Bioretention for stormwater quality improvement in Texas: Removal effectiveness of *Escherichia coli*

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**A B S T R A C T**

This study investigated the removal effectiveness of *Escherichia coli* by five pilot bioretention units with different vegetations. The bioretention units were originally planted or seeded with one of the following vegetation: shrubs, grass species specified for Texas highway applications, Texas native grasses, Bermudagrass (*Cynodon dactylon*), and none (control unit). To mimic highway conditions, the vegetation units were allowed to develop naturally outdoors and received no weed control. Longest hydraulic retention time (HRT) was observed in the control unit. Compared to the control unit, the vegetation caused 50% reduction of HRT in the shrub unit and 70–90% reduction of HRTs in the three grass units. Removal efficiency of *E. coli* of each unit, from high to low, was: control unit (97%) > the shrub unit (88%) > the unit originally seeded with Bermudagrass (76%) > the unit originally seeded with Texas highway grasses (57%) > the unit originally seeded with Texas native grasses (48%). Presence of common weeds, Giant Ragweed and Johnson grasses, might be responsible for the low *E. coli* removals observed in the grass units. The results provide fundamental and needed knowledge for designing bioretention that can effectively remove pathogens from highway stormwater runoff in semi-arid regions like Texas.

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1. Introduction

Urban and agricultural stormwater runoff often carries numerous pollutants, including suspended solids, organics, nutrients, heavy metals, and pathogens. Direct discharge of the runoff was unable to meet the discharge criteria of the water quality [1]. Of particular concern is the detection of numerous fecal coliform (FC) and pathogens in the urban stormwater runoff because epidemiological studies have linked waterborne illness to water bodies that were contaminated by stormwater runoff [2,3].

The United States Environmental Protection Agency has recommended bioretention as one of the best management practices (BMP) for stormwater runoff [4]. Bioretention was first invented in the late 1980s and later established for numerous residential and industrial applications, including residential gardens, parking lots, streets, and highways. A typical bioretention unit consists of (from top to bottom) water storage space, vegetation, mulch, soil filter media, and gravel layer. When passing through the bioretention unit, the pollutants in the runoff can be removed via sedimentation, filtration, and sorption on mulch and soil layers, plant uptake, and biodegradation by soil microorganisms [5].

Previous bioretention studies have been mainly focused on the removal of suspended solids, nutrients and metals from stormwater runoff [6–8]. Removal of FC bacteria and pathogens by bioretention is commonly limited to lab-scale column studies using culture-dependent methods [9,10].

Our previous study has examined the effects of vegetation on the performance of five bioretention pilot units under semi-arid conditions [11]. In the study, synthetic stormwater runoff containing nutrients, suspended solids, and heavy metals was supplied to the five different bioretention pilot units. Each unit was originally planted with one of the following vegetation types: shrubs, grass species specified for Texas highway applications, Texas native grasses, and Bermudagrass (*C. dactylon*), and no vegetation (i.e., control unit). Bioretention with shrub showed the best performance among the other units with vegetation. Interestingly, the control unit (no vegetation) outperformed those with vegetation (Table 1) [11].

This study was built upon our previous work to investigate the ability of bioretention to remove *Escherichia coli (E. coli)*, a type of FC bacterium. Quantitative molecular method, real-time PCR, was used to quantify *E. coli* as a means to assess the performance of the five bioretention units. The results of this study will provide information needed for bioretention design, particularly for highway stormwater runoff in the semi-arid regions like Texas.
2. Materials and methods

2.1. Preparation of E. coli

*E. coli* (ATCC#10798) was grown in *Luria–Bertani* (LB) Broth (10 g tryptone; 5 g yeast extract; 10 g sodium chloride; per 1 l adjusted to pH 7.0 with 10 N NaOH) at 30°C, 150 rpm overnight. The cell suspension was diluted to reach optical density (600 nm) between 0.7 and 0.8. When optical density was 0.8, the colony-forming unit (CFU) was equivalent to 10⁹ CFU/100 ml, based on a spread plate method (conducted at 35°C for 48 h on LB agar).

