THE REMOVAL OF INDICATOR MICROORGANISMS FROM PRIMARY TREATED WASTEWATER IN SUBSURFACE REED BEDS USING DIFFERENT SUBSTRATES

T. MANICOS1, E. L. STENTSFORD2 AND P. A. MILLNER3

1Department of Environmental Engineering, Technical University of Crete, Chania, 72100, Crete, Greece
2School of Civil Engineering, Leeds University, LS2 9JT, Leeds, UK
3School of Biochemistry and Molecular Biology, Leeds University, LS2 9JT, Leeds, UK

(Received 28 August 2003; Accepted 14 January 2003)

ABSTRACT

Subsurface, horizontal flow, experimental reed beds, were designed and built based on a combination of two design methodologies: that of the WRc and Severn Trent Water Plc and that of the USA, EPA. Four different growing media were used with a combination of top soil, gravel, river sand and mature sewage sludge compost, aiming to da aubstrate for enteric pathogen removal. Eight units were constructed, two for each material. One bed for each pair was planted with Typha latifolia plants commonly known as cattails. Primary treated domestic wastewater was continuously fed in to the bed for more than six months. All beds achieved a high reduction of Escherichia coli and faecal coliforms with the best results recorded in the gravel reed beds with an average removal above 3.5 log for E. coli and 2.0 for faecal coliforms. There was no significance difference in the performance of planted and unplanted reed beds.

Keywords: Reed beds, E. coli, faecal coliforms, wastewater, sewage sludge, compost, gravel, Typha latifolia.

INTRODUCTION

Constructed subsurface flow (SF) reed beds or wetlands are low cost, low technology systems able to treat a variety of wastewater [1]. For the past few years and especially in Europe such systems have been successfully used for treating mainly domestic sewage for small communities (less than 2,000 people equivalent) [2, 3]. In order to avoid clogging when using fine media, many engineers used larger rock sizes (10 - 15 cm) with larger void spaces and greater permeability. This approach did not solve the problem. The vegetation, which was used in these systems, normally grows in soil and the root network was not developed properly in the large void spaces. In addition the larger rocks provided a smaller surface area for the support of microbial growth compared with the smaller rock sizes [4].

WRc and Severn Trent Water Plc [3] in the first reed bed design and construction manual for the UK water industry, suggested the use of gravel with size varying from 3 to 12 mm. However no specific guidance was given for the way the substrate should be constructed or what would happen if a mixture of different sized gravel was used. On the contrary for the vertical systems (V3) the use of successive layers of gravel and sand in order to achieve maximum performance, was recommended. Those layers would be as follows, from top to bottom: 8 cm of sharp river sand, 15 cm of 6 mm washed pea gravel, 10 cm of 12 mm round washed gravel and 15 cm of 30 - 60 mm round washed gravel. The main purpose of the sand layer was to provide support for the plants vegetating the bed. The different diameter gravel would allow the wastewater to be treated by "different" filters each one with different characteristics and abilities. It was hoped that this variety of materials would be able to create an effective system operational from the first day of instalument, the pores of which will not be easily blocked with time. The potential use of such substrate in a horizontal flow SF reed bed has not until now been studied, even though similar principles apply in both systems. It is possible that this multi-size gravel substrate could be ideal for SF systems.

However, the use of gravel and sand, materials that often have to be transported from some distance, increases considerably the cost of construction. It was considered important to study the possible use of alternative materials that would provide the cheaper soil-based substrates with
additional mechanical support in order to sustain its original hydraulic characteristics. A number of researchers have used compost as part of SF systems aiming to remove heavy metals from water with considerable success [5, 6, 7]. However, none of them studied the possible use of such materials in SF beds treating municipal wastewater. Composted materials have been used in agriculture for a long period of time as a soil improver. Their main advantages are the provision of nutrients in to the soil, an increase in the content of organic matter and improvement in the soil structure [8]. Such structural ability could help to improve the hydraulic characteristics of soil-based substrates used in reed beds and maintain their original permeability during operation, minimizing short cuts and overflows.

The first objective of this research was to study the behaviour and performance of the multi-size, multi-layered gravel substrate recommended by the WRC and Severn Trent Water plc [3] for vertical flow systems in a horizontal flow subsurface reed bed. Comparing the performance of such substrate with soil-based substrates with or without compost comprised the second part of our research. The third and final objective was to determine the importance of Typha latifolia plants (commonly known as cattails) in the wastewater treating process in SF systems. The removal of indicator microorganisms, E. coli and temperate-resistant faecal coliforms, from primary treated wastewater was used as a companion tool.

