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Studying the effectiveness of Wetlands Built on an Upright Subsurface Flow in Reducing Organic Matter and Faecal Indicator Bacteria in Different Sizes of Gravel Substrate Aggregates

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ABSTRACT

Constructed wetlands (CWs) polish wastewater prior to discharge into aquatic receptacles. Size variability's of substrates used in CWS may have effects on the treatment efficiencies though there is scanty information regarding this aspect in VSSF CWs. To address this knowledge gap, a laboratory scalemicrocosm experiment was set up to investigate the potential of a VSSF CW to reduce organic matter andFIB using various gravel substrate aggregate sizes. This consisted of three gravel size treatment units; <12.5, 12.5-18 and 18-24 mm in triplicates. 70 liters of pre-treated wastewater from the final wastewater stabilization pond (WSP) the dairy farm sited near of Glocal University's WSPs system was added to the units, allowed to settlefor 6 weeks for development of biofilms, followed by periodic feeding of equal wastewater quantity on weekly basis and influent and effluent samples collected for 8 weeks for analysis. Results disclosed reduction efficiency of 95.2, 94.3 and 88.4 % for *E coli* in the fine, medium and coarse gravel aggregates respectively. Less than 20 % reduction efficiency was recorded for BOD_5 in all gravel aggregate sizes. There was no significant variation on performance of the three gravel aggregate sizes in reduction of bothFIB and BOD_5 ($p>0.05$). Poor performance in BOD_5 reduction was related to absence of wetland macrophytes in the study. The relatively high reduction efficiency for FIB was attributed to other factorsand processes such as predation, mechanical interactions, starvation, microbial interactions and natural die-offs. The study recommends assessing the combined effect of increasing the retention time, use of wetland macrophytes and incorporating various gravel aggregate sizes in order to increase the efficiencyof VSSF CW in reduction of FIB and organic matter.

Keywords: *Vertical Sub-Surface Flow Constructed Wetland; Faecal Indicator Bacteria; BiochemicalOxygen Demand; Mesocosm experiment; Wetland macrophytes.*

1.0 INTRODUCTION

Constructed wetlands (CW) have been conveniently used for post-treatment of wastewater for more than 100 years, mimicking the specialized treatment functions of natural wetlands. CW comprise of four major system components including wetland macrophytes, water column, living organisms and wetland substrate [1]. Wetland substrate plays a fundamental role in the treatment process, storing all biotic and abiotic components occurring in the wetland. Moreover, the substrate provides a larger surface area and biochemical support to plants and other living organisms, intercepting contaminants by the filtration, sedimentation, and adsorption processes [1]. As a result, the performance of a CW entirely depends on substrate characteristics and appropriate selection of granular substrate offers an opportunity to decrease the estimated wetland area and improve contaminant reduction efficiency [2]. The substrate selected for use in CW should be clean, hard, durable stone capable of retaining its shape and water permeability of the wetland over a long period of time [3].

The major substrate factors that influence the performance of CW are hydraulic conductivity and hydraulic retention time, which needs to be stabilized and maintained.Substrates of fine grain sizes decrease the Hydraulic Loading Rates (HLR), subsequently increasing the Hydraulic Retention Time (HRT). This ensures efficient contact and establishment of appropriate microbial community to degrade, transform and mineralizewastes thus increasing reduction efficiency of CW [1]. Previous studies on wetlands thatconsider soil-based substrates have demonstrated that grain size distribution largely influences soil hydraulics. Systems with fine and soil-based substrates have low hydraulic conductivity, while coarse sand and gravel-based substrates display higher hydraulic conductivity. Soil- based CW experience high level of clogging, which blocks interstitial spaces hence reducing the available volume within the substrate. The end effect is decreased HRT, increased flow velocities and short circuiting [1]. In comparisonto soil substrate, gravel has demonstrated to be the most preferable substrate since the void spaces allow through-flow of water from the start and serve as flow channels for wastewater throughout the system [4]. Nevertheless, this study focused on pebble gravelof different sizes consisting of fine, medium to coarse gravel aggregates following the grading by the International Organization for Standardization (ISO) 14688.

