Denitrification Enzyme Activity in Swine Wastewater Lagoons

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Introduction

Anaerobic lagoons are typically used for treatment of swine wastewater. Recent reports of high levels of di-nitrogen emissions and high levels of potential surficial oxygen transfer indicated that large amounts of nitrogen may be removed via denitrification in these anaerobic lagoons (Harper et al., 2004; Ro and Hunt, 2006; and Ro et al., 2006). While specific lagoon characteristics vary, conditions are generally thought to be favorable for some level of denitrification. If classical denitrification is occurring in these lagoons, the denitrification enzyme levels should be correspondingly high. Our objective was to quantify denitrification enzyme activity (DEA) in several lagoons.

Methods

Denitrification enzyme activity was measured on eight commercial swine wastewater lagoons. Wastewater samples were taken at four quadrants of each lagoon from the surface, midway to the bottom, and just above the bottom of the lagoon. Samples (1000 ml) were collected with a subsurface grab sampler, stored on ice, and transported to the laboratory. DEA was measured by the acetylene inhibition method (Tiedje, 1994). Dissolved oxygen, ORP, pH, conductivity, and temperature were measured on site with an YSI multi-parameter pH/ORP meter (YSI Incorporated, 1700 Brannum Lane, Yellow Springs, OH, USA). Wastewater analyses were performed according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998).

Results

Lagoon characteristics: The lagoons provided storage/treatment of wastewater for swine production facilities with 1000 to 9200 head and had surface areas of 0.54 to 2.68 ha. The lagoons included both finishing farms and farrowing-to-finishing farms. The physiochemical characteristics of the lagoons varied from low to high nitrogen loads (109 to 631 mg N L^{-1}) and from relatively reduced to highly reduced environments (ORP= +6 to -398 mV). Nitrate-N concentrations were generally low in the lagoon wastewater (0.1 to 2.0 mg L^{-1}). All physiochemical characteristics were typical for lagoons within this region.

DEA: Analysis of variance (ANOVA) statistics indicated that there was a significant difference in DEA between lagoons, between water depths, and between actual and potential DEA (Table 1).

Table 1. Analysis of Variance for DEA in Swine Wastewater Lagoons

Source	D.F.	Mean Square	F Value	Pr>F
Lagoon	8	198281	69.7	<0.0001
Depth	2	23624	7.9	0.0235
Lagoon x Depth	16	2793	1.4	0.1762
Treatment	3	307058	188.8	<0.0001
Lagoon x Treat.	24	35859	20.7	<0.0001
Depth x Treat.	6	4746	10.8	<0.0001

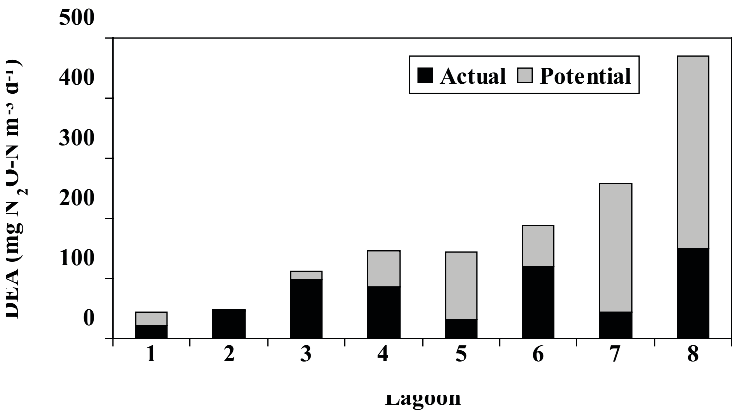
The depth at which wastewater samples were collected had a significant impact on DEA (Table 2). Wastewater samples collected near the lagoon bottom had higher actual and potential DEA than at the shallower depths. The higher DEA may have been due to a higher concentration of suspended solids and the associated microbial population in the depth just above the lagoon bottom.

Table 2. DEA as Impacted by Depth in Swine Wastewater Lagoons

Depth	Actual	Potential
	mg N_2O-N m^{-3} day^{-1}	
At Lagoon Surface	54	132
Midway to the Bottom	65	122
Above the Lagoon Bottom	73	189
$LSD_{0.05}$	16	28

Actual DEA ranged from 22 to 151 mg N_2O-N m^{-3} d^{-1} (Figure 1). With these levels of DEA, the amount of N being removed by denitrification in these lagoons would have been less than 3 kg N ha^{-1} d^{-1} (Figure 2). These levels of N removal are considerably lower than reported di-nitrogen emissions from lagoons (Harper et al., 2004). Furthermore, the potential for removal was very low (< 7 kg N ha^{-1} d^{-1}) when nitrates were provided (Figure 2). They are also lower than those projected to be possible with the potential surfical oxygen supply (Ro et al., 2006). One important question in evaluating these results is the determination of whether the method will detect high levels of DEA in swine wastewater. This question was resolved positively by the high levels of DEA found in the denitrification tank of a nitrification/ denitrification treatment unit in a swine wastewater treatment system; DEA was >55,000 mg N m^{-3} d^{-1} (Vanotti, 2004).

