REDUCTION OF FECAL INDICATOR BACTERIA COUNTS IN AN ECOLOGICALLY-ENGINEERED ACID MINE DRAINAGE AND MUNICIPAL WASTEWATER PASSIVE CO-TREATMENT SYSTEM[1]

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Abstract: Passive co-treatment of municipal wastewater and synthetic acid mine drainage in a laboratory-scale, four-stage continuous flow reactor system was examined for changes in fecal indicator bacteria counts. Synthetic acid mine drainage was mixed at a 1:2 ratio with raw municipal wastewater from the City of Norman, Oklahoma and introduced to the system. The municipal wastewater contained varying concentrations of total coliforms (TC), fecal coliforms (FC), *E. coli* (EC), and fecal streptococci (FS). Initial concentrations ranged from 6-13, 0.6-6, 3-5, and 0.1-0.7 million cfu/100 mL, for TC, FC, EC, and FS, respectively. During the 6.6-day system residence time, a 100% reduction of all indicator bacteria was observed. However, indicator bacteria exhibited evidence of sub-lethal injury with slower colony formation rates on standard growth media. Extending standard incubation periods resulted in higher concentrations of all indicator bacteria in each treatment stage, except the final stage where only EC and TC counts increased. Although this co-treatment regime effectively reduced indicator bacteria concentrations, much remains unknown about the potential for sub-lethal injury to indicator bacteria and its impact on the viability of co-treatment for pathogen removal.

Additional Key Words: acid mine drainage, wastewater, passive treatment, co-treatment, aqueous geochemistry, water quality, pathogen, coliform, pathogen removal, and sewage

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[1] Paper was presented at the 2009 National Meeting of the American Society of Mining and Reclamation, Billings, MT, ***Revitalizing the Environment: Proven Solutions and Innovative Approaches*** May 30 – June 5, 2009. R.I. Barnhisel (Ed.) Published by ASMR, 3134 Montavesta Rd., Lexington, KY 40502.

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Proceedings America Society of Mining and Reclamation, 2009 pp 1611-1626
DOI: 10.21000/JASMR09011611
http://dx.doi.org/10.21000/JASMR09011611

Introduction

Background

Acid mine drainage (AMD) and municipal wastewater (MWW) problems are ubiquitous and their effective treatment is central to maintaining water quality. Untreated AMD causes water quality degradation in mining regions worldwide (e.g., Bell and Donnelly, 2006). Discharges of untreated MWW degrade water resources in many developing nations (Gadgil, 1998; Kivaisi, 2001; Nelson et al., 2001). In developed nations, where MWW is generally effectively addressed via active means, treatment consumes considerable financial, material and energy resources. Conventional MWW and AMD treatment are energy-intensive with higher operational and maintenance costs than passive methods (Nelson et al., 2001; Younger et al., 2002). Treating AMD and MWW together could save resources and alleviate some of the infrastructural challenges of building separate treatment systems in areas where these two waste streams are prevalent.

Pathogen removal is a key aspect of MWW treatment. Total coliform (TC), fecal coliform (FC) and fecal streptococci (FS) are typical indicator organisms monitored to determine pathogenic risk. TC, FC and FS concentrations in untreated MWW are generally 10^5-10^6, 10^4-10^5 and 10^3-10^4 cells/mL respectively (Metcalf and Eddy, 1991). Pathogens are typically removed from MWW by chemical agents (e.g., chlorination), physical processes (e.g., heating), mechanical means (e.g., sedimentation) or radiation (e.g., ultraviolet disinfection) (Metcalf and Eddy, 1991). However, pathogens can be removed by exposure to other unsuitable growth circumstances, such as elevated concentrations of dissolved metals and extreme pH (Hackney and Bissonnette, 1978; Wortman and Bissonnette, 1985; Wortman et al., 1986; Wortman and Bissonnette, 1988).

The sterilization of domestic sewage by AMD was first documented in the 1930s. Roetman (1932) was the first to note this effect, specifically suggesting that wastewater treatment facilities take advantage of this phenomenon. Joseph and Shay (1952) found that populations of *E. coli* were rapidly decreased when exposed to AMD. More recent studies have noted that exposure to AMD causes death or widespread structural damage to *E. coli* and that extended incubation periods in specialized enriched medium are necessary to repair surviving cells (Hackney and Bissonnette, 1978; Wortman and Bissonnette, 1985; Wortman et al., 1986; Wortman and Bissonnette, 1988). Carlson-Gunnoe et al. (1983) determined TC, FC and FS counts to be

decreased by orders of magnitude within two hours following in-stream exposure of sewage to AMD. Keating et al. (1996) noted all strains of enteric bacteria tracked in a sewage-contaminated stream to be significantly and rapidly decreased to varying degrees during in-situ and laboratory bioassay exposure to AMD.

