting in the uncoupling of metabolic reactions.

The broad objective for this study was to compare microbial activity in a functional (actively bubbling, not odoriferous) to a non-functional lagoon (no active gas production, malodorous) in a microcosm study to evaluate methanogenesis function.

MATERIALS AND METHODS

Lagoon selection

Two anaerobic lagoons treating swine waste, one classified as functional and the other as non-functional were selected for this study. The functional lagoon is a single-stage system with semi-annual solids removal. The lagoon receives wastes that have been collected in a pit before being flushed and washed down. The water recycled from the lagoon is used to wash down the waste from the pit. The lagoon turns purple in the warm weather. The lagoon size is 65.5 m (L) x 58.8 m (W) with the depth range from 2.4 to 5.5 m (Figure 2). The non-functional study lagoon is the initial stage of a two-stage system without solid separation, recycle and solid removal. The first stage is a primary treatment lagoon where the solids accumulate, while the second stage receives overflow from the initial stage lagoon. The treatment lagoon size is 54 m

(L) x 21.6 -36.6 m (W) with the depth ranging from 0.3 to 2.7 m (Figure 3). The sludge depth varies from 0.3 to 1.2 m. The lagoon is grayish or black colored lagoon with high sludge accumulation. Groundwater containing approximately 37 mg/L sulfate is used to flush waste to this lagoon.

Sample collection

For sampling, each lagoon was divided into quarters, laterally and longitudinally. The intersection of these lines determined the sample locations. Depth profile samples, surface, middle, bottom and sediment were collected at each point. There were eight sampling locations for the non-functional lagoon and nine sampling locations for the functional lagoon (Figures 2 and 3). Samples were also taken from the waste inlet of each lagoon for waste characterization. Samples were taken at location 3, 5 and 7 for lab analyses, such as total solids/volatile solids (TS/VS), soluble chemical oxygen demand (SCOD), ammonia-N, ions (sulfate and chloride), and VFA concentrations. For microcosm studies, samples were collected at location designated #5 in each lagoon. Slurry and sediment samples were collected from a boat on a semi-annual basis. Surface, middle and bottom slurries were collected by using a Van Dorn-style water sampler (Cole Parmer, Vernon Hills, IL). Sediment was collected by using an Ekman dredge. Water samples used to prepare slurries were placed in Nalgene polyethylene bottles that were pre-rinsed on site with the respective lagoon liquid. Sediment samples were placed in 1 L glass jars. Samples were transported to the lab in a cooler and stored at $4^{o}C$ prior to analysis and microcosm studies. Samples for VFA analysis were frozen prior to analysis.

Onsite measurements

Onsite analysis, which included pH, temperature, dissolved oxygen (DO), oxidation reduction potential (ORP) and conductivity, were measured at the surface, middle and bottom depths of all sampling locations. These parameters were measured 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.

Microcosm studies

Microcosms were prepared within 24 hours after sampling. Autoclaved 160-mL serum bottles were filled with 100 mL of slurry or sediment and 0.1 ml of resazurin solution (10 g/L) in an anaerobic glovebag containing10% H_2:90%N_2 to determine the microbial activity. Microcosms were prepared in triplicate. 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 that was collected at least 4 days prior to the experiment was autoclaved three consecutive days for 30 minutes at 121^{o}C in order to prepare negative controls. The microcosms were incubated at 4, 10, 25, and 35 ^{o}C on shaker tables (Cole Parmer, Vernon Hills, IL) with rotation set at 52 rpm. Pressure, along with hydrogen and methane concentrations, was measured at 0, 24, 48, 72, 120, and 168 hours. Samples for soluble chemical oxygen demand (SCOD) and VFA in the slurry were measured at 0 and 168 hours.

Microbial Population

Methanogens, fatty acid oxidizing bacteria, and sulfate reducing bacteria were enumerated by using a most probable number (MPN) assay. A mineral basal medium was used [11] with resazurin added as a redox indicator. Acetate was provided as a carbon source for

methanogens. Acetate and lactate were provided as electron donors and sulfate salts (5.3 mM sulfate) as $FeSO_4.7H_2O$ and Na_2SO_4 were provided as electron acceptors for SRB growth. Propionate was added into the media for FAOB. A pure culture of methanogens (JF1 culture) was inoculated into culture tubes in order to scavenge the hydrogen produced by FAOB [11]. All MPN tubes were incubated in the dark at room temperature for one month.

Analytical methods.

