Performance of Retention Ponds and Constructed Wetlands for Attenuating Bacterial Stressors

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INTRODUCTION

Microbial contamination from fecal origins in stormwater runoff poses a risk to human health through the consumption of drinking water and recreational and bathing contact with surface waters. Indicator bacteria serve as the regulatory meter by which water quality is measured and water quality standards (WQS) must be met. Research on constructed wetlands inactivation of fecal indicators in secondary and animal wastewater is well documented (Bavor *et al*., 1987; Gersberg *et al.,* 1987; Ottová *et al*., 1997). Removals of fecal streptococci and coliforms generally exceeded 80% and 90%, respectively, in a review by Kadlec and Knight, 1996. Gersberg *et al.* (1987) and Garcia and Bécares (1997) concluded that extensively vegetated systems remove indicator bacteria at significantly higher rates from wastewater than unvegetated systems. However, because of the potentially high indicator bacteria concentrations in stormwater runoff, the untreated fraction in effluent from retention ponds and constructed wetlands may increase receiving water concentrations beyond WQS. This is in contrast to separate sanitary systems and combined stormwater and sanitary systems which, other than during sewer overflow conditions, chemically treat the wastewater routed to treatment plants.

Experiments to evaluate the use of the first-order decay function for predicting indicator bacteria concentrations in effluent from best management practices (BMPs) were designed and completed by U.S. EPA's Urban Watershed Research Facility in Edison, NJ. Two studies, one at the bench-scale and the other at the pilot-scale, were completed to determine similarities and differences in inactivation rate constants, coefficients, and affects of environmental conditions on bacterial indicator concentrations. The focus of this paper is on the results of the pilot-scale studies which specifically explored the environmental factors that influence the rate of microbial inactivation as urban stormwater passes through retention ponds and constructed wetlands. The mesocosms designed and constructed for this project offered a unique setting allowing many characteristics associated with stormwater and flow to be held constant (i.e., influent characteristics, residence time, and pollutant loading). By varying testing dates with climatic conditions experienced throughout the year, an assessment of the impact of the environmental change on

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bacterial inactivation rates could be assessed. The results allowed comparison of rates of inactivation with seasonal wet-weather events and were used to determine new inactivation coefficients based on environmental variables. More information is needed to determine whether models that use first-order decay functions when predicting bacteria effluent concentrations from field BMPs (usually as a point source) are accurately providing effluent predictions and concomitant loads. Results suggest, depending on how bacterial loading is modeled during total maximum daily load (TMDL) development, that first-order decay may not adequately consider background concentrations when calculating dry-weather loading. Similarly, predicting loading over longer durations while ignoring seasonal changes may result in less accurate predictions of indicator bacteria loading. Both outcomes may impact actual bacterial loading and risk to human health with exposure to stormwater runoff contaminated water.

Study Site and Experimental Design

Two rectangular mesocosms of the same size with separate stormwater BMP treatments (constructed wetland and retention pond) were constructed at the Urban Watershed Research Facility (UWRF) in Edison, New Jersey. The experimental designs are detailed in an EPA report (Struck *et al.*, 2006a). Figure 1 provides a picture of each mesocosm used in the study. Tanks had a length, width, and depth of 1.78 m, 0.74m, and 0.65 m, respectively with a stormwater volume of approximately 227 L. Both systems were constructed in August of 2002.

(A) **(B)**

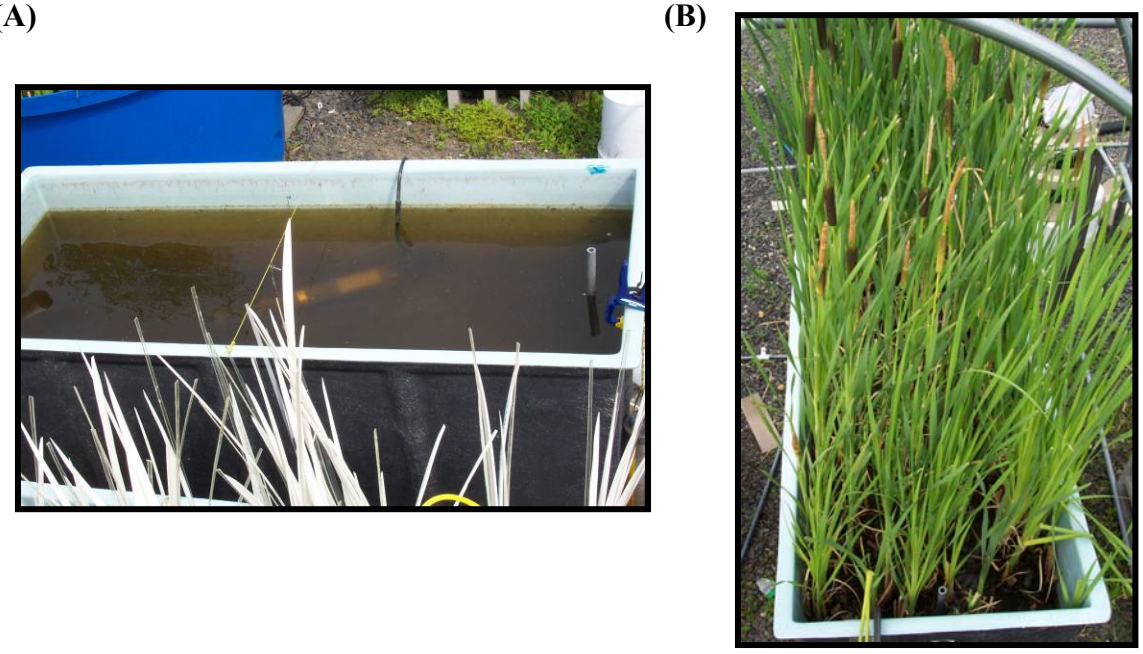


Figure 1. Pictures of the pilot-scale retention pond (A) constructed wetland (B) treatment systems.

RESULTS AND DISCUSSION

Physical and Chemical Properties of the Pilot-scale Systems

Physical and chemical parameters measured in the study are listed in Table 1. Water temperatures averaged 2.15ºC lower in the constructed wetland compared to the retention pond. This difference was likely due to shading from the macrophytic vegetation (*Typha latifolia*, average stem density = 39.3 stems/m^2). This temperature difference was more notable in the September and July sampling events (difference of 3.08ºC and 1.82ºC, respectively) compared to the November sampling event (difference of 1.0 ºC).

Dissolved oxygen (DO) was higher in the retention pond compared to the constructed wetland with the highest temperatures recorded in May, July, and September. The process of decomposition of organic matter in the constructed wetland may consume some of the DO, causing lower concentrations (sometimes near 0 mg/L) especially during the warmer periods in which greater rates of decomposition are expected. Also, diurnal fluctuations in DO and temperature tended to be reduced during initial storm event loading. Values for these parameters did not generally reach pre-event diurnal fluctuations until after 48 h of detention for most events.

Conductivity was nearly the same in the two systems while pH was neutral to alkaline in the retention pond but tended to be acidic in the constructed wetland. This pattern was observed by Mitch and Gosselink (2000) in constructed wetlands with mineral soils and in some lake sediments by Stumm and Morgan (1996). These differences were attributed to the organic matter build-up in sediments and corresponding decomposition causing greater quantities of organic acid in the constructed wetland system, reducing the pH. The oxidation-reduction potential (ORP) was much lower (and often negative) in the retention pond compared to the constructed wetland. The depth of inundation of the free water in the retention pond was generally three times that of the constructed wetland. A greater water depth and the lack of aquatic vegetation would substantially increase the potential for reducing conditions, resulting in lower ORP values, through both reduced oxygen diffusion and lower photosynthetic oxygen production.

Light intensity in the constructed wetland was consistently 9-10% of that measured in the retention pond. The difference in light intensity, recorded with a hand-held meter under several light intensities, was used to calculate a corrected irradiance expected at the surface of the constructed wetland to compare irradiance values between the wetland and retention pond systems.