Despite the amount of peripheral research regarding MWW and AMD treatment separately, only one system was found in the literature that has been constructed to simultaneously treat these effluents (Johnson and Younger, 2006). However, this system treated secondary MWW effluent and relatively weak (net-alkaline with 3 mg/L Fe) mine drainage and the study did not track indicator bacteria or pathogen removal performance. Pathogen removal performance during co-treatment of high-strength AMD and raw MWW has not been investigated and this is central to addressing the long-term feasibility of the approach. This study intended to assess the ability of these co-treatment systems to remove pathogens from MWW and explore the processes that may be responsible for this removal.

Methods

Experimental Design

The experimental setup involved three serial unit processes using four replications (Fig. 1 and 2). The first unit processes were primary clarifiers where the combination of MWW and

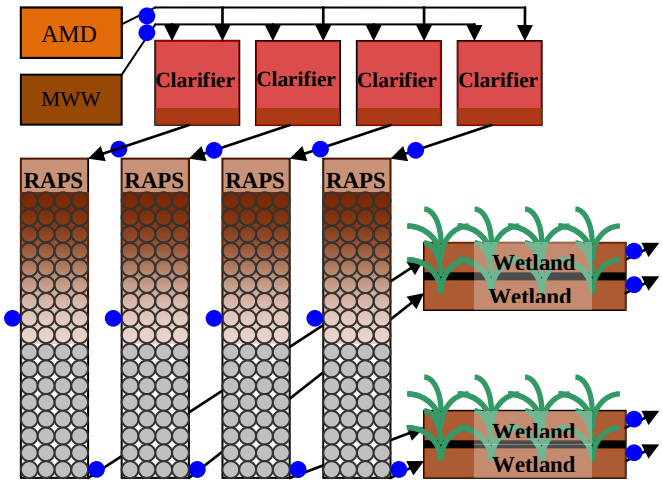

Figure 1: Conceptual experimental layout. Blue dots indicate sampling points. Acid mine drainage (AMD) and municipal wastewater (MWW) were pumped into first unit process. The system is subsequently gravity-fed. Unit processes include clarifier, reducing and alkalinity producing system (RAPS), and aerobic wetland.

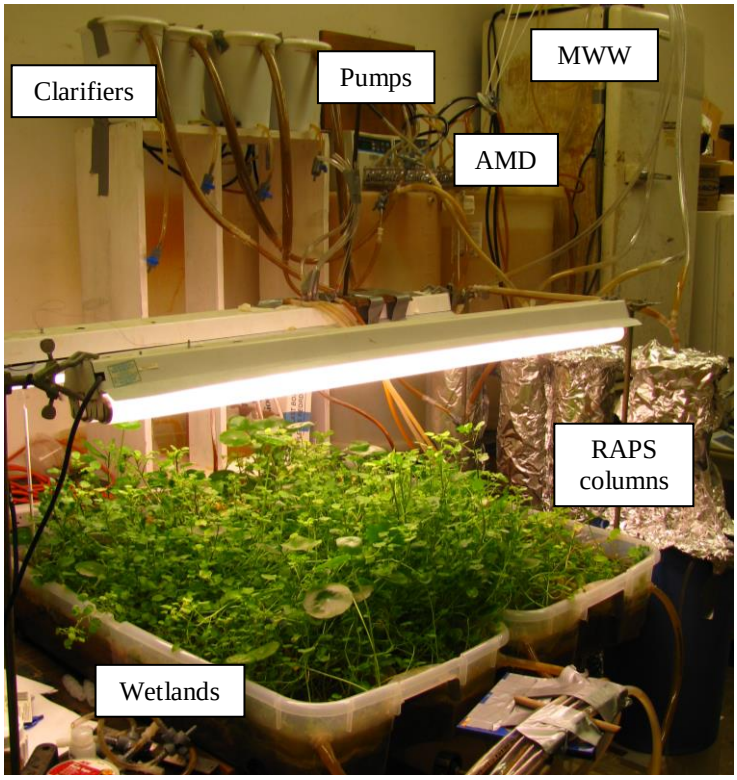

Figure 2: Photo of laboratory setup showing AMD and MWW reservoirs, peristaltic pumps, clarifiers, RAPS simulation columns, grow-lights and wetlands.

AMD react and solids settle. The second unit processes resembled a reducing and alkalinity producing system (RAPS) for alkalinity and metal sulfide generation. The final unit processes were aerobic, surface flow wetland mesocosms for Fe and then Mn oxidation and precipitation. Each unit process was connected to the next via clear vinyl tubing.

