

# Removal of pathogenic and indicator bacteria from dairy wastewater using an ecological treatment

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## Abstract

In the quest to improve the sustainability of water treatment options, plant-based systems, such as wetlands and ecological treatment systems, have become a promising alternative. To date, most of the research on ecological treatment systems has focused on removing excessive nutrients, turbidity and BOD from wastewater. However, wastewater is the primary source of fecal contamination in aquatic ecosystems; therefore another factor key to making these systems successful is ensuring that they remove pathogens. This study assessed the ability of an ecological treatment system to remove total coliforms and *E. coli* from dairy wastewater.

Total coliform and *E. coli* data was collected from the ecological treatment system located on Waterman Farm (WETS) at The Ohio State University. A three phase dosing experiment was conducted during the summer and early fall of 2005 to assess the system's ability to remove pathogens from wastewater. Wastewater was diluted with wellwater at a ratio of 1:3 during the month of July, in August the ratio of wastewater concentration to wellwater was increased to 1:1, and in September increased to 2:1. Regardless of wastewater concentration, total coliform concentrations were consistently reduced by at least 95% from influent to effluent of the WETS, with over 60% of the reduction occurring in the first two reactors. Pathogen concentrations were negatively correlated with DO and NO<sub>3</sub> concentrations and with TSS concentrations. These results indicate that ecological treatment systems have the potential to successfully remove pathogens from wastewater.

## Introduction

In the quest to improve the sustainability of water treatment options, plant-based systems, such as wetlands and ecological treatment systems, are a promising alternative. Traditionally, agricultural wastewater has been stored in lagoons, which can overflow or fail with drastic consequences to water quality (Innes 2000). As a result of lagoon failures and application of manure to crop fields, nutrients from animal wastes are the second most common cause of water pollution leading to reductions in dissolved oxygen and contributing bacteria that are harmful to humans and fish populations (USEPA 1993). Alternatively, ecological treatment systems, which rely on renewable resources, and combine anaerobic reactors, vegetated reactors, and wetlands, could be used to treat agricultural wastewater. Ecological treatment systems have successfully treated municipal and industrial effluents with reduced costs compared to conventional methods (Austin 2000). Nutrients, solids and BOD also have been effectively removed from liquid manure wastewater using ecological treatment systems (Lansing and Martin, in press). To date, most of the research on ecological treatment systems has focused on the ability of these systems to remove excess nutrients, turbidity and biochemical oxygen demand from wastewater. However, another key factor to making these systems successful is ensuring their ability to remove pathogens present in wastewater.

## Specific Objective

- The specific objectives of this study were to determine the bacterial pathogen removal efficiency of an ecological treatment system for dairy wastewater and to identify the primary removal mechanisms.
- We hypothesized that total coliforms and *E. coli* in the wastewater would be significantly reduced between the influent and effluent of the ecological treatment system, with the majority of the removal occurring as result of sedimentation in the first clarifier.

## Ecological Treatment System Description

The ecological treatment system is located at Waterman Farm (WETS) (40° N, 83° W), a working dairy farm, on The Ohio State University campus in Columbus, Ohio. The WETS is housed in a 9.1 m x 10.36 m polyhouse and consists of two identical treatment lines, each receiving waste from a 7.57 m<sup>3</sup> dosing tank. Dairy wastewater is collected from two feeding barns and the milking parlor and stored in a belowground wastewater reservoir. Wastewater is pumped from the reservoir to the WETS dosing tank, from which it is pumped into the polyhouse and distributed to two identical treatment lines. After exiting the WETS, a third pump returns the treated water to the wastewater reservoir.

Each treatment line is designed in the following manner: one 0.587 m<sup>3</sup> anaerobic reactor, one anoxic reactor (0.416 m<sup>3</sup>), one closed aerobic reactor (0.416 m<sup>3</sup>), one open aerobic reactor (0.416 m<sup>3</sup>), one clarifier (0.586 m<sup>3</sup>), one subsurface flow gravel wetland (1.2 m x 0.6 m x 1.2 m) (length x width x depth), two aerobic reactors (0.34 m<sup>3</sup>), one clarifier (0.34 m<sup>3</sup>), and two subsurface flow gravel wetlands (1.2 m x 0.6 m x 0.3 m) (length x width x depth) (Fig. 1). Two feedback lines return wastewater from the first clarifier to the anaerobic reactor and from the second clarifier to the second aerobic reactor, for further treatment. Wastewater is pumped into the system at a rate of 0.35 m<sup>3</sup> day<sup>-1</sup> line<sup>-1</sup>.