Most storm events had maximum initial total suspended solids (TSS) and turbidity values below 100 mg/L and 150 NTUs, respectively, upon stormwater loading to the mesocosms. As expected, turbidity and TSS values decreased with residence time in each system. Geometric mean turbidity values for sampling events before October

Table 1. Average Event *in-situ* Physical and Chemical Results

		Retention Pond						Constructed Wetland					
Date	Parameter	Valid N	Mean	Min.	Max.	Stnd. Dev.	Stnd. Err.	Valid N	Mean	Min.	Max.	Stnd. Dev.	Stnd. Err.
	Temp (ºC)	36	27.0	21.9	32.2	2.9	0.5	36	25.5	22.2	29.4	2.2	0.4
	Cond (mS/cm)	36	0.316	0.296	0.345	0.016	0.003	36	0.284	0.265	0.316	0.014	0.002
	D0 (mg/L)	36	5.8	1.9	11.4	2.6	0.4	36	3.6	0.8	9.2	2.3	0.4
Jun-04	pH	36	8.0	7.3	9.1	0.6	<0.1	36	6.7	6.6	7.0	0.1	<0.1
	ORP (mV)	36	364	200	465	93	15	36	465	317	529	67	11
	Turbidity (NTU)	36	11.6	5.1	31.1	5.7	0.9	36	10.3	6.5	23.8	4.8	0.8
	Irradiance (kJ/m^2)	12	43.9	<0.1	139.1	48.9	14.1	12	4.1	<0.1	12.8	4.5	1.3
	Temp (ºC)	36	22.8	18.7	27.3	2.8	0.5	42	19.4	16.1	22.1	1.8	0.3
	Cond (mS/cm)	36	0.217	0.196	0.237	0.011	0.002	42	0.204	0.190	0.237	0.013	0.002
	D0 (mg/L)	36	8.5	5.8	11.5	1.9	0.3	42	4.1	0.7	13.4	3.6	0.5
Sep-04	pH	36	7.9	7.5	8.3	0.2	<0.1	42	6.4	6.3	6.7	0.1	<0.1
	ORP (mV)	36	-288	-354	-15	95	16	42	555	524	591	19	3
	Turbidity (NTU)	36	11.0	7.9	20.7	3.2	0.5	42	6.6	3.7	18.5	3.8	0.6
	Irradiance (kJ/m^2)	12	31.6	<0.1	125.0	45.1	12.0	14	3.4	<0.1	11.5	4.7	1.3
	Temp (ºC)	42	11.1	5.6	16.1	3.3	0.5	42	10.1	4.6	15.9	3.2	0.5
	Cond (mS/cm)	42	0.189	0.159	0.216	0.018	0.003	42	0.179	0.130	0.208	0.021	0.003
	D0 (mg/L)	42	9.5	6.2	13.9	2.0	0.3	42	9.0	2.3	14.3	4.1	0.6
Nov-04	pH	42	7.2	7.0	7.3	0.1	<0.1	42	6.3	6.1	6.8	0.2	<0.1
	ORP (mV)	42	-285	-322	-211	29	4	42	403	376	452	19	3
	Turbidity (NTU)	42	7.8	4.0	11.5	1.9	0.3	42	8.4	0.4	27.6	6.5	1.0
	Irradiance (kJ/m^2)	14	19.0	<0.1	84.4	26.5	7.1	14	1.9	0.0	7.8	2.4	0.7
	Temp (ºC)	33	19.9	16.6	27.0	3.2	0.6	39	17.1	15.3	22.3	1.9	0.3
	Cond (mS/cm)	33	0.447	0.394	0.555	0.047	0.008	39	0.680	0.585	0.771	0.052	0.008
	D0 (mg/L)	33	10.5	8.5	15.6	1.8	0.3	39	1.4	0.1	4.4	1.2	0.2
May-05	pH	33	8.3	7.3	9.3	0.5	0.1	39	6.8	6.8	7.0	0.1	<0.1
	ORP (mV)	Not Recorded											
	Turbidity (NTU)	33	1.8	<0.1	11.2	2.8	0.5	39	4.4	2.5	12.0	2.4	0.4
	Irradiance (kJ/m^2)	12	26.9	<0.1	119.0	40.2	11.1	13	2.6	<0.1	17.4	5.6	1.6
	Temp (ºC)	24	26.7	24.0	31.2	2.2	0.4	24	24.5	23.1	27.1	1.3	0.3
	Cond (mS/cm)	24	0.253	0.235	0.297	0.018	0.004	24	0.259	0.197	0.371	0.054	0.011
	D0 (mg/L)	24	5.5	3.0	9.5	1.9	0.4	24	1.3	0.1	3.7	1.3	0.3
Jul-05	pH	24	7.5	7.2	8.2	0.3	0.1	24	6.4	6.2	6.6	0.1	<0.1
	ORP (mV)	Not Recorded											
	Turbidity (NTU)	24	26.0	6.5	92.0	20.2	4.1	24	61.0	13.5	167.2	50.9	10.4
	Irradiance (kJ/m^2)	8	29.4	<0.1	119.0	40.3	14.3	8	2.7	<0.1	10.9	3.7	1.3
	Temp (ºC)	48	14.6	12.6	17.6	1.6	0.2	48	14.7	11.6	18.4	1.7	0.3
	Cond (mS/cm)	48	0.156	0.066	0.240	0.048	0.007	48	0.184	0.092	0.273	0.046	0.007
	D0 (mg/L)	48	3.2	<0.1	6.3	1.6	0.2	48	2.8	<0.1	9.1	3.0	0.4
Oct-05	pH	48	7.0	6.1	7.5	0.4	0.1	48	6.0	5.8	6.5	0.1	<0.1
	ORP (mV)	Not Recorded											
	Turbidity (NTU)	48	849.1	<0.1	1236.5	396.9	57.3	48	937.4	11.2	2141.4	782.5	112.9
	Irradiance (kJ/m^2)	16	18.7	<0.1	135.3	39.8	10.0	16	1.9	<0.1	12.4	3.7	0.9

2005 are shown in Figure 2. Turbidity values were averaged for each time step and then over each sampling event.

The October 2005 experimental run had starting TSS values near 3,000 mg/L and turbidity values averaging 2,173 NTUs, which was 20-30 times greater than typical conditions for both parameters. Active construction in the watershed was evident in the stormwater runoff during this sampling event. Inclusion of this stormwater runoff greatly increased the variability in solids concentration, overwhelming the smaller concentrations found in the previous and subsequent runoff events. Thus some analyses occurred with the exclusion of this event as noted.

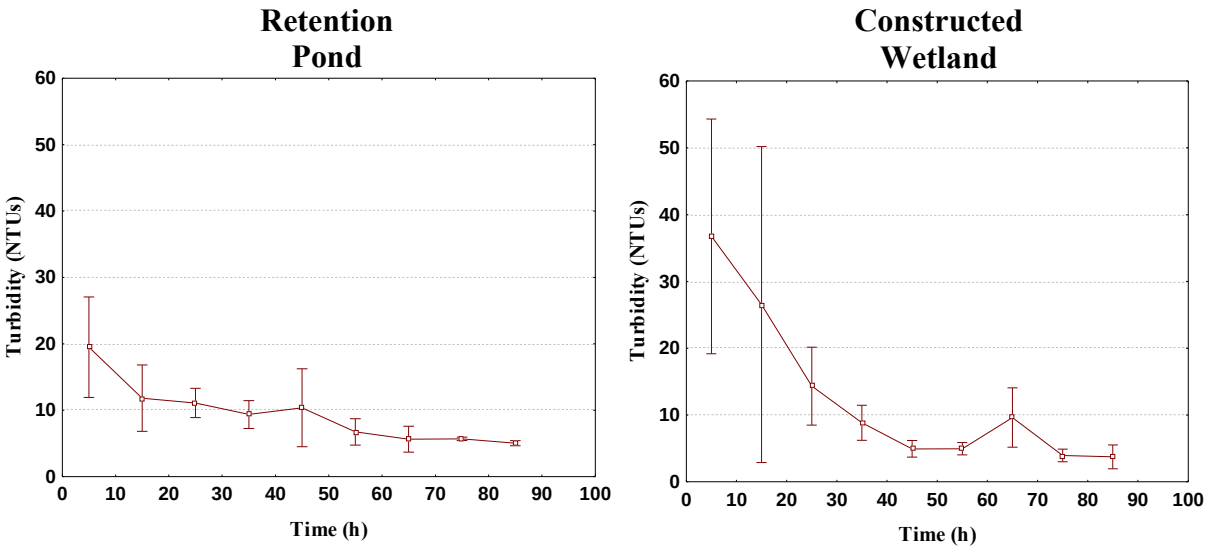


Figure 2. Mean turbidity (with 95% confidence intervals) in the retention pond and constructed wetland in all storm events except October 2005.