Total solids (TS) and volatile solids (VS) were measured by using standard methods [28]. Slurry, 15 mL, was centrifuged at 10,000 G for 10 minutes and filtered with 0.2µM nylon syringe filter (Whatman, Inc, Clifton, NJ) prior to SCOD, ammonia-N, VFA and salts analyses. Chemical oxygen demand (COD) was measured by using Hach Method 8000 (Hach, Loveland, CO). Ammonia-N in the slurry was analyzed by using Hach Method 10031 (Hach, Loveland, CO). The concentration of VFA (acetic acid, propionic acid, isobutyric, and butyric acid) in the slurry samples were analyzed by using gas chromatography (GC) with a flame ionization detector (FID) model 8610B (SRI Instruments, Torrance, CA). One micro liter injections of the supernatant were made on to a 15 m Nukol capillary column (0.53 m ID, 0.5 µm film, Supelco, Bellefonte, PA). The gradient program was started at 120 °C and ramped at 8 °C/minute for 5 minutes. The method detection limit (MDL) for each VFA analyzed was as follows: acetic acid, 0.86 mM, propionic acid, 0.46 mM, isobutyric acid, 0.64 mM and butyric acid, 0.41 mM. Sulfate and chloride was measured by using an ion chromatography (IC) (Model DX, Dionex, Sunnyvale, CA) equipped with an AS4A column and a AG4A guard column. An eluent solution of Na_2CO_3 (9 mM) was used. The headspace pressure was determined by using a PX26-100GV (0-100 psig) series pressure transducer (Omega, Stamford, CT) [29] 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). The injector, column, and detector were held at 100°C, 105°C, and 120°C, respectively. Nitrogen (80 psig, 30 ml/min) was used as a carrier gas. The FID was supplied with hydrogen (40 psig, 30 ml/min) and air (60 psig, 300 ml/min). A stainless steel 80/100 Porapak Q (6 ft by 1/8in) packed column (Supelco, Bellefonte, PA) was used to resolve methane. The headspace sample volume was 0.2 ml. The MDL of CH_4 was 13 μM. Headspace H_2 concentrations were measured by using a gas analyzer (Model 3000, Molecular Analytical, Sparks, MD). Nitrogen was used as a carrier gas at a 25 ml/min flow rate. The column temperature was maintained at 105°C and the detector at 265°C. Headspace samples (500 μl) were pulled from the microcosms and then diluted with purified nitrogen gas to 5 ml. The hydrogen concentrations in the slurry samples were calculated by using the Ostwald coefficient for each temperature [30] and with the assumption that the solvent was pure water.

RESULTS AND DISCUSSION

Influent and lagoon slurry SCOD and TS/VS concentrations were significantly different between the lagoons (Table 1). Solids accumulation was visually apparent in the non-functional lagoon. The organic loading rate calculated based on the influent VS, the flow rate and the estimated lagoon's volume ranged from 24.7 to 56.5 g of VS/m^3/day, and 100.7 to 188.9 g of VS/m^3/day for the functional and non-functional lagoon, respectively. Maximum loading rate recommended in Iowa, South Carolina and North Carolina are 61.8, 79.5 and 67.9 g of VS/m^3/day respectively [1, 4], and the non-functional lagoon exceeded these recommendations by approximately double. Although apparent organic overloading was observed in the non-

functional lagoon, a similar range of lagoon slurry pH was observed in both lagoons. The pH values of lagoon slurries were near neutrality and did not differ (Table 2). This indicated that both lagoons have well-buffered systems helping to maintain the activity of pH sensitive microorganisms, especially methanogens [14, 15].

There was a positive correlation between methane production rates and temperature for both lagoons. The non-functional lagoon microcosms generated more methane than the functional lagoon microcosms. However, when methane production was normalized to SCOD, subtle differences between the lagoons became apparent for the fall samples (Figures 4 and 5). The ratio methane/SCOD for sediment samples at 35^{o}C of the functional lagoon was slightly higher than that of the non-functional lagoon. The methane production per kg SCOD per day at 35 ^{o} C for fall samples ranged from 0.8 for surface slurry samples to 13.2 mol/kg/day for sediment samples for the non-functional lagoon and ranged from 2.6 for surface slurry samples to 16.0 mol/kg/day for sediment samples for the functional lagoon. The results indicate that the apparent organic overloading did not significantly inhibit methanogenesis in the lagoon. Lagoon samples from bottom and sediment layers produced significantly more methane than slurry taken from the surface and middle depths (Figures 4 and 5).

Hydrogen and acetate are important intermediates during the anaerobic degradation of organic waste and can serve as substrates for methanogens. However, hydrogen is a critical though transient intermediate in anaerobic environments. Degradation of fatty acids by FAOB thermodynamically requires low H_2 concentrations [11, 12, 13]. Hydrogen has to be maintained at a level low enough to allow exergonic H_2 production by fatty oxidizing bacteria while remaining high enough to allow methanogenesis from H_2 [12-15] The hydrogen concentrations in the microcosms reached an apparent low-level steady state for both functional and non-

functional lagoons. The steady state hydrogen concentrations at 25°C and 35°C ranged from 0.01to 0.03 μM for the functional lagoon and ranged from 0.02 to 0.05 μM for the non-functional lagoon (Figures 6 and 7). The range of steady state hydrogen concentrations was close to the value of hydrogen concentrations reported in methanogenic lake Mendota sediment (0.04 μM) [21], but low in comparison to the median value of hydrogen concentrations reported for landfill samples (1.9 μM) [13] and sewage sludge (0.2 μM) [19]. Volatile fatty acids were detected in the early summer in the non-functional lagoon as acetate, propionate and butyrate (Table 1), while there was no accumulation in the functional lagoon. The VFA analyses of all microcosms exhibited non-detectable concentrations. Our hydrogen and VFA analyses indicated that there was no sign of hydrogen and VFA accumulation to a level inhibitory to FAOB and methanogenic bacteria.

The estimated number of methanogens in the sediment of the non-functional lagoon was one order magnitude higher than those in the functional lagoon, while FAOB were approximately two orders of magnitudes higher in the non-functional lagoon than those in the functional lagoon (Table 3). These results suggested that the requisite microbes for methanogenesis were present in both lagoons. The MPN results for population of SRB were also one order magnitude higher in the sediment of the non-functional lagoon than those of the functional lagoon (Table 3). The average of influent sulfate concentration was 185 mg/L and 19 mg/L for non-functional and functional lagoons, respectively (Table 1). The results of sulfate concentrations in the lagoon slurry indicated that more sulfate reduction occurred in the non-functional lagoon than in the functional lagoon (Table 2). A lack of solids removal and use of groundwater containing a high concentration of sulfate to flush wastes may cause a proliferation

of SRB and generation of H_2S that produces a component of nuisance odors in the non-functional lagoon.

