Escherichia coli control in a surface flow treatment wetland

M. E. MacIntyre, B. G. Warner and R. M. Slawson

ABSTRACT

A field experiment showed that numbers of *Escherichia coli* declined significantly when floating *Lemna* spp. plants were removed to create open water areas in a typical newly constructed surface flow treatment wetland in southern Ontario. It is suggested that *E. coli* declined immediately after *Lemna* removal because the *Lemna* was shading the water column from penetration by natural UV radiation, it was providing favourable attachment sites for the *E. coli*, and it was not allowing effective free exchange of oxygen from surface winds to the water column to maintain high enough dissolved oxygen supplies for predator zooplankton populations. Operators of wetland systems must have the specialized skills required to recognize the cause and the appropriate maintenance requirements to maintain efficient operation of such unconventional systems should *E. coli* numbers increase during the course of operation.

Key words | Canada, *Escherichia coli*, Ontario, surface flow wetlands, wastewater treatment

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INTRODUCTION

Non-traditional biological treatment systems including wetlands are known to effectively remove enteric bacteria such as *Escherichia coli* from sewage waters (Karpiscak *et al.* 1996; Gerba *et al.* 1999; Perkins and Hunter 2000). Natural die-off, temperature shifts, unfavourable water chemistry, predation, sedimentation, and solar (UV) radiation are the primary mechanisms in wetland systems leading to removal of bacteria such as *E. coli* in the waste stream (Kadlec and Knight 1996). If any one or combination of these removal mechanisms is impaired for any reason, the wetland system will cease to effectively reduce bacterial numbers, and thereby fail to perform as expected.

During a detailed study aiming to characterize the performance of a newly constructed surface flow treatment wetland system in southern Ontario, numbers of *E. coli* were found to become unusually and unacceptably high towards mid-summer in year 2 of full operation, suggesting that natural removal mechanisms were not performing optimally. It was observed that growth of floating *Lemna minor* also greatly increased over the same time period. The dense growth of floating macrophytes has been found to impair quantities and composition of planktonic communities in the water column in natural wetlands (i.e. Cronk and Fennessy 2001). Therefore, it is possible that the *Lemna* was shading the water column in the treatment wetland system, cutting out natural UV penetration, and allowing *E. coli* to flourish. In an attempt to determine whether there was any relationship between *L. minor* and *E. coli* numbers, a field experiment was undertaken to identify whether *E. coli* numbers would change if *L. minor* was removed.

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MATERIAL AND METHODS

Study site

The study site is a surface flow or free-water surface flow treatment wetland located at the Ontario Power Generation Nanticoke Facility on the north-central shore of Lake Erie, Ontario (42°48′N, 80°04′W; 182 m a.s.l.). The wetland system was constructed in autumn 1999 and began operation in 2000. It was constructed to polish effluent

from facultative lagoons used for primary and secondary treatment of wastewater coming from washrooms, showers and the cafeteria kitchens at the facility.

The wetland system comprises two adjacent cells, each approximately 0.5 ha in area with a total working volume of about 3,000 m^3. Each of the two cells is about 0.3 m deep, and is interrupted by deep trenches (*c*. 42 m long, 7 m wide and 1.3 m deep) across the inflow, middle and outflow of each cell, which aids in distributing flow evenly across the entire wetland cell. The western cell was planted in *Scirpus acuminata* and *Typha latifolia*, and the eastern cell was planted in *T. latifolia* (Figure 1). Our study was confined to the eastern cell only. Our experiment was undertaken between 13 August and 4 September 2001.

Field and laboratory methods

A total of eight field-sampling stations were established across the eastern cell (Figure 1). Surface cover estimates of *Lemna* were made prior to beginning the experiment. Water samples for *E. coli* analysis were collected by inverting a Nalgene bottle below the water surface. Water was placed in pre-sterilized 250-ml Nalgene bottles. About 3 cm of headspace was left in each bottle, the capped bottle wiped with isopropyl alcohol, and placed on ice in a cooler for immediate transport to the laboratory. Water samples were analysed for *E. coli* and total coliforms within 24 hours of field collection using the Colilert test kit at the Kinectrics Laboratory, Toronto.