Bacteria Indicator Organisms

Samples were not analyzed for enterococci in the first sampling event (June 2004) but resumed for all subsequent events. Temperature appeared to affect bacterial indicator organism concentrations in the retention pond. The optimal temperature range that resulted in the greatest number of observed bacteria colony forming units was between 11ºC and 26ºC. A similar trend was noticed in the constructed wetland for a temperature range between 11º C and 23ºC (Figure 3). Conductivity and DO did not appear to significantly affect bacterial concentrations over the ranges observed. ORP may have moderately affected fecal coliforms and *E. coli* concentrations around 200 mV in the retention pond while densities of fecal coliforms, *E. coli*, and enterococci decreased between 500 and 600 mV in the constructed wetland. However, only three events were monitored for this parameter. Densities of fecal coliforms and *E. coli* decreased above a pH of 8.5 in the retention pond but remained unaffected over the range of observed pH values in the constructed wetland.

There was a distinct relationship between concentration of indicator organisms and turbidity during this study. While there was no visible trend in turbidity at concentrations less than 20 NTU, turbidities greater than 100 NTUs exhibited a predictable increase in bacteria organism concentrations with increasing turbidity in both the retention pond and constructed wetland (Figure 4). The United States Geological Survey (USGS) reported similar results in larger rivers in northern and central Virginia and by the EPA in smaller streams in northern Virginia (Hyer and Moyer, 2003; Struck *et al.,* 2006b). These solids can potentially affect rates of bacteria attenuation.

Overall bacteria indicator inactivation rates for all simulated storm events are shown in Table 2. Significant differences were observed between the constructed wetland and retention pond in eight of the bacteria indicators for six runoff events. The retention pond had significantly higher inactivation rates for total coliforms in June and July; *E. coli* in May; fecal coliforms in July and enterococci in May and November compared to the constructed wetland. However, the constructed wetland had significantly greater bacterial inactivation rates compared to the retention pond for fecal coliforms in June and enterococci in July. Both treatments had significantly greater inactivation rates compared to the light and dark controls in September and November for total coliforms, *E. coli*, and fecal coliforms. Retention pond inactivation rates were also greater than controls for total coliforms in June and July, *E. coli* in May and July, and fecal coliforms in May, June, and July. Constructed wetlands inactivation rates were greater for *E. coli* in July, fecal coliforms in May and June, and enterococci in July. Light controls were greater than dark controls in nine instances, including May and June for total coliforms and *E. coli*, July for fecal coliforms, and May, July, September, and November for enterococci. This suggests light does have an impact on bacteria indicator organisms.

The exponential regression coefficients shown in Figure 5 are the calculated inactivation rates from the data. A two step process of generating an overall inactivation value for each bacterial indicator organism from 0-50 h and from 50-100 h was used to generate a best fit relationship. This timeframe was determined by maintaining R^2 values of regressions greater than 0.70 while varying the time interval between 0 and 100 until the difference in slope (inactivation rate) was maximized for the majority of the bacteria indicator organisms.

In most instances, the R^2 values improved when dividing the duration of the experiment into the two timeframes, suggesting that inactivation rates vary as a function of time with greater rates of inactivation during the first 50 h timeframe compared to the second 100 h timeframe. Fecal coliforms and enterococci in the retention pond were an exception to this generalization. Several of the inactivation rates during the 50-150 h timeframe had values nearing zero suggesting that these organisms may have reached or nearly reached background concentrations after 50 h. This is supported by the average pre-event background concentrations in the retention pond and constructed wetland found in Table 3.

Table 2. Inactivation Rates for the Constructed Wetland, Retention Pond, and Dark and Light Controls for all Indicator Bacteria Organisms for each Sampling Event.

		Retention Pond				Constructed Wetland			
Month	Year	Total Coliforms	*E. coli*	Fecal Coliforms	Entero-cocci	Total Coliforms	*E. coli*	Fecal Coliforms	Entero-cocci
		(h^{-1})				(h^{-1})			
June	2004	0.2419*+	0.1484	0.1814+		0.1529	0.1651	0.3277*+	
Sept.	2004	0.144+	0.1164+	0.1192+	0.2030	0.1204+	0.1204+	0.1515+	0.1786
Nov.	2004	0.1653+	0.1164+	0.1485+	0.1730*	0.1235+	0.1157+	0.1137+	0.1245
May	2005	0.0949	0.3350*+	0.1417+	0.1717*	0.1090	0.0919	0.1233+	0.0852
July	2005	0.1811*+	0.1957+	0.2610*+	0.1240	0.0733	0.1894+	0.1025	0.2112*+
Oct.	2005	0.0437	0.0524	0.0566	0.0512	0.0427	0.0597	0.0536	0.0594

		Dark Control				Light Control			
Month	Year	Total Coliforms	*E. coli*	Fecal Coliforms	Entero-cocci	Total Coliforms	*E. coli*	Fecal Coliforms	Entero-cocci
		(h^{-1})				(h^{-1})			
June	2004	0.0247	0.0276	0.0249		0.1390♦	0.1502♦	0.0242	
Sept.	2004	0.0700	0.0563	0.0527	0.0773	0.0588	0.0789	0.0760	0.2027♦
Nov.	2004	0.0815	0.0480	0.0445	0.0711	0.0658	0.0724	0.0692	0.1787♦
May	2005	0.0258	0.0725	0.0514	0.0351	0.0679♦	0.1158♦	0.0828	0.0884♦
July	2005	0.0637	0.0509	0.0619	0.0944	0.0720	0.0712	0.1136♦	0.1681♦
Oct.	2005	0.0538	0.0605	0.0514	0.0194	0.0676	0.0862	0.0822	0.0316

* Indicates a significantly higher value between retention pond and constructed wetland
+ Indicates a significantly higher value between retention pond or constructed wetland values and control values
♦ Indicates a significantly higher value between light and dark control values

Table 3. In-situ Indicator Organisms Average Background Concentrations

Indicator Organism	Background Concentration (CFU/100 mL ± Standard Error)	
	Retention Pond	Constructed Wetland
Total Coliforms	$1.39x10^4 \pm 3.85x10^3$	$3.37x10^4 \pm 4.04x10^3$
E. coli	$6.42x10^0 \pm 7.22x10^0$	$2.55x10^1 \pm 6.21x10^0$
Fecal Coliforms	$1.02x10^4 \pm 2.55x10^3$	$8.09x10^3 \pm 1.12x10^3$
Enterococci	$3.70x10^1 \pm 8.29x10^0$	$2.01x10^1 \pm 5.46x10^0$

