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Seasonal Variation in Heat Fluxes, Predicted Emissions of Malodorants, and Wastewater Quality of an Anaerobic Swine Waste Lagoon

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Abstract The concentrations of *p*-cresol above a wastewater lagoon were modeled from February through June based on equations developed in a previous study. Using this model, in which *p*-cresol concentrations were calculated based on lagoon evaporation and net available radiation at the lagoon surface, predicted *p*-cresol concentrations were highest during the months of March and April and declined to very low levels thereafter. This was in accordance with observed emission patterns in the previous study. In the same period during which predicted emissions increased, wastewater concentrations of malodorants decreased. While other indicators of wastewater quality such as ammonium and chemical oxygen demand (COD) also decreased in concentration, the magnitude of their improvement was not as high as for the malodorants. There were no pronounced differences in bacterial populations between the cool and warm seasons based on molecular quantification of genes targeting total cells, *Bacteroides*, Clostridia, and methanogens. While the improvement in the concentrations of wastewater malodorants may be due to catabolism by lagoon bacteria, our findings indicate that evaporative losses that occurred as the lagoon warmed may also play a strong role.

Keywords *p*-Cresol · Emissions · Evaporation · Heat flux · Lagoon · Malodor · Wastewater

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1 Introduction

Anaerobic lagoons are an inexpensive means of waste treatment and/or storage, particularly in agriculture where economic considerations are relatively more important than is the case for municipal waste treatment. Although there is pressure to replace lagoons with more effective methods of waste treatment as evidenced by a ban on the construction of new lagoons in the state of

North Carolina (Williams 2007), thousands of waste treatment lagoons are still in operation and no such ban exists in most areas of the country.

The major concern in the use of waste treatment lagoons is the possibility of air and water pollution. In the case of air pollution, ammonia losses can lead to acidification of sensitive soils (Apsimon et al. 1987) and eutrophication of water (Paerl 1997). Small molecular weight polar compounds such as aromatics and volatile fatty acids (VFA) are malodorous and may lead to complaints in the vicinity of large animal rearing operations (Schiffman and Williams 2005). Due to these concerns, there has been a large deal of research into the physical, chemical, and biological factors that control the release of volatile pollutants from waste treatment lagoons.

Regardless of the focus of the research, however, it has been found that pronounced seasonality in emissions exists. For example, the emission of both ammonia and methane has been found to be much higher in warm than in cool seasons (DeSutter and Ham 2005; Bajwa et al. 2006). In the case of ammonia, this is largely due to high emission rates in warm weather, whereas microbial production of methane increases in the warm season. Especially in the case of the malodorous aromatics that are characteristic of animal wastes, though, there is no real understanding of the relative importance of emissive losses and microbial catabolism in controlling lagoon concentrations of these compounds. Understanding the processes controlling losses and concentrations of these pollutants is important in the design of lagoons, the development of protocols for waste loading recommendations, and in the manipulation of lagoon biology to minimize emissions.

Recently, we described emission of the aromatic malodorants *p*-cresol and *p*-ethylphenol from a swine waste treatment lagoon (Loughrin et al. 2011). The variables "evaporative water loss" and "net available radiation at the lagoon surface" were found to be important terms for predicting malodorant concentration above the lagoon surface. In the model, evaporation was negatively correlated with malodorant emission. This was due to the model describing malodorant concentrations over an extended period encompassing cool and warm weather conditions. During cool weather when evaporative losses from the lagoon were low, malodorant emissions were high due to high concentrations of these compounds in the wastewater. Conversely, during warm weather when evaporative losses from the lagoon were high, low wastewater concentrations of the malodorants led to relatively low air concentrations of the malodorants. Regardless of season, however, emission of the malodorants from the lagoon was highly dependent on solar radiation and hence heating of the lagoon surface.

Still, we were unable to determine whether emissive losses of malodorants from the lagoon during our monitoring period had been of sufficient magnitude to account for the decline in their wastewater concentrations. Monitoring was conducted from late winter to early summer, a period in which the concentrations of individual aromatic malodorants declined on average by over 90 %. In this report, we use the models developed in our previous work (Loughrin et al. 2011) to calculate seasonal changes in lagoon heating and evaporation and use these calculations to predict emission of *p*-cresol from the lagoon surface. In addition, we combine the broad range of data available to us including microbial community analysis to achieve insight into the factors affecting malodorant emissions from wastewater treatment lagoons.