The primary clarifier unit process (treatment stage C) was sized for a relatively high retention time of 32 hr (Table 1). Influent AMD and MWW were pumped separately into the 5-L low-density polyethylene (LDPE) clarifier at the water surface level. Secondarily, this unit process served to ensure MWW and AMD had ample time to mix and react before entering the RAPS simulation columns. While retention times of 1.5-2.5 hr are typical for MWW primary clarification systems (Metcalf and Eddy, 1991; Frigon et al. 2006), the clarifiers were intended to partially treat the AMD/MWW mixture through physical and chemical processes in order to prevent overloading the RAPS simulation column with high sulfates and metals, low pH, and abundant suspended solids. Additionally, retention times of around 6 hr or more commonly exist (Anderson, 1981; Gernaey et al. 2001). Single transverse baffles were installed to a depth of 4 cm to prevent preferential flow in the clarifier. A 2.5-cm radius semi-circular weir was used at

the clarifier outlet to prevent floating solids from exiting this unit process. Sludge was wasted from the bottom of the clarifiers under gravity flow with a barbed high-density polyethylene (HDPE) T-connector attached to clear vinyl tubing.

The RAPS simulation columns were 91.5 cm in height and 12.5 cm in diameter (Table 1). The bottom 38 cm of the columns were filled with high quality (>90% $CaCO_3$) limestone washed of all fines and separated by sieve analysis adapted from ASTM D422 with the fraction passing 2.54-cm sieve yet retained 1.27-cm sieve (treatment stage L). The remaining 53.5 cm of the columns were packed with Kaldnes K3 biofilm media to provide conditions that promote the growth of sulfate-reducing bacteria (SRB). The Kaldnes zone (treatment stage K) was inoculated with 100 mL of RAPS substrate from two mature passive AMD treatment systems, one in Pittsburg County and the other in Latimer County, OK following Pruden et al.'s (2007) findings of inoculation's importance to sulfate reducing bioreactor performance. Columns were wrapped in aluminum foil to emulate the lightless conditions in RAPS substrate.

The aerobic constructed treatment wetland mesocosms (treatment stage W) were two shallow, LDPE storage containers with a capacity of 20 L and a surface area of 5,100 cm^2 (Table 1). Each wetland was bisected longitudinally with an impermeable plastic barrier to create the necessary four treatment trains. Wetland soil was collected from an existing constructed mitigation wetland at the City of Midwest City, Oklahoma MWW Treatment Plant. The mesocosms were surface flow and planted with floating marshpennywort (*Hydrocotyle ranunculoides*) and watercress (*Nasturtium officinale*). These wetlands were placed under timed grow-lights on 12 hr/d.

Raw MWW collected after grit screening at the Norman, OK MWW treatment plant and synthetic AMD approximating that found at Cerro Rico de Potosí, Bolivia were introduced to the system at a 2:1 ratio with peristaltic pumps at a combined flow rate (3.8 L/d) to produce the recommended minimum 15-hr design residence time (Younger et al., 2002) in the limestone layer of the RAPS simulation unit process (Table 1). The system was gravity flow from the first (clarifier) to last (wetland) unit processes. MWW for the co-treatment influent was collected weekly, homogenized during pumping and refrigerated at 4°C before introduction to the system in a 50-L HDPE carboy with a mixing apparatus that switched on with the peristaltic pumps. The AMD was prepared weekly and stored at room temperature (20° C) until use. All unit

processes were maintained at room temperature throughout the experiment. Less than one percent of the total flow entering the clarifiers was wasted throughout the experiment.

Table 1. Design details and residence times for each unit process.

Unit Process	Surface Area (cm^2)	Total Volume (cm^3)	Porosity (-)	Void Volume (cm^3)	Residence Time (hr)
Clarifier (C)	410	5000	1	5000	32
RAPS-Kaldnes Zone (K)	120	6600	0.82	5400	34
RAPS-Limestone Zone (L)	120	4700	0.5	2300	15
Wetland (W)	5100	10600	1	10600	67
Overall	***5750***	***269000***		***23400***	***147***

The experiment consisted of two treatment regimens. First, each treatment train continuously handled the mixed influent from March 6 to July 21, 2008 (135 d), when pumping was stopped. Then, from July 21 to October 22, 2008 (91 d) the RAPS simulation columns were sealed and held at room temperature. Because sub-lethal damage to coliform cells from the mixture of AMD and MWW was suspected, extended incubation periods were employed and colony-forming units were counted every 24-48 hours for up to 244 hours from filtration.

