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# Tertiary treatment of abattoir wastewater in a horizontal subsurface flow-constructed wetland under tropical conditions

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**Abstract:** A horizontal subsurface flow-constructed wetland (HSSF-CW) system planted with *Cyperus papyrus* was used to polish abattoir wastewater pre-treated in anaerobic and aerobic sequencing batch reactors at City Abattoir, Kampala. The HSSF-CW was 13 m long, 5 m wide, and was filled with gravel up to a depth of 0.6 m, of which 0.55 m was saturated with wastewater at hydraulic retention time (HRT) of 1.16 days. The percentage removals for turbidity, ammonium-nitrogen ($NH_4$-N), total nitrogen (TN), orthophosphate (o-$PO_4$-P), total phosphorus (TP), chemical oxygen demand (COD) and faecal coliform were 76, 48, 46, 74, 63, 60, and 100, respectively. Among different plant tissues investigated (roots, culm, sheath and umbel), nitrogen concentration was highest in the sheath. The different parts of plants also had different concentrations of phosphorus, with the culm having the highest concentration. Compared to literature, the percentage removals for organic matter, nutrients and faecal coliforms reported in this paper were high. Therefore, *C. papyrus* based constructed wetlands could potentially be used for tertiary treatment of abattoir effluents.

**Keywords:** abattoir wastewater; nutrients; constructed wetland system; anaerobic-aerobic sequencing batch reactors; SBR; papyrus.

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## 1 Introduction

Effluent from bio-digesters treating industrial wastewater highly recalcitrant in inorganic nutrients and organic matter, such as abattoir wastewater do not usually meet national discharge limit requirements, necessitating tertiary treatment (Scholz, 2006). Utility of constructed wetlands to treat wastewater was first experimented by Seidel in Germany in the 1950s (Vymazal, 2005). The premise for use of constructed wetlands in wastewater treatment is in the functioning of natural wetlands. Wetlands remove inorganic and organic matter through different processes including filtration, adsorption, absorption, plant uptake and assimilation, microbial uptake and transformations (Gersberg et al., 1986). Effluent from a constructed wetland system should meet national discharge standards, be safe to discharge to the environment, with potential for use in irrigation or recycling within an industry for peripheral uses such as washing paved surfaces.

Constructed wetlands are a preferred choice for tertiary wastewater treatment because they are relatively cheap to operate and maintain, macrophytes grown therein can be harvested regularly for use as fuel source or making art craft, habitat for fauna, especially birds, pleasant landscape or scenery, and they sequestrate carbon dioxide (Merz, 2000). However, developing countries have been slow at adoption of constructed wetlands technology in wastewater treatment (Denny, 1997). Treatment performance of constructed wetland systems depend on several factors including design, shape, bed slope, length to width ratio, baffle, type of gravel media used, macrophytes, influent wastewater quality, flow rate, hydraulic retention time, and climatic conditions (Reed et al., 1995; Kadlec and Knight, 1996; Merz, 2000; Persson, 2000; US-EPA, 2002; Hedges et al., 2008). Sizing and design of constructed wetland systems are based on land availability, influent organic loading rate and discharge standards to be achieved during treatment (Kadlec, 2009).

Constructed wetlands are subdivided into free water surface (FWS) flow and subsurface flow (SSF), which are further divided into horizontal flow and vertical flow, depending on the direction of water flow through the system. In contrast to FWS flow systems, SSF flow systems are supplied with porous media such as sand, gravel and crushed stones, averaging in size between 4–16 mm (Vymazal, 1997) in which emergent macrophytes are planted. Subsurface flow systems are filled with gravel to a depth of about 450–750 mm, with wastewater reaching about 75–150 mm below the top of gravel (Coleman et al., 2001). When properly established, macrophyte roots percolate the gravel media up to 150–300 mm depth. To prevent loss of wastewater and contamination of underground water, the bottom bed and sides of SSF systems are usually lined by synthetic materials such as polyethylene. Compared to FWS, SSF flow systems provide higher contact area for wastewater, bacteria and substrates, thus improving treatment performance (Sleytr et al., 2007).