2 Experimental Methods

2.1 Site Description and Meteorological Measurements

The observational site was an approximately 60-m^2 lagoon that served as the primary waste recipient for a farrowing operation of approximately 2,000 sows housed in six buildings. Once a week, wastes were flushed from subfloor pits into the lagoon, and fresh water was used to partially recharge the pits. There was no pit recharge from the lagoon. Typically, the lagoon was partially pumped down twice yearly, in the fall and spring, for field application. During this study, the level of the lagoon was lowered by 10–20 % in April. Agitation was used during pump down to remove as much as of the sludge layer as possible. Volatile collections were performed 11 times from 6 February to 30 March, 2009 and again eight times from 3 June to 25 June, 2009. Lagoon depth was about 2.9 m during the first collection period and 3 m during June. Details of meteorological measurements and energy budget calculations have been given previously (Loughrin et al. 2011).

2.2 DNA Extraction and PCR Amplification

DNA was extracted from swine lagoon slurry samples (0.5 mL) in triplicate for each sampling day using the FastDNA® Spin kit for soils (MP Biomedical, Solon, OH, USA) according to manufacturer's specifications. Quantitative, real-time PCR (qPCR) using specific PCR primer sets were used to target the 16S rRNA gene of the total microbial population, the Clostridia/*Eubacteria* (CE) group, or the *Bacteroides*/*Prevotella*/*Porphyromonas* (BPP) group in the swine lagoon as previously described (Cook et al. 2010). Concentrations of methanogens were analyzed by targeting the highly conserved methyl coenzyme M reductase (MCR; mcrA) gene using primers and conditions as described by Hales et al. (1996). One to 10 ng of DNA extract was used as PCR template with the appropriate primer set (with 800 nM of each primer). Total and BPP groups were targeted in TaqMan-based qPCR assays using the Qiagen HotStarTaq® Master Mix (Qiagen, Valencia, CA) and primers and probes as previously described (Cook et al. 2010). The amplification mixture contained 3.0 mM $MgCl_2$, 600 nM each primer, 200 nM of probe and sample DNA or standard (from 10^2 to 10^8 copies). Cell concentrations were calculated by dividing the copy number per milliliter of slurry by 4.0 or 6.0, the average copy number of 16S rRNA genes in all cells or in *Bacteroides* cells, respectively (Klappenbach et al. 2000). qPCR analysis of the CE and for methanogens were carried out in a QuantiTect™ SYBR® Green (Qiagen, Valencia, CA) PCR reaction. The amplification mixture contained 800 nM each primer, sample DNA or standard (10^1 to 10^8 copies). Since the SYBR Green dye binds to all double-stranded DNA, a melting curve was performed after the reaction to ensure proper amplification had occurred. The melting curve parameters included temperatures ranging from 50 °C to 90 °C, with reads every 0.2 °C after a 1-s hold. qPCR assays were run on the DNA Engine Opticon 2 (MJ Research, Inc., Waltham, MA) in a total volume of 25 μL. Baseline values were set as the lowest fluorescence signal measured in the well over all cycles. The baseline was subtracted from all values, and the threshold was set to one standard deviation of the mean. All PCR runs included duplicates of standards and control reactions without template. Standard DNA consisted of plasmid PCR 2.1 vector (Invitrogen, Carlsbad, CA) carrying the appropriate insert for the given assay.

2.3 Wastewater and Air Analyses

Details of wastewater collections were given in Loughrin et al. (2010). Wastewater quality analyses and malodorant analyses are given in Loughrin et al. (2011).

3 Results and Discussion

3.1 Lagoon Energy Budget and Modeled Emissions

Table 1 presents average weather conditions during the cool (February and March) and warm (June) seasons. Average air temperature increased from 7.6 °C to 24.2 °C with a related increase in absolute humidity. The lagoon also underwent considerable warming between the two periods with sludge increasing from an average of 10.3° to 28.4°.

