DENITRIFICATION ENZYME ACTIVITY IN A MARSH-POND-MARSH WETLAND USED FOR SWINE WASTEWATER TREATMENT AS INFLUENCED BY ALTERNATE WETTING AND DRYING CYCLES

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ABSTRACT

Constructed wetlands with continuous vegetative cover (marsh) have been documented to be very effective for denitrification of nitrogen in swine wastewater. Generally, the limiting factor for denitrification in such wetlands is slow formation of nitrate via nitrification because anaerobic soil conditions are prevalent and ammonia is the major nitrogen component in swine wastewater. It has been postulated that the shallow pond section of constructed wetlands with marsh-pond-marsh (M-P-M) designs would promote nitrate formation and subsequently denitrification. However, experimental results have shown the marsh-pond-marsh wetlands to be less effective for removal of nitrogen. Thus, we investigated enhancing soil aeration and the associated nitrification by short interruptions of wastewater application. The procedure was a one week drying cycle followed by two, three, or four weeks of wastewater applications vs. continual application. The study was conducted in 2002 at North Carolina A&T State University on marsh-pond-marsh wetlands with flat bottoms and cattails vegetation in the marsh sections. We measured soil redox, nitrogen treatment efficiency, and denitrification enzyme activity. Soil redox condition was more oxidized in the 2:1 cycle, but the conditions were not sufficient to promote higher treatment efficiency. However, there were highly significant increases in DEA values from addition of nitrate. Furthermore, when nitrate was added, there was a good linear correlation between percentage of time in the drying cycle and the level of denitrification. Effective use of a pond section in constructed wetlands will likely require altered design.

INTRODUCTION

Constructed wetlands with continuous vegetative cover (marsh) have been documented to be very effective for denitrification of nitrogen in swine wastewater (Cathcart et al., 1994; McCaskey et al., 1994; Knight et al., 2000; Hunt et al., 2002). Generally, the limiting factor for denitrification in such wetlands is formation of nitrate via nitrification because 1) anaerobic soil conditions are prevalent and 2) ammonia is the major nitrogen component in swine wastewater (Hunt et al., 2003). It has been postulated that the shallow pond section of a M-P-M design would promote nitrate formation and subsequently denitrification (Cathcart et al., 1994). However, experimental results have shown that marsh-pond-marsh wetlands are less effective for removal of nitrogen (Poach et al., 2004). It seems that the lack of slope and the deep water in the M-P-M wetlands may be limiting the needed precursor-nitrification. Therefore, we investigated enhancing soil aeration and the associated nitrification by short interruptions of wastewater application.

MATERIALS AND METHODS

The study was conducted at the swine production facility on the campus of North Carolina A&T State University in Greensboro, North Carolina, USA. There were four marsh-pond-marsh constructed wetlands. The marsh sections (1 and 2) were vegetated with cattails (*Typha latigo-*

lia L.). Swine wastewater was applied from an anaerobic lagoon via gravity flow at the rate of 20 kg N ha^{-1} d^{-1}. In the first constructed wetland, swine wastewater was applied continuously throughout the summer (0% of the time in the dry cycle). In the second constructed wetland, swine wastewater was applied four of every five weeks during the summer (20% of the time in the dry cycle). In the third constructed wetland, swine wastewater was applied three of every four weeks during the summer (25% of the time in the dry cycle). In the fourth constructed wetland, swine wastewater was applied two of every three weeks during the summer (33% of the time in the dry cycle). The redox potential was measured with platinum electrodes. Soil samples were collected from the 0 to 2.5-cm depth of the two marshes in all four constructed wetlands. Denitrification enzyme activity was determined by the acetylene blockage technique (Tiedje, 1994). Field moist soil (10-15 g) was placed in 60 ml serum bottles (five bottles per sample). Each bottle received one of the following amendments: 1) 5 ml of chloramphenicol (1 g L^{-1}) to inhibit protein synthesis; 2) 5 ml of chloramphenicol with nitrate-N (200 mg NO_3-N L^{-1}); 3) 5 ml of chloramphenicol with glucose (2 g glucose-C L^{-1}); or 4) 5 ml of chloramphenicol with nitrate-N (200 mg NO_3-N L^{-1}) and glucose (2 g glucose-C L^{-1}). Bottles were capped with rubber septa, evacuated, and purged with nitrogen gas three times. Fifteen cc of acetylene was inserted into four bottles with a syringe. The fifth bottle, which also received amendment 4, did not receive any acetylene. The bottles were incubated on a horizontal shaker at 90 rpm. Samples of the headspace gases were removed after 1, 5, and 24 hours with a syringe (Becton Dickinson Plastipak syringe with slip tip needle) and placed in vials (borosilicate glass, crimp top with butyl septum). A Varian Model 3600 CX gas chromatography (Palo Alto, CA) with a 15-mCi^{63}Ni electron capture detector operating at 350^{o}C was used for measuring N_2O in the gas samples. A 1.8-m by 2-mm ID stainless steel column packed with Poropak Q (80-100 mesh) was used to separate CO_2, N_2O, and C_2H_2. The column and injector temperatures were 70^{o}C. Samples were injected into the column by a Varian 8200 auto-sampler.

