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 Mill Creek Wetlands, a natural treatment wetland facility, in the neighboring city of Chino to address local water quality management, improve water quality for users downstream, and also serve as a recreational area for the community's habitants. Fecal indicator bacteria (coliforms, *Escherichia coli*, *Streptococcus*, and *Enterococcus* spp.) have been testing higher in the outflow than 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 bacterial 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 SMAC-BCIG agar were used for isolation of *E. coli*, and BBL Enterococcosel 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 the *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 Chino. Water outflow is reintroduced to the Mill 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, but the United States Environmental Protection Agency (USEPA) considers all presence of enterococci in water as a an indicator of fecal pollution. Due to the difficulty in distinguishing between enterococci of fecal and non-fecal origin, we are focusing on *Enterococcus faecalis* and *Enterococcus 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 influent 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

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IDEXX Quanti-Tray system for mean probable numbers (MPN)/100 ml)

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Isolated *Enterococcus* & *E. coli* spp.

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PCR analysis using primers specific for *Enterococcus* spp. and *E. coli* spp.

↓

Gel Electrophoresis

Results

1. March 2018 MPN Data

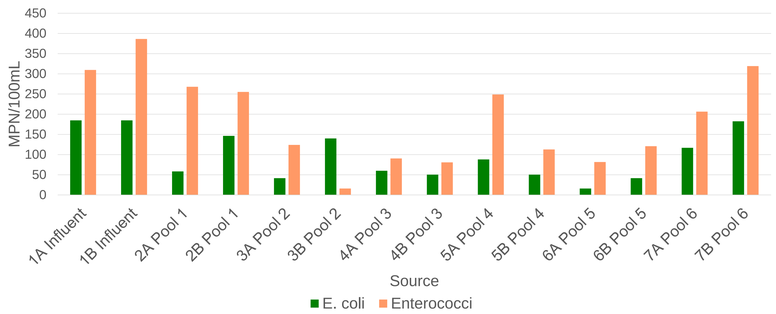

FIGURE 1. ***First data collection from the wetlands. MPN data is shown for the first 14 locations tested; Control Point and Outflow locations were not tested in the first trial***

2. July 2018 MPN Data

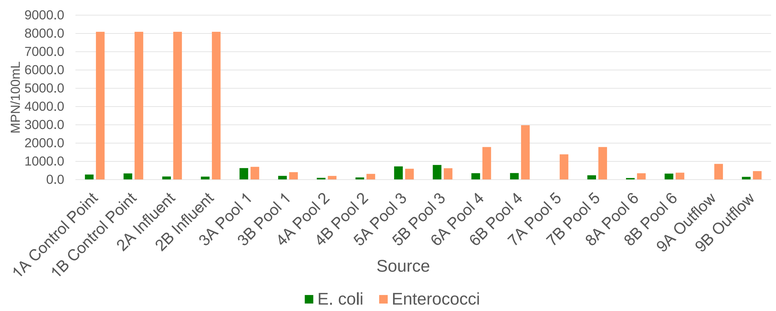

FIGURE 2. ***Data collection shown after a four-month progression into the project. MPN data is shown for all water collection points.***

3. September 2018 MPN Data

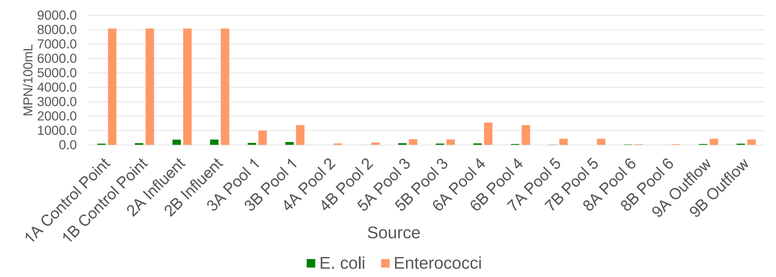

FIGURE 3. ***Latest data collection shown six-months after the start of the project. MPN data is shown for all water collection points.***