2.2. Construction and operation of bioretention pilot units

The five units, each with a dimension of 1.8 m long x 1.8 m wide x 1.2 m deep, were reconstructed from the metal garbage dumpsters. To prevent potential corrosion, the inner surfaces of the units were first coated with truck bed spray liner (40% polyurethane and 60% polyurea). After the coating, each box was built with an underdrain pipe, followed by a 20-cm large gravel layer, a 10-cm pea gravel layer, and a 61-cm deep compost-amended soil. The compost-amended soil (soil to compost ratio, 7:3) had 25 cm/h infiltration rate during the column test using 30-cm long and 10-cm PVC pipes. Four different vegetation types (Table 2): shrubs (hereafter referred as Unit-S), Texas native grasses (hereafter referred as Unit-N), grass species for highway roadside application in Texas (hereafter referred as Unit-H), and Bermudagrass (*C. dactylon*) (hereafter referred as Unit-B) were planted in the soil. One box with no vegetation was used as the control. The space above the soil media (30 cm in depth) was for detaining synthetic stormwater runoff. After the completion of our previous study, the four vegetation units (Unit-S, Unit-H, Unit-N, and Unit-B) were allowed to progress naturally outdoor without weed control during summer months. To maintain no vegetation condition in the control unit, weeds were manually removed on a weekly basis. No new soils were added or revegetation was performed in all five units. After summer vegetation secession, the bioretention units were then used for *E. coli* experiments.

2.3. Sampling and preservation

Influent was prepared by adding *E. coli* ATCC#10798 into potable water. The *E. coli* was supplied using a KDS-210 syringe pump (KDScientific, Holliston, MA) in a small mixing container before being applied to the influent wells.

### Table 1

<table>
<thead>
<tr>
<th>Pollutants Removal (%)</th>
<th>Unit-S (shrubs)</th>
<th>Unit-H (highway grass mix seed)</th>
<th>Unit-N (Texas native grass mix seed)</th>
<th>Unit-B (Bermuda grass)</th>
<th>Control (no vegetation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>–25</td>
<td>–43</td>
<td>–13</td>
<td>–19</td>
<td>37</td>
</tr>
<tr>
<td>Zn</td>
<td>81</td>
<td>82</td>
<td>30</td>
<td>47</td>
<td>68</td>
</tr>
<tr>
<td>Pb</td>
<td>80</td>
<td>77</td>
<td>76</td>
<td>80</td>
<td>84</td>
</tr>
<tr>
<td>TSS</td>
<td>68</td>
<td>36</td>
<td>–6</td>
<td>36</td>
<td>80</td>
</tr>
<tr>
<td>NO₂–N</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NH₄–N</td>
<td>96</td>
<td>92</td>
<td>88</td>
<td>81</td>
<td>77</td>
</tr>
<tr>
<td>TP</td>
<td>–3251</td>
<td>–3062</td>
<td>–1134</td>
<td>–963</td>
<td>–954</td>
</tr>
</tbody>
</table>

ND = not detected. Below NO₂–N detection limit of IC.

### Table 2

Grass and shrub species planted in the bioretention units.