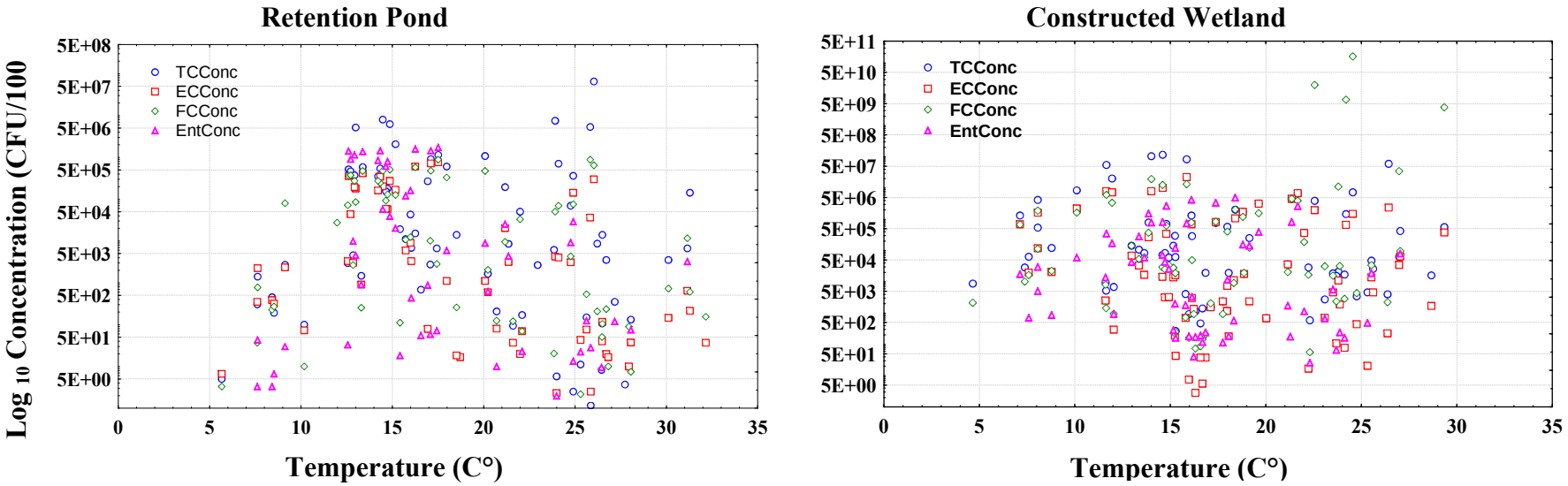


Figure 3. Effluent concentrations of indicator organisms with temperature.

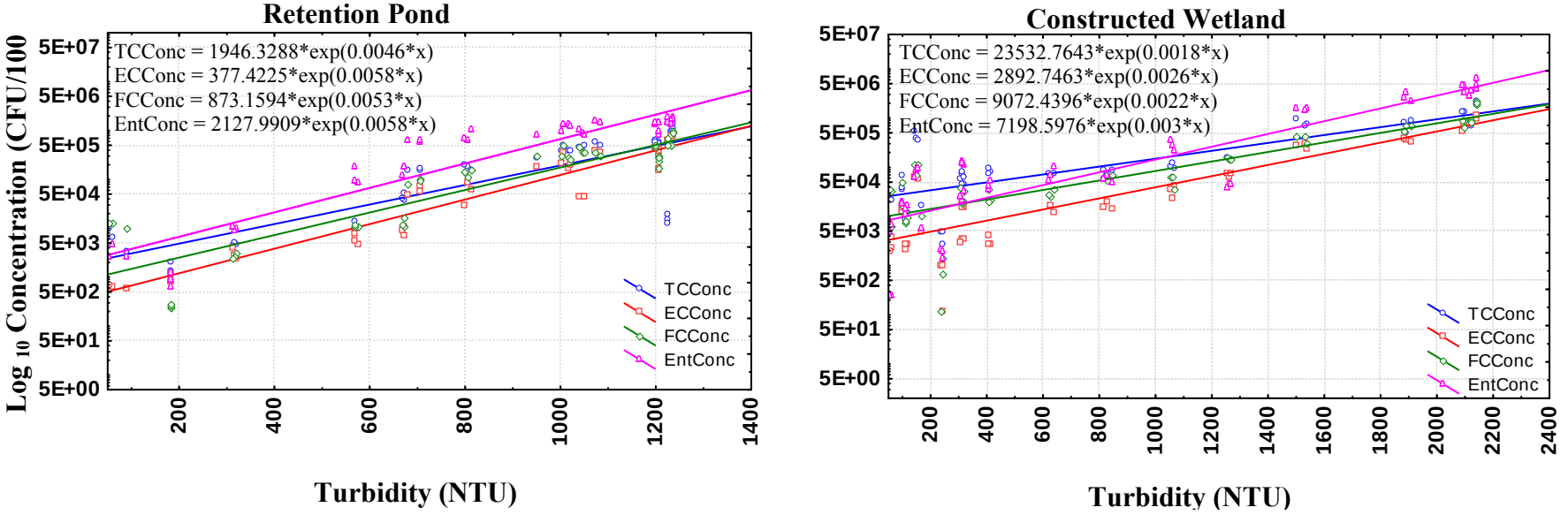
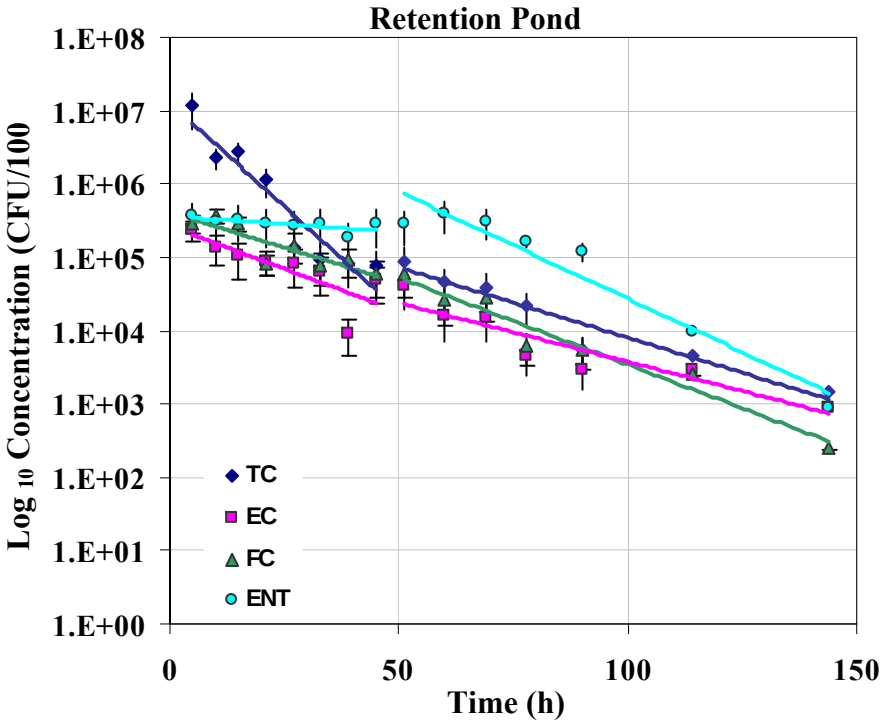
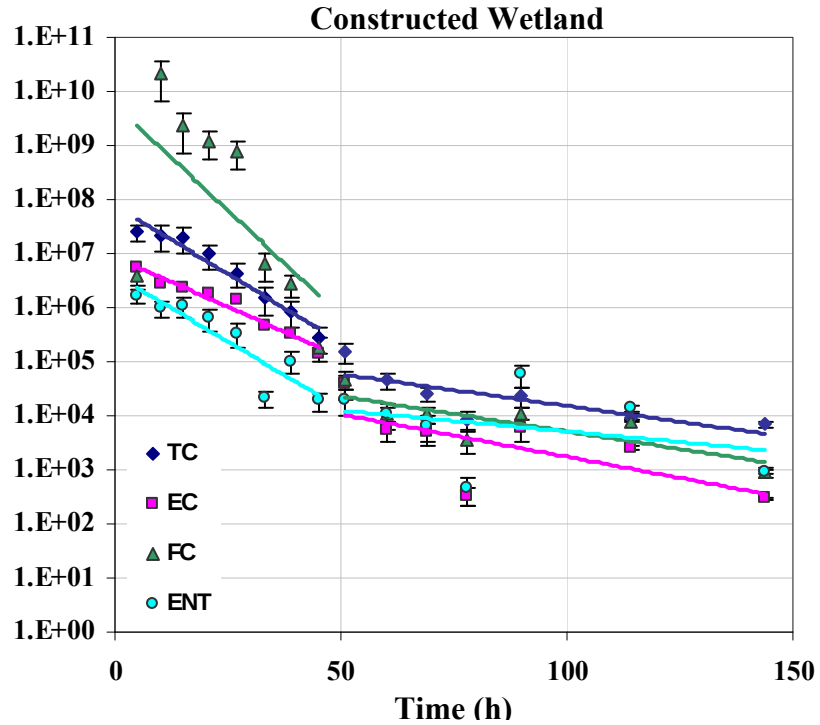


Figure 4. Effluent indicator bacteria concentrations with *in-situ* turbidity.