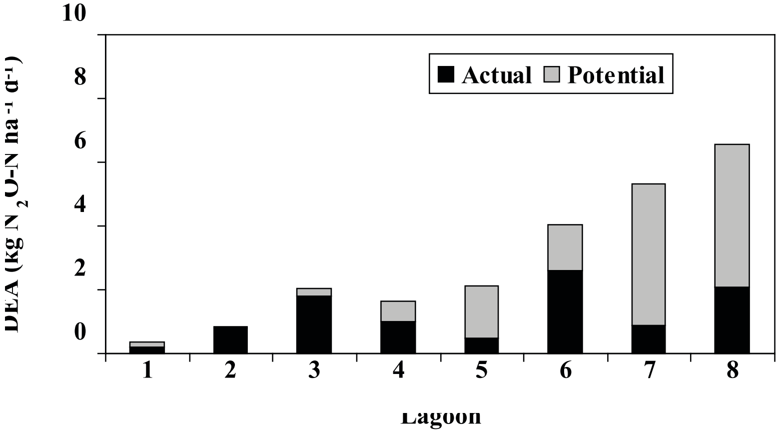
Figure 1. Actuali and Potential DEA (mg N_2O-N m^{-3} d^{-1}) of lagoons



Actual DEA appears to be influenced by the interaction between wastewater N concentrations and ORP. Lagoon 6 and 8, which had the highest actual DEA, had high N concentrations (>500 mg L^{-1}) with moderate ORP (-120 and -169mV). Generally, DEA was lowest in lagoons with ORP lower than -200 mV and/or N concentrations <500 mg L^{-1}.

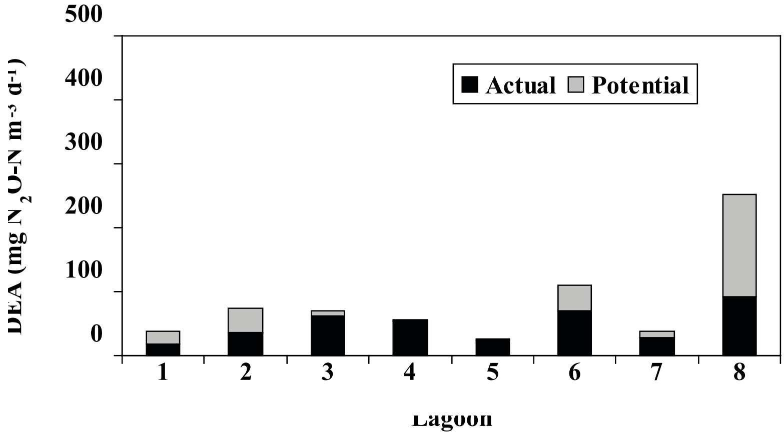
Potential DEA ranged from 24 to 470 mg N_2O-N m^{-3} d^{-1} (Figure 1). Even with an abundance of the nitrate substrate, denitrification was relatively low. The lagoons (7 and 8) with the highest potential DEA had ORP values of -132 to -169. Lagoon 2, which had a high ORP (+6 mV), did not have a higher denitrification potential.

Figure 2. Actual and Potential DEA (kg N_2O-N ha^{-1} d^{-1}) of Lagoons



Incomplete denitrification may be a source of nitrous oxide, a greenhouse gas. In these lagoons, actual and potential incomplete denitrification was relatively low (Figure 3). These results indicated that these lagoons would not be a significant emitter of nitrous oxide.

Figure 3. Actual and Potential Incomplete DEA of Lagoons



Relationship of lagoon characteristics to DEA: Stepwise regression analyses were performed on actual DEA (Table 3) to ascertain the contribution of each wastewater variable. Total N was the best correlated variable to actual DEA, but it only had a model R^2 = 0.455. None of the other wastewater variables were well correlated to actual DEA.

Table 3. Stepwise Regression for Actual DEA

Variable	Partial R^2	Model R^2	C(p)	Pr>F
Total N	0.455	0.455	19	<0.0001
NH_3-N	0.039	0.494	11	0.0027
NO_x-N	0.023	0.517	7	0.0172
ORP	0.024	0.541	3	0.013

Stepwise regression analyses were performed on potential DEA (Table 4) to ascertain the contribution of each wastewater variable. Total N and NO_x-N were the best correlated variable to potential DEA with a model $R^2 = 0.670$. None of the other wastewater variables were correlated to actual DEA.

Table 4. Stepwise Regression for Potential DEA

Variable	Partial R^2	Model R^2	C(p)	Pr>F
Total N	0.413	0.413	261	<0.0001
NO_x-N	0.257	0.670	97	<0.0001

Wastewater versus Sludge: In contrast to the lagoon wastewater the lagoon sludge had higher values of DEA (1008- vs 37-mg N_2O-N $m^{-3}day^{-1}$ for sludge and wastewater, respectively). This was particularly true when nitrate was added (3096-vs 48 mg N_2O-N $m^{-3}day^{-1}$). Thus, we have an increased interest in ascertaining a more complete understanding of the sludge DEA. Furthermore, we are currently conducting microbial community analyses.

Conclusions

- Although lagoons have significant surficial oxygen transfer to potentially produce precursors for denitrification, there was very little DEA measured in these eight commercial lagoons.
- The oxygen in these commercial lagoons may have been used to oxidize BOD.
- Also, the oxygen might have been used to form precursors that supported alternate nitrogen removal processes such as ANAMMOX.
- Further research needs to be conducted on DEA, enzyme activation, and microbial communities from lagoons in order to better understand the nitrogen cycling process of swine wastewater lagoons.

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