<table>
<thead>
<tr>
<th>Box</th>
<th>Species (botanical name)</th>
<th>Seeding rates (kg/ha)</th>
<th>Pure live seed (%)</th>
<th>Survival observed after 14 months of development**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit-S (shrub)</td>
<td><em>Ilex vomitoria</em> ‘Stokes Dwarf’</td>
<td>3 counts</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td><em>Morella cerifera</em></td>
<td>3 counts</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Leucophyllum frutescens</em> ‘Bertstar Dwarf’</td>
<td>3 counts</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>Unit-H (highway grass seed mix)*</td>
<td><em>Cynodon dactylon</em></td>
<td>1.7</td>
<td>95.0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Eragrostis curvula</em></td>
<td>0.7</td>
<td>92.7</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td><em>Eragrostis trichodes</em></td>
<td>0.7</td>
<td>88.7</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td><em>Leptochloa dubia</em></td>
<td>0.3</td>
<td>93.0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td><em>Paspalum notatum</em></td>
<td>8.4</td>
<td>95.0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td><em>Coreopsis lanceolata</em></td>
<td>1.1</td>
<td>89.6</td>
<td>–</td>
</tr>
<tr>
<td>Unit-N (native grass seed mix)</td>
<td><em>Bouteloua curtipendula</em></td>
<td>11.2</td>
<td>89.6</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td><em>Leptochloa dubia</em></td>
<td>5.6</td>
<td>93.0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td><em>Sclerochyrium scoparium</em></td>
<td>5.6</td>
<td>67.2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td><em>Eragrostis trichodes</em></td>
<td>5.6</td>
<td>88.7</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td><em>Desmanthus illinoensis</em></td>
<td>7.9</td>
<td>96.9</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Chamaecrista fasciculata</em></td>
<td>5.6</td>
<td>91.8</td>
<td>–</td>
</tr>
<tr>
<td>Unit-B (bermudagrass)</td>
<td><em>Cynodon dactylon</em></td>
<td>18.6</td>
<td>95.0</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>No vegetation</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Standard highway grass seed mix used by the Bryan District of the Texas Department of Transportation.
** The data provided by suppliers.
*** –, No detection; *, 0–25% surface coverage; **, 25–50% surface coverage range; **+, 50–75% surface coverage; ++++, 100% surface coverage.
entering the bioretention units. The bioretention units received different influent flow rates over time (Table 3). Influent flow was supplied to the unit for 3 h only. The influent concentration of *E. coli* was consistently maintained as $10^2–10^3$ CFU/100 ml. The influent samples were collected manually every 1 h and effluent samples were collected using a Teledyne ISCO 6712 water sampler every 30 min for 4 h. For the control unit, the effluent samples were taken for seven hours, the time when the effluent flow subsided. The effluent flow rate was measured every 30 min after the influent was supplied to the boxes. The flow rates were used to determine the hydraulic detention time for each unit. Collected samples were preserved at $-20^\circ$C before analysis.

### 2.4. DNA extraction from liquid samples

Liquid sample (300 ml) was first poured in a beaker and stirred with a magnetic stir bar at a speed of 600–700 rpm. A 100 ml subsample was taken from the middle of the beaker using a wide-bore pipette [13]. The subsample was filtered through a 0.45 μm-pore size filter membrane (Osmonics Inc., Westborough, MA) to collect *E. coli* in the membrane. The genomic DNA of *E. coli* in the filter membrane was extracted using the FAST DNA Spin Kit for Soil (MP, Solon, OH). The extracted DNA was preserved at $-20^\circ$C before use.

### 2.5. Quantification of 16S rRNA of *E. coli* in water samples

The iCycler iQ™ 5 Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA) was used to quantify the amount of 16S rRNA of *E. coli* in samples. Amplification and detection were carried out in 96-well plates with SYBR-Greens PCR 2X Master Mix. A region of 16S rRNA of *E. coli* was amplified using *E. coli* species-specific forward primer (5’-CATGCGGCGTGATGAGAAGA-3’, base pairs 395–414) and reverse primer (5’-CGGGTAACGTCAATGAGCAAA-3’, base pairs 470–490) [14,15]. The specificity of these primers has been vigorously tested against 48 bacterial species [15]. Only 16S rRNA of *E. coli* was amplified by the primer sets. Each reaction was run in a final volume of 25 μL with 1X final concentration of SYBR-Greens PCR 2X Master Mix, 300 nM forward and reverse *E. coli* species-specific primers, and 1 μL of whole DNA templates. The PCR products were cloned into the vector pCR4-TOPO (TA cloning; Invitrogen, Carlsbad, CA) according to manufacturer’s protocol. Clones with inserted plasmid were grown overnight in 10 ml of LB broth with kanamycin. Extracted plasmids were purified using Wizard Plus SV Minipreps (Promega, Madison, WI) and the concentration of plasmid DNA was determined using Nanodrop 2000. The copy numbers of the plasmid DNA were determined as described by QuantiTect SYBR Green PCR Handbook [17]. Standard curves, ranging from $1.47 \times 10^3$ to $1.47 \times 10^8$ copies of the 16S rRNA gene, were generated and used for assessing *E. coli* concentrations in samples.

