Mosquito Control and Pollutant Removal in Constructed Wetlands: Subsurface Flow Cells vs. Periodically Dry Surface Flow Cells

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Summary: Three groups of constructed wetland (CW) mesocosms (0.7 m$^2$ each) were operated treating dairy wastewater. Group 1 consisted of free water surface (FWS) cells filled with plants that had been established for three years, Group 2 consisted of newly established FWS cells, and Group 3 consisted of newly established subsurface flow (SSF) cells. The SSF cells were loaded continuously with wastewater, while the FWS cells were periodically dried to prevent emergence of adult mosquitoes associated with FWS systems. Total nitrogen, ammonia nitrogen, and chemical oxygen demand were monitored to compare pollutant removal among the groups. Preliminary data indicate that periodic drying of FWS CW systems successfully kills larvae and pupae, thereby preventing adult mosquito emergence, as long as there is no significant rainfall within the first two days of the drying phase. Preliminary data also indicate that nitrogen removal is significantly greater in Group 1 than in both other groups, suggesting an important role for dense vegetation in nitrogen removal mechanisms.

Keywords: Constructed wetland, mosquito control, treatment performance, dairy wastewater.

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C. R. Mayhew and D. R. Raman

ABSTRACT

Both subsurface flow (SSF) and free water surface (FWS) constructed wetland (CW) systems have been used for partial treatment of wastewater from animal production systems. SSF systems are considerably more expensive, especially with regard to capital costs. However, the less expensive FWS systems have tremendous mosquito production potential; a factor which cannot be ignored by design engineers, especially given increasing public concern about pathogen transmission from concentrated animal wastes.

One promising method of reducing mosquito production in FWS CW systems is periodic drying. By disrupting the development of larval mosquitoes, this strategy may prevent the emergence of adult mosquito species associated with FWS CW systems. To test the efficacy of this method, experiments were conducted on 12 CW mesocosms (0.7 m$^2$ surface area each), divided into three groups. Each group received dairy wastewater, pretreated by a high-rate anaerobic filter (AF), and all mesocosms were populated by cattail (Typha latifolia). Group 1 consisted of FWS cells filled with plants that had been established for three years, Group 2 consisted of newly established FWS cells, and Group 3 consisted of newly established SSF cells. The SSF cells were loaded continuously with wastewater, while the FWS cells received wastewater periodically: 7-d on, 7-d off. While receiving flow, each mesocosm was operated at a 7-d hydraulic retention time (HRT); two mesocosms in series thus had a net HRT of 14 d.

Emergence of adult mosquitoes from FWS cells was monitored during the drying phase. The removal rates of total nitrogen, ammonia nitrogen, and chemical oxygen demand were monitored in all cells, to compare pollutant removal between groups.

Preliminary data indicate that periodic drying of FWS CW systems successfully kills larvae and pupae, thereby preventing adult mosquito emergence, as long as there is no significant rainfall within the first two days of the drying phase. Preliminary data also indicate that nitrogen removal in Group 1 was significantly greater than in both other groups, suggesting an important role for dense vegetation in nitrogen removal mechanisms. COD removal was significantly greater in Group 1 than in Group 2, again suggesting that plant densities can affect pollutant removal in CW.

INTRODUCTION

Agricultural production, specifically livestock production, has evolved in such a way that there are fewer producers, larger average herd sizes, and a higher concentration of herds and production facilities on less land area (Ohio State University, 1992). While this evolution has certain benefits, it has also changed the distribution and characteristics of production byproducts, such as animal waste. For example, the confinement of dairy cattle has concentrated dairy waste. To avoid environmental problems, these concentrated wastes must be handled, treated, and recycled or disposed of appropriately.

With increasing pressure for ownership of environmental pollution and contaminants, the challenge facing many agricultural producers today is to install and operate a reliable, effective, and economical waste management system. One relatively new technology being applied to agricultural wastewater is that of constructed wetland (CW) systems (Ancell et al., 1998; Cheng et al., 1998; Moore et al., 1995).