Data Collection

Samples were collected for the assessment of indicator bacteria for this study. Strosnider et al. (2009) reported system performance for other water quality parameters. Untreated MWW and AMD were examined and are characterized in Tables 2 and 3. In this study, two treatment regimes were examined. During the first treatment regimen, sampling events occurred every two weeks, with exception at the end of the first treatment regimen when weekly samples were taken at the last two sampling events. Samples were taken from points at the end of each unit process (Figure 1). Sterilized lab-grade glassware was used to obtain samples that were analyzed upon collection. Samples from each location were replicated once and examined at varying dilutions during each sampling event. The membrane filter technique (APHA, 1998) was employed to estimate TC, FC, FS, and EC concentrations in these water samples. Difco™ growth media was prepared according to the manufacturer's instructions in 47-mm Millipore® disposable culture dishes prior to filtration. MI agar was used in culture dishes for TC and EC. FS and FC culture dishes used m-Enterococcus and m-FC media, respectively. Water samples were filtered through 47-mm Millipore® 0.45-µm membrane filters. Following the designated incubation period,

colony forming units were counted on each culture dish and recorded. In addition, culture dishes were incubated past their prescribed incubation periods. Colony forming units were counted and recorded after the prescribed incubation period every 24 or 48 hours for up 120 hours.

Table 2. Mean influent AMD and MWW physiochemical properties and sulfate concentrations. n=10 for all except where noted (Strosnider et al., 2009)

		pH	DO	SC	Alkalinity	Net Acidity$^{\delta}$	Net Acidity$^{\tau}$	SO_4^{2-}
		s.u.	mg/L	uS/cm	mg/L as CaCO3 equivalent			mg/L
MWW		7.67	0.98	951	288	-287	-268	70
	s.d.	*0.12*	*0.49*	*66*	*20*	*20*		*16*
AMD		2.60	7.69	3010	0	1870	1810	1920
	s.d.	*0.04*	*0.64*	*112*	*0*	*91*		*140*

$^{\delta}$ Calculated with dissolved metal concentrations
$^{\tau}$ Calculated with total metal concentrations (n = 2)

Table 3. Mean influent AMD and MWW dissolved$^{\delta}$ (n=10) and total$^{\tau}$ (n=2) metal concentrations (Strosnider et al. 2009).

	Al	As	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Zn
								mg/L						
MWW$^{\delta}$	0.11	<0.022	40	0.0010	0.0036	0.0067	0.32	16	21	0.056	74	0.010	0.015	0.045
s.d.	*0.02*		*4.5*	*0.0003*	*0.0045*	*0.0021*	*0.09*	*0.58*	*3.0*	*0.010*	*3.3*	*0.002*	*0.005*	*0.040*
MWW$^{\tau}$	0.69	<0.022	40	0.0009	0.007	0.029	0.72	17	18	0.063	67	0.005	0.014	0.53
AMD$^{\delta}$	46	0.25	83	2.0	0.027	0.0052	290	0.46	26	55	<0.0006	0.14	1.2	390
s.d.	*3.2*	*0.14*	*3.5*	*0.083*	*0.034*	*0.0028*	*24*	*0.62*	*1.3*	*3.0*		*0.026*	*0.10*	*22*
AMD$^{\tau}$	46	0.38	91	2.3	0.012	0.088	270	0.02	26	54	<0.0006	0.19	1.3	390

During the second treatment regimen, one sampling event occurred 91 days after sealing the RAPS simulation column. The same methods used in the first treatment regimen for analyzing indicator bacteria concentrations were used in the second treatment regimen. Culture dishes were not incubated past their prescribed incubation periods for this treatment regimen.

Data Analysis

Means of indicator bacteria concentrations after the standard incubation period for each stage were compared for both treatment regimens using Student's *t*-test. A type I error rate of $\alpha = 0.05$ was used for all statistical analyses. Data points from each sampling event and replication were treated as separate observations. FC and FS were examined throughout the experiment.

However, TC and EC were examined in only the last half of the study period. Consequently, the number of observations varied between indicator bacteria for each treatment stage. Treatment means among stages during standard incubation periods were compared as two-tailed, unpaired, and heteroscedastic.

Averages of indicator bacteria concentrations after extended incubation periods were compared between treatment stages at each colony enumeration. Concentration means were compared between treatment stages after each incubation period using Student's *t*-test assuming unpaired and unequal variance existed between samples. These treatment means were compared using a one-tailed test because only the treatment stage concentrations that increased from the previous stage were of interest.