**MATERIALS AND METHODS**

Eight pilot reed beds were set up containing four different mixtures of gravel, river sand, top soil and mature sewage sludge compost. For each material two beds were constructed, one of them was planted with Typha latifolia commonly known as cattails, where the other remained free of any vegetation. In the results the symbol (+) is used to identify these beds planted with cattails. The ratios (by volume) of each of the materials for each growing medium were as follows:

- **Material A**, (beds A and A+): 25% compost, 25% river sand and 50% top soil per volume.
- **Material B**, (beds B and B+): 50% compost, 10% river sand and 40% top soil per volume.
- **Material C**, (beds C and C+): 50% river sand and 50% top soil per volume.
- **Material D**, (beds D and D+): was constructed in layers. The bottom 15 cm was a 30 mm washed gravel layer, the next 10 cm was a 12 mm washed gravel. The final two layers were 10 cm of 6 mm gravel and 5 cm of river sand.

FVC tanks were used, each 65 cm long by 45 cm wide and 60 cm deep. The media depth was 40 cm, occupying a total volume of 117 litres and giving a surface area of approximately 0.29 m² and a cross-sectional area of 0.18 m². The mature sewage sludge compost used was produced by Thames Water, using an initial mix of sludge and straw 1:1 by volume. There were a large variety of different composts that could have been used for the experiments in this study. However it was decided to use sewage sludge and straw compost for a number of reasons:

- the research group had extensive experience in the use of such materials for a variety of experimental purposes;
- large quantities of high quality, mature sewage sludge compost were widely available;
- the contamination of such material with heavy metals decreases their potential use in agriculture, increasing the need for alternative uses;
- the existence of straw would increase the structural characteristics of the compost; and
- such material contains a large variety of microorganisms which it was hoped would affect positively the beds' performance especially against pathogenic microorganisms.

The feeding of the bed was continuous. The design of the beds was based on a combination of equations developed by the WRC and Severn Trent Water plc [3] and the EPA [9] of the USA:

\[ \text{As} = \frac{Q(I_{\text{InC}} - I_{\text{Co}})}{k_{\text{BOD}}} \]  

where:

- \(\text{As}\) = surface area of the system (m²)
- \(Q\) = average flow rate through the system (m³ d⁻¹)
- \(I_{\text{Co}}\) = influent value of BOD₃ (mg l⁻¹)
- \(C_e\) = effluent value of BOD₃ (mg l⁻¹)
- \(k_{\text{BOD}}\) = temperature depended rate constant (m³ d⁻¹)

The rate constant \(k_{\text{BOD}}\) would normally be expected to be between 0.007 and 0.1 m d⁻¹. The WRC and Severn Trent Water plc [3] manual recommends as the best value for the UK 0.1 m d⁻¹ which was also used in the design of the experimental reed beds.

If the following values are used with equation (1): \(C_e = 150\) mg l⁻¹, \(C_o = 20\) mg l⁻¹, \(k_{\text{BOD}} = 0.1\) m d⁻¹ and \(\text{As} = 0.29\) m² then the permissible flow would be 14.5 l d⁻¹ (approximately 15 l d⁻¹). This flow would give to the soil-based beds a designed retention time of 72 hours and 42 hours for the gravel bed. Despite the efforts made in designing and constructing the beds to ensure that all the substrate volume would be available to the flowing wastewater, their hydraulic performance required checking. This was done by measuring the retention time of the beds, to give an estimate of the degree of channelled flow taking place. A tracer study using rhodamine was carried out [5]. The rhodamine test is based on adding a "spike" to the inlet and then measuring the
concentration of the rhodamine in the effluent using a fluorometer. Based on the literature it was expected that there would be a considerable difference between the designed and the real retention time for both soil and gravel based beds [10, 11].

Analyses for E. coli (incubation in 37 ± 0.5 °C) and faecal coliforms (incubation in 44 ± 0.5 °C) were based on the Standard Methods for the Examination of Water and Waste Water [12]. The significance of the effect of cattails in the indicator's removal was measured by using the t-test for paired samples as presented by Snedecor and Cochran [13].

RESULTS

The ambient temperature during the experimental period ranged from 8 to 21 °C providing good growing conditions for the microorganisms and the plants in the beds. The pH value for Materials A, B and C was near neutral, where gravel and sand used for Material D were well washed. The flow of wastewater was constant (15 day⁻¹ per bed) during the 165 days of the experiment. The cattails (Typha latifolia) were well developed in the planted beds, whereas no vegetation was allowed to grow in any of the unplanted beds. Approximately two months into the experiment the retention time was measured using the rhodamine tracer test [5]. For beds A+, A, B+, B, C+, C, D+ and D the estimated retention time was 10, 8, 12, 14, 11, 14, 24 and 18 hours respectively.