Although, there was apparent organic overloading, there was no indication methanogenesis was inhibited in the non-functional lagoon. The hydrogen and VFA analyses as well as MPN results for population of methanogens and VFA oxidizers were in agreement with this finding. Anaerobic lagoons are considered electron donor rich environments and electron acceptor poor as are landfills and sewage digesters. As previously reported for landfills and anaerobic digesters, organic overloading can cause an accumulation of H_2 and VFA resulting in low pH values and reduced methanogenic activity [11, 31]. However, this pattern was not observed in our study where the organic loading in the non-functional lagoon exceeded recommended maximum loading rate for a typical swine lagoon. Due to the influx of sulfate in the rinse water, this lagoon was not electron acceptor poor and microorganisms other than methanogens, such as sulfate reducing bacteria, were mineralizing the carbonaceous wastes. Hydrogen and VFA can serve as electron donors for these organisms.

The organic overloading and lack of solids removal practices in the non-functional lagoon resulted in a solids build up and effectively reduced the liquid volume of the lagoon. Reduced liquid volume of the lagoon can have the same effect as an increase in loading rate resulting in a further decrease in the retention time, thereby reducing the efficiency of treatment. Significant anaerobic degradation was observed in the non-functional lagoon, however, with higher biomass and substrates, the kinetic limitations were pushed to a maximum growth rate for bacteria. The kinetic limitations can lead to incomplete COD reduction. This decreased treatment efficiency can cause an increase in odor frequency as observed in the non-functional lagoon. This ineffective treatment, along with H_2S generation by SRB leads to an odor problem.

As a lagoon stabilizes and desired bacterial populations develop, the color of the lagoon changes from brown to pink or purple during the late spring. Gas bubbling from high biogas production can also be visualized. Pink or purple colored lagoons indicate the presence of phototrophic purple bacteria. Photosynthetic purple bacteria consume odor compounds such as hydrogen sulfide, ammonia and excess VFA [20-22]. Therefore, pink or purple lagoons with uniform gas bubbling have low offensive odor. In contrast, black colored lagoons typically possess unpleasant odors and sludge can build up at a high rate [4, 32]. It appears that a black color is also an indication of organic overloading [33]. The non-functional lagoon studied here was a black colored lagoon with sludge accumulation. The phototrophic purple bacteria were not quantified in this study; however, it was obvious that the non-functional lagoon displayed no signs that photosynthetic purple bacteria were present. One probable reason is that organic overloading can inhibit the purple photosynthetic bacteria in the lagoon. An organic load at 3.5 g [dry weight]/ L decreased the population of photosynthetic bacteria in sewage treatment [34]. Additionally, reduced light penetration due to high solids content in the slurry can inhibit the growth of photosynthetic purple bacteria. Turbidity data in the non-functional lagoon slurry was higher than that of the functional lagoon (Table 2). Previous research established that an organic loading rate at 111 g of VS/m^3/day will produce a significant odor near lagoons 60 % of the time [1, 4]. Therefore, the odor problem in the non-functional lagoon may be due to higher H_2S production and ineffective treatment of organics in this system. Promotion of conditions conducive to the development of a population of purple sulfur bacteria might reduce the offensive odors.

In addition, high ammonia concentrations can negatively impact anaerobic metabolism and lead to odor problems. Influent N-ammonia concentrations were 295 – 504 mg/L for non-

functional lagoon and 125 – 292 mg/L for functional lagoon (Table 1). It is reported that total ammonia nitrogen in lagoons should be kept below 1500 mg/L [4] and that ammonia concentrations of 1100 mg/L and above can cause inhibition on methanogenesis in anaerobic digester [27, 28]. As the ammonia nitrogen levels in the lagoons studied were lower than those guidelines, ammonia probably was not a contributor to the noxious odor at the non functional lagoon.

Another potential impact to lagoon microbiota is high concentrations of salt. The conductivity measured in the lagoon slurry ranged from 4600 to 5900 μmho/cm for functional lagoon and ranged from 3600 to 3710 μmho/cm for functional lagoon (Table 2). Conductivity, a measure of the level of salts can also be monitored to observe lagoon performance. Conductivity range of 2000 to 8000 μmho/cm indicates non-inhibitory levels of salts [4, 6, 27]. As with the ammonia concentrations measured in the studied lagoons, the salt levels were also not inhibitory to the anaerobic microbial activity in these systems.

CONCLUSIONS

The apparent organic overloading in our selected non-functional lagoon did not appear to inhibit methanogenesis. Hydrogen reached apparent steady state concentrations while there was no VFA accumulation in the microcosms. Higher concentrations of methanogens, sulfate-reducing bacteria, and fatty acid oxidizing bacteria were observed in the non-functional lagoon indicating the presence of the requisite microbes for anaerobic metabolism. There was no accumulation of ammonia or salts to inhibitory levels. Though the non-functional lagoon did not perform optimally with respect to sludge buildup and odor production, significant anaerobic

degradation still occurred. However, this lagoon is probably kinetically limited due to both high loading rates and solids accumulation, leading to incomplete COD reduction. The odor issue perceived from the non-functional lagoon is partially associated with hydrogen sulfide generated by SRB populations that are proliferated by the high sulfate concentration of flush water. Management practices such as solids removal and using recycled water may improve the lagoon performance, especially in controlling odor and lowering sulfide and COD levels.