Constructed wetlands offer the most cost-effective alternative in wastewater treatment in comparison to the more technical and expensive tertiary processes especially for Sub- Saharan Africa [5]. Along Western margin of the Gregory Rift, gravel is one of the readilyexposed Turkana grits, formed as a result of the sub-volcanic and intra-volcanic sediments deposited during the early tectonic events in the region, thus offering an opportunity to investigate its suitability to serve as a potential granular substrate for CW [6]. Constructed wetlands are categorized differently, resulting in three main designs: Hybrid Systems (HS), Free Water Surface (FWS) and Sub-Surface Flow (SSF) which is further divided into Horizontal Sub-Surface Flow (HSSF) and Vertical Sub-Surface Flow[7]. Use of CW by most researchers has focused on HS, FWS and HSSF CW, documentinglittle information on potential use of VSSF CW in reduction of FIB and organic matter. Furthermore, most studies have emphasized on use of aquatic macrophytes in CW due to their ability to enhance the uptake of nutrients in wastewater [7]. However, the combined effect of non-planted VSSF CW and substrate size has not been studiedextensively. In view of the foregoing, the current study investigated the use of a VSSF CW filled with various gravel substrate sizes to reduce FIB and organic matter.

More often, CW are constructed with coarse and fine gravel. The upper layers are constructed with fine gravel in the size range of 12-15 mm while the bottom layers are constructed with coarse gravel in the size range of 30 - 40 mm, allowing the void spaces in the media to serve as flow channels for wastewater [1]. The fine gravel in the upper layers ensures maximum organic matter retention, less clogging effect and good oxygenpenetration [8]. In addition to that, the fine gravel supports root penetration in case wherewetland macrophytes are employed, making the upper layers the most biologically activepart of the CW [9]. According to [3], the most commonly used gravel sizes for CW ranges from 13 – 38 mm, with a depth between 30 cm to 90 cm. In the current study, the gravel sizes adopted were <12.5 mm (fine), 12.5-18 mm (medium) and 19-24 mm (coarse) to a depth of 60 cm.

Many institutions and small towns in india that generate high domestic waste with a range of physicochemical to biological contaminants are currently adopting the use of CW for domestic wastewater treatment [10]. However, lack of innovative approaches to wastewater management and poor understanding of this technology is continuously slowing down the adoption [11] . This therefore informed the aim of the current study to investigate the efficiency of an operational set up that mimicked the wastewater purification processes in a VSSF CW, to reduce FIB and organic matter under varied sizes of pebble gravel substrate aggregates of volcanic origin. It was hypothesized that fine gravel substrate increased the reduction efficiency of contaminants in comparison to coarse gravel substrate.

2.0 MATERIALS AND METHODS

2.1 Development and operational set up of VSSF CW

A laboratory scale VSSF CW system was set up at the dairy farm sited near of Glocal University's. Before setting up the operational units, gravel substrate was washed to reduce silt and other organic impurities and air-dried. Using sieves of various mesh sizes, the air- dried gravel was graded into fine, medium and coarse- sized aggregates using ½'', 3/4''and 1'' sieves respectively, before filling eachsubstrate into respective tanks to a depth of 60 cm. The mesocosm subsurface CW consisted of three cylindrical operational units (Height = 100cm, Diameter = 30 cm, Volume= 70.69 liters) to ensure the substrate is fully submerged [9]. The first, second and third operational units consisted of fine (<12.5 mm), medium sized (12.5-18 mm) and coarse (19-24 mm) gravel aggregates respectively. Each of the units was replicated three times, and fitted with an outlet tap ½ inch, 10 cm from the ground. The units were randomly arranged outdoor to account for potential variability in micro environmental conditions such as exposure to sunshine, shading and rainfall. A similar system was developed without addition of gravel substrate that served as a control.

Before being subjected to working conditions, all units were filled with 70 liters of wastewater from the final WSP at the dairy farm sited near of Glocal University WSP system. In order to mimicthe properties of a VSSF CW, the contents in the operation units were allowed a period of 6 weeks for establishment, acclimation and stabilization of microbial community to allow proper system functioning. This also contributed to the establishment of a compactbed for wastewater treatment. The units operated on an assumption that a microbial community formed within biofilms surrounding the gravel substrate and in the interstitial water. During the acclimation period, the chemical constituents were considered as food for the microorganisms, hence anchoring and creating fixed biofilmsthrough secretion of extracellular polymeric substances. This anabolic action of biofilm and microbial mass creation persisted, which led to formation of pore spaces and allowedwastewater to take a different hydrological regime and hence an overall flow-path. Eventually, after the acclimation phase, the biofilms became well distributed, heterogeneous and stable. In this operation, wetland plants were not included, as the study sought to investigate the reduction of contaminants using other processes rather than those initiated by presence of wetland macrophytes. The physico-chemical parameters and FIB loading before and after establishment of microbial community in the units were recorded. Henceforth, the VSSF CW was operated as a batch reactor wherewater samples were collected from each of the processing units after a 7-days retention period for 8 weeks for various physico-chemical parameters, organic matter and faecal indicator bacteria (FIB). Immediately after every sampling, the units were mechanically refilled with equal amount of wastewater from the same source in preparation for the next sampling episode.