Results and Discussion

Co-treatment of AMD and MWW in this ecologically engineered system resulted in 100% removal of indicator bacteria in the last two stages of treatment (Fig. 1). There was no significant difference between the limestone zone in the column (L) and wetland (W), for FC, FS, or EC. A significant ($\alpha < 0.001$) difference between the treatment means of the L and W stages occurred for TC. Significant differences existed between all other stages of each indicator bacteria as exponential decreases in concentrations were observed through each stage.

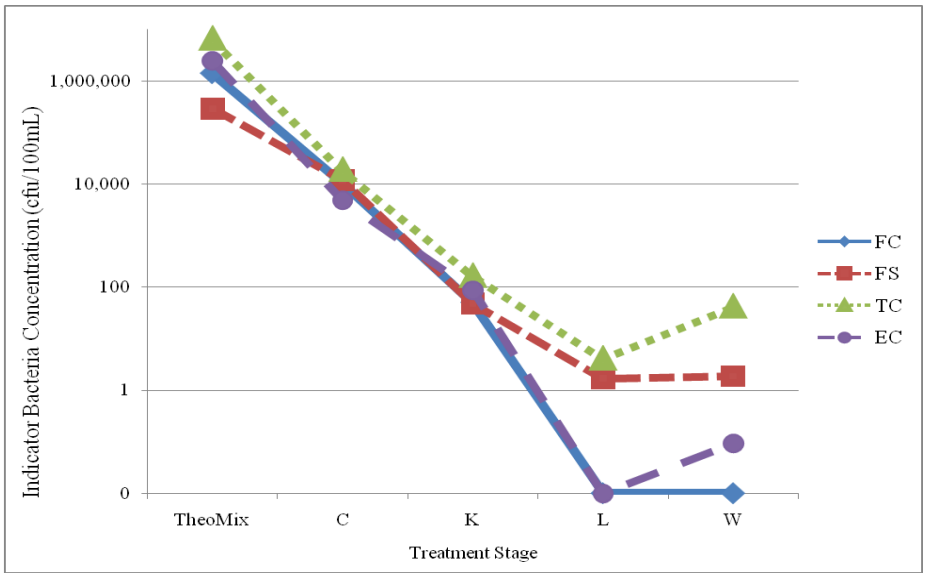

Figure 3: Indicator bacteria concentrations means in each treatment stage. TheoMix refers to the 2:1 ratio of MWW to AMD in the system influent. Standard errors of the means were too small to be perceptible on this figure and thus excluded. The number of samples for each treatment stage and indicator bacteria was highly variable.

The observed removal efficacy shows that this system has the ability to decrease indicator bacteria concentration by 100% during the first three treatment stages. This reduction occurs within 81 hours of the introduction of AMD and MWW to the treatment system. The significant increase in TC from stage L to the outflow of the wetland (W) indicates coliforms may proliferate in the wetland, despite close proximity to UV light from the grow lights, which has been shown to destroy indicator bacteria (Metcalf and Eddy, 1991). However, the other fecal indicator bacteria tracked did not exhibit a significant increase in the wetland during standard incubation in culture dishes.

Many municipal wastewater treatment plants (WWTPs) discharge treated effluent with substantial concentrations of fecal indicator bacteria (Rose et al., 1996; Koivunen et al., 2003; Zhang and Farahbakhsh, 2007; Kay et al., 2008; Suh et al., 2009). In a study of 12 WWTPs across the United Kingdom, Kay et al. (2008) document no significant elimination of FC or TC in primary clarification. However, Kay et al. (2008) noted FC decreases from $10^{7.23}$ to $10^{5.63}$, $10^{5.45}$, and $10^{5.20}$ and TC decreases from $10^{7.59}$ to $10^{6.15}$, $10^{5.89}$ and $10^{5.83}$ cfu/100mL as MWW flows through primary clarification and trickling filters, activated sludge, or rotating biological contactors, respectively. Ultraviolet radiation decreased FC and TC concentrations to $10^{2.45}$ and $10^{3.18}$, respectively. This study shows fecal indicator bacteria removal in co-treatment systems may be higher than removal in active municipal wastewater treatment systems.

The co-treatment system outperforms other passive MWW treatment systems, such as free-water surface (FWS), subsurface flow (SSF) constructed wetlands, and soil filters (Table 4). In a compilation of FWS constructed wetland treatment data from several across the globe treating secondary and tertiary MWW, many of which have residence times days greater than the co-treatment system, Kadlec and Wallace (2009) document FC, FS, *E. coli*, and TC reductions in the approximate range of 10^5 to 10^3, 10^4 to 10^2, 10^5 to 10^1, and 10^7 to 10^5 cfu/100mL, respectively. Co-treatment achieves more complete and rapid removal of indicator bacteria than that documented in SSF constructed wetlands (Ottová et al., 1997; Green et al., 1997; Vymazal et al., 2001, 2005; Meuleman et al., 2003, Garcia et al., 2008; Kadlec and Wallace, 2009). The constructed soil filters examined by Kadam et al. (2008) removed indicator bacteria rapidly, however, less completely than co-treatment.

