Treatment of Bio-Oil Refinery Storm Water by a Simulated Constructed Wetland: A Sustainable Management Alternative

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Abstract

Contaminated storm-water discharge is a growing concern in the United States owing to a steady increase of harmful pollutants entering freshwater sources. This study remediated contaminated storm-water runoff from a bio-oil conversion facility through a simulated constructed wetland. A six-phase series of constructed wetlands was contaminated with varying dilution levels of bio-oil process water. The study concluded that there was a significant decrease in biological oxygen demand (BOD) and microtoxicity over a 10-day remediation cycle within the constructed wetlands for the lower levels of contaminated storm water. The higher levels of contamination changed very little in total volume of pollution. A comparative screening of the bacterial community within the wetlands during the contamination process showed a similar trend in species richness and composition for the first three phases of contamination. There was a shift in richness and diversity for the final three phases of contamination after 10 days. The constructed wetlands were successful at lowering BOD and toxicity levels and achieving permissible pH levels at dilutions higher than 500 times. When the concentrations of contaminated water were lower than 830 mL of contaminated bio-oil wastewater for every 240 liters of rainwater, the constructed wetlands were successful only at achieving permissible pH discharge levels. Better results for high-level contamination may be achievable with longer residence time in the wetlands.

The quality of storm-water discharge is a growing concern in the United States owing to a steady increase of harmful pollutants entering freshwater. Many congressional mandates that require local governments to reduce the impact of storm-water discharge on the environment have greatly increased the need for economically and environmentally viable solutions to pollution reduction. One such solution is that of constructed wetlands. Wetlands naturally function as a water-purifying system that removes contaminants such as organic material, suspended solids, pathogens, nutrients, and heavy metals and that lowers biological oxygen demand (BOD) levels (Pastor et al. 2003). Storm water and wastewater have conventionally been treated in off-site locations by different physical, chemical, and biological systems; however, for larger industrial units, it has become more cost-effective and environmentally beneficial to treat storm water on-site with constructed wetlands (Environmental Protection Agency [EPA] 2009). The use of constructed wetlands for large industrial facilities allows for more sustainable means of removing harmful pollutants on-site before they enter the local water source rather than sending the water to the regional water treatment facility (Campbell and Ogden 1999). The growth in consumption of forest products for biofuels has led to increased waste by-products generated from the bio-oil manufacturing processes. This waste is often found in the storm-water runoff from these facilities and can have harmful effects on the environment if not treated properly. A rapid development of widely applicable, low-cost methods for treatment of this type of wastewater is a high priority because several bio-oil refineries will start operating throughout the United States in the next few years (KiOR 2013). An efficient way to deal with the resulting wastewater produced needs to be examined.

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Since the passing of the Clean Water Act in 1972, the country has made great efforts in reducing the amount of pollution released into surface waters. For this study, the contaminants that were treated in the constructed wetlands originated from the thermochemical processing of biomass. Hydrothermal processing occurs when wet biomass undergoes thermal treatment at high pressures to produce liquid hydrocarbons (Mohan et al. 2006). The source of the wastewater for this study comes from hydrothermal processing of biomass.

Industrial biomass conversion sites contain a number of contaminants that are found in the storm-water runoff. These include aromatic hydrocarbons, such as benzene, toluene, ethylbenzene, and xylene, as well as other residual chemicals derived from petroleum. The other pollutants found in the contaminated wastewater derived from the bio-oil process include aldehydes, ketones, carboxylic acids, phenols, and furans (Klass 1998, Basu 2010, Caldeira-Pires et al. 2013).

There are many ways of dealing with contaminants that are often found in water, such as filtration, absorption through plant uptake, volatilization, and the microbial activity within a constructed wetland (Baker and Herson 1994). Some of the dominating organisms that are often found in a constructed wetland system are those that can break down hydrocarbons, aldehydes, ketones, carbolic acids, and phenols (Vymazal 2011, Weaver et al. 2012, Zhao et al. 2012). These include but are not limited to *Rhodococcus aetherivorans, Archaeoglobus, Borrelia burgdorferi,* and *Achromobacter* (Nelson and Wolverton 2011, Ansola et al. 2014).