Organism	$K_{T\ 0\text{-}50h}$ (h^{-1})	R^2	$K_{T\ 50\text{-}150h}$ (h^{-1})	R^2
TC	-0.1308	0.895	-0.0044	0.937
EC	-0.0534	0.628	-0.0369	0.876
FC	-0.0440	0.464	0.0547	0.956
ENT	-0.0044	0.765	-0.0672	0.937

Organism	$K_{T\ 0\text{-}50h}$ (h^{-1})	R^2	$K_{T\ 50\text{-}150h}$ (h^{-1})	R^2
TC	-0.1164	0.959	-0.0270	0.636
EC	-0.0853	0.960	-0.0361	0.475
FC	-0.1797	0.383	-0.0298	0.664
ENT	-0.1142	0.824	-0.0180	0.116

Figure 5. Indicator organism concentrations with time. (Regressions fits are for time = 0-50 h and 50-150 h. Regression coefficients (k-values) of the exponent (slope) are shown in the tables.)

Bacteria Concentrations in Sediment

Results from the sediment bacteria indicator concentrations collected and analyzed one day before and two days after the November 2004 storm event are shown in Table 4. Sediment bacteria increased substantially for total coliforms and *E. coli* after the storm event. However, concentrations of enterococci decreased over the experiment. The initial bacteria indicator organism concentrations measured before the storm event were considered as background concentrations as the previous input of indicator organisms through stormwater runoff was more than two months prior to the November event.

Table 4. Sediment Bacteria Indicator Organisms Sampled in November of 2004

Indicator Organism	Indicator Organism Concentrations BEFORE Stormwater Loading (MPN)	Indicator Organism Concentrations AFTER Stormwater Loading (MPN)
	Retention Pond	
Total Coliforms	2.25×10^4	1.94×10^5
E. coli	<MDL	7.41×10^4
Enterococci	1.62×10^4	1.88×10^3
	Constructed Wetland	
Total Coliforms	3.70×10^4	$>2.41 \times 10^5$
E. coli	2.37×10^4	$>2.41 \times 10^5$
Enterococci	2.02×10^5	8.67×10^3

Predation

Groups of organisms identified in samples collected from each system in August, 2005 are shown in Table 5. The average number of organisms enumerated (invertebrate density) in the constructed wetland was 777 and in the retention pond was 442 organisms. However, due to the high variance in the samples, this difference was not significant.

The difference in the number of organisms present (invertebrate taxa richness) was significant between the constructed wetland and retention pond, averaging 15.75 organisms and 7.5 organisms, respectively. These groups often had more than one species in a taxonomic group represented in each system as shown in the parenthesis of table 5. Ostrocods were not speciated below this subclass.

Cladocerans were the dominant species present both in richness (3 species) and in density (83%) in the retention pond. Ostrocods (11%), ephemeroptera (2%), rotifers (2%), copepods (1%), and oligochaetes (<1%) made up the rest of the composition within the retention pond. The dominant invertebrate species in the constructed wetlands were

collembola, ostrocods, copepods, and oligochaetes composing 51%, 25%, 9%, and 7% of the invertebrate species present, respectively.

Table 5. Macroinvertebrate Groups Identified in the Retention Pond and Constructed Wetland

Taxa	Retention Pond	Constructed Wetland
Oligochaetae	1	231 (3)
Chironomidae	0	12
Cladocerans	1479 (3)	190 (2)
Coleoptera	0	9
Collembola	0	1591 (2)
Copepoda	22 (2)	269 (2)
Ephemeroptera	42	0
Hemiptera	0	8 (2)
Hydracnidia	0	9 (2)
Ostrocoda	186	782
Rotifera	39	1

*Parentheses indicate the number of taxa identified in that group

EFFECT OF ENVIRONMENTAL VARIABLES

Results from the pilot-scale studies show environmental conditions affect indicator bacteria concentrations in retention ponds and constructed wetlands. Attempts have been made to single out environmental variables such as temperature, sunlight, settling, and predation. Other variables were considered as a group as data did not support their separation.

Effects of Temperature

Generally, the results from the pilot-scale experiments are similar to those of other studies (e.g., Easton *et al.*, 2005; Ferguson *et al.*, 2003; Geldreich *et al.*, 1968; Medema *et al.*, 1997; and Canteras *et al.*, 1995). Calculated inactivation rate constants increased with increasing temperature. Similarly, inactivation rates were lower at lower temperatures. This trend was most notable during the October 2005 sampling event. Selvakumar *et al.* (2004) noted that concentrations of organisms did not change significantly when the samples were stored at 4^{o}C beyond the standard holding time of 24 h. Geldreich *et al.* (1968) noted that organism persistence remained at higher levels at 10^{o}C compared to 20^{o}C. In the pilot-scale experiment the optimal temperature range for growth (as indicated by overall indicator bacteria concentrations) was similar to values reported in the literature with indicator concentrations increasing with temperature, reaching a maximum concentration from 20^{o}-25^{o}C in both the retention pond and constructed wetland. Medema *et al.* (1997) found that inactivation was faster at 15^{o}C than at 5^{o}C. Canteras *et al.* (1995) noted a clear positive correlation between inactivation

and temperature. In their study, when test conditions were at 10°C, 36 h was necessary to reduce the population of *E. coli* to 10% of the original as opposed to 8.4 h at 42°C. Greater inactivation was also noticed in the range between 10 and 18°C than between 18 and 42°C.

The study also found indicator organism inactivation rates were much greater over the first 50 h as compared to the following 100 h. Although over a longer period of time, Easton *et al.* (2005) reported that the 0-7 day inactivation rates were much larger than the 7-21 day rates. It may be possible that during the 0-7 days studied by Easton *et al.* (2005) there have been varying rates of die-off. In this study, the temporal resolution as measured 16 times over 150 h compared to 4 times over 168 h (seven days) by Easton *et al.* (2005), the changes in bacteria inactivation may be more easily observed.

Effect of Sunlight/Light Intensity

Many studies have shown that sunlight is an important factor in bacteria indicator inactivation (Sinton *et al.,* 1994; Canteras *et al.,* 1995). In this study, statistically lower inactivation rate constants in the dark control compared to the light control for total coliforms and *E. coli* were found for the months of May and June. June had the greatest irradiance of any of the dates sampled while May ranked fourth in light intensity (because of cloudy conditions during the experiment) (Table 2). Enterococci showed the greatest difference in inactivation rate constants between light and dark controls followed by total coliforms and *E .coli*. The primary difference between these controls was the exposure to sunlight. The difference in rate constants, up to 0.12 h^{-1} for enterococci and *E. coli*, are substantial.