2.2 Wastewater sample collection scheme

Physico-chemical parameters (temperature, Dissolved Oxygen, pH and conductivity) were recorded in situ using a calibrated HQ 40d (HACH) multi-meter probe. Wastewatersamples were transported to the dairy farm sited near of Glocal University's for analysis of total suspended solids (TSS), Biochemical Oxygen Demand (BOD) and FIB (Total coliforms and *Escherichia coli).*

2.3 Determination of TSS

The TSS were estimated gravimetrically using glass micro-fiber filter paper method (Whatman GF/C filters with pore size 0.45µm) [9]. A known volume of wastewater sample was filtered using pre-weighed Whatman GF/C filter and then dried at 95°C to aconstant weight. TSS were estimated according to [9] formula:

$$\text{TSS} = (\text{mg}/)\,\frac{((Wf-Wc)10^6)}{V}$$ Where:

TSS = Total Suspended Solids

Wc = Weight of pre-combusted filter in grams Wf = Constant weight of filter + residue in grams
V = Volume of wastewater sample filtered in ml

2.4 Determination of BOD

According to [13], 30 ml of wastewater was added to BOD bottles with a capacity of 300 ml and topped up with distilled water. Initial DO concentration was measured using a calibrated HQ40D (HACH) multi-meter probe. The BOD bottles were capped tightly andincubated in a cabinet whose room temperature ranged between 20 °C to 25 °C for 5- days. For this experiment 5-day BOD was used and hence determined as BOD_5. After this incubation period, the final DO concentration was determined using the formula for unseeded water by [14]:

2.5 Microbiological analysis of wastewater samples

Wastewater sample processing was undertaken using the Membrane FiltrationTechnique (MFT). Ten times (10X) serial dilutions of wastewater were prepared using sterile 0.1 % bacteriological peptone solution to end dilution of 10-7. 5ml of the sample diluent was introduced aseptically into a sterile stainless-steel filtration multi-channel apparatus containing a sterile membrane filter (47 mm diameter, 0.45 µm pore size) in each funnel. indicator organisms. Visually identifiable typical colonies appearing pink and dark bluewere identified as coliforms and the blue colonies counted as *Escherichia coli*. These colonies were counted using the Fisher Accu-lite colony counter model 133-8002A. The results were expressed in numbers of “Colony Forming Units’’ (CFU) per 100 ml of the original water sample.

2.6 Determination of substrate size efficiency in reduction of organic matter and FIB The overall reduction efficiency was calculated based on difference between the influentand effluent mean concentrations relative to the influent mean concentration of measuredparameters (BOD, *E. coli* and TC) using the reduction efficiency formula [15]:

2.7 Data Analysis

Data was analysed using Sigma plot v.14 statistical package software for data analysis. Data on the physico-chemical characteristics of wastewater was described andsummarized. The averages in BOD_5 concentration, TC and *E. coli* counts, were subjectedto One Way ANOVA test to determine any significant differences between the various substrate sizes in reducing FIB and organic matter. Means were separated using Tukey'sHSD post hoc test. All statistical tests were conducted at the 95 % significance level.