Hammer (1993) defines constructed wetlands as consisting “of former terrestrial environments that have been modified to create poorly drained soils and wetlands flora and fauna for the primary purpose of contaminant or pollutant removal from wastewater.” This definition primarily serves to set constructed wetlands apart from natural, created, or restored wetlands. However, it should be noted that constructed wetlands have many of the same ancillary benefits and drawbacks associated with these other types of wetland.
In recent years, as more large scale CW systems have been built and brought online, it has become apparent that the potential for mosquito production – not the effectiveness of the waste treatment – may determine their feasibility (Mortenson, 1982; Martin and Eldridge, 1989; Stowell et al.; 1985). This is because wastewater in a CW, with its high nutrient concentrations, can serve as an excellent habitat for the immature stages of some mosquito species. To further complicate the problem, location of a CW near human, equine, and fowl species increases the chances of mosquito problems, both as disease vectors and as pests (Mortenson, 1982). The severity of the problem was described by Martin and Eldridge (1989), who found that five out of nine pilot CW plants built in California between 1974 and 1989 were taken out of operation due to mosquito problems. Stowell et al. (1985) named mosquito production problems as the major drawback of CW systems and called for further research in design, operation, and mosquito control measures as they relate to mosquito production.

One solution to the problem of mosquito production in CW systems is to use subsurface flow (SSF) instead of free water surface (FWS) cells. In SSF CW systems, wastewater flows through a porous media planted with aquatic macrophytes. The water remains below the level of the media, and SSF cells therefore lack the free water surface habitat required for mosquito larval and pupal development. In the case of domestic wastewater, SSF CW systems are reported to achieve the same treatment efficiencies in one-forth the space that a FWS CW would require (Tchobanoglous, 1997). However, this advantage is neutralized by the mean capital cost for SSF systems, which is four times as much per acre than that of FWS systems (Tchobanoglous, 1997). In agricultural applications, where producers frequently have ready access to earth-moving equipment, the cost differential between FWS and SSF systems may be even greater.

Various control methods have been attempted in suppressing mosquito production in FWS systems. The use of mosquito fish (Gambusia affinis) is frequently suggested (Kadlec and Knight, 1996; Knight, 1993; Martin and Eldridge, 1989; Hammer, 1993). However, use of mosquito fish is unsuccessful in habitats with excessively low dissolved oxygen (DO), where there are dense stands of emergent macrophytes restricting fish movement and sheltering larvae, or where hot weather elevates water temperatures or dries the cells (Kadlec and Knight, 1996; Knight, 1993; Martin and Eldridge, 1989; Hammer, 1993; Dill, 1989). Such conditions can frequently occur in CW systems treating agricultural wastewaters, where extensive primary and secondary treatment have typically not occurred prior to wetland treatment.

Larvaeicides, particularly the bacterial larvaeicides Bacillus thuringiensis israelensis H-14 (Bti) and Bacillus sphaericus (Bs), have been studied for their effects on mosquito populations in CW systems, but with limited success. For example, Tennesen (1993) reported disappointing results from insecticide treatments. Typically, mosquito larvaeicides are considered effective if a 95% or better reduction rate is achieved. However, the high mean larval densities associated with CW systems do not approach acceptable threshold densities even after a 95% reduction is achieved. Furthermore, owing to the adsorption of toxicants by organic matter, diluting and degrading action by the CW treatment process, and the short life cycle of mosquitoes, insecticides are not a satisfactory long-term control for mosquito production in treatment wetlands (Tennesen, 1993).

In contrast to the biological and chemical approaches mentioned above, some investigators have considered hydraulic means of limiting mosquito production. For example, Steiner and Freeman (1989) suggested that mosquito control may be one of the benefits of having a CW configuration of multiple parallel cells wherein individual cells are drained periodically and allowed to dry. To date, not much information is available regarding the long-term effectiveness of this method. Toward this end, we are investigating the long-term feasibility of periodically drying FWS CW systems for mosquito control, with the ultimate goal of determining the costs and benefits of such an operation. At the time of this writing, our experimental system has been operational for three months, thus, while preliminary data is reported here, a cost-benefit analysis has not yet been conducted.