Effects of Sedimentation, Sorption, and Filtration

Sedimentation, sorption, and filtration processes are generally accepted as the dominant mechanisms for the removal of solids and other sediment-related stressors such as heavy metals. Settling velocity has been used as an approximation of the overall removal (settling) rate constant in stormwater treatment systems (Wong and Geiger, 1997). Other environmental factors such as non-ideal flow conditions, though, could increase solids in the water column through resuspension. In addition, higher density vegetation could reduce flow rates or increase particle pathway length thereby increasing the rate of the settling constant (Wong and Geiger, 1997). In this study, effluent concentrations of solids were similar between the wetland and retention pond systems. Settling velocities appeared to be greater in the constructed wetland with the higher (>100 NTUs) sediment loading as observed in the October 2005 sampling event.

The difference in indicator bacteria concentrations and the inactivation rate constants between the constructed wetland and retention pond in this study supported settling as a contributing but not primary factor in bacterial inactivation. It may be possible that a large fraction of the influent indicator bacteria were unassociated (free) with solids or associated with only very fine particles and would not settle during the duration of the sampling. This may be a real phenomenon or an artifact of the manner in which the enriched stormwater was created. The turbid, even colloidal nature of the solids may also

occlude light penetration or prevent predation by bacteriovores through limiting access and harboring the indicator bacteria that are near or agglomerated to the solids resulting in less bacterial inactivation through settling.

Wong and Geiger (1997) suggest, when selecting an appropriate *K* value for sedimentation, filtration, and sorption, using the settling velocity of the fiftieth percentile sediment grade with adjustments for increased effectiveness for wetlands having higher vegetation density. However, as experienced in this study, this may not adequately predict the effluent concentrations of stormwater runoff passing through passive treatment systems if bacteria are either unassociated with settleable particles or if they are associated with the fine particle fraction, i.e., less than 2 μm in size (Davies and Bavor, 2000).

Bacteria indicator organisms present in the sediments have the potential to be resuspended in the water column with turbulent flow or disturbance and may contribute to increased effluent bacteria indicator organism concentrations in the future.

Effects of Predation

Previous research has suggested bacterivory can significantly reduce indicator bacteria organism concentrations (Green *et al*., 1997; Mandi *et al.*, 1993; Decamp and Warren, 1998; Pretorius, 1962; Fernandez *et al.,* 1992; and Troussellier *et al.*, 1986). There were a variety of invertebrates present in the constructed wetland and retention ponds during this study. While the retention pond and constructed wetland had different taxa represented in each of these systems, dominant invertebrates in both systems have been shown to consume large quantities of indicator bacteria based on the population size of both predators and prey. The major difference in species richness between the systems was the retention pond was dominated by cladocerans while the constructed wetland had populations of oligochaete, collembola, copepod, and ostrocod invertebrates. The difference in predatory effects of the dominant species on indicator bacteria concentrations in each system cannot be adequately quantified. Characteristics of the constructed wetland and retention pond do suggest why there may be different invertebrate communities between the different systems.

The constructed wetland systems generally had taxa that are associated with greater organic matter (derived from the macrophytic vegetation) (i.e., oligochaetes, collembola, copepods, and ostrocods) (Peckarsky *et al*., 1990). Collembola and ostrocods are reported to feed on detritus algae, fungi, and dead animal matter with collembolan having special mouthparts for consuming the surface film or underlying bacterial populations (Peckarsky *et al.,* 1990). Therefore, their numerical importance in the constructed wetlands was not surprising. Cladocerans and copepods can affect bacterial populations in both wetlands and open water systems. Both taxa have been shown to consume greater than 25% of the bacterial populations in near shore areas of lakes (Heath *et al*., 1999). The association of collembolans with detrital organic matter and cladocerans with more open water habitat may explain the relative densities of these invertebrates in both the retention pond and constructed wetland environments.

Again, the identification and enumeration of the taxa did not provide quantifiable results to determine the predatory contribution of invertebrates on indicator bacteria concentrations. However, their presence and abundance provided substantial evidence that invertebrates may contribute to the reduction of indicator bacteria in natural systems. This study did not directly measure bacteria indicator inactivation rates due to predation, but likely included the overall effects of predation by incorporating this effect into the cumulative inactivation rates of collective environmental factors as discussed below.

Effects of Other Potential Factors

The many other factors (i.e., DO, pH, conductivity, ORP) that may contribute to inactivation rates of indicator bacteria were not directly assessed in this study. Instead, rather than individually assessing these factors, their effects were grouped into the overall inactivation rates and treated as collective environmental factors.

Inactivation Rates Due to Collective Environmental Factors

Geldreich *et al.* (1968) reported the data on inactivation rates for microorganisms in stormwater and effects of natural factors on survival rates. A decay rate of 0.061 h^{-1} at 20^{o}C for fecal coliforms was determined in that study. This is a similar result to the inactivation rate constant of fecal coliforms at 20^{o}C obtained in the 0-50 h and 50-150 h rates for fecal coliforms observed in the retention pond.

Overall, total coliforms had a much slower inactivation rate than other indicator organisms. Traditional indicators (total coliforms and fecal coliforms) had lower inactivation rates than the alternate indicators (*E. coli* and enterococci) suggesting that when using traditional indicators, higher concentrations may be predicted, compared to alternate indicators. Depending on the stressor(s) for which the BMP is designed, this could affect the necessary retention time calculated when designing stormwater BMPs.

Conductivity with *E. coli* and enterococci with DO were significantly correlated in the retention pond when correlational analyses were performed on the chemical and physical parameters with overall inactivation rates. The constructed wetland had no significant physical or chemical correlations with inactivation rates. Conductivity of the *in situ* water was found to be a good surrogate for total dissolved solids. However, standard methods suggest the relationship is not constant (APHA *et al.*, 1998). The relationship between total dissolved solids and conductivity is a function of the type and nature of the dissolved cations and anions in the water (i.e., the ability of the water to carry a charge). Some total dissolved solids measuring devices measure the conductivity of the water with the assumption that the primary dissolved minerals are either a combination of NaCl or KCl. Other anions and cations, such as sodium sulfate, sodium bicarbonate, or possibly some organic molecules with ionic and cationic charges can contribute to the conductivity in water samples suggesting total dissolved solids, while not directly measured in the experiment, may be correlated with *E. coli* concentrations in the retention pond if other mineral or organic compounds are present.

Though, the bacteria inactivation generally followed the first-order rate equation, a jackknife relationship showing a greater rate constant for the first 50 h compared to longer periods, as in Figure 5, was appropriate for most indicator bacteria. This relationship was also observed by Thomann and Mueller (1987) for bacteria distributions in rivers with resistant strains. In addition, with indicator species concentrations having an average predicted background concentration of 10^1–10^4 organisms/100 mL and *in situ* background concentrations ranging from $6.42x10^0$-$3.37x10^4$ organisms/100 mL, reasonable support exists for changes to the first-order rate equation in these wetland and retention pond BMPs.

Kadlec and Knight (1996) suggest that because of residual indicator bacteria populations present in wetlands, bacteria removal efficiency is a function of the inflow bacteria concentrations. Removal efficiency typically is higher at high inflow concentrations, but declines to low or negative values when inflow concentrations are lower than the *in situ* bacteria production rates. However, during periods when influent flow rates are turbulent, causing resuspension of the previously settled solids, removal efficiency may not depend on influent concentrations alone. Because these settled sediments are associated with *in situ* bacteria populations, there may be an increase in effluent concentration of indicator bacteria with turbulent or high flow or when sediments are disturbed by other means (i.e., waterfowl, muskrats, etc.) compared to the influent concentration. Similarly, sediment resuspension may be more likely to occur in wetland and retention pond BMPs that were poorly designed, have reached their design life, or are not maintained and may contribute to lower or negative indicator bacteria inactivation rates (and removal efficiencies).

EVALUATION OF THE FIRST-ORDER DECAY EQUATION

Recalling that one of the primary objectives of this research was to evaluate the affect of typical environmental conditions on the first-order decay equation for predicting bacteria indicator concentrations to further investigate the effects of typical environmental conditions (similar to expected field BMP conditions). This pilot-scale study demonstrated the first-first order decay function adequately models indicator bacteria concentrations in the short term. However, during longer periods, the first-order decay equation may not provide an accurate prediction of indicator bacteria (or other stressors of concern) in effluent from these types of BMPs. This equation also fails to account for background concentrations that may limit attenuation of stressors to levels predicted. Literature has reported that the assumptions for a first-order decay function (i.e., steady flow conditions) may seldom be met in studies concerning stormwater runoff in constructed wetlands and retention ponds (Wong and Geiger, 1997).