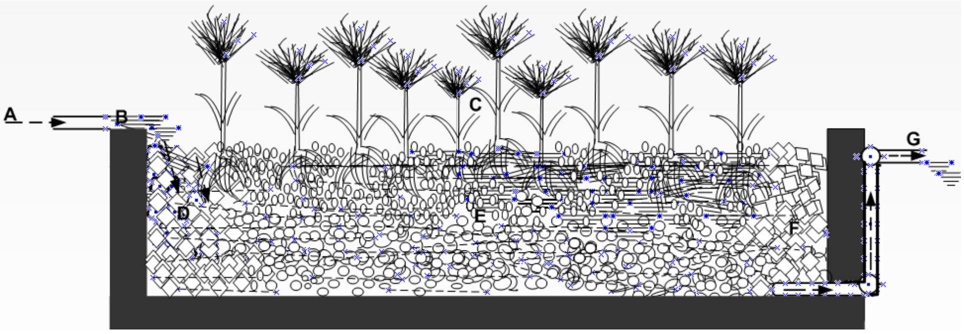
Outside Africa, a few authors have used constructed wetland systems to treat abattoir wastewater (for example Gutierrez-Sarabia et al., 2004; Soroko, 2005). However, none of the constructed wetlands in Eastern Africa (for example Sekiranda and Kiwanuka, 1998; Okurut et al., 1999; Nyakango and van Bruggen, 1999; Mashauri et al., 2000; Nzengy'a and Witshitemi, 2001; Kyambadde et al., 2004; Kyambadde, 2005; Abira et al., 2003; Bojcevska and Tonderski, 2007) had been tested for abattoir wastewater treatment. Therefore, this study investigated the potential use of horizontal subsurface flow-constructed wetland (HSSF-CW) as an integral part of a system comprising of anaerobic and aerobic/anoxic sequencing batch reactors (SBR) to polish abattoir wastewater. The objective of the study was to determine the removal efficiency for nutrients, organic matter and faecal coliforms in the HSSF-CW.

## 2 Materials and methods

### 2.1 System design and planting of *C. papyrus*

The HSSF-CW system was trapezoidal in shape (Figure 1) and made of concrete, with size dimensions of 13 m × 5 m. The walls were 50 mm thick and inclined at an angle of 60°. The HSSF-CW bed had a gradient of 1% towards the outlet. It was filled with gravel up to a depth of 0.6 m, of which 0.55 m was saturated with wastewater. The bulk density of gravel used was 1.52 g/cm$^3$, and its porosity was estimated to be 35% (Senzia et al., 2002). A weir (0.15 m wide and 0.15 m deep) was used to distribute influent wastewater evenly across the HSSF-CW system. Both inlet and outlet pipes were 75 mm in diameter. At the inlet and outlet zones, the HSSF-CW bed was filled with gravel of 50–200 mm in diameter (Figure 1). The rest of the wetland bed was filled with gravel of 25 mm in diameter. The HSSF-CW was planted with *C. papyrus* at a planting density of 1 clone per square metre, each weighing about 500 g. This low planting density was meant to allow for ample space for persons while pruning and cleaning the wetland cell of debris.

**Figure 1** Sketch drawing of HSSF-CW at City Abattoir, Kampala (see online version for colours)



Notes: A inlet pipe
B weir
C *C. papyrus*
D inlet distribution zone
E →: direction of wastewater flow
F effluent zone
G effluent pipe

The HSSF-CW was fed with pre-treated abattoir wastewater from anaerobic and aerobic/anoxic SBR, at an average flow rate of 10.8 m$^3$/day giving HRT of 1.16 days. The HRT was calculated using the formula that follows (Vymazal et al., 1998).

$$\text{HRT} = phlw/q$$

where

$p$ porosity of gravel media (0.35)

$h$ effective depth of constructed wetland unit (0.55 m)

$l$ length of constructed wetland unit (13 m)

$w$ width of constructed wetland unit (5 m)

$q$ mean flow rate (10.8 $m^3$/day).