3.0 RESULTS

3.1 Physico-chemical parameters and FIB loadings before and after establishment of microbial community.

The freshly collected wastewater sample had an average temperature of 18.6 °C, which reduced by 3 °C to averagely 15 °C after exposure to the treatment for 6 weeks in all effluents of the 3 different gravel substrate sizes (Table 1). Dissolved Oxygen, similarly, showed a similar trend, dropping from 7.9 mg/l in the raw wastewater to 0.97 mg/l in thefine gravel units and 2.5 and 2.3 mg/l in the medium and course gravel sized units respectively. There was an increase in electrical conductivity of wastewater whereby, theinfluent recorded 697 µS/ cm, which increased to 726.7, 852.8 and 768.5 µS/ cm for the effluents of fine, medium and coarse gravel aggregates respectively. There was novariation in pH in both the influent and the effluent samples with both cases depicting alkaline conditions of up to a pH of 8. The initial concentration of TSS in the influent sample was 382.05 mg/l which reduced to 142.53, 81.49 and 132.44 mg/l in the fine, medium and coarse sized gravel units respectively. In the influent, the *E. coli* load was 2.9×10^7 CFU/ 100 ml while in the effluent, the load reduced to 6.6×10^5, 4.0×10^5 and 7.3×10^6 CFU/ 100 ml in the fine, medium and coarse gravel units respectively. Additionally,TC counts reduced as well with freshly collected wastewater depicting a high load of 4.7×10^9 CFU/ 100 ml while, after treatment, the results recorded were 8.3×10^4, 1.1×10^5 and 8.6×10^6 CFU/ 100 ml for the fine, medium and coarse gravel units respectively.

Table 1: Physico-chemical parameters and FIB loadings before and after establishment of microbial community in the operational units for the 6 weeks

		Effluent samples		
Parameter	**Influent sample**	**fine gravel**	**medium gravel**	**coarse gravel**
Temp (°C)	18.6 ± 2.0^{a}	15.0 ± 0.8^{b}	15.6 ± 0.9^{b}	15.4 ± 0.9^{b}
DO (mg/l)	7.94 ± 2.5^{a}	0.97 ± 0.02^{b}	2.5 ± 0.7^{b}	2.3 ± 0.6^{b}
Cond (µS /cm)	697 ± 142^{a}	726.7 ± 162^{b}	852.8 ± 102^{b}	768.5 ± 93^{b}
pH range	8.7-8.9	8.0-8.3	8.1-8.4	8.5-8.7
TSS (mg/l)	382.05 ± 192.1^{a}	142.53 ± 88.0^{b}	81.49 ± 32.4^{b}	195.82 ± 52.7^{b}
E. coli (CFU/100 ml)	$2.9 x 10^{7} \pm 1.2 x\ 10^{5a}$	$6.6\ x\ 10^{5} \pm 2.0\ x\ 10^{3b}$	$4.0 x 10^{5} \pm 1.2\ x 10^{3b}$	$7.3\ x\ 10^{6} \pm 2.4\ x 10^{4b}$
TC (CFU/ 100 ml)	$4.7 x\ 10^{9} \pm 2.1 x 10^{6a}$	$8.3\ x\ 10^{4} \pm 3.4 x 10^{1b}$	$1.1 x 10^{5} \pm 1.4 x 10^{2}$ b	$8.6\ x\ 10^{6} \pm 3.5 x 10^{3b}$

Physical-chemical parameters and FIB loadings of wastewater; temperature, DO, EC TSS, *E. coli* and TC measured before (influent sample) and after exposure (effluent samples) where microbial community wasallowed to establish for 6 weeks and allow growth of biofilms. FIBs are presented as CFU/ 100 ml, temp as°C, DO as mg/l, EC as µS /cm and TSS as mg/l. All parameters are presented as means ± SD, except pH presented as range. For each individual parameter, different superscript letters indicate significant differences while similar letters indicate **no** significant differences at α=0.05, 95% confidence level.

3.2 Physico- chemical parameters of the operational units.

As outlined in table 2, influent water temperature was 17.7 °C, but the effluent temperature decreased in all the effluent units to averagely 14 °C. Temperature varied significantly in all the tanks ($F_{(4,\ 100)} = 46.21$, $p < 0.05$). Tukey's HSD test revealed a significant variation in temperature between the influent samples and all the other effluent samples ($p<0.05$), while no significant variation occurred among different gravelaggregate sizes. Influent DO concentration was 3.1 mg/l, which reduced to 1.3 mg/l and 1.8 mg/l in fine and medium gravel aggregates respectively.