Table 4. Passive MWW treatment system mean influent (I) and effluent (E) indicator bacteria concentrations (in $\log_{10}$ cfu/100mL) for systems handling primary and secondary or tertiary MWW.

	Primary/Secondary/Tertiary		Secondary/Tertiary					
	Co-Treatment		FWS		SSF		Soil Filter	
	I	E	I	E	I	E	I	E
FC	6.19	-[A]	5.21[B]	3.28[B]	6.04[B]	3.72[B]	7.70[F]	4.76[F]
FS	5.49	-	5.01[C]	3.62[C]	5.27[D]	2.27[D]		
E. coli	6.40	-	5.14[C]	3.72[C]	7.67[E]	1.27[E]		
TC	6.79	1.60	6.76[C]	4.91[C]	6.35[B]	4.23[B]	8.44[F]	6.41[F]

[A] "-" indicates 0 cfu/100mL detected.
[B] Kadlec and Wallace (2009) (7.7- and 5.8-d residence time for FC and TC, respectively)
[C] Molleda et al. (2008) (312-hr residence time)
[D] Garcia et al. (2008) (3-d residence time)
[E] Meuleman et al. (2003)
[F] Kadam et al. (2008) (0.5-2.0 hr residence time)

Extended incubation of culture dishes gave further evidence of sub-lethal damage to indicator bacteria cells (Fig. 4). Although average concentrations of FS in AMD or MWW did not exhibit

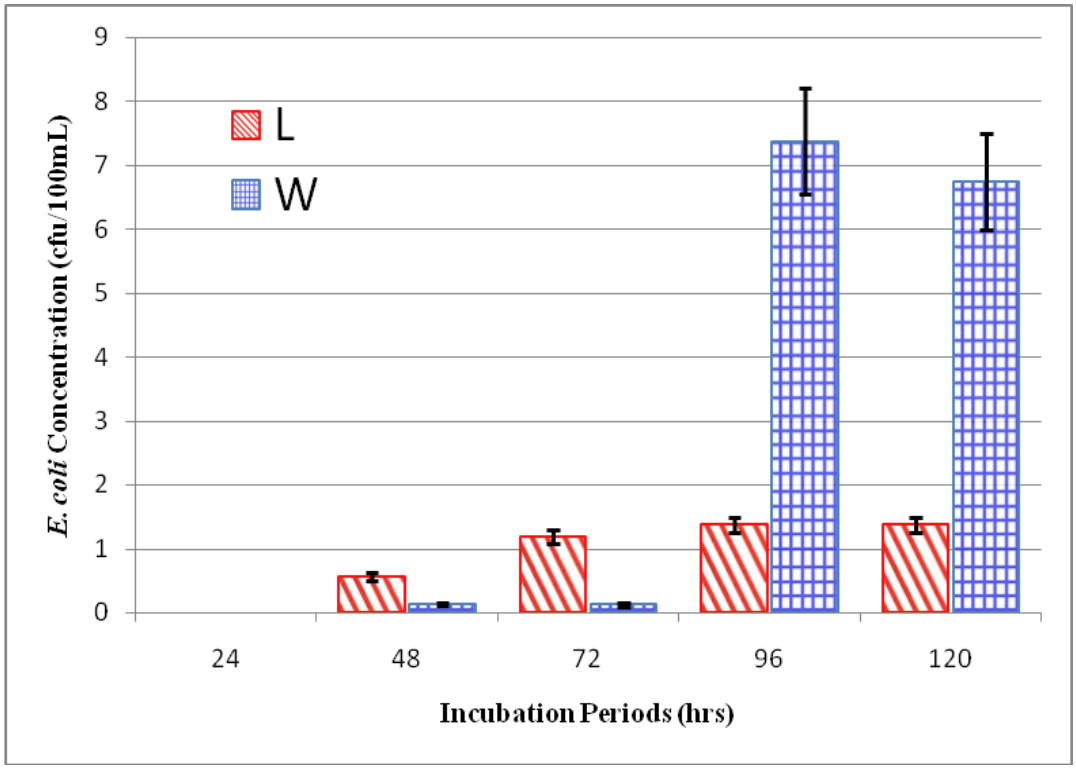

Figure 4: Average concentrations of E. coli in treatment stages L and W after extended incubations. Error bars show standard error of the means. Average concentrations after standard incubation were significantly different in both treatment stages after 72 hours of incubation.