Other researchers have suggested using surface area based models for wetlands constructed for secondary treatment of wastewater (Kadlec and Knight, 1996). One of these models, the *K*-C* model, incorporates a concentration term, C*, that represents the background concentration often present in natural systems. The formula is:

$$\frac{(C_{out} - C^*)}{(C_{in} - C^*)} = e^{-K} \quad (1)$$

Where: C_{out} = effluent concentration; C_{in} = influent concentration; C^* = background concentration; and, K = rate constant for the water quality parameter being treated based on time of detention.

However, Wong and Geiger (1997) point out that the stochastic nature of stormwater-related systems introduces significantly different system functions compared to wastewater treatment. These authors formulate a procedure that incorporates the use of the *K*-*C** model and the interaction between the requirements for wetland storage for inflow stochasticity and stormwater treatment.

They recommend an adaptation of Kadlec and Knight's *K*-*C** model to:

$$C_{out} = C^* + (C_{in} - C^*)e^{-KA/Q} \quad (2)$$

Where: C_{out} = effluent water quality target; C_{in} = influent event mean concentration; C^* = background concentration; K = rate constant for the water quality parameter being treated; A = constructed wetland or retention pond area; and, Q = steady state flow.

It should be noted that equations 1 and 2 have attempted to incorporate conditions that meet the assumptions for the first-order decay equation or include environmental realities such as background concentrations of indicator organisms (C*) to improve prediction of this stressor. The rate constant *K,* which governs inactivation rate determinations in the first-order decay equation, is the only means of incorporating environmental variables to better predict effluent concentrations in surface water models. To provide more information on the effects of environmental variables, the present study estimated inactivation rate constants for indicator bacteria from environmentally influenced systems. Further, constant coefficients were calculated to improve predictions of the effects that environmental factors have on overall indicator bacteria inactivation rates.

Khatiwada and Polprasert (1999) and Canteras *et al.* (1995) proposed the following formula for overall inactivation rate constants:

$$K_{overall} = K_{20}\Phi_T^{T-20} + \Phi_l I + K_f + K_p \quad (3)$$

Where: $K_{overall}$ = overall inactivation rate constant; K_{20} = inactivation rate constant due to temperature at 20°C; Φ_T = temperature coefficient; Φ_l = light proportionality coefficient; l = light intensity (mW/cm^2); K_f = inactivation rate constant due to other factors such as sorption, filtration, and sedimentation; and, K_p = inactivation rate constant due to predation.

With the inability to separate sorption, sedimentation, predation, and other environmental factors in the study, the variable K_{other} was substituted for K_f and K_p. As a result, the inactivation rate equation from (3) can be written as:

$$K_{overall} = K_{20}\Phi_T^{\,T-20} + \Phi_l I + K_{other} \qquad (4)$$

Where definitions are as above and K_{other} = inactivation rate constant due to other factors such as sorption, filtration, sedimentation, predation, pH, DO, conductivity, ORP, etc.

Table 6 lists coefficients for light and temperature from the light and dark controls of this study. Using the light and dark control inactivation rates, the inactivation rate due to other parameters was calculated as the measured $K_{light\ +temperature}$ value. Subtracting the $K_{temperature}$ value from the $K_{light\ +temperature}$, resulted in a calculated K_{light} value. The K_{other} value was then calculated by subtracting K_{light} and $K_{temperature}$ from the $K_{overall}$ values that weres measured for the retention pond and constructed wetland. All K values for the retention pond and constructed wetlands are in Tables 7 and 8, respectively.

Table 6. Inactivation Rate Coefficients from the Mesocosm Studies

Indicator Organisms	Φ_L (cm^2/ mW-h)	K_{20} (h^{-1})	Φ_T	Φ_L (cm^2/ mW-h)
TC	0.0016	0.066±0.007*	1.005±0.011	0.0092
FC	0.0130	0.053±0.006*	1.017±0.004*	0.0047
EC	0.0025	0.057±0.008*	1.013±0.002	0.0022
ENT	0.0076	0.054±0.006*	1.050±0.014	0.0070

* Coefficient is statistically significant at α=0.05

The $K_{light\ +temperature}$ values for the constructed wetland were not directly measured but were calculated. To calculate these values, the K_{light} value from the light control was multiplied by the weighted average of light intensity expected at the surface of the constructed wetland. The weighted average was calculated as 10% of the light that reached the retention pond surface (based on light meter measurements) for six hours (h) out of 24 h of effective light exposure, multiplied by 18 h out of 24 h in which the exposure was relatively the same as in the retention pond. This resulted in a multiplication factor of 0.775 •K_{light} of the retention pond. All negative calculated values were assumed to be a propagation of error and were therefore expected to be within the range of error for the respective inactivation rate constant.

Inactivation rate constants had differing values throughout the year based on different effects of environmental factors. With the exception of enterococci, the combination of "other factors" had the greatest impact on inactivation rates in the retention pond. Temperature was found to have a greater effect than light on inactivation rates of indicator bacteria, with the exception of enterococci in which light had the greatest influence. Light, however, was still a statistically significant factor and should be considered when using the first-order equation.

In the constructed wetland, temperature was found to have the greatest effect on inactivation rates for the indicator bacteria, with the exception of fecal coliforms. The combination of "other factors" had the greatest influence on fecal coliforms and was similar or slightly greater in influence to light for the other indicator bacteria. Again, enterococci was an exception to this, where light and temperature had a similar influence on inactivation rates.

Application of the inactivation rate constants found in Tables 7 and 8 can provide an overall inactivation rate constant incorporating temperature, light intensity, and a lumped factor for other environmental variables. The overall inactivation rate constant can be applied to equation 2 to determine the required area necessary to achieve water quality standards when designing constructed wetland or retention ponds.

Table 7. Retention Pond Overall, Temperature, Sunlight, and Other Factors Rate Coefficients

Indicator Organism	Month/Year	$K_{overall}$ (measured)	K_{temp} (measured)	K_{light} (calculated)	K_{others} (calculated)
				(h^{-1})	
TC	June 2004	0.242	0.025	0.114	0.103
	September 2004	0.144	0.070*	-0.011*	0.085
	November 2004	0.165*	0.082*	-0.016*	0.010
	May 2005	0.095	0.026	0.042*	0.027
	July 2005	0.181	0.064*	0.008*	0.109
	October 2005	0.044*	0.054*	0.014*	-0.024
	Annual Average	0.145	0.054	0.025	0.052
FC	June 2004	0.181	0.025	-0.001	0.157
	September 2004	0.119	0.053*	0.023*	0.043
	November 2004	0.149*	0.045*	0.024*	0.079
	May 2005	0.142	0.051*	0.033	0.059
	July 2005	0.261	0.062*	0.052*	0.147
	October 2005	0.057*	0.051*	0.031*	-0.025
	Annual Average	0.152	0.048	0.027	0.077
EC	June 2004	0.148	0.028	0.122	-0.002
	September 2004	0.116	0.056*	0.023*	0.038
	November 2004	0.116*	0.048*	0.024*	0.044
	May 2005	0.335*	0.073	0.043	0.219
	July 2005	0.196	0.051*	0.020*	0.125
	October 2005	0.052*	0.061*	0.025*	-0.034
	Annual Average	0.161	0.053	0.043	0.065
ENT	September 2004	0.203	0.077*	0.126*	0.001
	November 2004	0.173*	0.071*	0.108*	-0.006
	May 2005	0.172	0.035*	0.053*	0.083
	July 2005	0.124	0.094*	0.074*	-0.044
	October 2005	0.051	0.019*	0.013	0.019
	Annual Average	0.145	0.059	0.075	0.011

* Coefficient is statistically significant at α=0.05

If a background concentration exists, the overall rate constant can also be applied to equations 1 and 2 to predict the expected event mean concentration of BMP effluent. The first-order decay equation is most accurate when used with the inactivation rates and uncertainties in short-term models to predict stormwater runoff effluent quality from constructed wetland and retention pond BMPs to improve or prevent further degradation of water quality. Longer-term modeling would benefit from applying separate inactivation rates for periods immediately following stormwater runoff and periods unaffected by stormwater runoff.