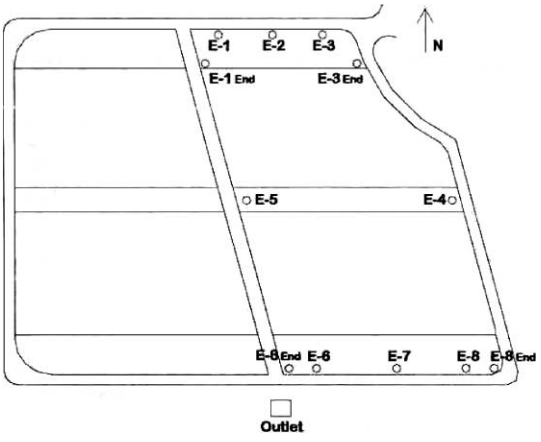

Figure 1 | Sketch of the wetland system at the study site showing the location of the deep zones. Stations E1, E2 and E3 are in the deep zone closest to the inflow, stations E4 and E5 are in the central deep zone and stations E6, E7 and E8 are in the deep zone closest to the outflow.

Heavy-duty, long-handled pool skimmers were then used to remove *Lemna* spp. and other floating debris (i.e. *Typha* leaves and roots, and some algae) in the deep zone closest to the inlet. It took two people nearly three full days to clear about 95% of the wetland water surface. Water samples for *E. coli* analysis were taken in early morning (prior to any *Lemna* removal) and late afternoon on day one (during the course of *Lemna* removal) and then early morning each day thereafter. The magnitude and practicality of the task involved to remove the *Lemna* and debris made it impossible to remove all *Lemna* across the whole wetland cell. Our experiment focused, therefore, on the first deep zone closest to the inlet where *Lemna* coverage was greatest.

RESULTS AND INTERPRETATION

Lemna coverage was greatest (100%) in the deep zone closest to the inlet. It was less dense across the middle deep zone with about 65% coverage, and about 45% coverage in the deep zone closest to the outlet (Figure 2). *Lemna* productivity was so thick nearest the inflow that wind was not an effective mechanism for moving the floating plants and debris to the edges. However, in the middle deep zone and the deep zone closest to the outlet where *Lemna* density was lower, wind continued to accumulate floating *Lemna* and debris in flotsam piles around the edges of the wetland, thereby contributing to more open water in these parts of the wetland.

Prior to manually removing any floating plants and debris (morning of 13 August 2001), *E. coli* numbers were

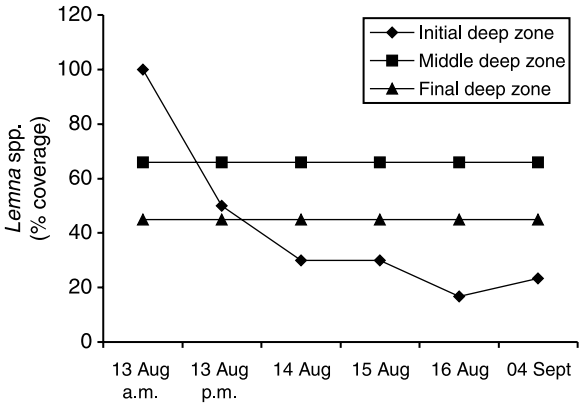

Figure 2 | Estimates for coverage of *Lemna* spp. of the open water areas of the three deep zones in the eastern cell of the study site.

found to be greatest closest to the inlet deep zone and were significantly less in the middle zone and only slightly less again close to the outlet (Figure 3). While removal of *Lemna* and debris continued for the next two days (14 and 15 August 2001), *E. coli* numbers did not change significantly in either the inlet deep zone and elsewhere in the wetland. When nearly all *Lemna* and debris had been removed from the inlet deep zone, *E. coli* numbers showed a decrease in numbers by 16 August and had dropped significantly within a week, by 4 September, while *E. coli* numbers remained largely unchanged elsewhere in the wetland.

Midday near-surface water temperatures during the period of the experiment averaged 26–27°C. Dissolved oxygen content was low (i.e. 0.5–0.6 mg l^{-1}) on the morning of 13 August prior to any *Lemna* removal, and had increased to values as high as 2.6 on 4 September.