significant differences between incubation periods of 48, 72, or 120 hours, FS in treatment stage L significantly increased from the standard incubation period of 48 hours to and extended incubation periods of 120 hours (3.2 cfu/100mL to 11 cfu/100mL). In addition, all incubation periods resulted in significantly higher concentrations of FS in the K stage than in the L stage. Because a layer of biofilm was present on the Kaldnes K3 media in the stage, it is possible this stage promoted higher microbial activity and higher tolerance of FS to metals. *Staphylococcus epidermidis* proliferates in biofilms in industrial, clinical, and environmental settings (Baker-Austin et al. 2006). Baker-Austin et al. (2006) point out biofilms may encourage co-selection of resistance to antibiotics and metals.

Extended incubation of EC culture dishes also resulted in increased concentrations. Average EC concentrations in treatment stages L and W significantly increased after extended incubation from 0 cfu/100mL each to 1.4 and 6.8 cfu/100mL, respectively. Because the residence time is over 4 times as long in treatment stage W than stage L, there may be greater potential for metal tolerant bacteria to thrive in the wetland through increased exposure to resistance genes. Metal tolerant *E. coli* has been found to proliferate in municipal wastewater at circumneutral pH (Gikas, 2008). Perhaps, after the limestone treatment increased the pH of the system, *E. coli* cells were impeded in growth rather than destroyed. Increased concentrations at extended incubations periods in the wetland treatment stage may be due to horizontal transfer of metal tolerance genes (Abskharon et al., 2008).

Nies (1999) presented minimal inhibitory concentrations (MIC) of metals that depress the growth of *E. coli*. Only Zn exceeded the MIC of *E. coli* (65.4 mg/L) in this system and did so in all treatment stages (at $\geq$ 80 mg/L) except the L and W. Although the MICs from the Nies (1999) study were determined for metals singularly (i.e.: not in combination), it is possible the lower Zn concentrations in the L and W treatment stages allowed sub-lethally damaged *E. coli* to survive. All metal concentrations decreased from the influent to treatment stages L and W (Strosnider et al., 2009). Co-selection of metal and antibiotic resistance could explain the observed metal tolerance of indicator bacteria in this study. Because many of these bacteria may have been exposed to antibiotics in the wastewater conveyance system, they could be inclined to tolerate metals. The same genes that promote antibiotic resistance may also promote metal tolerance and may be passed to cells via horizontal gene transfer (Baker-Austin et al., 2006) prior to introduction to the co-treatment system.

In the second treatment regimen, indicator bacteria concentrations were determined at days 1 and 91 in the column. On these days, only one-indicator bacteria (TC) exhibited significantly greater average concentrations over the extended column residence time. Fecal streptococcus significantly decreased in the K treatment stage from 21 to 0 cfu/100mL. No significant changes occurred in the L treatment stage during the 91-day incubation period in the columns.

Coliform indicator bacteria concentrations were not affected to a large extent by extended residence time in the sealed columns. *E. coli* and FC concentrations showed no significant difference at days 1 and 91 in K and L treatment stages after standard incubation in culture dishes. Total coliform concentration increased significantly from an average of 0 to 0.75 cfu/100mL in the K treatment stage over the 91-day incubation period in the columns. While *E. coli* concentrations did not significantly differ under standard incubation periods (24 hrs), significant increases in bacterial counts were seen after 120 hours of incubation on culture dishes (Fig. 5). This delayed growth of *E. coli* could have been caused by sub-lethal damage done to cells exposed to metals in the column.