Objectives

This study proposed to amend the waste-contaminated storm-water runoff from biomass conversion to a bio-oil facility through a simulated constructed wetland. The hypothesis of this study was that the contaminated rainwater can be remediated by constructed wetlands and safely released back into the native waterways. The results of this study help determine if wetlands can rapidly reduce the levels of bio-oil–contaminated storm-water runoff. This study also examined what levels of bacterial microbial communities occur during contamination and how they fluctuate during the breakdown of toxins. Previous studies at the Department of Sustainable Bioproducts at Mississippi State University have demonstrated the feasibility of kenaf fiber and wood shavings to remove toxins and crude oil from the bio-oil wastewater (Moghbeli et al. 2014). This study provides a foundation to future research on bio-oil wastewater–contaminated storm water and its remediation by constructed wetlands.

Methods

The experimental constructed wetlands consisted of sediments of pea gravel at the bottom, sand, and wetland clay soil on top, layered in equal volumes in six 87-liter plastic bins. The bins were connected to each other in a closed system, with the first bin attached also to a small-scale aquatic pump used for initial delivery of contaminated water into the system. Native wetland plants were randomly planted in five bins, with the first bin containing only gravel and sand, being left without any plants or soil, thus serving as a filtration system. The plant species included bulrush (*Scirpus californicus*), softrush (*Juncus effusus*), water hyacinth (*Eichhornia crassipes*), duckweed (*Lemna minor*), pickerelweed (*Pontederia*), duckpotato (*Sagittaria lancifolia*), native canna (*Canna* spp.), buttonbush (*Cephalanthus occidentalis*), cutgrass (*Leersia oryzoides*), and native lotus (*Nelumbo lutea*).

The contaminated storm water consisted of rainwater subsequently polluted at predetermined levels with bio-oil wastewater acquired from a biomass conversion facility. Four levels of contamination, representative of storm-water runoff that would occur on-site, were obtained by mixing 230 mL, 630 mL, 830 mL, and 2.5 liters of wastewater with 240 liters of rainwater (1,000×, 500×, 300×, and 100× dilution, respectively). The first three dilutions were circulated through the bin system once, and the lowest dilution was applied three times, thus providing the opportunity to measure the effects of increased contamination levels through Phases 1 to 3 and the steady, highest, repeated levels of contamination in Phases 4 to 6. After Phase 3 of the sampling, the new wetland plants were reestablished for Phases 4 to 6. Three repetitions of each sample were taken for all the tests. Sampling of water and soil was performed on the first and tenth day of each phase. In addition, control samples—unpolluted rainwater and contaminated storm water—were analyzed along with the samples from each bin (i.e., microcosm). The samples were analyzed for (1) BOD, (2) microtoxicity, (3) pH, (4) microbial counts, and (5) microbial bacterial community of both soil and water. All results were processed within 1 or 2 days of the sampling event. The BOD analysis were performed according to the standardized test by Enviro-Lab, Inc., in Starkville, Mississippi (EPA 2009). Microtoxicity was tested according to the Microtox Model 500 (M500) 100 percent toxicity procedure. Microbial counts were performed by streaking 10^4 dilutions of water samples on agar plates after 5 days, and the bacterial community was analyzed through terminal restriction fragment length polymorphism (T-RFLP) according to the method of Kirker et al. (2012).

The results were pairwise compared using SAS, and nonmetric multidimensional scaling (NMDS) of T-RFLP data was performed in PC-ORD software. For NMDS analysis, the data were analyzed for difference based on the Jaccard distance.