Acknowledgements The research was supported by the U. S. Environmental Protection Agency (XP-99795901-0). The authors thank Matthew Satterfield, Kevin Morrissey and Michael Richards for their assistance in both the field and laboratory. The authors also thank the CAFO operators for permitting and assisting with this research activity at their facilities.

References

1. 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.

2. U. S. EPA. 1996. Swine cafo for odors: Guidance for environmental impact assessment U. S. EPA Region 6. Dallas, Texas, p. 5-6.

3. Sweeten, J. M. 1980. Waste treatment: State-of-the-art. In: Lifestock Waste: A Renewable Resource. Proceedings of the 4th International Symposium on Livestock Wastes. ASAE. St. Joseph, MI, p. 334-338.

4. Miner J. R., F. J. Humenik, and M. R. Overcash. 2000. Managing livestock wastes to preserve environmental quality. Iowa State University Press. Ames.

5. Barker, J. C. and L. B. Drigger. 1985. Pit recharge system for managing swine under floor manure pits. In: Agriculture Waste Utilization and Management. Proceedings of the 5th International Symposium on Agriculture Wastes. ASAE. St. Joseph. MI, p. 575-581.

6. Speece, R. E. 1996. Anaerobic biotechnology for industrial wastewaters. Archae Press Nashville. Tennessee.

7. Mc Carthy, P. L. and D. P. Smith. 1986. Anaerobic wastewater treatment. *Environ. Sci. Technol.* 20, p.1200-1206.

8. North Carolina Agriculture Research Service. 1998. Control of odor emissions from animal operations. North Carolina State University. pp.43

9. Williams, A. J. 1984. Indicators of piggery slurry odour offensiveness. Agriculture Wastes. 10, p. 15 36.

10. Galvin, G., K. D. Casey, E. J. McGahan, S. A. Lowe and M. G. Atzen. 2002. Effect of season and loading rate on odour emission from piggery anaerobic lagoons in Australia. ASAE Annual International meeting/CIGR XVth World Congress July 28 - 31. Chicago.

11. Mormile, M., K. Gurijala, J. Robinson, M. McInerney and J. Suflita. 1996. The importance of hydrogen in landfill fermentations. *Appl. Environ. Microbiol*. 62, p.1583-1588.

12. Conrad, R. 1999. Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiology Ecology*. 28, p.193-202.

13. Schink, B. 1997. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews*. 61(2), p. 262-280.

14. Vogels, G. D., J. T. Keltjens 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, p. 469-586.

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

16. Postgate, J. R. 1979. The Sulphate-Reducing Bacteria. Cambridge University Press. New York, 151 p.

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

18. Fennel, D. E., J. M. Gosset and S. H. Zinder. 1997. Comparison of butyric acid and ethanol, lactic acid, and propionic acid as hydrogen donors for the reductive dechlorination of tetrachloroethene. *Environ. Sci. Technol.*. 31(3), p. 918-926.

19. Conrad, R., T. J. Phelps and J. G. Zeikus. 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, p. 595-601.

20. Lotringen, J. M. V. and J. B. Gerrish. 1978. H_2S removal by purple sulfur bacteria in swine waste lagoons. Proceeding of the 32nd Industrial Waste Conference. Purdue University, p. 440-448.

21. Kobayashi, H. A., M. Stenstrom and R. A. Mah. 1983. Use of photosynthetic bacteria for hydrogen sulfide removal from anaerobic waste treatment effluent. *Water Res*. 17(5), p. 579-587.

22. Do, Y.S., M. T. Schmidt, J. A. Zhan, E. S. Boyd, A. de la Mora and A. A. Dispirito. 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), p. 1710-1720.

23. Masse′, R. L., R. L. Droste, K. J. Kennedy, N. K. Patni, and J. A. Munroe. 1997. Potential for the psychrophilic anaerobic treatment of swine manure using a sequence bacth reactor. *Canadian Agricultural Engineeri*ng. 39 (1), p. 25-33.

24. Mc Carthy, P. L. 1964. Anaerobic waste treatment fundamentals. *Public Works*. 95(11), p. 91-94.

25. Hansen, K. H., I. Angelidaki and B. K. Ahring. 1998. Anaerobic digestion of swine manure: inhibition by ammonia. *Wat. Res*. 32(1), p. 5 – 12.

26. Hilpert, R., J. Winter and O. Kandler. 1984. Agricultural feed additives and disinfectants as inhibitory factors in anaerobic digestion. *Agricultural Wastes*. 10, p. 103-116.

27. Poels, J., P. Van Assche and W. Verstraete. 1984. Effects of disinfectants and antibiotics on the anaerobic digestion of piggery waste. *Agricultural Wastes*. 9, p. 239-247.

28. APHA. 1992. Standard Methods. 18th edition.

29. Suflita, J. M and F. Concannon. 1995. Screening tests for assessing the anaerobic biodegradation of pollutant chemicals in subsurface environments. Journal of Microbiological Methods 21:267-281.)

30. Letterman R. D. ed. 1999. Water quality and treatment: A handbook of community water supplies. 5th ed, McGraw-Hill, New York, NY, USA, p. 58.