The influent and effluent E. coli concentration values (cfu/100 ml), for the duration of the experiment, are presented in Figure 1 for beds A+, A, B+ and B and Figure 2 for beds C+, C, D+ and D. Respectively Figures 3 and 4 present the faecal coliforms (FC) concentration values (cfu 100 ml⁻¹). In order to compare the removal performance between the different substrates for both E. coli and faecal coliforms the difference in the log values of the concentrations were used. Table 1 presents the log reduction for E. coli where Table 2 presents the log reduction for faecal coliforms. The missing

![Figure 1](image.png)

**Figure 1.** Effluent E. coli (EC) concentration for beds A+, A, B+, B in comparison with the influent (●)
Figure 2. Effluent E. coli (EC) concentration for beds C+, C, D+, D in comparison with the influent (○).

Figure 3. Effluent faecal coliforms (FC) concentration for beds A+, A, B+, B in comparison with the influent (○).
Figure 4. Effluent faecal coliforms (FC) concentration for beds C+, C, D+ and D in comparison with the influent (●).

Table 1. Log reduction for E. coli for all eight beds during the experimental period.

<table>
<thead>
<tr>
<th>Day</th>
<th>A+</th>
<th>A</th>
<th>B+</th>
<th>B</th>
<th>C+</th>
<th>C</th>
<th>D+</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.12</td>
<td>2.06</td>
<td>2.48</td>
<td>2.04</td>
<td>1.99</td>
<td>2.14</td>
<td>3.39</td>
<td>3.46</td>
</tr>
<tr>
<td>3</td>
<td>3.10</td>
<td>2.41</td>
<td>2.15</td>
<td>2.91</td>
<td>2.10</td>
<td>2.09</td>
<td>2.58</td>
<td>2.37</td>
</tr>
<tr>
<td>12</td>
<td>2.96</td>
<td>2.75</td>
<td>2.35</td>
<td>2.74</td>
<td>2.77</td>
<td>3.06</td>
<td>2.82</td>
<td>3.10</td>
</tr>
<tr>
<td>18</td>
<td>2.74</td>
<td>2.89</td>
<td>2.03</td>
<td>2.39</td>
<td>3.50</td>
<td>2.60</td>
<td>3.21</td>
<td>2.46</td>
</tr>
<tr>
<td>24</td>
<td>2.34</td>
<td>3.90</td>
<td>3.13</td>
<td>3.90</td>
<td>3.03</td>
<td>3.13</td>
<td>3.13</td>
<td>3.54</td>
</tr>
<tr>
<td>30</td>
<td>1.96</td>
<td>2.31</td>
<td>2.42</td>
<td>3.91</td>
<td>2.77</td>
<td>3.91</td>
<td>4.66</td>
<td>3.47</td>
</tr>
<tr>
<td>48</td>
<td>1.96</td>
<td>2.31</td>
<td>2.42</td>
<td>3.91</td>
<td>2.77</td>
<td>3.91</td>
<td>4.66</td>
<td>3.47</td>
</tr>
<tr>
<td>82</td>
<td>2.54</td>
<td>2.71</td>
<td>2.23</td>
<td>1.89</td>
<td>2.50</td>
<td>3.04</td>
<td>3.60</td>
<td>3.83</td>
</tr>
<tr>
<td>101</td>
<td>2.52</td>
<td>2.71</td>
<td>1.85</td>
<td>1.85</td>
<td>2.29</td>
<td>2.46</td>
<td>2.85</td>
<td>3.62</td>
</tr>
<tr>
<td>138</td>
<td>2.07</td>
<td>2.34</td>
<td>2.43</td>
<td>1.88</td>
<td>2.60</td>
<td>3.56</td>
<td>4.04</td>
<td>4.69</td>
</tr>
<tr>
<td>127</td>
<td>2.05</td>
<td>1.71</td>
<td>1.36</td>
<td>1.36</td>
<td>1.80</td>
<td>1.97</td>
<td>2.82</td>
<td>2.69</td>
</tr>
<tr>
<td>136</td>
<td>2.06</td>
<td>2.30</td>
<td>1.99</td>
<td>2.03</td>
<td>2.13</td>
<td>2.51</td>
<td>2.51</td>
<td>3.85</td>
</tr>
<tr>
<td>152</td>
<td>1.70</td>
<td>1.91</td>
<td>2.03</td>
<td>1.68</td>
<td>1.76</td>
<td>2.15</td>
<td>2.51</td>
<td>2.68</td>
</tr>
<tr>
<td>161</td>
<td>2.23</td>
<td>2.05</td>
<td>2.27</td>
<td>1.86</td>
<td>2.80</td>
<td>2.69</td>
<td>3.27</td>
<td>3.43</td>
</tr>
<tr>
<td>Average</td>
<td>2.39</td>
<td>2.54</td>
<td>2.20</td>
<td>2.34</td>
<td>2.46</td>
<td>2.73</td>
<td>3.42</td>
<td>3.32</td>
</tr>
</tbody>
</table>
Table 2. Log reduction for faecal coliforms for all eight beds during the experimental period.