In addition to mosquito control, periodic draining of FWS CW systems will presumably have an impact on pollutant removal. To address this issue, we are examining the pollutant removal performance of the FWS and SSF CW cells in our experimental system. Because of other ongoing research in our lab (Hawkins & Raman, 1999) we are interested in the feasibility of discharging effluent from a high-rate anaerobic digester to CW cells. We are particularly interested in the impact of an anaerobic effluent on plant health, and on nitrogen removal. Because nitrification rates limit denitrification rates, an aerobic pretreatment is generally advised to promote the oxidizing conditions needed by nitrification (Jenssen, et.al. 1997). The periodic draining of FWS CW cells might actually enhance oxygen availability and thus nitrification (Qiu and McComb, 1996).
MATERIALS AND METHODS

This study used outdoor laboratory-scale components consisting of a downflow anaerobic filter (DFAF), SSF CW cells, and FWS CW cells. Dairy wastewater was initially treated by the anaerobic filter and then pumped into the constructed wetlands for additional treatment.

Site Description
The DFAF/CW facility was located at the Tennessee Agricultural Experiment Station, Knoxville Station, on an easterly-facing slope with a grade of approximately 10%. The area is included in USDA climate zone 7 at roughly 35.97° latitude and –83.88° longitude. For safety and convenience, the experimental CW cells were situated on level 4 x 10-m pad, constructed from exterior grade lumber and gravel. A 2.5 x 3-m aluminum shed was constructed adjacent to the west edge of the pad to house the wastewater storage tank, the anaerobic digester, and electronic equipment. Temperature, precipitation, and solar radiation data from an existing weather data monitoring station situated approximately 30 m from the pad was used to characterize site atmospheric conditions.

Wastewater Source
Liquid dairy wastewater was obtained from a local dairy in Blount County, TN. A 1900-L tank trailer was used to collect liquid waste after it was screen-separated from solids. Obtaining wastewater in this way ensured a realistic wastestream, with components – including milk, cleaning solutions, and antibiotics – that an artificially generated wastewater would not have contained. Disadvantages of this approach were the natural treatment that occurred prior to waste collection, and the inherent variability of the feedstock. Once collected, the wastewater was stored at ambient temperature in a 750-L tank within the shed. Stored wastewater was transferred by submersible centrifugal pump to the DFAF.

Downflow Anaerobic Filter (DFAF)
The DFAF received wastewater from the storage tank within the shed. The reactor vessel was constructed of 30.5-cm (12 in.) diameter schedule 40 PVC pipe, with a liquid level height of 104-cm. The reactor was filled to a height of 94-cm with rock lava media (60% porosity) to serve as microbial attachment sites. At the design flow of 72 L d⁻¹ the DFAF operated on a 0.58 d hydraulic retention time (HRT).

DFAF effluent was routed to a sump where it accumulated until a level switch activated a submerged pump to drain the sump. (Accumulation time was approximately 1 h.) Sump effluent was routed to a flow divider that split the flow into four equal parts (aliquots). The four aliquots flowed by gravity into selected wetland mesocosms.

Constructed Wetlands
The 12 CW mesocosms used in this experiment had been used in past thesis projects and experiments at UTK (Benham, 1995; Bowling, 1996; Raman et al., 1997). They consisted of rectangular boxes constructed from exterior grade plywood with 5 x 10 cm (2” x 4”) bracing around the sides, reinforced and waterproofed with resin coated fiberglass. Interior dimensions were 150 x 47 x 60 cm giving each mesocosm cell a 0.7-m² surface area.

The 12 cells were divided into three groups consisting of four mesocosms each (Figure 1). Group 1 consisted of FWS CW mesocosms planted with cattails (Typhia latifolia). At the start of our experiment, these cells had been in use for three years receiving concentrated manure wastewater (average COD between 5,000–10,000 mg/L) on a weekly basis (Raman et al., 1997). Group 2 consisted of newly established FWS CW mesocosms, also planted with T. latifolia. Soil for Group 2 cells was obtained from the UTK Experiment Station Plant Science Farm along the bank of the Tennessee River at the same site where soil was collected when Group 1 was established. Cattails were transplanted into these cells in September 1998 by using 10 x 10-cm cuttings from colonies used in a previous study (Raman et al., 1997). The cuttings were planted on 15-cm centers resulting in approximately nine cuttings per CW mesocosm. Group 3 consisted of newly established SSF CW mesocosms with 2.5-cm river rock as the substrate and cattail transplants planted at the same time and in the same fashion as Group 2. All of the wetlands had a 15-cm PVC stand pipe for effluent collection, surrounded by a 5-cm layer of river rock.

