Pathogen Removal by Agricultural Constructed Wetlands in Cold Climates

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ABSTRACT. Constructed treatment wetlands have been found to remove fecal coliform (FC) through a variety of mechanisms. This research evaluated the removal of FC in both warm and cold seasons from surface flow treatment wetlands in Bible Hill, Nova Scotia. Two wetlands (100 m\textsuperscript{2}), of differing depths were monitored over a 17 month period. The wetlands were loaded with dairy wastewater (average inlet FC concentration of 7438 CFU) at a rate of 2.2×10\textsuperscript{7} CFU ha\textsuperscript{-1}d\textsuperscript{-1}. Weekly samples were collected at the wetland inlet and outlet for FC using the MPN method. Removal rates and mass reductions ranged from 96.8 to 99.7% over the entire monitoring period. Fecal coliform discharge levels were below guidelines for recreation (< 200 CFU 100 mL\textsuperscript{-1}) and irrigation (< 100 CFU 100 mL\textsuperscript{-1}) purposes the majority of the time, indicating that removal of FC in these systems was sufficient in both warm and cold seasons, even when ice conditions existed.

Keywords: Agricultural wastewater, cold climates, constructed wetlands, pathogen removal, surface flow wetlands

1. Introduction

Fecal coliforms (FC) are the most commonly used indicator bacteria to quantify health risks from waters. Because of this, the presence of FC often leads to boil orders for drinking water, beach closures, and shellfish bans, adversely impacting communities from a human health perspective, as well an economic one (Perkel, 2002). The presence of FC bacteria in aquatic systems indicates a high probability that water has recently been contaminated with fecal material from warm blooded species (Hammer, 1992). Often agricultural sources, such as runoff from manure storage, runoff from field applied manure, and contributions from livestock located in close proximity to waterways, contribute to the presence of FC in aquatic systems (Hammer, 1992; Kadlec and Knight, 1996).

The survival, fate and distribution of microorganisms in wetlands depend on the type of wetland and the associated phenomena that influence their death, losses and growth (Khatiwada and Polprasert, 1999). Constructed wetlands are effective in removing bacteria and viruses from wastewater (Duncan and Groffman, 1994; Ottova et al., 1997; Khatiwada and Polprasert, 1999). They act as biofilters through a combination of physical, chemical and biological processes (Hammer, 1992; Kadlec and Knight, 1996; Werker et al., 2002). In wetlands, FC attach to suspended solids that are then trapped by vegetation (Hemond and Benoit, 1988). Quantifying such processes however, have been difficult when FC are used as indicators because birds and mammals living in and adjacent to wetlands also contribute to loading (Hemond and Benoit, 1988; Werker et al., 2002).

Wastewater is a hostile environment for pathogenic organisms and factors such as natural die-off, temperature, ultraviolet radiation, unfavourable water chemistry, predation and sedimentation cause pathogen populations to be reduced (Tanner et al., 1995; Ottova et al., 1997). These conditions often exist in wetland environments and help to purify water, especially during warm periods. Such conditions reduce eutrophication and pollution of aquatic systems and even waterborne diseases such as typhoid fever, bacterial gastroenteritis and hepatitis A (Tanner et al., 1995; Ottova et al., 1997). The factors which affect the reduction of FC in wetlands are however, less understood.

Since FC is an indicator of fecal contamination in water systems, federal and provincial guidelines have been established in relation to water-use. The Canadian Council of Ministers of the Environment have recommended a FC limit for body-contact recreation to be < 200 CFU’s per 100 mL, < 100 CFU’s per 100 mL for irrigation and 0 CFU per 100 mL for drinking water (CCME, 1999).

Although wetlands have been found to reduce FC levels to safe dischargeable levels, (Duncan and Groffman, 1994; Ottova et al., 1997; Khatiwada and Polprasert, 1999), limited information exists for cold climates. Before they can be promoted as effective FC treatment systems for northern climates, the survivability of these organisms must be closely examined. Although they have shown promise in warmer regions with removal rates ranging from 70 to 99% (Cronk, 1996), it is uncertain whether they can provide effective FC treatment throughout the year in regions where temperatures fall below

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0°C. For this reason, the objective of this study was to (i) evaluate the removal of FC from two surface flow constructed wetlands in Atlantic Canada for a 17 month period (November 2000 to March 2002) and (ii) to evaluate FC discharge levels in relation to Canadian water quality guidelines.