31. Harper, S. R., and F. G. Pohland. 1986. Recent developments in hydrogen management during anaerobic biological waste treatment. *Biotechnology and bioengineering*, p. 558-562.

32. Paing, J., B. Picot and A. Rambaud. 2000. Sludge accumulation and methanogenic activity an anaerobic lagoon. *Water Science and Technology*. 42(10-11), p. 247-255.

33. USDA. July 1996. Agriculture waste characteristics. Part 651 Agriculture Waste Management Field Handbook, rev. 1.

34. Siefert, E. R. L. Igrens, R. L. and N. Pfenning. 1978. Phototrophic purple and green bacteria in a sewage treatment plant. *Appl. Environ. Microbiol*. 35, p. 38-44

Figure legends

Figure 2. Diagram of functional lagoon. Numbers represent sampling locations. Effluent was taken from recycle line. Arrows indicate where waste inlet and outlet are located.

Figure3. Diagram of non-functional lagoon. Numbers represent sampling locations. Build up of solids is also indicated. Arrows indicate where waste inlet and outlet are located.

Figure 4. Ratio of CH_4(mmole)/$SCOD_0$(mg) in functional lagoon microcosms. Error bars indicate standard deviations.

Figure 5. Ratio of CH_4(mmole)/$SCOD_0$(mg) in non-functional lagoon microcosms. Error bars indicate standard deviations.

Figure 6. Hydrogen concentrations in functional lagoon microcosms at 25^{o}C (A) and 35^{o}C (B). Error bars indicate standard deviations.

Figure 7. Hydrogen concentrations in non-functional lagoon microcosms at 25^{o}C (C) and 35^{o}C (D). Error bars indicate standard deviations.

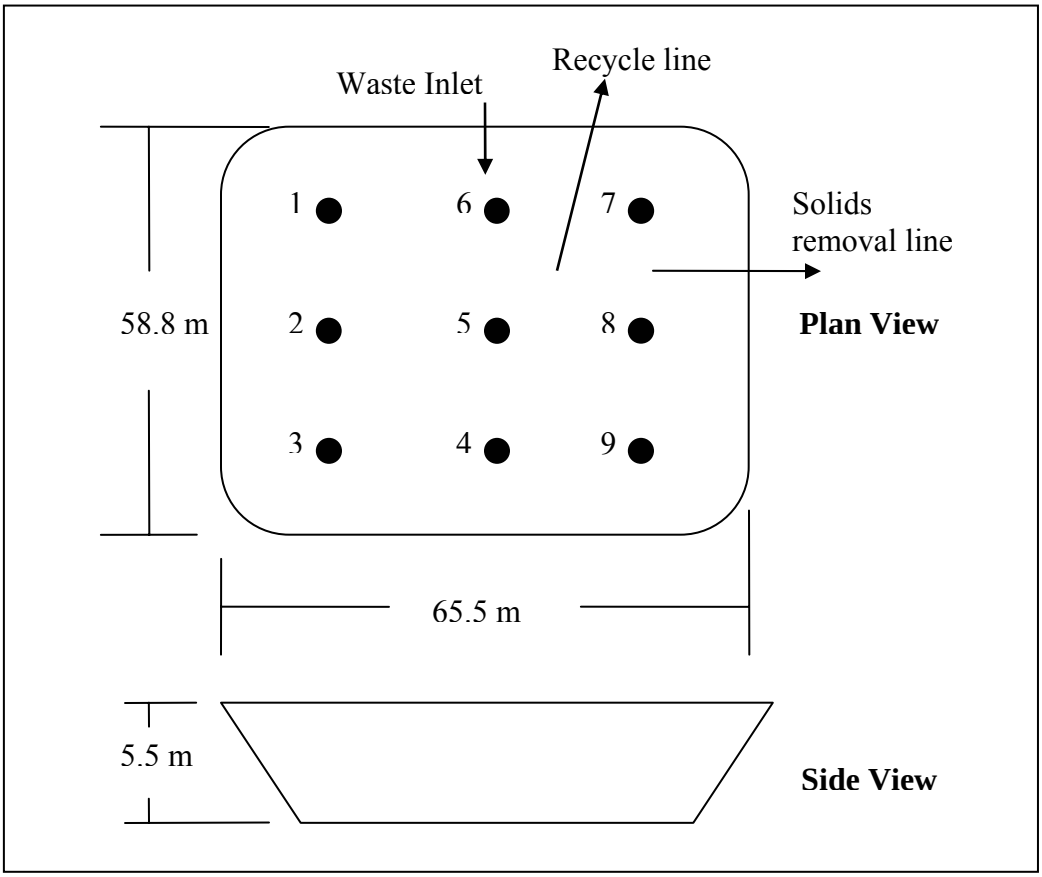

Waste Inlet
Recycle line
1
6
7
Solids
removal line
58.8 m
2
5
8
Plan View
3
4
9
65.5 m
5.5 m
Side View