<table>
<thead>
<tr>
<th>Days</th>
<th>A±</th>
<th>A</th>
<th>B±</th>
<th>B</th>
<th>C±</th>
<th>C</th>
<th>D±</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.62</td>
<td>1.71</td>
<td>2.21</td>
<td>1.69</td>
<td>1.65</td>
<td>1.50</td>
<td>3.07</td>
<td>3.07</td>
</tr>
<tr>
<td>12</td>
<td>2.71</td>
<td>3.07</td>
<td>2.27</td>
<td>3.53</td>
<td>2.65</td>
<td>2.65</td>
<td>3.84</td>
<td>3.84</td>
</tr>
<tr>
<td>18</td>
<td>2.72</td>
<td>3.43</td>
<td>3.26</td>
<td>4.47</td>
<td>3.98</td>
<td>3.34</td>
<td>3.92</td>
<td>3.85</td>
</tr>
<tr>
<td>24</td>
<td>1.57</td>
<td>3.22</td>
<td>2.12</td>
<td>1.80</td>
<td>3.22</td>
<td>2.75</td>
<td>2.26</td>
<td>1.33</td>
</tr>
<tr>
<td>30</td>
<td>2.81</td>
<td>2.83</td>
<td>2.83</td>
<td>1.70</td>
<td>3.12</td>
<td>3.12</td>
<td>4.05</td>
<td>3.65</td>
</tr>
<tr>
<td>48</td>
<td>2.89</td>
<td>2.29</td>
<td>2.49</td>
<td>3.18</td>
<td>2.34</td>
<td>2.08</td>
<td>2.26</td>
<td>2.55</td>
</tr>
<tr>
<td>82</td>
<td>2.38</td>
<td>2.92</td>
<td>3.06</td>
<td>3.18</td>
<td>3.01</td>
<td>2.92</td>
<td>3.08</td>
<td>3.35</td>
</tr>
<tr>
<td>127</td>
<td>1.94</td>
<td>2.25</td>
<td>2.52</td>
<td>2.25</td>
<td>2.42</td>
<td>2.45</td>
<td>3.01</td>
<td>3.21</td>
</tr>
<tr>
<td>152</td>
<td>2.34</td>
<td>2.67</td>
<td>2.63</td>
<td>2.75</td>
<td>2.57</td>
<td>2.56</td>
<td>3.11</td>
<td>2.99</td>
</tr>
</tbody>
</table>

value in day 48, was due to the fact that stored samples were mistakenly disposed of by the laboratory technician before the necessary analysis had been completed.

For all four substrates the t-test was used in order to estimate the significance difference in the performance of planted versus unplanted beds [13]. The critical point for the t-test (a = 5%) and for the samples analysed for each bed was t_{0.05} = 2.16 for E. coli and 2.26 for faecal coliforms. In all four correlations (A± vs. A, B± vs. B, C± vs. C and D± vs. D) the value of t was lower than the critical point (for both E. coli and faecal coliforms) indicating that there was no significant effect in the removal of pathogens from the treated wastewater due to the presence of Typha latifolia.

DISCUSSION

The retention time of the beds, as estimated using the rhodamine test, was lower than that originally designed (72 hours for beds A±, A±, B±, B± and C± and 42 for beds D± and D). In the soil-based beds channel flow explains the low retention time [10, 11]. Soil and compost are easily compacted and their hydraulic characteristics were affected by the handling of the materials while the beds were constructed. The fact that the beds containing Materials A and B and beds C± and C± presented similar retention times suggests that there was no improvement in the mechanical characteristics of the substrate’s matrix in the soil-based systems by the addition of compost. This was one of the original assumptions supporting the use of compost in the soil-based substrates.