As a consequence of the lagoon warming, heat storage into the lagoon (*G*) was strongest during March and April (Fig. 1). During both months, lagoon heating was highest about midmorning approaching

Table 1 Average weather conditions during the cool and warm seasons

Parameter	Cool season	Warm season
Average solar radiation (W m^{-2})	131.1	240.8
Daily cumulative precipitation (mm)	2.1	4.5
Average absolute humidity (g m^{-3})	6.1	17.3
Average air temperature (°C) (maximum, minimum)	7.6 (25.6, −13.5)	24.2 (34.0, 9.0)
Average lagoon surface temperature (°C) (maximum, minimum)	10.7 (31.6, −3.0)	29.5 (37.1, 24.8)
Average lagoon sludge temperature (°C) (maximum, minimum)	10.3 (17.1, 3.7)	28.4 (31.5, 24.6)

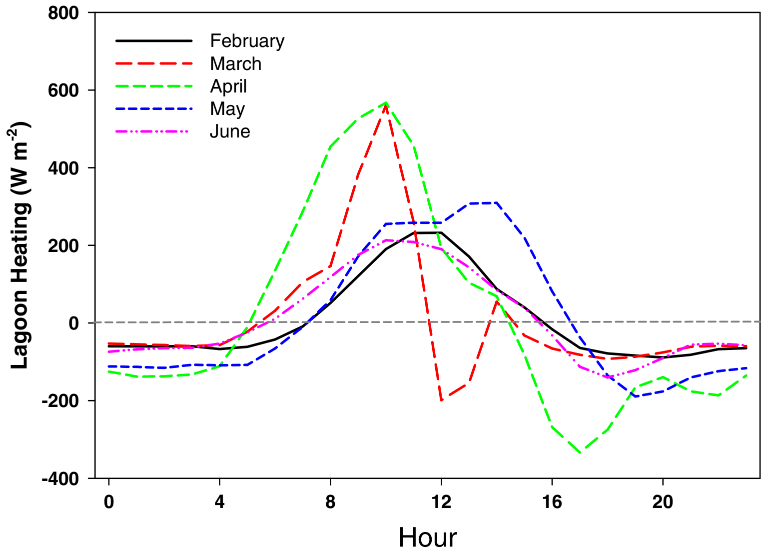


Fig. 1 Diurnal averages for lagoon heating from February through June

600 $W m^{-2}$. Heating did not approach these rates during May or June, since more heat was being lost from the lagoon in the form of evaporation (Fig. 2). During February, evaporative losses from the lagoon were relatively low with maxima about 0.5 mm h^{-1}, whereas during June evaporative losses peaked at about 1.3 mm h^{-1}. These values were similar to, if somewhat higher than, the values we obtained in an earlier study of the same lagoon (Quintanar et al. 2009). Evaporative losses exhibited strong diurnal behavior with the majority of evaporation occurring during the daylight hours through early evening.

Regardless of month, more energy was lost from the lagoon in the form of evaporation than due to sensible heating of the atmosphere as may be appreciated in Fig. 3 where the average daily Bowen ratio for each month is plotted. The average Bowen ratio was substantially higher in February than in the other months. As the atmosphere warmed and atmospheric water holding capacity thereby increased, Bowen ratios decreased as the lagoon lost progressively more energy through evaporation.

Previously, we found concentrations of the malodorant *p*-cresol 1.5 m above the lagoon and the transition