Therefore, HRT = 1.16 days

### 2.2 Performance evaluation for nutrient, organic matter and faecal coliform removal

The macrophyte, *C. papyrus* planted in the HSSF-CW was allowed to establish and acclimatise for one month before starting performance evaluation for wastewater treatment. Wastewater quality of influent and effluent was monitored from the month of June to September 2011. *In situ* measurements for temperature, pH, electrical conductivity (EC) and total dissolved solids (TDS) (mg/L) were determined using Eutech instruments, CyberScan, model PC 300, supplied by Wagtech Company (UK). Water samples were analysed for ammonium-nitrogen ($NH_4$-N) following direct nesslerisation method (APHA, 1995). A diazotisation method was used to analyse nitrite-nitrogen ($NO_2$-N), while nitrate-nitrogen ($NO_3$-N) and total nitrogen (TN) were analysed by cadmium reduction method and TN using the same method after digestion (APHA, 1995). Ortho-phosphate (*o*-$PO_4$-P) and total phosphorus (TP) were analysed following the ascorbic acid method and for TP using the same method after digestion. Chemical oxygen demand (COD) was determined according to standard procedures (APHA, 1995). All spectrophotometric determinations were made using an Aquamate spectrophotometer (Thermo Electron Corporation, UK Model No. 300). At the end of the study period, replicate samples (n = 9) of *C. papyrus* tissue were harvested to determine nitrogen and phosphorus content. The concentration of nitrogen and phosphorus in *C. papyrus* tissues (roots, umbel, sheath and culm) were determined as described by Novozamsky et al. (1983). Chromocult coliform agar (Merck KGaA, Darmstadt, Germany) was used to determine faecal coliform numbers.

### 2.3 Determination of C. papyrus productivity

Productivity of *C. papyrus* was determined as described by Baldwin et al. (2005). Culm girth (n = 30) were measured using callipers to determine their diameter at culm base. The 30 culms were thereafter harvested for biomass determination as described elsewhere (Kyambadde et al., 2005).

### 2.4 Statistical analysis

MINITAB Release 13.1 statistical software package for Windows was used, and tests included analysis of variance (ANOVA), F-test and Levene's test for homogeneity of variance and normality, and Tukey's multiple comparisons for differences between means. A significance level of $p \leq 0.05$ was used.

# 3 Results

Results of performance evaluation of HSSF-CW treating pre-treated abattoir wastewater are presented in Table 1.

**Table 1** Mean ± standard error of mean values (n = 15) of physicochemical and faecal coliforms variables determined for influent and effluent abattoir wastewater over a period of 15 weeks

| *Parameter* | *Range of influent* | *Range of effluent* | *Mean of influent* | *Mean of effluent* | *Percent removal* |
|---|---|---|---|---|---|
| EC (µS/cm) | 1904–3640 | 1444–3270 | 3157±172 | 2372±173 | 24.86 |
| Temp (°C) | 23.10–23.20 | 22.50–26.80 | 23.653±0.20 | 24.01±0.31 | –1.52 |
| Turbidity (FAU) | 114.84–133.52 | 11.49–69.74 | 121.16±1.49 | 29.24±5.89 | 75.86 |
| TDS (ppm) | 1090–9580 | 136–1630 | 2668±724 | 1190±106 | 55.41 |
| $NO_2$-N (mg/l) | 0.00–6.85 | 0.10–3.32 | 0.933±0.544 | 0.78±0.22 | 16.75 |
| $NO_3$-N (mg/l) | 0.00–11.96 | 0.10–7.13 | 1.081±0.792 | 1.70±0.48 | –57.40 |
| $NH_4$-N (mg/l) | 10.82–59.59 | 4.30–39.94 | 28.19±4.08 | 14.78±3.00 | 47.56 |
| TN (mg/l) | 13.34–60.54 | 6.42–55.82 | 44.56±3.96 | 23.93±4.24 | 46.31 |
| o-$PO_4$-P (mg/l) | 6.70–33.39 | 2.71–9.10 | 22.84±2.16 | 5.89±0.48 | 74.22 |
| TP (mg/l) | 24.12–95.76 | 4.52–54.56 | 85.53±4.48 | 31.58±5.32 | 63.08 |
| COD (mg/l) | 181.4–467.9 | 27.80–187.90 | 262.1±16.7 | 103.9±14.4 | 60.37 |
| Faecal coliforms (CFU/100 ml) | $1.0 \times 10^4 - 1.4 \times 10^5$ | 0.00–0.00 | $5.1 \times 10^4 \pm 1.0 \times 10^4$ | 0.00±0.00 | 100 |

## 3.1 Treatment of abattoir wastewater in HSSF-CW

The effluent pH value was in the neutral range (7.65±0.04), while the value of EC was reduced by 25%. Turbidity and TDS were reduced by an average of 76 and 55%, respectively. The values of nitrite-nitrogen ($NO_2$-N) and nitrate-nitrogen ($NO_3$-N) in influent and effluent wastewater were < 2 mg/l. TN and ammonium-nitrogen ($NH_4$-N) removal in HSSF-CW was by an average of 48 and 47 percent, respectively. Orthophosphate (*o*-$PO_4$-P) and TP were removed by an average of 74 and 63%, respectively. COD removal averaged 60%. The HSSF-CW completely (100%) removed faecal coliforms.