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

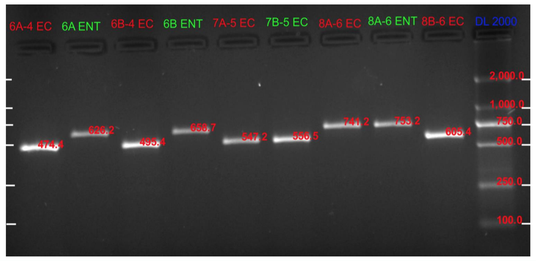

FIGURE 4: ***Detection of E.coli and Enterococcus spp. utilizing primers targeting the 16S and uidA gene sequence.***

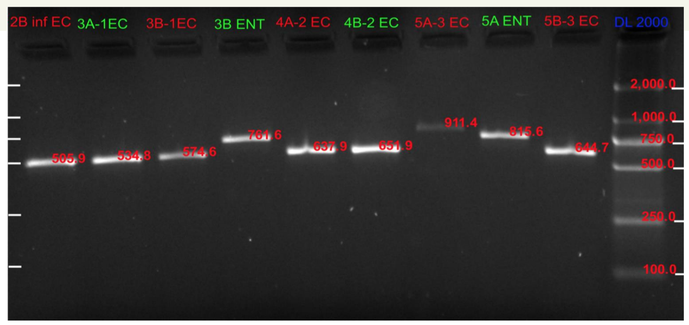

FIGURE 5:

Detection of E.coli and Enterococcus spp. using primers targeting the 16S and uidA gene sequences.

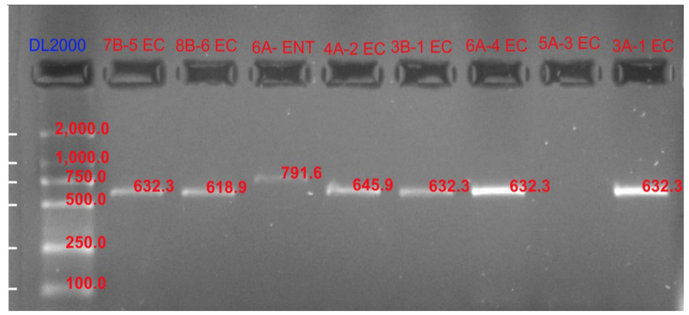

FIGURE 6:

Detection of STEC targeting stx genes

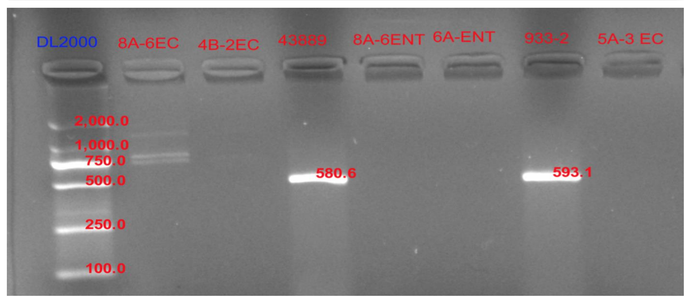

FIGURE 7:

Detection of stx2 genes. Positive controls STEC 933-2 and 43889.

Conclusion

- Microbial MPN counts continued to decrease with each monthly sample
- Achieved successful identification of *E. coli* and *Enterococcus* spp. utilizing 16SE1, 16SE2 (r), *uid*298F, *uid*884R, and STEC using *stx1*-F and *stx1*-R primers
- Igem 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

Gronewold, A., Borsuk, M., Wolpert, R., & Reckhow, K. (2008). An Assessment of Fecal Indicator Bacteria-Based Water Quality Standards. *Environmental Science & Technology*, 42(13), 4676 4682.

Maheux, Bissonnette, Boissinot, Bernier, Huppé, Bérubé, Bergeron. (2011). Method for rapid and sensitive detection of Enterococcus sp. and Enterococcus faecalis/faecium cells in potable water samples. *Water Research, 45*(6), 2342-2354.

Igem, P., & Glantz, S. (2013). Http://ljournal.ru/wp-content/uploads/2017/03/a-2017-023.pdf. *Colony PCR*. doi:10.18411/a-2017-023