Most of the potentially pathogenic bacteria in wastewater are of enteric origin and, as such, are relatively ill equipped to increase in numbers outside of the gut of suitable mammalian or avian hosts. In constructed wetlands, the removal process is due to a number of phenomena like photolysis, sedimentation, predation and natural die-off [14, 15]. In SF systems most of the pathogens are eliminated through the sedimentation of solids, on the surface of which the majority of microorganisms are developed, and natural die-off [14]. As such the ability of an SF to remove pathogens should be correlated with its ability to retain solids and at the same time provide adequate time (retention time) for the natural processes to occur and natural die-off to be completed [15–18].

According to Marinos et al. [19, 20] when compost is used as part of a SF substrate, leaching of organic matter takes place resulting in an increase in the effluent COD and NH₄-N values compared with the influent where at the same time solids concentration is reduced [21]. This leaching, considerably affecting the beds’ performance for organic matter and nutrients removal, did not seem to affect the beds’ performance for indicator microorganisms removal. This is explained by the fact that the nature and well composted material used in the beds did not contain any amount of either E. coli or faecal coliforms. Most of the pathogens were probably removed through the retention of solids [15, 21] and natural die-off [15, 16, 18]. In these experiments solids removal was similar among all six soil-based beds [21] as was the retention time (directly correlated with natural die-off as mentioned by Green et al. [15], Korn et al. [16], Ramos-Cormenzana et al. [18]). However the fact that beds A±, A±, B±, B± and C± presented similar behaviour for the removal of indicators, does suggest that there was no or minimum effect of the microorganisms existing in the compost in the removal of E. coli and faecal coliforms through antagonistic phenomena [8, 22].

For the gravel beds (due to the low plasticity of the materials) the deviation from the designed retention time was not as considerable. Beds D± and D did managed to remove larger amounts of pathogens compared with the six soil-based beds. This could be explained through both or either the larger retention time presented by the beds D± and D [15, 16] and the greater retention of solids [15, 18, 21]. For bed D± the total suspended solids (TSS) mean percentage removal was 96.6 ± 4.1% producing an effluent with mean concentration of 5.2 mg L⁻¹, where for bed D± the values were 96.1 ± 5.0% and 8.1 mg L⁻¹ respectively (the mean influent value for TSS was 128 mg L⁻¹). However the retention times recorded for beds D± and D were low, compared with similar systems in relevant publications [15, 17, 18]. This could have affected considerably their ability to remove pathogens compared
with other gravel based beds with larger retention periods. Instead their performance was considerable and steady, producing all the time an effluent containing less than 10^6 cfu/100 ml. For bed D+ the average log removal for E.coli and faecal coliforms was 3.42 and 3.19 respectively, where for D the values were 3.32 and 2.99 respectively. These results suggest that the use of different size gravel in layers could be an optimum substrate for SF systems. Manios et al. [19, 20, 21] presented data showing a good performance of this substrate in the removal of COD, TSS and NH3-N also.

The presence of cattails (Typha latifolia) did not produce a significant difference between the vegetated beds' performance and their twin unplanted beds as suggested by the t-test used in all four pairs. The t value for couple A+ and A, couple B+ and B, couple C+ and C and couple D+ and D was 0.34, 0.26, 0.57 and 0.21 respectively for E.coli. For faecal coliforms the t value for the same couples was 0.71, 0.86, 0.32 and 0.26 respectively, with the critical point been 5.99 = 2.16 for E.coli and 2.16 for faecal coliforms respectively (α = 5%). It is a common belief that the role of plants in the removal of pollutants is small or even non-existent in a constructed SF [15, 18, 23]. Work by Neralla and Weaver [24] suggest some effect of Typha latifolia plants in the removal of pathogens but only under specific circumstances and environmental conditions.

CONCLUSIONS

The two beds containing gravel performed better than any of the other six beds in removing pathogens. Their performance was similar to other SF gravel systems as presented in relative literature. The reason for the good performance of gravel was due in part to the reduced short circuiting and channeling in the bed. Both beds had the longest retention times compared with the other beds. From the experience gained when working with the seed beds short circuiting and channeling is a greater problem with the soil-based systems, with and without compost. In our experiments this resulted in a decrease in the volume of the bed accessed by the main influent flow to less than one third. The addition of compost failed to improve the hydraulic characteristics of the beds. At the same time there was no increase of antagonistic phenomena against the pathogens, from the microorganisms existing in the compost. There was no significant effect in the removal of either E.coli or faecal coliforms due to the presence of plants (Typha latifolia).

REFERENCES

3. WRc, and Severn Trent Water. Reed Beds & Constructed Wetlands for Wastewater Treatment, 1996, Swindon, UK.


