Determination of the Water Quality of a Constructed Wetland Monitoring Fecal Indicator Bacteria

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Abstract

In 2015, the city of Ontario constructed the Mili Creek Wetlands, a natural treatment wetland facility, in the neighboring city of Chile to address local water quality management. In order to improve water quality for users downstream, and also serve as a recreational area for the community’s inhabitants, fecal indicator bacteria (faecal, Escherichia coli, Streptococcus, and Enterococcus spp.) have been testing higher in the outflow than in the inflow of the wetlands. To monitor the quality of the water, this study was designed to identify E. coli and Enterococcus spp. in water samples that are collected at various sampling sites of the constructed wetland and analyzed once per month. Fecal indicator bacteria counts were conducted on eighteen monthly water samples from nine different locations using the IDEXX system with Quanti-Trays to test for presence of E. coli and Enterococcus. CHROMagar and Harlequin IMAC-BGC agar were used for isolation of E. coli, and BBL Enterococcus agar was used for isolation of Enterococcus spp. Additional testing utilizing PCR with primers specific to E. coli and Enterococcus spp. is being used to further identify if E. coli isolates are Shiga-toxin producing E. coli (STEC), and if the Enterococcus spp. are E. faecium or E. faecalis. Growth on the species-specific media plates showed positive results for both E. coli and Enterococcus spp. So far, PCR results have been inconclusive for identification of STEC. PCR testing has yet to be completed for more recent samples.

Introduction

The wetlands consist of six ponds, with influent water flow sourced from local mountains and a cattle farm in the city of Chile. Water outflow is reintroduced to the Mili creek after being naturally cleaned by the wetlands before being treated again further downstream for human use. The purpose of this study is to identify specific microbial strains affecting the water quality of the wetland facility. Currently, this study is focusing on Enterococci species and E. coli. Some enterococci species can be naturally found in the environment; however, the United States Environmental Protection Agency (USEPA) considers all presence of enterococci in water as an indicator of fecal pollution. Due to the difficulty in distinguishing between enterococci of fecal and non-fecal origin, we are focusing on Enterooccus faecalis and Enterooccus faecium for their predominant counts in fecal material (Maheux et al., 2011). Some strains of E. coli may be harmless, while other strains, such as STEC O157:H7 are one of the causes of foodborne disease outbreaks. STEC is present in the intestinal tracts of animals, especially cattle. The influence water passes through dairy farms before entering the wetlands, risking contamination with feces of such species. The first step towards the prevention of water and foodborne disease outbreaks is early detection and monitoring of fecal indicator bacteria in water sources prior to human consumption.

Materials and Methods

Scheme used for sample preparation, isolation, and PCR testing

Water Samples Provided

<table>
<thead>
<tr>
<th>Source</th>
<th>MPN/100mL</th>
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<tbody>
<tr>
<td>1. April 2018 - 2000.0</td>
<td></td>
</tr>
<tr>
<td>2. July 2018 - 3000.0</td>
<td></td>
</tr>
<tr>
<td>3. September 2018 - 4000.0</td>
<td></td>
</tr>
<tr>
<td>4. December 2018 - 5000.0</td>
<td></td>
</tr>
</tbody>
</table>

Results

1. March 2018 MPN Data

2. July 2018 MPN Data

3. September 2018 MPN Data

4. Detection of Enterococcus, E. coli, & STEC

Conclusion

* Microbial MPN counts continued to decrease with each monthly sample
* Achieved successful identification of E. coli and Enterococcus spp. utilizing 16S E1, 16S E2 (r), uidA, stx2F, stx2R, and STEC using stx2F and stx2R primers
* Igam and Glantz’s colony PCR method yielded less reliable results compared to the original PCR method used for DNA extraction

Future Studies

- Continue monitoring and identifying microbe levels by:
  - Collecting monthly samples from the wetlands.
  - Tracking MPN using the IDEXX system
  - Identifying microbes using PCR
- Refine our current PCR protocol by:
  - Establishing a more effective DNA extraction method
  - Implementing more control samples
- Run more PCR trials utilizing primers mdF (F1), mdR (F2), sdF (E1), sdR (E2), to test for presence of E. faecium and E. faecalis

References