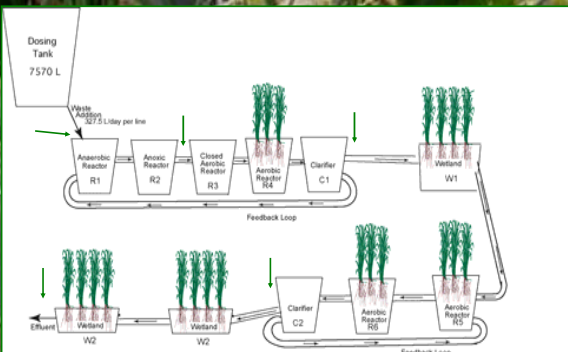


Fig. 1 Layout of a single treatment line in the WETS. Green arrows indicate pathogen sample locations.

## Methods

An experiment was conducted over the 2005 summer to assess the capacity of the WETS to remove pathogens from wastewater at three increasing concentrations.

- July 1 part wastewater to 3 parts well water
- August 1:1
- September 2:1
- Samples were serially diluted down to 10<sup>-7</sup> and then spread plated on (Fig. 2):
  - MacConkey agar for total coliform identification
  - MacConkey agar containing 4-methylumbelliferyl-beta-D-glucuronide (MUG) for *E. coli* identification
- Plates were incubated for 18-24 hrs at 37 C, following which colony counts were made.
- Total coliforms were identified as pink colonies (Fig. 3)
- E. coli* colonies on MUG plates were identified by fluorescence under a UV lamp (Fig. 4)



Fig. 2 Spread plating on MacConkey agar.



Fig. 3 Total coliform colonies.



Fig. 4 Mug agar fluorescence of *E. coli*.

Water quality monitoring:

- Dissolved oxygen (DO) and temperature were monitored weekly using a handheld YSI probe.
- Samples were collected from influent and effluent twice a week and from internal reactors once per dosing regime.
  - Vacuum filtered onto a 0.45 um filter for total suspended solids (TSS) analysis
  - Filtered samples analyzed for NO<sub>3</sub> on a Flow Injection Analyzer

## Results and Discussion

- Over 60% of the pathogen reduction occurred in the first two reactors (Fig 5 and 6).
- Total coliform and *E. coli* did not significantly differ at a particular location, from one dosing regime to the next, implying that the system treated wastewater at a constant rate and was able to handle the increased wastewater concentration.
- Although reductions were substantial, USEPA discharge requirements for *E. coli* of 126 cfu 100 ml<sup>-1</sup> were not met.

- Pathogen concentration was significantly positively correlated with TSS during the 1:1 and 2:1 WW concentrations (Fig. 7).
- About 25% of pathogens in wastewater treatment system effluent are hypothesized to be attached to suspended matter > 3-5 um (George et al. 2002).
- Therefore, sedimentation in anaerobic and clarifier reactors is an important pathogen removal mechanism.

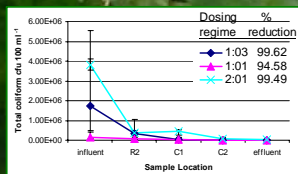


Fig. 5 Total coliform reductions for each dosing regime.

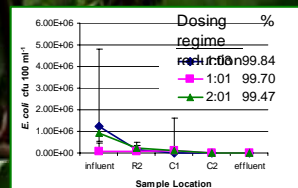


Fig. 6 *E. coli* reductions for each dosing regime.

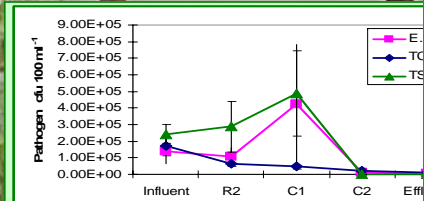


Fig. 7 Correlation between average TSS and pathogen reduction

- DO was negatively correlated with TC and EC (Fig. 8)
- Studies have found declines in pathogens with elevated DO
- Correlation between DO and TC was sig. during 1:3 dosing regime (p = 0.000)
- Correlation between DO and EC was always significant (p-value = 0.041, and 2:1 dosing regimes (p = 0.000)
- Enteric pathogens are facultative anaerobic bacteria that can use terminal electron acceptor.
- Nitrate concentration in animal wastewater is generally low.
- During the 1:3 and 2:1 dosing regimes NO<sub>3</sub> and DO concentrations in wastewater were very low, and pathogen concentrations were dramatically reduced in anoxic

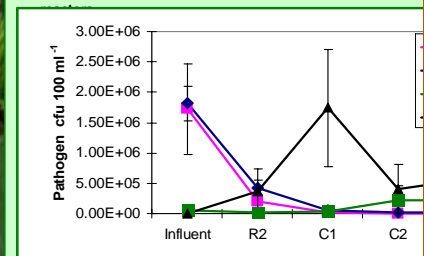


Fig. 8 Change in average NO<sub>3</sub>, DO and pathogen concentration

## Conclusions

- The WETS achieved substantial reductions in pathogen concentration regardless of dairy wastewater concentration.
- Majority of the reduction occurred in the first two reactors, and respectively.
- While reductions were substantial, discharge requirements of 126 cfu ml<sup>-1</sup> were not met.
- Improvements in TSS removal may increase pathogen removal to meet discharge requirements.

## Acknowledgements

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