Results

pH of water samples

According to the EPA (2009) and the Clean Water Act (1972), permissible discharge limits for storm-water pH levels should remain within a range of 6.5 to 8.5. A majority of the 24 samples of the pH results measured in each microcosm were found to meet permissible discharge levels after 10 days within the wetlands. During Phases 1 to 3, the pH levels increased from acidic values to neutral levels within the first 10 days in the wetlands. The same was observed in Phases 4 to 6 despite a significantly higher contamination level. Data in Table 1 represent average values of all six microcosms.

BOD of water samples

The BOD levels of the first three phases were substantially reduced for all treatments after 10 days. However, control samples exposed in open containers also decreased

Table 1.—Water quality results measured over time in six phases of the study.[a]

	Phase 1		Phase 2		Phase 3		Phase 4		Phase 5		Phase 6	
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10
pH	5.2 A	6.8 B	5.6 A	6.4 B	5.6 A	6.4 B	5.1 A	6.9 B	5.1 A	6.3 B	4.9 A	6.0 B
Biological oxygen demand (mg/liter)	54 A	2 D	238 B	7 D	233 B	26 D	329 C	334 E	323 C	313 C	308 C	436 F
Microbial colonies (colonies $\times 10^4$)	66.9 A	11.9 B	35.8 A	22.5 B	33.3 A	32.8 A	2.4 A	45.5 B	1.7 A	62 B	54 A	91 B
Microtoxicity (% toxicity)	51.4 A	8.9 B	80.4 A	49.5 B	49.2 A	41 B	100 A	75.9 B	97.2 A	93.6 B	100 A	95.6 B

[a] Values represent an average of all three repetitions for each of the six microcosms at each sampling event for each phase. Within each row, values with different letters denote significant differences at the 0.05 level.

BOD levels, likely due to volatilization of volatile contaminants. As opposed to the first three phases, Phases 4 and 5 did not significantly decrease BOD, whereas Phase 6 increased BOD levels after 10 days, as shown in Table 1. We conclude that relatively unchanged levels of BOD in Phases 4 and 5 after 10 days were not affected by volatilization owing to the high contaminant concentrations. This was also seen in the control samples with high contamination levels. It has been shown that the volatility of contaminants does depend on concentration in addition to vapor pressure, adsorption reactions, temperature, solubility, etc. (Spencer and Cliath 1972).

Microbial colony counts of water samples

The microbial community counts showed a similar growth pattern for the first three phases. The microbial growth was high at the initial extraction stage and then declined after 10 days because of microbial consumption of contaminants. The opposite was true for phases of high contaminant levels. During these phases, the microbial colonies lagged at initial sampling and then adjusted and thrived during the later stage.

Microtoxicity results of water samples

The microtoxicity test indicated a steady decline in water toxicity over time for all six phases, with the first two phases showing a much greater decrease. During Phases 1 to 3, toxicity levels also steadily decreased across the six microcosms, while the toxicity levels varied across the microcosms in the latter three phases.

Bacterial fragment data of soil and water samples

The T-RFLP analysis identified 6,429 individual bacterial fragments in the soil and the water samples that were representative of 209 distinct taxonomic units (phylotypes). A total of 3,171 individual fragments were detected in the water, and 3,258 fragments were identified in the soil bacteria DNA samples. The T-RFLP data were exported into PC-ORD version 5.0 to determine species richness and diversity.

The microbial species richness in the soil was similar on Days 0 and 10 (Table 2). However, there was a shift in bacterial phylotypes among the phases. Phase 1 was similar in richness to Phases 4 and 5 even though the levels of contamination were substantially different. Phases 2 and 3 were similar in richness and were the only phases to have a higher level of richness on Day 0 than on Day 10. No significant differences were observed in bacterial community richness among the six microcosms.

The results from the water microbial species richness test (Table 2) displayed a trend similar to what was seen in the microbial colonies test. Microbial richness was high at the initial extraction and then declined after 10 days in Phases 1 and 3. The opposite was again true for phases of high contamination levels. During these phases, the richness increased with time.