There was a comparatively slight increase in DO to 2.0 mg/l in coarse gravel aggregate and 2.7 mg/l in the control. Statistical test revealed a significant difference in DO concentration among the units (Kruskal-Wallis; H=20.04; df=4; $p < 0.05$). A post hoc test showed the influent was significantly different from fine gravel aggregate ($p< 0.05$) but did not vary significantly with medium and coarse gravel aggregate ($p>0.05$). Additionally, DO concentration did not vary significantly among the various gravel sizes, ($p>0.05$). High mean electrical conductivity of averagely 800 µS/ cm (fine-866±120, medium-870±94 and course-859±100µS/ cm) was recorded in effluent of all the units in comparison to the influent samples (583.8±246 µS/ cm). Electrical conductivity in the effluent was significantly different from of all the three substrate sizes (F (4, 100) = 14.611; $p<0.05$). However, electrical conductivity did not vary with fine, medium and coarse gravel aggregates ($p>0.05$). In the current study, untreated influent sample had a pH range of 5.6-10.7 and, subject to treatment in the various units, the pH increased to a range of 7.2-11.5. The influent pH was highly variable in range and so was the effluent pH. TSS in mid-sized gravel aggregate unit had the lowest effluent mean of 92.9 ± 72.8 mg/l while fine and coarse sized gravel aggregates recorded means of 161.9 ± 79.5 and 147.6 ± 99.7 mg/l respectively. The influent, on the other hand, had the highest TSS mean concentration of 340.5 ± 273.5 mg/l. These results translated into reduction efficiency of 52.4, 72.7 and 56.6% for medium,fine and coarse gravel aggregates. A significant variation existed in TSS concentration among the operational units (Kruskal-Wallis; H=22.874; df=4; $p<0.001$). A post hoc pairwise comparison test revealed that the influent significantly differed with the fine, mid and coarse gravel aggregates (Tukey's HSD test; $p<0.001$) while no significant variation existed among the three operational units with various gravel aggregate sizes (Tukey's HSD test; $p> 0.05$).

Table 2: Physical - chemical characteristics of wastewater in the operational units over the study period

Effluent samples					
Parameter	**Influent samples**	**Fine gravel**	**Medium gravel**	**Coarse gravel**	**Control**
Temp (°C)	17.7 ± 1.0^{a}	14.1 ± 0.7$^{\mathbf{b}}$	14.4 ± 0.7$^{\mathbf{b}}$	14.8 ± 0.6$^{\mathbf{b}}$	15.8 ± 1.3
DO (mg/l)	3.1 ± 2.3$^{\mathbf{a}}$	1.3 ± 0.9$^{\mathbf{b}}$	1.8 ± 1.1$^{\mathbf{b}}$	2.0 ± 0.6$^{\mathbf{b}}$	2.7 ± 0.7

Cond (µS/ cm)	583.8 ± 246^{a}	866 ± 120^{b}	870 ± 94^{b}	859.8 ± 100^{b}	763 ± 94
pH range	5.6 - 10.7	7.2 -10.1	7.9 -11.5	7.8 -10.1	7.9 - 9.5
TSS (mg/l)	340.5 ± 273.5^{a}	161.9 ± 79.5^{b}	92.9 ± 72.8^{b}	147.6 ± 99.7^{b}	354.76±229.5

Physical- chemical parameters were measured on weekly basis for 8 weeks and results for both influent and effluent samples are presented as averages ± SD with exception of pH presented as range. Temp is presented in °C, DO in mg/l, EC in µS/ cm and TSS in mg/l. For each individual parameter, different superscript letters indicate significant differences while similar letters indicate **no** significant differences atα=0.05, 95% confidence level.

3.3 Performance of operation units in the concentration and reduction efficiency of organic matter and FIB (Table 3)

Prior to treatment, BOD$_5$ concentration was 148.5 ± 29.2 mg/l. Fine gravel aggregates recorded the lowest BOD$_5$ concentration of 124.9 ± 9.03 mg/l, which increased to 133.7 ± 4.9 mg/ l in the mid-sized gravel and finally a further slight increase in concentration to 135.9 ± 14.8 mg/l in the coarse gravel aggregate. Statistically, there was no significant variation amongst the various gravel aggregate sizes in terms of concentration (One WayANOVA; F ($_{4,\ 100}$) =1.399; p = 0.239). In terms of reduction efficiency, very low values (lessthan 20 %) were observed for all gravel aggregate sizes; fine = 15.9 %, medium = 8.5 %, and coarse = 9.9 %.The mean concentration of the influent was 3.3 x 10^{9} ± 1.2 x 10^{10} CFUs / 100 ml and 5.3 x 10^{10} ± 6.7 x 10^{10} CFU/ 100 ml for *E. coli and* TC respectively. After treatment, the counts went down to 1.6 x 10^{8}±2.6 x 10^{8}, 1.9 x 10^{8} and 3.8 x 10^{8} ± 1.4 x 10^{9} CFUs / 100 ml for *E. coli* and 2.5 x 10^{9} ± 3.6 x 10^{9}, 5.0 10^{9} ± 1 x10^{10} and 5.9 x 10^{9} ± 1.6 x 10^{10} CFUs / 100 ml for TC in the fine, medium and coarse gravel aggregates respectively. *E. coli* percentage reductionefficiency was computed as 95.2 %, 94.2 %, 88.4 % and 29.3 % for fine, medium, coarse gravel aggregate and control respectively while that of TC was recorded as 95.3 %, 90.4 %, 88.8 % and 32.1 % for fine, medium, coarse gravel aggregate sizes and control respectively.Just like in *E. coli* reduction, fine gravel aggregate was found to perform better than medium and coarse gravel aggregates. A post hoc analysis revealed a significant variation between the influent and all the other gravel aggregate sizes ($p<0.05$). However, no significant variation was noted in reduction efficiency among the various gravel aggregate sizes.