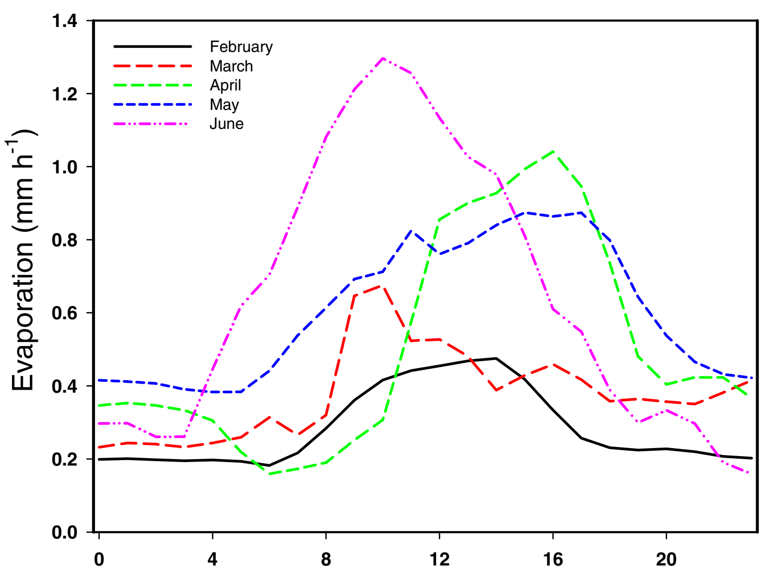


Fig. 2 Diurnal averages for evaporation from February through June

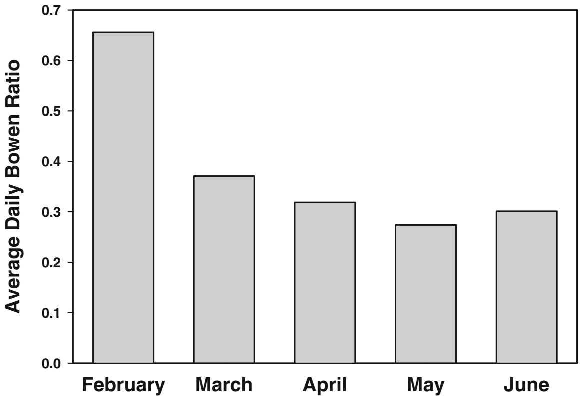


Fig. 3 Average daily Bowen ratio from the lagoon from February through June

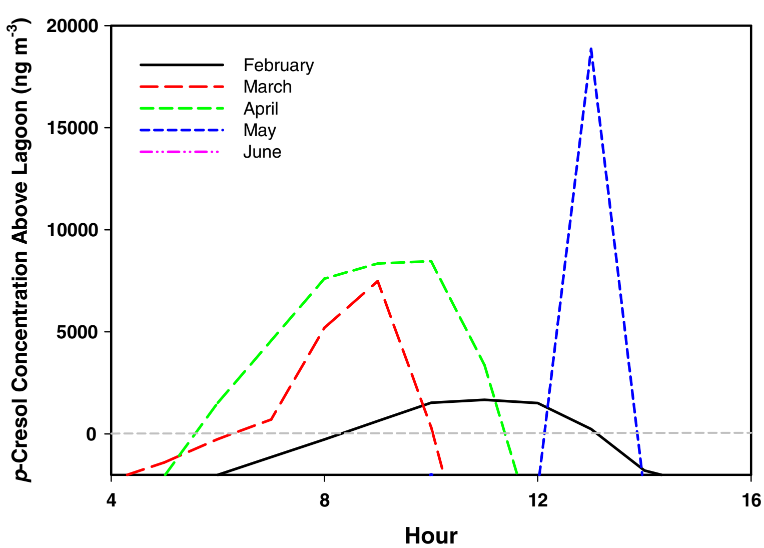
from cool to warm season could be described by the equation:

$$\text{Concentration} = -166.4 + (-1,212 \times \text{evaporation}) + (22.0 \times R_n) \quad (1)$$

where R_n equals net radiation at the lagoon surface. This is an empirical model based on observations that evaporative losses from the lagoon increased as the weather turned warmer resulting in the negative coefficient for the evaporation term. As the season progressed, however, the R_n term began to dominate. The model consequently predicted the highest emission of malodorants during the months of April and May with no emissions during the month of June (Fig. 4). This agreed relatively well with actual air measurements above the lagoon surface in which *p*-cresol concentrations 1.5 m above the lagoon averaged 10,700 ng m^{-3} during the cool season and 680 ng m^{-3} during the warm season with *p*-cresol concentrations reaching as low as 17 ng m^{-3}. Due to a spike in average R_n during the afternoon in May, the model predicted high emissions of malodorants during afternoons of this month. Although it is unlikely that such a pronounced difference in emission patterns existed between morning and afternoon hours, further sampling would be needed to confirm this.