The four cells in each group were divided into two sets of two cells in series (Figure 1). The FWS cells operated at 18-cm water depth, while the SSF cells operated at a 44.5-cm water depth (porosity = 0.40), giving each cell a 7-d HRT, and two cells in series a 14-d total HRT. Wastewater flowed along the long axis of each cell to the standpipe...
Figure 1. Diagram of experimental setup, showing all three groups of mesocosms. $P =$ pump, $DL =$ datalogger, $AF =$ anaerobic filter.

at the effluent end, where it was pumped (by a Teel Industrial Series epoxy-encapsulated pumps, Model 2P873A) into the next cell in the series, or into an effluent collection reservoir. Each pump had a floss filter over its inlet to act as a physical barrier preventing transfer of mosquito larvae between CW cells. Pumps were wired to three position switches. One switch setting provided continuous 120V power, the second setting provided power controlled by a datalogger (Model 21X, Cambell Scientific), allowing computer control of the pumps, and the third setting was off.

**Drying Schedule**

In each group of FWS cells one set was full while the other set was drying. When a set was to be drained, the operator routed tubing from the full cell to the dry cell next to it. Pumps were then manually activated to drain the wetland. Upon completion of draining, the pumps were switched back to computer-controlled operation, and the tubing was routed accordingly.
Mosquito Surveillance

Free surface wetlands were monitored for mosquito larvae and pupae using the dip method. A standard mosquito dip sampler (Clark Mosquito Control Products, Inc., Roselle, IL) was used to dip a water sample from the water surface of each full wetland. A standard operating procedure derived from techniques described by Service (1976) ensured consistency of sampling efforts between sampling events. Samples were taken to a laboratory and quantified by species, quantity of each instar (1$^{\text{st}}, 2^{\text{nd}}, 3^{\text{rd}},$ and $4^{\text{th}}$) within a species, and quantity of pupae.

Drained CW cells were monitored to capture adults that had emerged from pupae left to desiccate in the CW. Two emergence traps were placed into each of the drained mesocosms and checked daily for adults. Traditional floating emergence traps made from solid sheets of plastic (Service, 1976) were unsuitable for our work, because they would prevent ventilation of the soil surface. We therefore constructed and used a novel emergence trap (Figure 2), with a design based loosely on traditional floating emergence traps. Specifically, we employed a cylindrical PVC frame surrounded by screen mesh (Figure 2), to allow evaporation from the CW surface. A 1-L widemouth jar with an inverted funnel entry was attached at the top of the frame to direct flying mosquitoes into the collection container. When adults were collected, they were counted and identified by species. In addition, soil surface moisture (i.e. moist, moderately dry, very dry) was also noted whenever adults were observed.

**Figure 2.** Mosquito emergence trap designed to collect flying adults while allowing surface ventilation. Frame was constructed from PVC wrapped with screen mesh. A 1-L widemouth glass jar lid with 6-cm dia. hole was attached (inverted) to the top of the frame. An inverted funnel was fastened over the hole, and the glass jar was screwed into the attached lid.