Table 3: FIB and BOD^5 concentration and reduction efficiency over the study period

Effluent samples					
FIB	**Influent Samples**	- **Fine gravel**	**Medium gravel**	**Coarsegravel**	**Control**
BOD$_5$ (mg/l)	148.5±29.2	124.9±9.03	133.7±4.9	135.9±14.8	143.6±27.9
% Reduction	-	**15.9**	**8.5**	**9.9**	**0.03**
E.coli **(**CFUs/100 ml)	3.3 x 10^9	1.6 x 10^8	1.9 x 10^8	3.8 x 10^8	2.3 x 10^9
	±1.2x 10^{10}	± 2.6 x 10^8	± 3.6 x 10^8	± 1.4 x 10^9	±6 x 10^9
% Reduction	**-**	**95.2**	**94.3**	**88.4**	**29.3**
TC (CFUs/100ml)	5.3 x 10^{10}	2.5 x 10^9	5.0 x 10^9	5.9 x 10^9	3.6 x 10^{10}
	±6.7 x10^{10}	± 3.6 x10^9	± 1 x 10^{10}	± 1.6 x 10^{10}	±1.8 x 10^{10}
% Reduction	**-**	**95.3**	**90.4**	**88.8**	**32.1**

*Averages and SD of BOD*5*, E. coli and Total Coliform counts presented as mg/l, CFU/ 100 ml and reduction efficiencies presented as percentages for both influent and effluent samples*

4.0 DISCUSSION

4.1 Temperature

Temperature is one of the key parameters that influence both bacterial and organic matterreduction rates as it plays a role in the chemical and biochemical reactions as well as many other processes in a CW. A drop in temperature was noted between untreated influent and treated effluent samples. This could be attributed to the shed provided to prevent direct effects of potential variability in micro environmental conditions such as direct exposure to sunlight. Moreover, influent water temperature was slightly higher compared to outlet temperature in all the units because the influent water source was a shallow and open maturation pond exposed to direct sunlight, which experienced uniform temperature due to mixing during the day. The effluent temperature in this operation is believed to have had little contribution to microbiological and organic matterreduction in comparison to other physical processes that are not temperature sensitive [7]. A study by [16] reveals the negative impacts associated with low temperature in reduction of organic matter. In the current study, BOD was reduced by less than 20 % ata low temperature range between 14-17˚C. This study is in agreement with the study by [18], who found out that at low temperatures during winter, there is a comparatively lower performance of BOD reduction in sub surface CW ranging between 44-88 % while in summer, the performance increased to 60-90 %.

4.2 Dissolved Oxygen

DO has been cited as a major environmental factor that controls degradation of organic matter as well as influence the reduction rate of microbes. In the current study, low DO levels recorded for both influent and effluent samples were attributed to absence of wetland macrophytes in the operation units. [17] notes that wetland macrophytes contributes towards creation of an oxygenated environment, through internal transfer via air spaces in plants [19]. Additionally, plant roots improve DO conditions in CW hence supporting the aerobic processes [20]. Moreover, the low DO concentration could be because of decomposing organic matter in the units that required high oxygen demandfor biodegradation to effectively occur. The gravel substrate could have become water logged as well, which contributed to anoxic conditions that inhibited processes like removal of organic matter and nitrification [21]. Low DO in CW, however, does not always portray anoxic conditions. In their study, [22] suggested that there are anoxic zones within CW where denitrification occurs, and this contributes to faster degradation of microbes in comparison to nitrification process. Even with the low DO concentration in all the units, there was diffusion of atmospheric oxygen inside the matrix pores, whichcontributed to fostering of biodegradation pathways as observed by [23].