Table 8. Constructed Wetland Overall, Temperature, Sunlight, and Other Factors Rate Coefficients

Indicator Organism	Month/Year	$K_{overall}$ (measured)	K_{temp} (measured)	K_{light} (calculated)	K_{others} (calculated)
				(h^{-1})	
TC	June 2004	0.153	0.025	0.088	0.040
	September 2004	0.120	0.070*	-0.009*	0.059
	November 2004	0.124*	0.083*	-0.013*	0.054
	May 2005	0.109	0.026	0.033*	0.050
	July 2005	0.073	0.064*	0.006*	0.003
	October 2005	0.043*	0.054*	0.011*	-0.022
	Annual Average	0.104	0.054	0.019	0.031
FC	June 2004	0.328	0.025	-0.001	0.304
	September 2004	0.152	0.053*	0.018*	0.081
	November 2004	0.114*	0.045*	0.019*	0.050
	May 2005	0.123	0.051*	0.025	0.047
	July 2005	0.103	0.062*	0.040*	0.001
	October 2005	0.054*	0.051*	0.024*	-0.021
	Annual Average	0.146	0.048	0.021	0.077
EC	June 2004	0.165	0.028	0.095	0.042
	September 2004	0.120*	0.056*	0.018*	0.046
	November 2004	0.116*	0.048*	0.019*	0.049
	May 2005	0.092*	0.073	0.033	-0.014
	July 2005	0.189*	0.051*	0.016*	0.123
	October 2005	0.060*	0.061*	0.019*	-0.020
	Annual Average	0.124	0.053	0.033	0.038
ENT	September 2004	0.179*	0.077*	0.098*	0.004
	November 2004	0.125*	0.071*	0.084*	-0.030
	May 2005	0.085*	0.035*	0.041*	0.009
	July 2005	0.211*	0.094*	0.057*	0.060
	October 2005	0.059*	0.019*	0.010	0.030
	Annual Average	0.132	0.059	0.058	0.015

* Coefficient is statistically significant at $\alpha=0.05$

Scaling Consideration

Mesocosms have a history of use as a research tool for ecological studies of aquatic and terrestrial ecosystems (Grice and Reeve, 1982; Odum, 1984; Lalli, 1990; Adey and Loveland, 1991; Beyers and Odum, 1993; Kangas and Adey, 1996). They have been used in commercial scale applications, such as in wastewater treatment, food production (Kangas and Adey, 1996), and in ecosystem restoration (Callaway *et al*., 1997). Use of mesocosms, particularly in wetland science, has become more common as a research tool for use in studies of the fate and effect of pollutants, biogeochemical cycles, and the effects of nutrients on ecosystem dynamics.

When comparing mesocosms to natural ecosystems, ecological complexity is to some degree reduced or lost in microcosm or mesocosm studies depending on the size of the mesocosms being used relative to large ecosystem-scale research. Scale can change nutrient cycling, the number of trophic levels, number of species within trophic levels, habitat types, and connectivity between habitats (Beyers and Odum, 1993). Because of this, some caution needs to be used when one extrapolates mesocosm results to larger systems. Once models created using mesocosms are validated in the field, application of model results at a larger scale can be made.

CONCLUSIONS

This study demonstrated that the concentration of the tested indicator organisms decrease exponentially with time. The first-order decay model is a simple and efficient means of predicting indicator bacteria concentrations in stormwater runoff effluent from BMPs such as retention ponds and constructed wetlands for shorter durations (about 50 h). Results from this study provide new data on inactivation rate constants coefficients, and uncertainties used in this model. The factors of light, time, and temperature influence processes in all retention ponds and wetlands constructed to mitigate the effects of stormwater runoff on the receiving waters. A combination of other factors (e.g., predation, sedimentation, sorption, filtration, pH, BOD, pH, and DO) can also contribute significantly to the inactivation of indicator bacteria in constructed wetland and retention pond BMPs. Reliable rates, coefficients, and the uncertainties expected in the reported values will add to the accuracy of surface water quality models. Water quality models are a primary tool for evaluating permit applications (e.g., National Pollutant Discharge Elimination System (NPDES) permits) and have an important regulatory role in developing TMDL allocations. These models should incorporate the affects of BMPs to better model their potential for improving water quality in the watershed. The incorporation of simple reliable models is an important step in assuring that the models used in determining bacterial TMDL loading and allocations meet the state of the science.

BMPs were originally designed to control runoff volumes and rates by attenuating the flow. The attenuation increases the time between the rainfall-generated runoff and the water reaching the receiving water. The time lag serves to reduce the concentration of these indicator organisms. Structural BMPs then can be effective in reducing indicator bacteria concentrations contained in stormwater runoff. Low inactivation rates may

occur in BMPs where inflow bacterial concentrations are lower than the *in situ* bacteria productions rates, or turbulent flow through the BMPs causes resuspension of sediments.

Quantitative microbial partitioning estimates can represent critical inputs in areas where sedimentation is a primary mode of indicator organism inactivation when modeling the location and severity of impaired waters. The lack of reliable partitioning information currently leads most surface water modeling efforts to assume that microbes exist in the free phase. The presumption of only free-phase organisms biases model results to greater dispersion and shorter microbial longevity. However, from the results obtained from this project, factors such as temperature and light intensity have been shown to be as, or more important to, indicator bacteria inactivation rates. This would suggest that when attempting to mitigate bacteria in runoff, watershed managers should construct BMPs to maximize the temperature increase from solar exposure. Similarly, the added effects of light, even at constant temperature, can increase inactivation rates, improving BMP performance. The extent to which shading in constructed wetlands, due to vegetation or the deeper water of retention ponds, attenuates the effect of incident light will vary with runoff and *in situ* water properties (e.g., turbidity, color) in the BMP. It is also important to recognize that bacteria loading seldom acts as a single environmental stressor of concern. The watershed manager must consider the effects of the increased effluent temperature on the receiving waters, particularly when the receiving water is a low-order cold water stream. Also, the results from this study suggest that the regulatory indicator selected can influence BMP design. The apparent insensitivity of coliforms to light levels suggests that the shading effects may be reduced when this is selected as the water quality indicator. When the monitored indicator organism is *E. coli* or enterococci, the effect of light would be expected to be greater than for coliforms.

It is accepted that placement of appropriate BMPs in watersheds can lead to improvements in receiving water quality by reducing the overall load of pollutants to receiving waters. If watershed managers can reduce microbial loads in waterbodies using the range of possible BMPs, verification of these stormwater management tools will help MS4 Phase I and Phase II communities reduce microbial loadings and meet requirements set out by the TMDL process. Long-term microbial load reductions will improve the overall water quality and could potentially lead to increased consumption of fish and shellfish, increased use of recreational waters, reduced beach closures, and increased protection of source water used as drinking water sources.

The limitations of BMP effectiveness in reducing bacterial loading to WQS must be recognized. In most natural treatment systems there will be an irreducible concentration that is often maintained in system sediments. Dilution of BMP effluent likely plays a significant role in attaining WQS in receiving water. However, elimination of bacteria indicators may require chemical treatment. In addition, overall effectiveness and efficiencies of BMPs hinge on proper design and maintenance of these systems.

Disclaimer: Any opinions expressed in this paper are those of the author(s) and do not, necessarily, reflect the official positions and policies of the U.S. EPA. Any mention of products or trade names does not constitute recommendation for use by the US EPA.

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