Figure 2

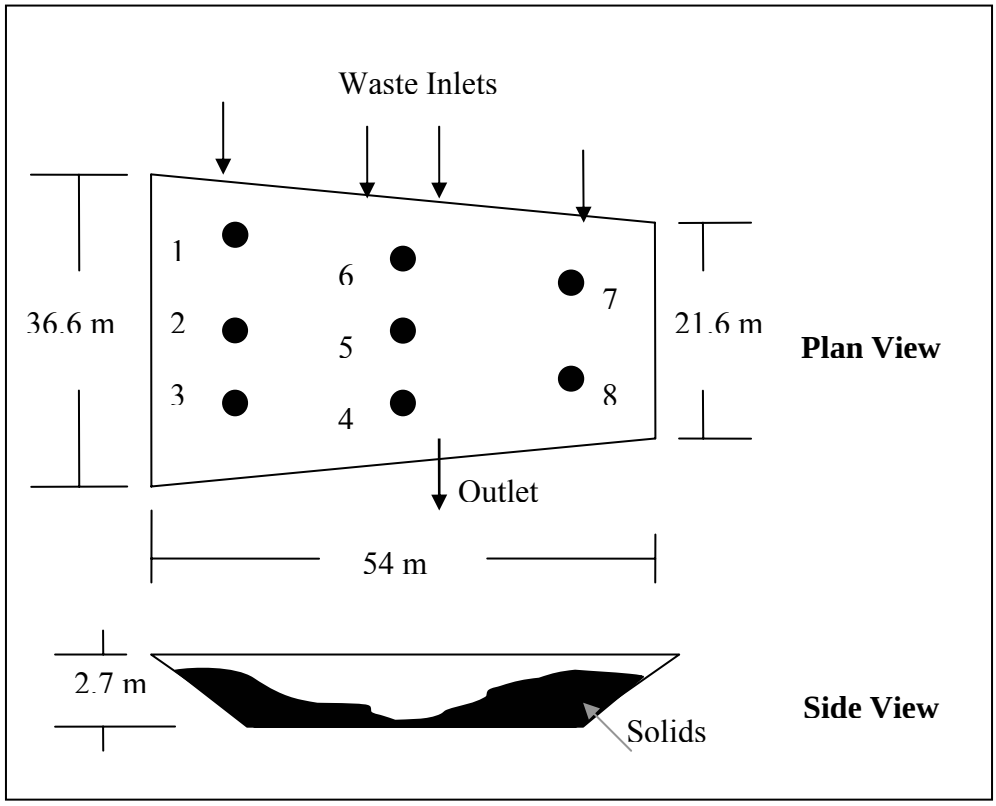

Waste Inlets
1
2
3
4
5
6
7
8
36.6 m
21.6 m
Plan View
Outlet
54 m
2.7 m
Side View
Solids

Figure 3

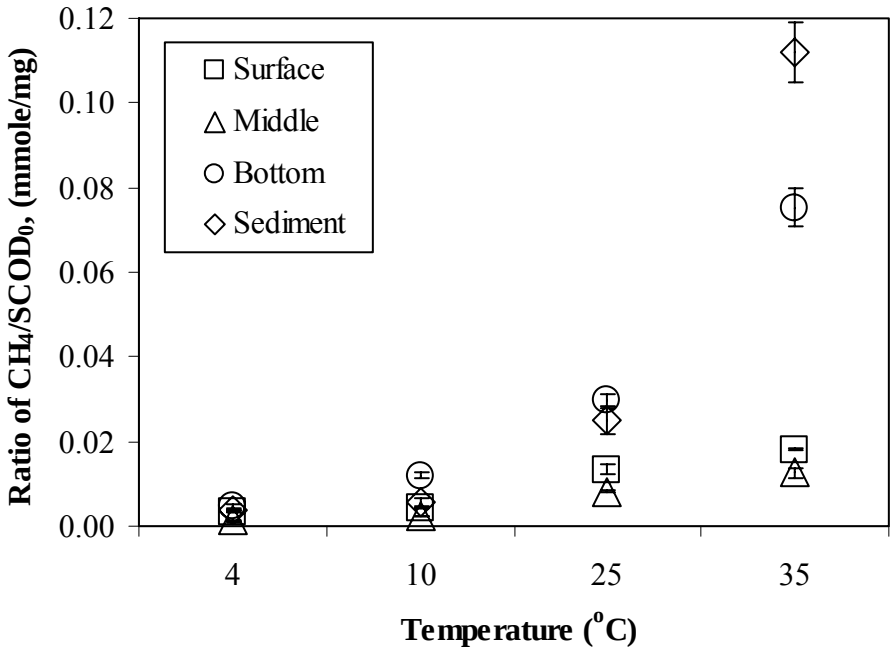

0.12
0.10
0.08
0.06
0.04
0.02
0.00
Surface
Middle
Bottom
Sediment
Ratio of CH4/SCOD0, (mmole/mg)
4
10
25
35
Temperature (°C)