The drying schedule was selected so that few, if any, mosquitoes emerged as adults. To prevent emergence, the wetland surface must have dried sufficiently to desiccate and kill larvae and pupae before the cell was refilled. Failure to kill the larvae and pupae during the drying period resulted in their continued development when the CW was refilled. In preliminary laboratory experiments, larvae and pupae survived >2 days when kept on a damp paper towel. Pupae were observed to emerge as adults up to two days after introduced to this condition. Therefore, we reasoned that a high-degree of drying of the wetland surface must occur to successfully control the adult mosquito production. It is unknown at this time how long, on average, a CW should remain empty to achieve reliable desiccation of mosquito larvae and pupae. This time will depend on factors such as evapotranspiration (ET), rainfall, wastewater hydraulic loading, and soil surface temperature. Since these parameters are generally quantifiable, it should be possible to develop an equation that would guide operators in selecting their drying intervals. The development of such an equation is one of the ultimate goals of this work, but is not addressed in this paper.
The principle mosquito species expected to be found in our mesocosms was the so-called “filth breeder” *Culex pipiens*. During the summer of 1998, larvae were collected from the established FWS CW cells, reared to adults and identified as *Cx. pipiens*. Additionally, adults collected from a standpipe in the spring of 1999 were identified as *Cx. pipiens*.

Rueda et al. (1990) reported temperature dependent development rates for *Culex* sp. to be 31.35, 12.07, 10.52, 7.07, 7.21, and 7.08 days for development from egg hatch to adult emergence at constant temperatures of 15, 20, 25, 27, 30, and 34 °C, respectively. Temperature dependent development rates for egg hatch to time to complete the 4th instar are 25.35, 9.53, 7.95, 5.38, and 5.31 days at constant temperatures of 15, 20, 25, 27, and 30 °C, respectively. A simple two-parameter model was fit to this data, to allow computation of time-to-develop (TD) from knowledge of temperature (T), as follows:

\[
TD = TD_{\text{max}} \left( e^{-k(T-15)} \right)
\]

where \(TD_{\text{max}}\) is maximum time to develop (d), \(k = 0.16 \, \text{°C}^{-1}\), and T is temperature (°C). We used equation (1) to estimate TD during summer months when H2O temperature varies cyclically from 15°C to 27°C, arriving at a value of TD = 7 d from time of egg hatch to completion of the 4th instar. Completion of the 4th instar was selected as the critical point for starting a drying phase due to observed hardiness of pupae in semi-moist conditions in preliminary laboratory experiments.

**Water Quality Monitoring**

A water quality monitoring schedule allowed assessment of the CW treatment efficiencies. Sample points include system influent, AF effluent, and each CW effluent. Grab samples were collected from the sample points on a routine schedule for laboratory testing. For the first three months, sampling events occurred twice weekly. Based on low variability between same-week samples, this schedule was changed to once each cycle for the FWS cells, and weekly for the SSF cells.

All samples were analyzed for total nitrogen (TN), chemical oxygen demand (COD), pH, temperature, and DO. TN and COD were analyzed using digestion-spectrophotometric methods (DR/2000 Direct Reading Spectrophotometer, HACH, Loveland, CO. Handheld DO and pH meters (Model HI 9142, Hanna Instruments Limited, Bedfordshire, UK, and Model 63, YSI, Yellow Springs, OH, respectively), were used to obtain DO and pH at the standpipe of each mesocosm. Type T thermocouples, placed in the first CW of each series, were used to record average hourly temperatures of the wastewater.

Results of water quality testing were used to compare pollutant removal between SSF and periodically dried FWS cells, between newly planted vs. established FWS cells, after 7-d and 14-d of treatment.

**RESULTS AND DISCUSSION**

**Mosquito Surveillance**

Dip sampling for mosquito larvae and pupae began in early spring (mid March, 1999). The first appearance of larvae occurred in mid-April. Results from the first trap-week were promising with no adult mosquitoes emerging from either of the FWS groups. However, during the second trap-week, flying adults were found in Group 2 cells (Table 1). These differences may be explained by the differences in rainfall during the two trap-weeks. During first trap-week, total rainfall was 1 cm, the majority of which fell on the sixth day of the drying phase. During the second trap-week, 3 cm of rain fell in the first day. Two days later, standing water in Group 2 cells was removed to enhance drying. (Interestingly, Group 1 cells were drier, probably because the denser vegetation caused higher ET in these cells.) Despite the draining, adults were collected in the Group 2 cells two days later. No additional adults were collected after this time.