2. Methodology

Two surface flow wetland systems (100 m²) were established in the spring of 2000 at the Nova Scotia Agricultural College in Bible Hill, Nova Scotia, Canada (Figure 1). Each single celled wetland contained two deep and shallow zones. The shallow zones were 0.15 m deep, while the deep zones were 1 m. The shallow zones were covered with 0.30 m of loamy sand topsoil to act as a bed for cattails (*Typha latifolia*). Cattails were only planted in the shallow zones at 2 cattails m⁻². Cattail density in both wetlands during the first growing season (2000) was 7 cattails every m². The cattail density increased during the second growing season (2001) with a density of 10 cattails every m² in both W1 and W2.

The wetlands were loaded with fresh water after construction in May 2000 and were allowed to stabilize until November 2000. At this time dairy wastewater (manure and milkhouse washwater-based) was loaded into each wetland at a rate of $2.2 \times 10^7$ CFU ha⁻¹d⁻¹ (0.3 m³ d⁻¹ flow rate, Table 1). Wastewater characteristics entering the systems are provided in Table 2. The wastewater was equally distributed to each wetland by gravity from holding tanks through a sampling hut (Figure 1), where inflow rates were measured using a calibrated tipping bucket and recorded using a CR10X datalogger (Campbell Scientific, Edmonton, AB). The wetlands were managed differently. The first wetland remained at a constant water depth (0.15 m) during the winter months, while the water level in W2 was altered during the time of freezing to achieve an insulating effect and prevent total ice formation throughout the winter.

### Table 1. Average Daily Concentrations and Loading Rates Cumulated for Each Wetland System

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>FC (CFU 100 mL⁻¹)</th>
<th>Flow (m³ d⁻¹)</th>
<th>Average Daily Load (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet</td>
<td>7438</td>
<td>0.299</td>
<td>$2.2 \times 10^7$</td>
</tr>
<tr>
<td>Wetland 1</td>
<td>20</td>
<td>0.420</td>
<td>$8.6 \times 10^4$</td>
</tr>
<tr>
<td>Wetland 2</td>
<td>23</td>
<td>0.540</td>
<td>$12.7 \times 10^4$</td>
</tr>
</tbody>
</table>

Isco Model 6700 Portable Autosamplers were used to collect multiple composite wastewater samples for the 17 month (November 2000 through March 2002) monitoring period at both the inlet and outlets. Outlet sampling frequency depended on the prevailing weather conditions. For example, if the winter was extremely cold for long periods of time there was little to no outflow, therefore fewer samples were collected. Generally, samples were collected on a weekly basis. Samples were collected and sent to the lab for analysis the same day they were obtained. Samples (inlet and outlet) were analyzed for FC using the MPN procedure outlined in Standard Methods for the Examination of Water and Wastewater.
(APHA, 1998). The measurement of fecal coliform is usually expressed as the number of colony-forming units (CFU’s) per 100 mL of water (APHA, 1998).

Table 2. Characteristics of Dairy Wastewater Entering the Wetlands

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wastewater characteristics (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.8</td>
</tr>
<tr>
<td>BOD₅</td>
<td>1491.4</td>
</tr>
<tr>
<td>TSS</td>
<td>716.0</td>
</tr>
<tr>
<td>TKN</td>
<td>172.9</td>
</tr>
<tr>
<td>NO₃-N</td>
<td>2.4</td>
</tr>
<tr>
<td>NH₃-N</td>
<td>147.1</td>
</tr>
<tr>
<td>TP</td>
<td>44.4</td>
</tr>
<tr>
<td>SRP</td>
<td>39.0</td>
</tr>
<tr>
<td>FC</td>
<td>7438</td>
</tr>
</tbody>
</table>