Figure 4

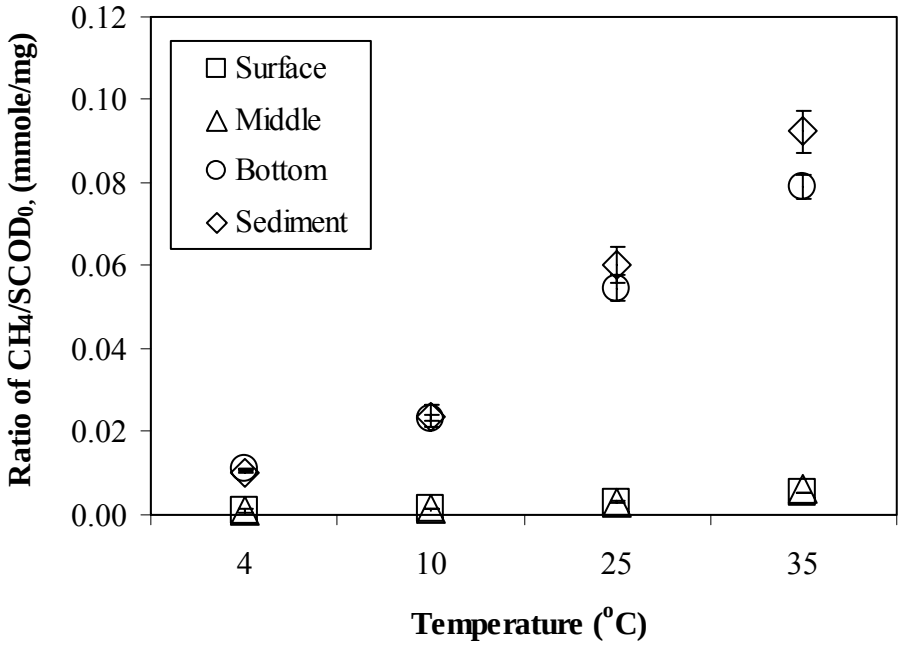
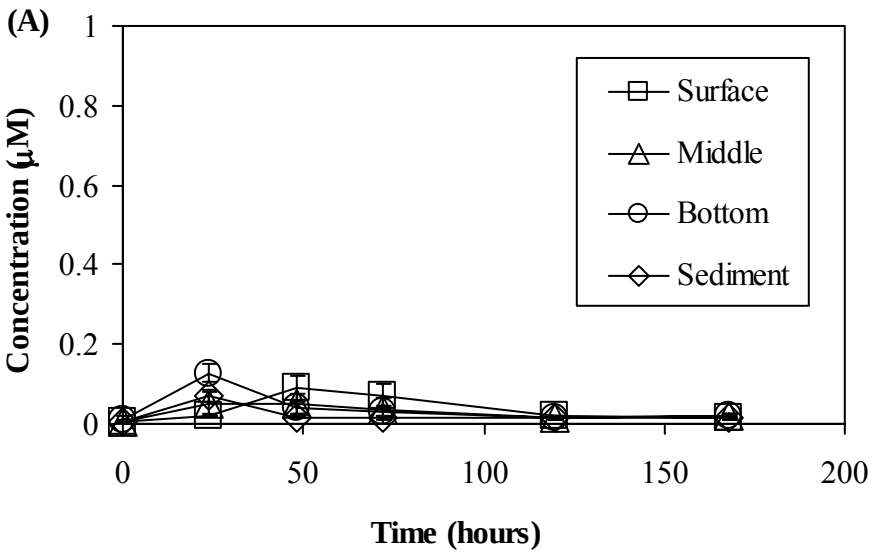

0.12
0.10
0.08
0.06
0.04
0.02
0.00
Surface
Middle
Bottom
Sediment
Ratio of $CH_4/SCOD_0$, (mmole/mg)
4
10
25
35
Temperature (^oC)

Figure 5


(A)
Concentration (µM)
Time (hours)
Surface
Middle
Bottom
Sediment
0
0.2
0.4
0.6
0.8
1
0
50
100
150
200

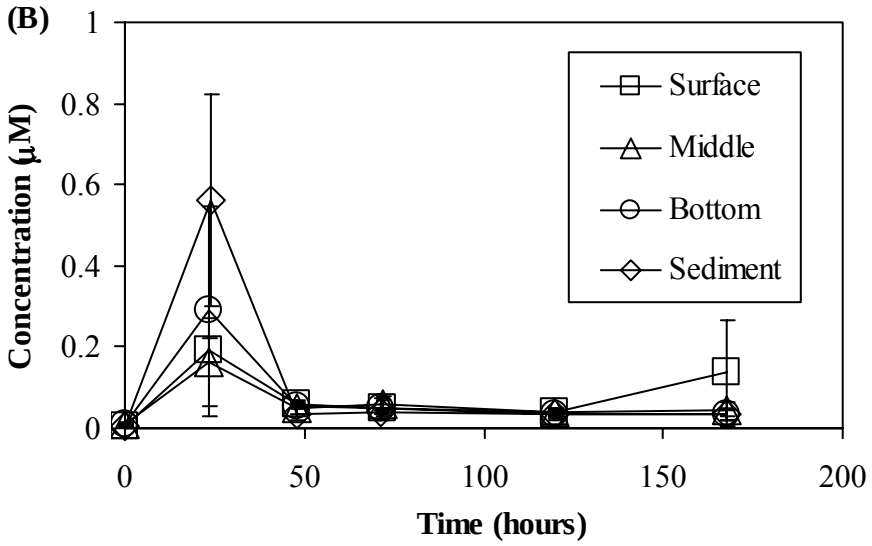
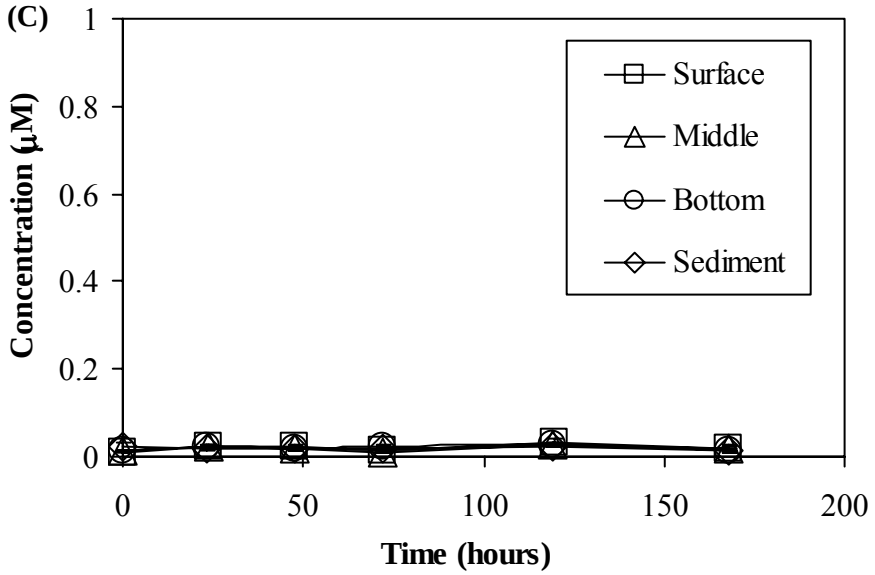