The third trap-week was similar to the second trap-week, with 5 cm of rain within the first 24 h of the drying phase. Standing rainwater was removed one day later to enhance drying. Despite this effort, adults were collected in both Group 1 and Group 2 cells.
Table 1. Total number of adults observed in emergence traps during subsequent trap-weeks.

<table>
<thead>
<tr>
<th>ID</th>
<th>Trap Week 1</th>
<th>Trap Week 2</th>
<th>Trap Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trap 1</td>
<td>Trap 2</td>
<td>Trap 1</td>
</tr>
<tr>
<td>FWS 1 – 3 yr @ 7-d HRT</td>
<td>0</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>FWS 1 – 3 yr @ 14-d HRT</td>
<td>0</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>FWS 2 – 3 yr @ 7-d HRT</td>
<td>*</td>
<td>*</td>
<td>0</td>
</tr>
<tr>
<td>FWS 2 – 3 yr @ 14-d HRT</td>
<td>*</td>
<td>*</td>
<td>0</td>
</tr>
<tr>
<td>FWS 3 – New @ 7-d HRT</td>
<td>0</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>FWS 3 – New @ 14-d HRT</td>
<td>0</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>FWS 4 – New @ 7-d HRT</td>
<td>*</td>
<td>*</td>
<td>1</td>
</tr>
<tr>
<td>FWS 4 – New @ 14-d HRT</td>
<td>*</td>
<td>*</td>
<td>1</td>
</tr>
</tbody>
</table>

* Not applicable.

Pollutant Removal
Thirty-five days of water quality data (representing between 11 and 22 sampling events per cell) were used for statistical evaluation of pollutant removal. SPSS for Windows, Release 8.0.0 (Statistical Product and Service Solutions, Chicago, IL) was used to perform GLM Type III analysis giving partial tests on each factor while adjusting for others in the model. Custom contrasts were performed to compare mean effects. Statistical hypotheses tested were $H_0: \mu = 0$ for each of the following comparisons: 1) 3-yr FWS vs. New FWS @ 7-d HRT; 2) 3-yr FWS vs. New SSF @ 7-d HRT; 3) New FWS vs. New SSF @ 7-d HRT; 4) 3-yr FWS vs. New FWS @ 14-d HRT; 5) 3-yr FWS vs. New SSF @ 14-d HRT; and 6) New FWS vs. New SSF @ 14-d HRT.

Mean influent wastewater concentrations of $350 \text{mg/L} \ \text{TN}, \ 300 \text{mg/L} \ \text{NH}_3\text{-N}, \text{and} \ 1800 \text{mg/L} \ \text{COD} \text{ were observed. Influent and effluent concentrations are summarized in Figures 3, 4 and 5, with error bars representing standard errors. Also included in each figure is a dotted line, representing the mean influent concentration of the pollutant in question. Results of custom contrasts with contrast estimates, significance, and 95% confidence intervals on the estimate for TN, NH3-N, and COD are reported in Tables 2, 3, and 4 respectively.}

Total Nitrogen
Custom contrasts indicate significant differences among the three groups of CW mesocosms, both at 7-d and 14-d HRT (Figure 3, Table 2). In particular, at 7-d HRT the Group 1 cells outperformed both Group 2 and Group 3 cells, removing $54 \text{mg/L}$ and $89 \text{mg/L}$ more TN, respectively. This trend continued at 14-d HRT (refer to Table 2 for details). At 7-d HRT, the Group 2 cells outperformed the Group 3 cells, removing $35 \text{mg/L}$ more TN. The performance of Group 1 is more impressive when the bias in concentration measurements introduced by ET is considered. Cells with higher rates of ET will have artificially increased concentrations – the Group 1 cells, had the highest ET, thus, they are even more effective at mass removal than the concentration numbers suggest. Mass removals can be computed, rather than only concentration changes for each cell. While such analyses have not been performed in this preliminary work, we will ultimately use this technique.

Table 2. Contrast results indicating contrast estimates for TN (mg/L) with F-values, significance levels, and 95% confidence intervals for the difference.