### 2.7. Data analysis

Because influent and effluent flow rates vary over time, the event mean concentrations of pollutants do not correctly represent the performance of bioretention. Thus, the removal of *E. coli* from each box was calculated by the following equation:

$$\text{Removal efficiency} = 1 - \frac{\Sigma(C_{out,t} \times \text{Effluent flow rate}_t \times \Delta t)}{\Sigma(C_{in} \times \text{Injection flow rate}_t \times \Delta t)}$$

![Fig. 1. Schematic of a bioretention unit [11.](image)](image)
where, \( C_{in} \) is the concentration of \( E. coli \) in the stock solution (16S rRNA copy #/100 mL). Injection flow rate, \( t \) is the flow rate of \( E. coli \) stock solution entering the small mixing tank, \( C_{out,t} \) is the effluent concentration at time \( t \) in \( E. coli \) 16S rRNA copy #/100 ml, and \( \Delta t \) is the time interval of flow data, i.e., one minute.

3. Results

3.1. Vegetation secession in the pilot bioretention units

Vegetation secession was observed in the bioretention units (Table 2). For Unit-S, \textit{Leucophyllum frutescens} ‘Bertstar Dwarf’ (also called Texas Sage) was the dominant species (75% surface area of the unit (i.e., 3.24 m²)), followed by \textit{Morella cerifera} (1% surface area of the unit). However, \textit{Ilex vomitoria} ‘Stokes Dwarf’ did not survive during the summer of Texas. Approximately, 25% surface area of the unit was covered with three different types of weeds: Yellow Nutsedge, Prostrate Spurge, and Pigweed. \textit{C. dactylon} (Bermudagrass), covered 25% of the surface area of the unit, was the only surviving grass species in Unit-H. For Unit-N, \textit{Desmanthus illinoensis} was the surviving species with a 15% coverage of surface area. While Bermudagrass was still present, it only covered 5% surface area of Unit-B. Giant Ragweed and Johnsongrass were two dominant weed species, covering 75–95% of the surface of the grass units. For Unit-H, 70% of the surface area was Giant Ragweed and 5% was Johnsongrass. The surface of Unit-N was covered with 50% Giant Ragweed and 35% Johnsongrass. For the surface of Unit-B, 25% was Giant Ragweed and 70% was Johnsongrass.

3.2. Hydraulic retention times of pilot bioretention units

Different hydraulic retention times were observed in five different bioretention units (Fig. 2). The control unit (without vegetation) showed the longest retention time (141.6 min). The detention time was consistent with our previous study[11]. For units with vegetation, a much shorter retention time was observed. The retention times were 67.2 min for Unit-S, 42.6 min for Unit-H, 18.3 min for Unit-N, and 16.2 min for Unit-B. Much shorter retention times of vegetation units than the non-vegetation unit (control unit) might be due to the change of soil porosity and granulation caused by the roots of vegetation.

3.3. Removal of \( E. coli \) by the pilot bioretention units

The bioretention units could remove \( E. coli \) with a wide range of removals (Fig. 3). The highest \( E. coli \) removals were observed both in the control unit (97%). The removal efficiency in vegetation units, from high to low, is Unit-S (88%) > Unit-B (76%) > Unit-H (57%) > Unit-N (48%). The high removal of \( E. coli \) in control unit was similar to our previous study[11]; the control outperformed other units with vegetation in removing copper, lead, and TSS. Similarly, the shrub unit outperformed the other three grass units.