The percentage (%) removal was calculated as follows:

\[
\text{Removal} = \frac{\bar{C}_{\text{in}} - \bar{C}_{\text{out}}}{\bar{C}_{\text{in}}} \times 100\% \tag{1}
\]

where \( \bar{C}_{\text{in}} \) = average inlet concentration (CFU per 100 mL), and \( \bar{C}_{\text{out}} \) = average outflow concentration (CFU per 100 mL). The % mass reduction (CFU) was also determined by:

\[
\text{Mass Reduction} = \left( \frac{\sum Q_{\text{in}}(\bar{C}_{\text{in}})}{\sum Q_{\text{out}}(\bar{C}_{\text{out}})} \right) \times 100\% \tag{2}
\]

where \( \bar{C}_{\text{in}} \) = average monthly inflow concentration (CFU per 100 mL), \( \bar{C}_{\text{out}} \) = average monthly outflow concentration (CFU per 100 mL), \( Q_{\text{in}} \) = sum of the monthly flow volume into the wetland (L), and \( Q_{\text{out}} \) = sum of the monthly outflow volume out of the wetland (L).

3. Results and Discussion

The average loading rate for the 17 month monitoring period was 2.2 × 10⁷ CFU ha⁻¹ d⁻¹ (Table 1). Average FC concentrations entering the wetlands were 7438 CFU 100 mL⁻¹, with substantial variability (Figure 2). Outlet concentrations were 20 and 23 CFU 100 mL⁻¹ for W1 and W2, respectively (Table 2). Removal rates of 99.7% were achieved for both systems over the entire monitoring period (Figure 2).

Similar FC removals (99%) were found for five wetlands in the Czech Republic (Ottova et al., 1997). Removals also compared with those for dairy wastewater treatment from a subsurface flow wetland in East Germany (95.8%) (Kern et al., 2000). For surface flow wetlands, FC removal rates have been found to range from 84 to 94%, from June through September (Kern et al., 2000). When winter (December 22 - March 20) and non-winter periods were separated removal rates were still high for both periods. Both wetlands achieved average removal rates of > 98% for both periods (Table 2). These results suggest that season and managing W2 did not affect FC removals and that these systems were capable of high FC treatment in both warm and cold periods. In the present research, W1 and W2 had similar retention times (95 d) (Smith et al., 2005) and vegetation status and both demonstrated aerobic conditions (data not shown). As a result, similar removals were achieved for both systems (Figure 2), indicating that wetlands are capable of FC removal even during winter conditions (Table 3).

Werker et al. (2002) suggested that subsurface flow wetlands may have an advantage in colder climates, because treatment occurs below the surface, therefore helping to insulate indigenous bacterial populations from the frigid air conditions making them more readily abundant to aid in treatment processes. In the present investigation removals exceeded 99%, indicating that surface flow wetlands can also effectively remove FC even with temperatures < 0°C. Werker et al. (2002) indicated that some studies have determined that wetland plants exhibit little to no effect on FC removal. Others have found that plants provide a higher rate of FC removal compared to unplanted beds (Kadlec and Knight, 1996; Werker et al., 2002).

Inflow and outflow volumes play a key role in determining mass removals in wetlands and provide an estimate of treatment performance independent of dilution effects from precipitation. Mass loads for FC are provided in Figure 2. The 17 month FC mass reductions were 99.1% and 98.8% for W1 and W2, respectively. When examining winter and non-winter periods, mass reductions were still high for both wetlands. Wetland 1 had a mass reduction of 97.1% during the winter and 98.1% during the non-winter period. Wetland 2 had similar reductions for both winter and non-winter at 98.1% and 98.6%, respectively (Table 3).

Table 3. Removal (%) and Mass Reductions (%) for Wetland 1 (W1) and Wetland 2 (W2) for Both Winter and Non-Winter Periods (Data from the 17 month Monitoring Period of November 2000 to March 2002)

<table>
<thead>
<tr>
<th>Period</th>
<th>W1</th>
<th>W2</th>
<th>W1</th>
<th>W2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal (%)</td>
<td>99.6 ± 3.3</td>
<td>99.7 ± 3.4</td>
<td>97.1 ± 4.1</td>
<td>98.1 ± 3.3</td>
</tr>
<tr>
<td>Mass Reduction (%)</td>
<td>99.7 ± 3.3</td>
<td>99.8 ± 2.1</td>
<td>98.1 ± 3.2</td>
<td>98.6 ± 3.4</td>
</tr>
</tbody>
</table>