Species diversity of soil is shown in Figure 1. Examining patterns among the phases, NMDS analysis showed species communities distributed in very distinct trends. Phases 1 to 3 clustered together, indicating a similar microbial community. Phases 4 and 6 clustered away from each other, with Phase 5 transitioning between the two clusters, indicating a gradual shift in species with concentration.

NMDS analysis of water species diversity (Fig. 2) showed a somewhat similar trend to soil species diversity. Phases 1 to 3 had a similar composition with some overlap with Phases 5 and 6. However, Phase 4 showed a transitional cluster between Phases 5 and 6 owing in part to the replanting that occurred after Phase 3. This indicates a dissimilarity of Phase 4 that could be because of the new plant species added to the soils at that phase.

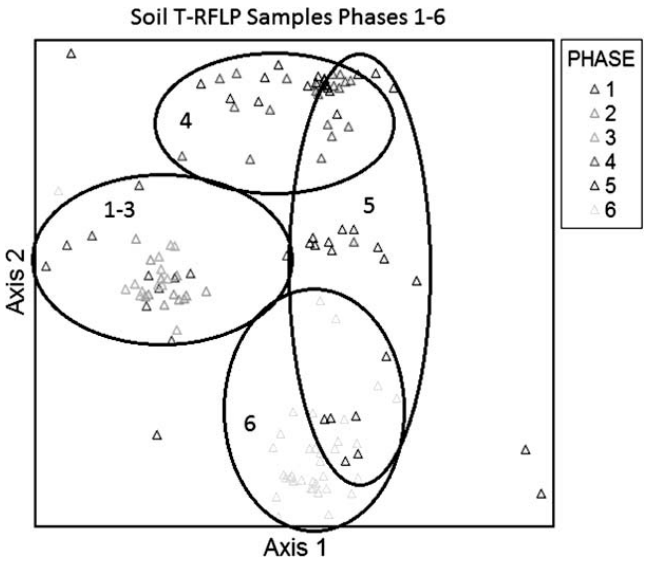

Figure 1.—Species diversity of soil samples as examined by terminal restriction fragment length polymorphism. The results are compared over time (Days 0 to 10) and contamination levels (Phases 1 to 6). The numerical values denote phases.

Table 2.—Species richness measured in water and soil samples by terminal restriction fragment length polymorphism.[a]

	Phase 1		Phase 2		Phase 3		Phase 4		Phase 5		Phase 6	
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10
Soil	23.7 A	25.2 A	34.5 B	31.3 C	32.5 B	30.3 C	21.9 A	22.3 A	20.5 A	21.9 A	16.6 D	17.4 D
Water	17.5 A	15 A	33 B	35.3 B	38.3 B	30 B	18.4 A	19.1 A	15.7 A	17.4 A	11.9 C	18.4 A

[a] The results are compared over time (Days 0 to 10) and by levels of contamination (Phases 1 to 6). Within each row, values with different letters denote significant differences at the 0.05 level.

Discussion

Development over the six phases of contamination

The six phases of contamination can be separated into two distinctive contamination levels: Phases 1 to 3 and Phases 4 to 6. Phases 1 to 3 had relatively low levels of contamination (albeit different from phase to phase), and the results of each experiment in these phases were very similar. Phases 4 to 6 had the same high levels of contamination and exhibited almost identical test results from phase to phase. Although none of the phases produced a sufficiently high contamination level to cause plant death, the first three phases were the most successful in reducing the amount of contamination in the storm water. Phases 4 to 6 maintained high levels of contamination and were relatively unchanged by the wetlands throughout the 10 days of the experiment. Therefore, the maximum level of contamination while still achieving high levels of reduced pollution occurred in Phase 3, consisting of 830 mL of contaminated bio-oil wastewater for every 2.4 liters of rainwater. The data from the bacterial community analysis revealed similar microbial communities for the lower levels of contamination (Phases 1 to 3), perhaps a result of a relatively low contamination level that never altered the microbial population of rainwater used in this experiment. During Phases 4 to 6, the communities were separated according to phase, indicating that a shift in community diversity occurred at the higher levels of contamination and over time.