4. Discussions

Many factors, including vegetation types, vegetation secession, and different climates, are expected to contribute to the overall performance of bioretention. However, these aspects have not been fully examined. To our knowledge, this is the first study to examine the removal \( E. coli \) by pilot bioretention units that underwent vegetation secession in hot and semi-arid climate. Higher \( E. coli \) removal (88%) was observed for the shrub unit than the three grass units (with an average of 60% removal). Interestingly, the highest \( E. coli \) removal (97%) was observed in the control unit that was with no vegetation. Our results (from 48–88% removal), as expected, were much lower than that observed in well-controlled laboratory soil columns (~91% removal)[9]. Interestingly, despite
that our bioretention units are constructed and developed under semi-arid climate, our E. coli. removals were comparable to those reported (~70% removal) from a field bioretention unit that was developed under warm and humid climate [10]. Unlike our bioretention units that were subject to natural vegetation secession, their field unit was revegetated with a mixture of shrubs and grasses before experiments were initiated. Our results would more likely be representing a real-world scenario for highway applications. Regrowth of E. coli. in bioretention units could potentially affect the overall removal of E. coli. As warm and humid conditions are favorable for microbial regrowth, it is expected that the regrowth of E. coli. might not be significant for bioretention units in hot and semi-arid climate and with short testing periods, like the experiments conducted in this study.

Straining (during the filtration) and sorption have been suggested to be two major removal mechanisms; however, the major removal mechanism of E. coli. in bioretention units has not been fully understood. E. coli. is a rod-shaped bacterium with a diameter of 2–6 μm and a length of 1.1–1.5 μm. Due to the small size of E. coli., it is most likely that adsorption, not straining, is the major removal mechanism for E. coli. in porous medium in the bioretention units [18]. In addition to the relative size of the porous medium to E. coli, many factors, such as the texture of porous media, the presence of organic matter and biofilm, temperature, flow rate, ionic strength, pH, hydrophobicity, chemotaxis, and electrostatic charge, can affect the sorption of bacteria in the bioretention units [9].

In this study, higher E. coli. removals were observed in the units with longer hydraulic retention times (i.e. the control and Unit-S), suggesting a positive correlation between E. coli. removal and hydraulic retention times. As different vegetation has various root growth, rooting depth, and nutrient metabolism, it is expected that the bioretention with different vegetation would create different rhizosphere environments, soil porosity, and even the development of preferential paths [11]. Particularly, any changes of soil porosity and the presence of preferential paths will reflect on the changes in hydraulic retention times in the bioretention units. This statement is supported by the results of this study and our previous study [11]. Our previous study reported better TSS and metal (copper and lead) removals by the control unit, which exhibited the longest hydraulic retention time [11]. The long hydraulic retention of the control unit is most likely due to lack of vegetation effects, i.e., uniform and smaller soil porosity and less preferential paths. When straining and surface sorption are the major removal mechanisms for pollutants, like TSS and metals, the longer the hydraulic retention times the more likely it is to link to a better removal efficiency. This statement was further supported by the observation of much shorter hydraulic retention times in the three grass units. Shorter hydraulic retention time and poorer E. coli. removals were observed for the three grass units (Unit-H, Unit-N, and Unit-B) than the shrub unit (Unit-S). These differences might be caused by different types of vegetation: shrubs, grasses, and weeds. More studies are needed to delineate the rooting effects of different vegetation on performance of bioretention units. Nevertheless, the length of hydraulic retention time in the bioretention units may be a useful and practical parameter to predict the removal effectiveness of pollutants that are removed by straining and surface sorption mechanisms, not by biological reactions.

Acknowledgments

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References


Fig. 3. Removal of E. coli by five bioretention units.