<table>
<thead>
<tr>
<th>Custom Contrast</th>
<th>Contrast Estimate TN (mg/L)</th>
<th>F value</th>
<th>Sig.</th>
<th>95% Confidence Interval for Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>3-yr FWS vs. New FWS @ 7-d HRT</td>
<td>-53.8</td>
<td>13.010</td>
<td>0.001*</td>
<td>-84</td>
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<tr>
<td>3-yr FWS vs. New SSF @ 7-d HRT</td>
<td>-89.3</td>
<td>48.009</td>
<td>0.000*</td>
<td>-115</td>
</tr>
<tr>
<td>New FWS vs. New SSF @ 7-d HRT</td>
<td>-35.5</td>
<td>7.607</td>
<td>0.007*</td>
<td>-61</td>
</tr>
<tr>
<td>3-yr FWS vs. New FWS @ 14-d HRT</td>
<td>-103</td>
<td>48.007</td>
<td>0.000*</td>
<td>-133</td>
</tr>
<tr>
<td>3-yr FWS vs. New SSF @ 14-d HRT</td>
<td>-151</td>
<td>120.163</td>
<td>0.000*</td>
<td>-178</td>
</tr>
<tr>
<td>New FWS vs. New SSF @ 14-d HRT</td>
<td>-47.5</td>
<td>11.917</td>
<td>0.001*</td>
<td>-75</td>
</tr>
</tbody>
</table>

* The difference is significant at the 0.05 level.
Figure 3. Mean Total Nitrogen concentrations (mg/L) plotted with standard error (SE) for each type of CW and each HRT. The horizontal dashed line represents mean TN concentration (m/L) of influent wastewater.

NH₃-N
Mean effluent NH₃-N concentrations and standard error (mg/L) for each CW type are shown in Figure 4. The trends observed are identical to those seen in the TN data. Mean effluent concentrations, as well as statistical results are provided in Table 3. Taken together, the superior performance of the Group 1 mesocosms in both TN and NH₃-N removal strongly suggest that plants play a critical role in wetland pollutant removal. This is an important result, as published design guidelines for constructed wetlands do not account for plants in any way (EPA, 1997). The enhanced removal may reflect increased plant uptake in the Group 1 cells, due to higher plant densities, as well as enhanced plant-assisted microbial nitrification and denitrification in the Group 1 cells.

Table 3. Contrast results indicating contrast estimates for NH₃-N (mg/L) with F-values, significance levels, and 95% confidence intervals for the difference.

<table>
<thead>
<tr>
<th>Custom Contrast</th>
<th>Contrast Estimate NH₃-N (mg/L)</th>
<th>F value</th>
<th>Sig</th>
<th>95% Confidence Interval for Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>3-yr FWS vs. New FWS @ 7-d HRT</td>
<td>-43.9</td>
<td>8.250</td>
<td>0.005*</td>
<td>-74</td>
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<tr>
<td>3-yr FWS vs. New SSF @ 7-d HRT</td>
<td>-99.1</td>
<td>56.350</td>
<td>0.000*</td>
<td>-125</td>
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<tr>
<td>New FWS vs. New SSF @ 7-d HRT</td>
<td>-55.3</td>
<td>17.517</td>
<td>0.000*</td>
<td>-82</td>
</tr>
<tr>
<td>3-yr FWS vs. New FWS @ 14-d HRT</td>
<td>-67.0</td>
<td>19.284</td>
<td>0.000*</td>
<td>-97</td>
</tr>
<tr>
<td>3-yr FWS vs. New SSF @ 14-d HRT</td>
<td>-160</td>
<td>129.326</td>
<td>0.000*</td>
<td>-188</td>
</tr>
<tr>
<td>New FWS vs. New SSF @ 14-d HRT</td>
<td>-93.1</td>
<td>43.725</td>
<td>0.000*</td>
<td>-121</td>
</tr>
</tbody>
</table>

* The difference is significant at the 0.05 level.
**Figure 4.** Mean NH$_3$-N concentrations (mg/L) plotted with standard error (SE) for each type of CW and each HRT. The horizontal dashed line represents mean NH$_3$-N concentration (mg/L) of influent wastewater.