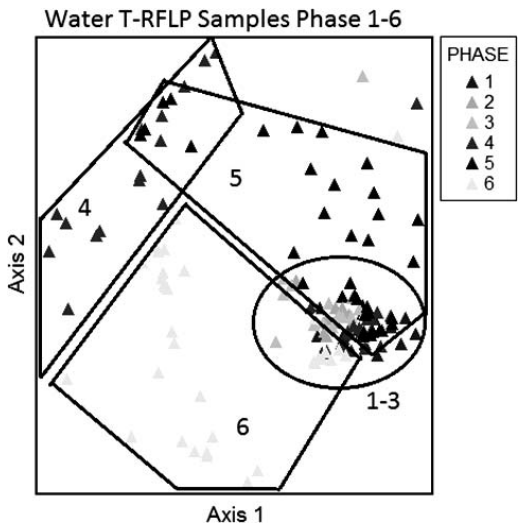

Figure 2.—Species diversity of water samples as examined by terminal restriction fragment length polymorphism. The results are compared over time (Days 0 to 10) and contamination levels (Phases 1 to 6). The numerical values denote phases.

The six microcosms had an overall similar trend of reduced contamination throughout all six phases of the study. In almost every sampling event, a marked change was observed from the first microcosm to the sixth. This was seen to be most prevalent in the pH values. BOD discharge limits were met after the fifth microcosm during Phases 1 to 3 and were never met during Phases 4 to 6. The microtoxicity in Phases 1 to 3 was significantly reduced at the sixth microcosm. T-RFLP results were the only ones with no significant trend when comparing microbial populations between microcosms.

All the samples exhibited some form of reduction in contamination from the first to the sixth microcosm after the 10-day sampling event. An increase was also seen over time in microbial count and species richness during Phases 4 to 6. This indicates that specific microbial communities thriving under the relatively low levels of contamination of Phases 1 to 3 diminished as the contamination levels dropped, while the high levels of contamination in Phases 4 to 6 may have initially shocked the microbial community, but they increased population size once they had adapted to the new levels of contamination. This trend of increase also occurred in species richness for both the soil and the water samples of Phases 4 to 6, suggesting that the microbial population was never fully exterminated by the high levels of contamination and that different communities adapted differently to the changes in contamination.

Conclusions

- The constructed wetlands were successful at lowering BOD and toxicity levels and achieving permissible pH levels when the concentration of contaminated storm water was less than or equal to 300× dilution. Much of the BOD reduction was a result of volatilization of the contaminated wastewater.
- When the concentration of contaminated water exceeded 300× dilutions, the constructed wetlands were successful only at achieving permissible pH discharge levels. Better results may be achievable with the longer residence time in the wetlands.
- The microbial colonies from Phases 1 to 3 thrived during initial contamination and waned after contamination levels were depleted. During Phases 4 to 6, the microbial colonies thrived in growth after adjusting to the high levels of contamination for 10 days. This is due in part to particular bacteria communities that flourish on the consumption of particular contaminants and other bacteria communities that cannot thrive under those specific conditions. There is potential research for determining which populations thrive under higher bio-oil wastewater contamination and which prosper under lower levels.

- The majority of bacterial communities compared were similar in composition for Phases 1 to 3 and different for Phases 4 to 6. Additional studies will be required to identify the individual species linked to bio-oil wastewater degradation.

It is acknowledged that the results for Phases 4 to 6 may improve in reduced levels of contamination should the storm water remain in the constructed wetlands longer. However, the amount of contamination lost through volatilization may increase as well.

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