(B)
Concentration (µM)
Time (hours)
Surface
Middle
Bottom
Sediment
0
0.2
0.4
0.6
0.8
1
0
50
100
150
200

Figure 6


(C)
Concentration (µM)
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
Time (hours)
Surface
Middle
Bottom
Sediment

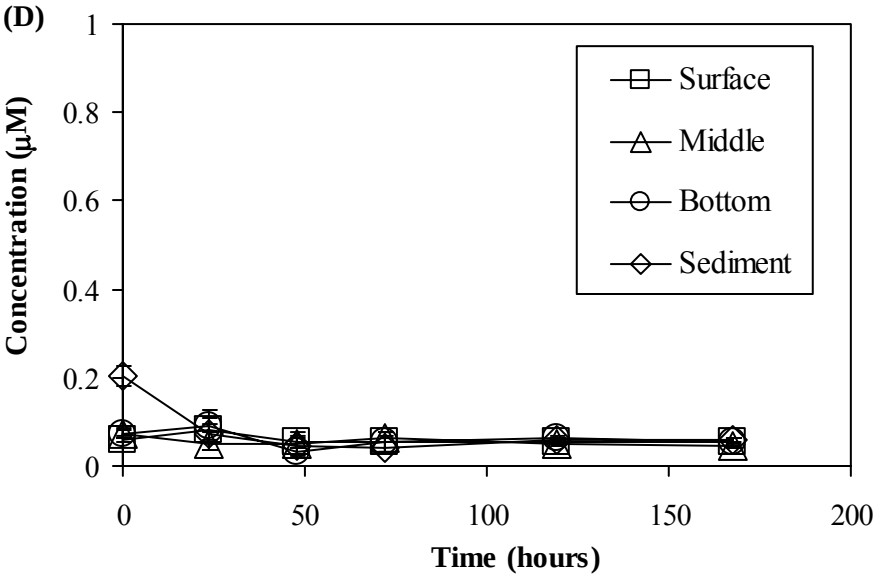

(D)
Concentration (µM)
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
Time (hours)
Surface
Middle
Bottom
Sediment

Figure 7

Table 1. Waste characterization of influent into lagoons

Component	Functional	Non-functional
pH	7.7 – 7.9	7.5 – 7.8
VS (mg(dry weight)/mL)	0.9 – 2.0	4.0 – 7.5
Conductivity (mS/cm)	3.70 – 3.79	2.69 – 3.40
SCOD (mg/L)	600 – 2800	2000 – 7000
Average sulfate (mg/L)	18.8	184.8
N-ammonia (mg/L)	125 – 292	295 – 504
VFA (mM)		
Acetate	nd	1.40 -1.53
Propionate	nd	0.50 – 0.61
Butyrate	nd	0.35 – 0.04

The values are the range of the lowest and the highest values (n=6). nd= non detect.

Table 2. Lagoon slurry characterization, fall 2002

Component	Functional	Non-functional
pH	7.39 ± 0.33	7.25 ± 0.32
ORP (bottom → surface) (mV)	−310 → −137	−430 → −237
Conductivity (mS/cm)	3.65 ± 0.17^{a}	5.34 ± 0.41^{b}
[c]TS (mg/L)	1811 ± 96^{a}	3578 ± 20^{b}
[c]VS (mg/L)	617 ± 44^{a}	2410 ± 20^{b}
SCOD (mg/L)	164 ± 39^{a}	917 ± 50^{b}
Sulfate (mg/L)	6 ± 3^{a}	27 ± 25^{b}
Chloride (mg/L)	147 ± 3^{a}	191 ± 4^{b}

The values are means ± standard deviations (n=9).

[a, b] Values are significantly different ($P<0.01$

[c] Values are from surface and middle slurries (n=6).

Table 3. MPNs of selected lagoon populations, fall 2002

Microbial community	Functional				Non-functional			
	Surface (CFU/ml)	Middle (CFU/ml)	Bottom (CFU/ml)	Sediment (CFU/g)	Surface (CFU/ml)	Middle (CFU/ml)	Bottom (CFU/ml)	Sediment (CFU/g)
Methanogens	2.4×10^3	4.6×10^3	4.6×10^4	1.1×10^5	2.4×10^5	2.1×10^4	2.4×10^5	1.1×10^6
SRB	2.4×10^5	4.6×10^6	4.6×10^6	1.5×10^6	2.4×10^7	2.4×10^7	2.1×10^7	2.4×10^7
FAOB	2.3×10^4	2.4×10^4	2.4×10^4	4.6×10^4	2.4×10^5	1.1×10^5	4.6×10^5	1.1×10^6

1