DISCUSSION

The results of this field experiment confirm an apparent relationship between *Lemna* growth and *E. coli* numbers. *E. coli* numbers dropped significantly once *Lemna*, floating debris and some suspended particulate matter was removed. It appears the floating material is shading the water column and inhibiting natural UV radiation from reaching and killing suspended *E. coli*. UV radiation has been shown elsewhere

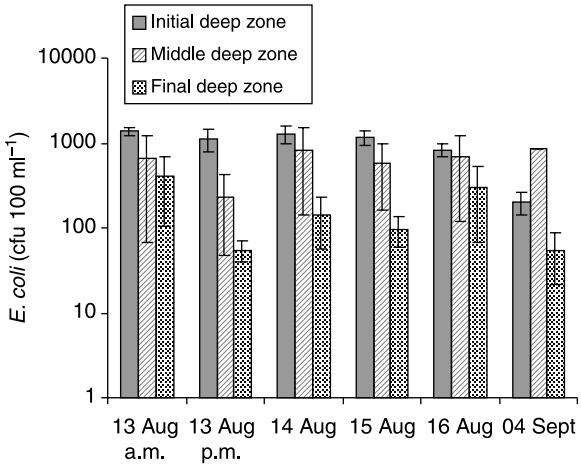

Figure 3 | *Escherichia coli* numbers (cfu 100 ml^{-1}) during the course of the experiment. Sampling on 13 August 2001 a.m. was prior to any removal of *Lemna*. Removal of *Lemna* was conducted on 13–15 August 2001. Numbers are averaged for each of the respective sections of the wetland cell. Error bars = 0.95 confidence intervals.

to contribute to bacterial mortality in water bodies (McCambridge and McMeekin 1981; Flint 1987). The floating material was probably serving as attachment sites for *E. coli* colonization and, once removed, this would be another contributory factor for the reduction in *E. coli* numbers.

As a facultative anaerobe, *E. coli* can thrive in waters both high and low in dissolved oxygen (DO). However, aerobic conditions with higher DO are less favourable for *E. coli* because predators such as zooplankton are better able to thrive in abundance and graze on *E. coli*. Our observed increase in DO values in the inlet zone after *Lemna* removal and values of DO as high as 13 mg l^{-1} in the middle and outlet deep zones show there is free exchange of oxygen by surface winds in open water areas. Our observations that the water column has higher DO values in the absence of *Lemna* than when it is covering the water surface in the wetland suggest that there are probably well-established zooplankton predator populations. Clearly, the presence of *Lemna* and other floating debris maintained hospitable conditions for *E. coli*. Our results show that so long as open water is maintained, even 20 days after removal of *Lemna* and other debris, conditions for *E. coli* remained less favourable; there was no significant decline in temperature in wetland waters over the same period to account for the low *E. coli* numbers.

The observations of this experiment generate some important lessons for effective operation and maintenance of such wetland systems. Unconventional operation and maintenance procedures are required for these unconventional treatment systems. It is important to ensure that open water areas exist and are maintained in surface flow wetland systems if they are to continue to effectively reduce *E. coli* and other pathogenic bacteria. Field maintenance staff must be trained to recognize and be prepared to incorporate such practices as regular clearance of floating macrophyte growth and other debris into maintenance routines to maintain high quality performance of such wetland treatment systems and to ensure the wetland systems remain in regulatory compliance. Wetlands are biological systems that change and such minor irregularities as unfavourably high *E. coli* populations should be expected if other biological components of the system are not held in check. Operators without the specialized skill set may become frustrated and be too quick to dismiss the whole

system as a failure, not thinking about the merits that led to the choice of adopting an unconventional treatment option offered by wetlands.

Other corrective measures other than simple *Lemna* control may be possible, such as altering flow rates and volumes in the system, or changing operation water levels. While these measures may be effective and suitable, one should be cautious because these hydrological alterations to the systems may have negative feedback, which could cause some other problem. Unusually high *E. coli* numbers is a biological phenomenon, and so biological tools should be the first option in trying to 'fix' the problem.

ACKNOWLEDGEMENTS

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