RESUSTS AND DISCUSSION

With the flow continuously on, the soil was very reduced with redox potentials generally < 0 mV. When the drying cycle was increased to 33% of the time, redox potential was somewhat

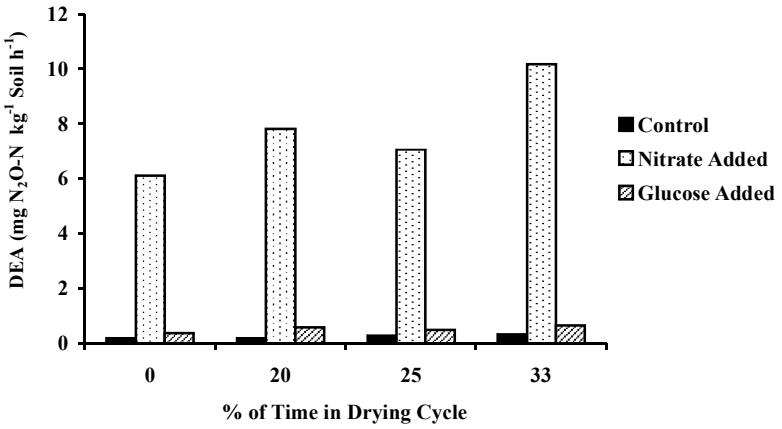

Figure 1. *Denitrification enzyme activity in Marsh 1 with different drying cycles and amendments.*

oxidized in the drying cycle. In this cycle, denitrification enzyme activity (DEA) in marsh 1 was slightly higher in the control treatment (no amendment, Fig 1). With the addition of glucose, DEA increased for all dry/wet cycles. With the addition of nitrate, the DEA was even higher for the wetland. These results indicate a microbial population with a higher denitrification activity. Even with 33% of the time in the drying cycle, DEA in marsh 2 was not significantly influenced in either the control treatment or glucose-amended treatments (Fig 2). However with the addition of nitrates, DEA in marsh 2 responded similarly to marsh 1. Furthermore, there was a good correlation between the percent of time the wetlands were in the drying cycle versus DEA with the addition of nitrate-N for marsh 1 (Fig. 3). None of the wet/dry cycles were adequate to significantly improve soil redox potential and the associated nitrification and denitrification. This was likely related to their fairly flat slope. However, the results from the wetlands that were in the drying cycle for 33% of the time indicate that this approach might be promising if there was more slope for rapid drainage.

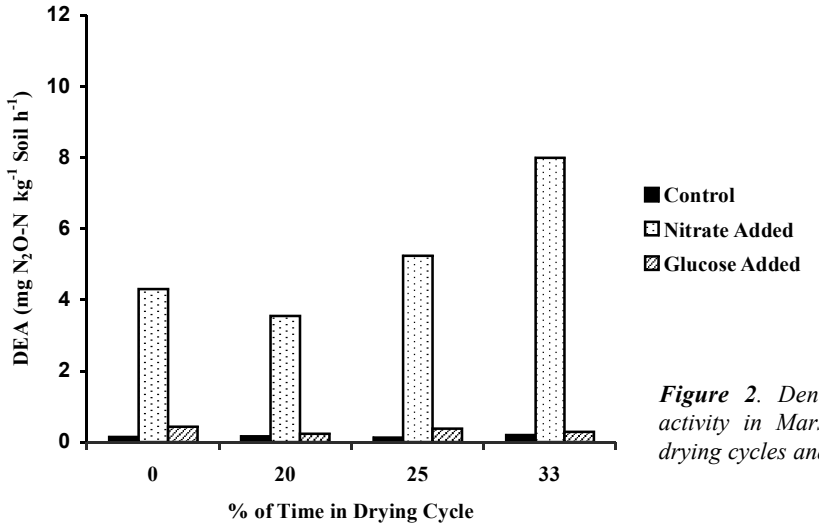

***Figure 2**. Denitrification enzyme activity in Marsh 2 with different drying cycles and amendments.*

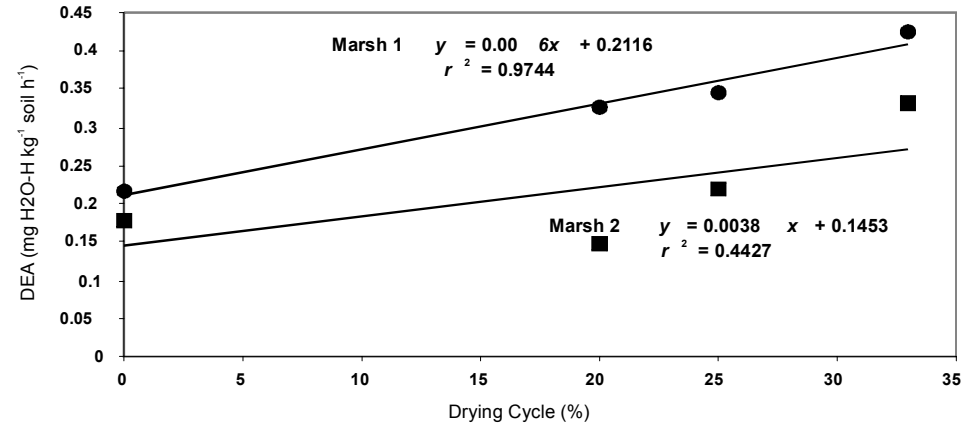

***Figure 3**. Regression of denitrification enzyme activity with time in drying cycle.*

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