4.3 Electrical Conductivity

Electrical conductivity is a useful indicator of water quality in terms of dissolved materials. It is used to denote the suitability of water for use, e.g., for irrigation and drinking, as well the level of ionic pollution [24]. In the current study an increase in electrical conductivity over the one-week wastewater retention period could be linked toevaporation from the units. Additionally, this could also be as a result of sedimentation hence the units acted as ion deposits.

4.4 pH

According to [25], the pH of water should range between 6.5-8.5, but, in the current study, there was a narrow range of pH in the influent as well as in the effluent. In both cases,pH was above neutral depicting alkaline conditions. This could be attributed to rapid photosynthesis by diverse algal population that was seen floating on the surface of the tanks, which raised the pH to values above neutral. Furthermore, the high pH consumedCO_2 faster than it could be replenished by bacterial respiration in the units, leading to dissociation of carbonate and bicarbonate ions. The resulting CO_2 was probably fixed byalgae and the hydroxyl ions dissociated, which raised pH values to high levels that bacteria could not withstand hence contributed to their increased mortality rate [26]. A study by [27] contradicts the above results, where he observed a slight decrease in effluent pH in CW, and attributed this to metabolism of sulphates, phosphates and nitrogenous compounds.

4.5 Total Suspended Solids

Reduction of suspended solids in CW has been attributed to the presence of wetland macrophytes and longer retention periods. [28] confirmed this in his study where he employed *P. australis* in a CW that corresponded to reduction of 79.7, 81.8 and 87.3 % for3.96, 4.56 and 5.4 days respectively which are results similarly obtained by [29]. Nevertheless, the current study did not employ wetland macrophytes and the recorded performance could be attributed to other processes including sedimentation, filtration and chemical precipitation with the 7-day reduction efficiency.

4.6 Organic matter

Both Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are associated with the amount of DO in water. The current study focused of BOD because sewage formed the bulk of raw influent hence organic matter was determined as BOD_5. Poor performance of the operational units (<20 % reduction efficiency) was largely attributed to the absence of wetland macrophytes, since there was no significant difference between the influent and the effluent in all the three gravel substrate sizes (p=0.241). Previous studies show that sufficient BOD_5 reduction is associated with wetland macrophytes in the treatment process by providing habitat for many decomposing microbes in the root zone [30], and, moving the oxygen to the roots and rhizomes [31]. According to [32], better treatment performance was obtained in CW cellsplanted with *T.*

latiolia and *Colocasia esculenta* in comparison to non- planted cells in BOD_5 treatment in anaerobically pretreated wastewater. Other studies that agree with the current study in view that vegetated CW demonstrate high BOD_5 reduction efficiency incomparison to non-vegetated ones include, and [33], [34] and [35]. Furthermore, low BOD_5 reduction efficiency could also be associated with the short 7-day retention time. Studies indicate that the combined effect of longer retention period and wetland macrophytes produces the best results. This is an argument presented by [36] whose results indicated BOD_5 reduction efficiency of 65.32 % and 91.9 % for 8 days retention time in *B. reptans* and 80.67 % in *T. portulacastrum* respectively. Also, [37] and [7] recordedup to 90 % BOD_5 reduction efficiency with >8 days HRT. However, even with longer retention times, BOD reduction rate may slow down in cases where there is accumulationof organic solids [38] and non-biodegradable compounds [39] that decompose slowly in the substrate interstices.