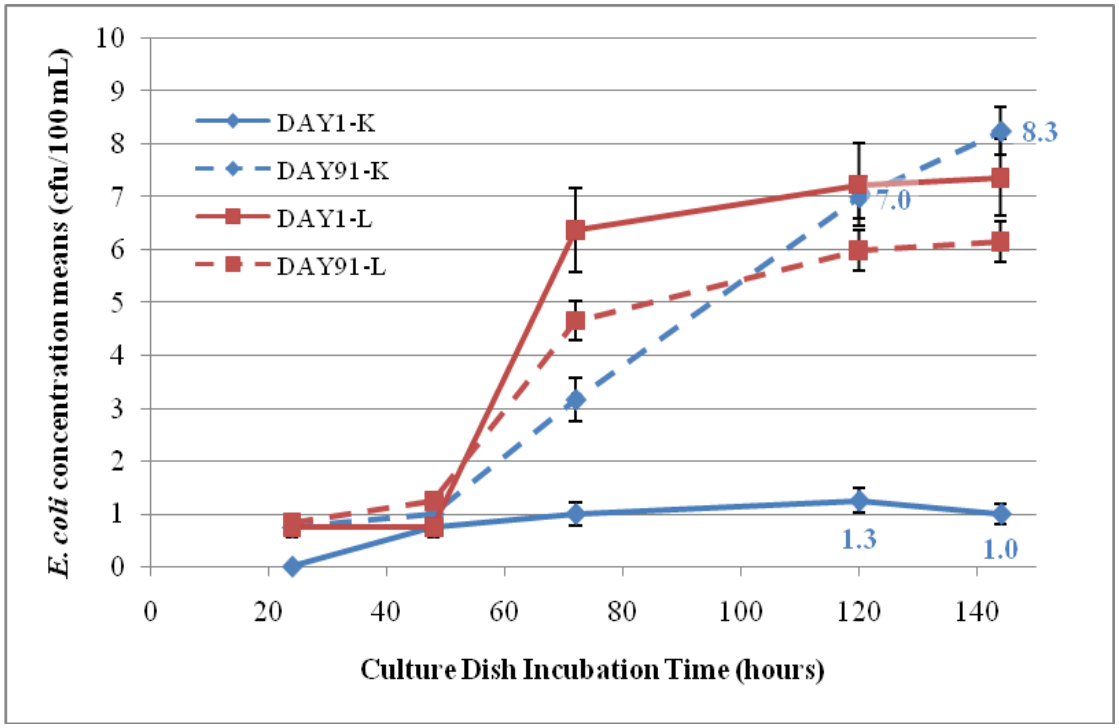

Figure 5: Average *E. coli* concentrations at treatment stages K and L at days 1 and 91 in extended incubation of culture dishes from extended column incubation period. Data points with labels indicate a significant difference existed between *E. coli* concentrations at days 1 and 91. P-values for the difference in *E. coli* concentrations in the K treatment stage between days 1 and 91 for extended incubation in culture dishes at 120 and 144 hrs were 0.042 and 0.020, respectively. Error bars represent standard error of the means.

Previous studies have documented greater AMD resistance by FS than by coliform bacteria (Hackney and Bissonnette, 1978; Carlson-Gunnoe et al., 1983; Keating et al., 1996). This suggests FS was able to thrive while continuously exposed to AMD in the column. However, when the columns stopped receiving effluent on day 1, perhaps TC and EC were able to grow in lieu of FS as AMD weakened in the columns over time. TC and EC increased over the extended residence time, which may indicate sub-lethal cell damage by the high metals concentrations and low pH observed in this system by Strosnider et al. (2009). Over time, without perpetual exposure to the waste stream from the clarifiers, some coliform bacteria were able to proliferate in the columns, perhaps by outcompeting FS in the absence of high-strength AMD. Although this system removed indicator bacteria more efficiently than other passive treatment systems (Table 4), the impacts of sub-lethal damage to these indicator bacteria needs to be further investigated.

Conclusions and Recommendations

Results suggest that passive AMD and MWW co-treatment is a viable ecological engineering approach for the developed and developing world that can be optimized and applied to improve water quality with minimal use of fossil fuels and refined materials. Because nearly complete removal of fecal indicator bacteria occurred, AMD may act as an effective disinfectant. However, extended incubation in culture dishes revealed sub-lethal damage to bacteria cells that may generate metal resistant *E. coli* in constructed wetlands.

This study could be followed by a more controlled, side-by-side comparison of identical systems treating AMD, MWW, and AMD+MWW. This experiment would provide further insight regarding the performance of co-treatment vs. traditionally separating the waste streams. Because these systems could be used to treat waste streams in communities in developing countries, it would be useful to track specific pathogens through these systems. These pathogens could be tested for expression of metal tolerance and antibiotic resistance genes. The link between antibiotic resistance and metal tolerance has been shown in several studies. While it is unknown whether these systems have the potential to increase the transfer of resistance to antibiotics and heavy metals, they may provide a means to consolidate resistant genes in order to remove them before they enter the environment.

Acknowledgments

Special thanks are extended to Kristina Johnson Strosnider for laboratory assistance. In addition, comments provided by reviewers are deeply appreciated for providing many helpful observations that significantly improved this paper. Funding was provided by U.S. EPA Agreement FY04 104(b)(3) X7-97682001-0, USGS Agreement 04HQAG0131 and the University of Oklahoma Center for Restoration of Ecosystems and Watersheds.

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