There are numerous references to spring "turnover" of lagoons which are the cause of nuisance odor emissions (e.g., National Small Flows Clearinghouse 1997; Pfost and Fulhage 1999). This represents turnover in the sense that, as the lagoon warms up, microbial activity dramatically increases resulting in gas ebullition and considerable roiling of the lagoon surface, which may help volatilize malodorants. Therefore, at least anecdotally, it has been noted that malodorous emissions tend to be high during the spring months. There is a possibility, therefore, that the declines in wastewater concentrations of malodorants that we noted (Loughrin et al. 2011) were largely the result of evaporative losses as the lagoon warmed. On the other hand, it is possible that the strong lagoon heating that occurred during April and May in particular (Fig. 1) may have been sufficient to induce significant microbial activity and the degradation of malodorous compounds.

Fig. 4 Hourly predicted *p*-cresol concentrations at 1.5 m above the lagoon surface



3.2 Wastewater Quality

Table 2 presents data on wastewater quality. Wastewater was more alkaline during the warm season than the cool season, but the biggest differences were seen in ammonium and chemical oxygen demand (COD) concentrations which declined by about 35 and 58 percent, respectively. All other wastewater parameters were similar between the cool and warm seasons with only slight declines in the levels of inorganic ions such as sodium and phosphate from the cool to warm season. While the decrease in COD was doubtless a result of microbial degradation of organic matter, this decrease was not of the same order as noted for decreases in malodorant concentrations (Table 3) although it has been shown that wastewater strength as measured by COD correlates with malodors (Aitken and Okun 1992; Loughrin et al. 2006).

Ammonia losses from wastewater lagoons are well known to be due to evaporative losses which increase in warmer weather (Bajwa et al. 2006; Aneja et al. 2008). The seasonal decline in wastewater ammonium concentrations was not of the same magnitude as the declines in the concentrations of organic malodorants either, however (Table 3). The levels of aromatic malodorants declined by an average of over 90 % with *p*-cresol and indole declining by 95 and 97 %, respectively. VFA declined so that by the warm season, only propionate occurred in concentrations sufficient for quantification. We have previously noted rapid declines

Table 2 Composition of swine waste lagoon in cool and warm seasons

Parameter	Cool season	Warm season
pH	7.65±.09	7.87±0.07
Chemical oxygen demand (mg L^{-1})	5,240±3,030	2,190±1,060
Total suspended solids (mg L^{-1})	966±262	1,070±706
	Concentration (mM)	
Ammonium	51.8±5.6	33.6±7.9
Nitrate	[a]	0.064±0.08
Nitrite	0.281±0.497	0.040±0.077
Sulfate	0.130±0.05	0.134±0.085
Organic phosphate	1.41±0.28	0.46±0.29
Inorganic phosphate	2.48±0.070	1.70±0.29
Sodium	9.66±2.4	5.94±0.9
Calcium	2.94±0.3	1.67±0.3
Potassium	22.8±3.3	18.7±1.6
Manganese	0.011±0.003	0.006±0.002
Magnesium	0.421±0.15	0.363±0.07
Iron	0.104±0.03	0.064±0.007

Data represent the mean of eight determinations for cool season measurements and six determinations for warm season measurements ± standard deviation of the mean

[a] Occurred below level of quantification

Table 3 Malodorant concentrations in swine waste lagoon during cool and warm seasons

Parameter	Cool season	Warm season
	Aromatics concentration (μM)	
Phenol	43.2±21.8	3.92±2.3
p-Cresol	246±69.1	13.5±6.5
p-Ethylphenol	30.2±6.2	2.71±0.96
Indole	2.79±0.5	0.11±0.09
Skatole	8.24±2.2	0.74±0.4
	VFA concentration (mM)	
Acetate	23.2±7.27	[a]
Propionate	13.7±0.3	3.37±0.39
2-Methylpropionate	2.79±1.0	[a]
Butanoate	3.51±1.1	[a]
3-Methylbutanoate	8.79±3.1	[a]

Data represent the mean of eight determinations for cool season measurements and six determinations for warm season measurements ± standard deviation of the mean