**COD**

The trend in N removal was not observed as strongly in COD. Mean effluent COD concentrations and standard error (mg/L) for each CW type are illustrated in Figure 5. At 7-d HRT, Group 1 achieved significantly greater pollutant removal rates than Group 2 (130 mg/L less COD), while Group 1 and Group 3 were not statistically different from one another (Table 4). Surprisingly, the trend changes at 14-d HRT, with Group 1 having significantly greater COD removal (ca. 300 mg/L) than either Group 2 or Group 3. Groups 2 and 3 were not significantly different from one another at 14-d HRT.

**Table 4.** Contrast results indicating contrast estimates for COD (mg/L) with F-values, significance levels, and 95% confidence intervals for the difference.

<table>
<thead>
<tr>
<th>Custom Contrast</th>
<th>Contrast Estimate COD (mg/L)</th>
<th>F value</th>
<th>Sig.</th>
<th>95% Confidence Interval for Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-yr FWS vs. New FWS @ 7-d HRT</td>
<td>-131</td>
<td>5.778</td>
<td>0.019*</td>
<td>-239 to -22</td>
</tr>
<tr>
<td>3-yr FWS vs. New SSF @ 7-d HRT</td>
<td>-15.7</td>
<td>0.111</td>
<td>0.740</td>
<td>-109 to 78</td>
</tr>
<tr>
<td>New FWS vs. New SSF @ 7-d HRT</td>
<td>115</td>
<td>5.984</td>
<td>0.017*</td>
<td>21 to 209</td>
</tr>
<tr>
<td>3-yr FWS vs. New FWS @ 14-d HRT</td>
<td>-311</td>
<td>32.735</td>
<td>0.000*</td>
<td>-419 to -202</td>
</tr>
<tr>
<td>3-yr FWS vs. New SSF @ 14-d HRT</td>
<td>-311</td>
<td>38.410</td>
<td>0.000*</td>
<td>-411 to -211</td>
</tr>
<tr>
<td>New FWS vs. New SSF @ 14-d HRT</td>
<td>0.185</td>
<td>0.000</td>
<td>0.997</td>
<td>-99.7 to 100.1</td>
</tr>
</tbody>
</table>

* The difference is significant at the 0.05 level.
Figure 5. Mean COD concentrations (mg/L) plotted with standard error (SE) for each type of CW and each HRT. Dashed line represents mean COD concentration (mg/L) of influent wastewater.

CONCLUSIONS

Preliminary data indicate that periodic drying of FWS CW cells can help control adult mosquito populations. However, the first 1 to 2 d of the drying phase is critical, and significant rainfall during this time can negate the effect of the drying period. It may be possible to overcome this limitation by always activating the drain pumps when rain occurs during a dry-phase; we will experiment with this control strategy as the experiment progresses.

Preliminary data also indicate that FWS CW cells with established plants achieve greater pollutant removal than newly established cells. This result warrants further examination, as the impact of green plants on pollutant removal in constructed wetlands is generally ignored.

It has been reported that SSF CW cells achieve areal removal rates four times greater than FWS CW cells. This result has not been confirmed in our experiments. Rather, our preliminary results show FWS and 3-yr old FWS CW's achieving similar removal rates as their SSF counterparts. Two caveats must accompany this observation. First, the FWS cells in our study actually receive half the organic loading as their SSF counterparts, due to the 50% duty cycle on which they operate. Secondly, long-term observation is needed to determine if this trend will continue beyond start-up.

Finally, this study demonstrates the possible synergism of high-rate anaerobic digestion and constructed wetlands, as treatment systems for dairy wastewater. Overall pollutant reductions of 83% (TN), 87% (NH₃-N), and 55% (COD) were observed for the combined DFAF – Group 1 system, operating at a total hydraulic retention time of just under 1 month. (True HRT is estimated as 28.6 d, based on doubling the 14-d HRT of the wet-dry cells to account for their inactivity 50% of the time.) Furthermore, the plants are thriving in the DFAF effluent, with greatest plant growth occurring in the cells directly receiving DFAF effluent, rather than the 2nd stage cells. Such a system may have practical application, if issues of mosquito control, maintenance requirements, capital costs, and phosphorous removal can be successfully addressed.

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REFERENCES