A number of factors may influence FC removal in wetlands. For example, the rate of effluent flow, die-off, rate of removal by filtration and sedimentation, rate of addition from animal sources, and the rate of predation (Kadlec and Knight, 1996; Perkins and Hunter, 2000). Many of these factors however, may be interrelated. For instance, effluent flow rate may potentially influence contact time with soil and vegetation and, in turn influence the opportunity for predation, filtration and sedimentation (Perkins and Hunter, 2000). In the present study overall outflow rates were higher than inflow rates (Table 1). This can be attributed to heavy rainfall, as well as...
snow melt events. Perkins and Hunter (2000) reported that FC removals declined at times of heavy effluent flow (following heavy rainfall). It seems as though this may have been the case in this investigation. This can be demonstrated by the increased FC outlet concentration in the months following the April 2001 and January 2002 tracer studies that were conducted on these wetlands as an additional study (Figure 2) (Smith, 2002). It was during these times that the inflow rate into each system increased from 0.3 to 1.0 m$^3$d$^{-1}$. At this time retention time for W1 and W2 were 15 and 18 d, respectively.

A more detailed explanation and examination of the tracer studies and retention times for these wetland systems can be found in Smith et al. (2005).

Although, predation, filtration and sedimentation were not the direct focus of this investigation, it appears by the high removal rates and CFU reductions that they all did at some point play a major role in FC removal in both wetlands. It is conceivable that the plants and soil in each wetland may have increased bacterial removal by providing a larger surface area for bacterial entrapment as wastewater flowed through.
Results demonstrated that FC removals were high in both the warm and cold seasons. Effluent FC levels met recreational guidelines 94% and 95.5% of the time in W1 and W2, respectively (Table 4). Effluent water was also suitable for irrigation 94% and 93.3% of the time, for W1 and W2, respectively (Table 4). It should be noted that one of the goals of an agricultural wetland is to reduce FC to recommended dischargeable levels, not necessarily to meet Canadian Drinking Water Quality Guidelines. In this case, the detection limit for FC using the MPN method (APHA, 1998) was < 2 CFU 100 mL⁻¹, making it difficult to see if the treated water reached the Canadian Drinking Water Quality Guideline of 0 CFU 100 mL⁻¹. Outflow FC levels however, were < 2 CFU 100 mL⁻¹ 80 and 64% of the time in W1 and W2, respectively (Table 4).

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>#2 CFU 100 mL⁻¹</th>
<th>&lt; 100 CFU 100 mL⁻¹</th>
<th>&lt; 200 CFU 100 mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet</td>
<td>2.2</td>
<td>8.9</td>
<td>15.5</td>
</tr>
<tr>
<td>Wetland 1</td>
<td>80.0</td>
<td>94.0</td>
<td>94.0</td>
</tr>
<tr>
<td>Wetland 2</td>
<td>64.0</td>
<td>93.3</td>
<td>95.5</td>
</tr>
</tbody>
</table>

It would be useful to compare these FC loading rates to other agricultural wastewater treatment systems. The lack of published literature in this area makes it difficult to put in perspective these loadings with other agricultural wastewater treatment and drainage systems. Due to this, it is not possible to compare such inflow and outflow loading rates. However, when comparing removal rates and mass reductions to other studies these wetlands act favorably to other agricultural systems, even under Atlantic Canada’s cold winter season.

4. Conclusions

Constructed wetlands are an effective method for reducing FC in both warm and cold seasons, even when ice conditions have been found to exist. High removals and mass reductions ranging from 97.1% to 99.8% demonstrate the potential that these small-scale systems have when used for agricultural wastewater treatment. Water discharged from the wetlands typically met recreational, irrigation and animal watering purposes. A need still exists however, for further research effort into the overall dynamics of FC removal in a constructed wetland system. While advancement in constructed wetland design has been made in the last few decades, there are still gaps in understanding how these systems can achieve sustained levels of water quality improvement, especially with regards to pathogen removal.

References