[a] Occurred below level of quantification

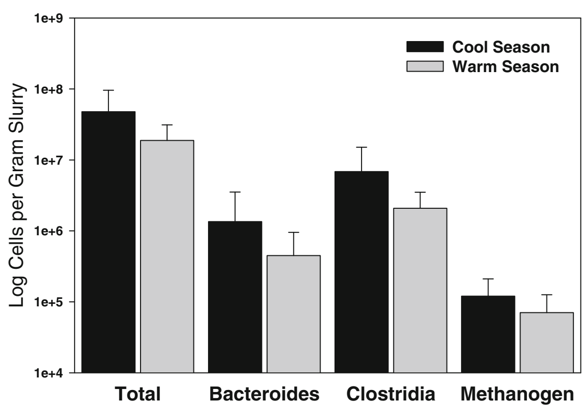


Fig. 5 Log10 of the concentration of bacterial cell numbers as determined by quantitative, real-time PCR (qPCR) targeting total cells (16S rRNA gene), Bacteroides, Clostridia, and Methanogen groups. Values are in cells per milliliter of slurry. *Error bars* represent the standard deviation of triplicate samples for each sampling data averaged for warm (n=11) and cool (n=7) seasons

in lagoon wastewater malodorant concentrations occurring during the spring months (Lovanh et al. 2009; Loughrin et al. 2010); the same period during which emissions from the lagoon undergo a dramatic, although short-term, increase. Again, from the limited amount of data available to us, whether evaporative losses of malodorous emissions during the spring months are of a magnitude sufficient to account for the wastewater malodorant declines is unknown.

3.3 Bacterial Populations

Although the concentrations of bacteria in both warm and cool seasons were variable, some trends were apparent (Fig. 5). In both the cool and warm seasons, Clostridia were the dominant portion of the bacterial population. From the cool season to the warm season, there was 60–70 % decrease in total bacterial, *Bacteroides* and Clostridia 16s RNA numbers, whereas there was no significant change in the population of methanogens. This is similar to what we found in an earlier study of the same lagoon except that we found no warm season decline in the population of *Bacteroides* (Cook et al. 2010).

Specific microbial groups and consortia have been suggested to be important for the degradation of malodorants in anaerobic lagoons. For instance, it is known that methane production from wastewater lagoons greatly increases during warm months (Wenke and Vogt 1981; DeSutter and Ham 2005), and methanogenic consortia are known to be capable of degrading malodorous compounds including substituted indoles (Gu and Berry 1991). In another instance, it is has been shown that purple sulfur bacteria can degrade malodorants and the timing of their blooms can be linked to declines in malodorant concentrations (Do et al 2003).

Other anaerobic bacteria are also metabolically versatile and, therefore, capable of degrading structurally diverse compounds including the malodorants characteristic of swine waste and structurally similar compounds. Clostridia, for instance, are capable of fermenting amino acids (Elsden and Hilton 1979; Fonknechten et al. 2010), completely mineralizing methoxylated aromatic compounds (Mechichi et al. 2005), and other groups such as *Bacteroides* are resistant to the antimicrobial effects of tannins and capable of degrading hydrolysable tannins and their phenolic monomers (Smith and Mackie 2004).

So, although there were no marked changes in bacterial populations in the lagoon between the cool and warm season, this does not necessarily reflective of microbial activity in the lagoon. It is likely that increased microbial activity that occurred as the lagoon warmed up was responsible for some of the decreases in malodorant concentrations.

4 Conclusions

We modeled emissions from a wastewater lagoon during a period in which the lagoon warmed, estimated emissions increased transiently, and biological activity presumably increased as wastewater strength declined. Improvements in wastewater malodorants were likely due to a combination of these two factors: mineralization and evaporative losses. Wastewater lagoons serve dual functions as reservoirs and waste treatment systems. In the latter case, however, they only effectively treat waste when ambient temperatures are sufficient to support significant biological activity. However, as the lagoon warmed, there was an unavoidable increase in malodorous emissions. This may be perhaps one of the biggest drawbacks of anaerobic treatment lagoons. As alternative means of waste handling become more widely available, improvements in air quality as well as other environmental benefits should ensue.

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