4.7 Faecal Indicator Bacteria

The findings indicate that gravel substrate characteristics have a potential impact on pollutant reduction in wastewater. Previous studies have elucidated use of different types of substrates in CW viz; organic wood [40], rice husks [41], zeolite [40], peat [42] among others. However, the current study utilized gravel substrate, which is the most commonly used media in most CW as researched by [4]. Fine gravel aggregates in the current study performed better than all other substrate sizes in reducing the concentration of *E. coli* and TC followed by medium and finally coarse gravel aggregate.The fine grain sizes ensured a larger surface area, better physical filtration of solids, morebiofilm growth hence pathogen inactivation. The microbial attachment to the gravel surface formed biofilms which developed capacity for filtration and adsorption of FIB [5]. A study by [43] indicated that greater filtration efficiency of a CW bed media overtime was attributed to acclimation phase where biofilm growth occurred on the bed media, and this is in agreement with the current study. When it comes to sediment size, [44] points out that fine gravel (2-13 mm) possesses higher reduction efficiency as compared to coarse gravel (5-25 mm). In addition to that, fine media in the range of 4-8 mm is moreefficient in reduction of faecal coliforms in comparison to course media ranging between10-20 mm [45]. Moreover, [46] shows the ability of small grain size having a larger specificsurface area for interactions, hence increased reduction efficiency for FIB. As earlier on mentioned, substrate size plays a significant role in pathogen inactivation and [44]. Moreover, the 7- day HRT provided maximum contact time between FIB reduction and the biofilm, short enough to prevent low hydraulic conductivity that couldlead to clogging and long enough to decrease their concentration in wastewater. Longer HRT exposes bacteria to unfavorable conditions. A study by [47] agrees with the currentstudy, where bacteria was exposed to HRT of 2.0, 3.0, 5.5, and 7 days in a CW and corresponded to reduction efficiency of 76.2, 79.4, 92.1 and 95 %. The results of the currentstudy as well agrees with a two-stage vertical flow CW in Denmark, where reduction efficiency of FIB increased from fine to coarse gravel aggregate [48].

The current study did not employ wetland macrophytes, although FIB reduction efficiencies ranging between 88 % and 95 % were registered translating to 1 log unit. Thisis attributed to other processes such as predation by other microbes e.g., grazing by protozoa, microbial interactions, mechanical filtration and natural die-offs as suggested by [49]. Most studies have stongly attributed higher reduction efficiencies to presence ofwetland macrophytes, including [50] and [49], who strongly disagree with the current study that attributes the high reduction efficiency to other mechanisms rather than the influence of wetland macrophytes. The results from the current study suggests that wetland macrophytes only play a minor role in reduction of microbes in VSSF CW. In a separate study that involved filters planted with *P. australis,* lower reduction efficiencies were recorded in comparison with non-planted ones [8]. Additionally, [47] and [51] notednotes that no significant variation exists between planted and non-planted systems. Other environmental factors like temperature and DO have been observed to improve reduction efficiency. The reduction efficiency for the current study ranged between 88 %and 95 % in comparison to a similar study whose reduction efficiency was 94 % to 99 %. In the later study, higher DO and temperature contributed to the good performance as they provided a suitable aerobic environment [7]. However, in the current study, a drop in the effluent temperature in all units indicated anaerobic environment, which demonstrates how this provides anaerobic conditions that prolong the survival of bacteria. In comparison to the current study without wetland macrophytes where reduction efficiency ranged between 88 to 95 %, [52] recorded overly a low reduction efficiency of 80 % in a tropical sub surface flow CW planted with both T*ypha sp* and *Scirpus lacustris* and lined with gravel substrate. The shortfall in the later treatment system was attributed to low frequency of dredging the CW and harvesting of macrophytes, suggesting that frequent maintenance of a CW could contribute to higher reduction efficiency of FIB.

5.0 CONCLUSION

The current study established the ability of CW to reduce FIB and organic matter in a mesocosm operational set up mimicking a VSSF CW. Various gravel aggregate substrateswere used as treatments without wetland macrophytes while a control was set up undersimilar conditions without any substrate. Both the treatments and the control were subjected to intermittent loading of pretreated wastewater on weekly basis for 2 months.The major conclusions in the study are:

- The operation units performed poorly in reduction of organic matter (<20 % BOD_5)reduction efficiency, a phenomenon largely attributed to lack of wetland macrophytes and a short HRT of 7 days. Going forward, incorporation of wetlandmacrophytes and increased HRT (>8 days) is recommended for improved performance.

- Among the three gravel substrate sizes adopted in the study, fine gravel substrate(<12.5 mm) showed higher potential in terms of reducing the counts of FIB in comparison to medium and coarse gravel. However, no statistical difference was obtained in reduction efficiency of the various gravel substrate sizes. Incorporation of the three gravel aggregate sizes in a CW and increasing the HRTcould be explored to verify their potential in increasing reduction efficiency for FIB.
- A 1-log unit was achieved in reductio of FIB, attributing it to other factors and processes rather than wetland macrophytes, including predation mechanical filtration, microbial interactions, starvation and natural die-offs.

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