One-way ANOVA showed significant removal of TN, TP, COD, $NH_4$-N and conductivity ($p < 0.013$) in the HSSF-CW. Although TDS was removed by 55%, one-way ANOVA did not detect significant removal ($p = 0.053$). Figures 2, 3 and 4 show the removal of TN, TP and COD, respectively in HSSF-CW over a period of 15 weeks.

**Figure 2** Influent and effluent values of TN over a period of 15 weeks

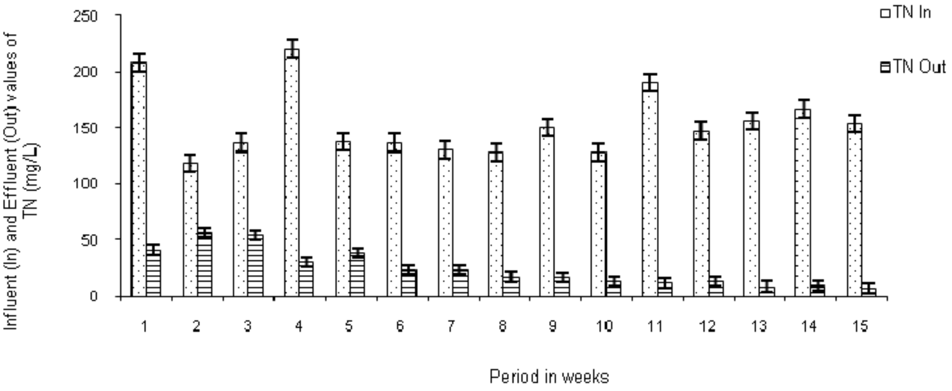


**Figure 3** Influent and effluent values of TP over a period of 15 weeks

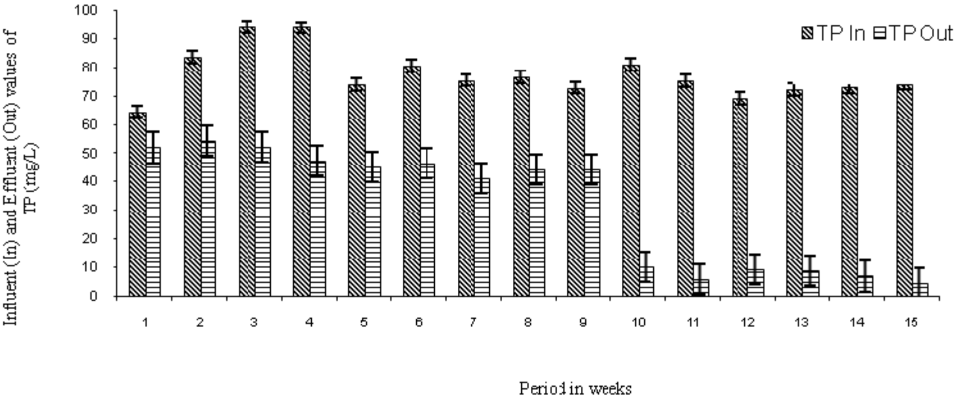
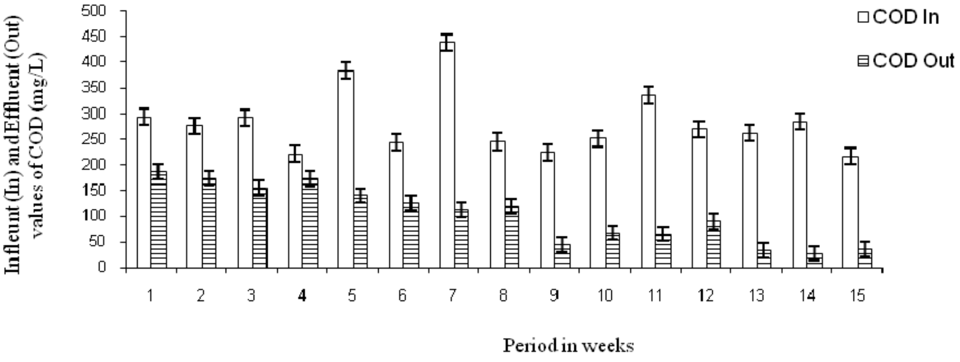


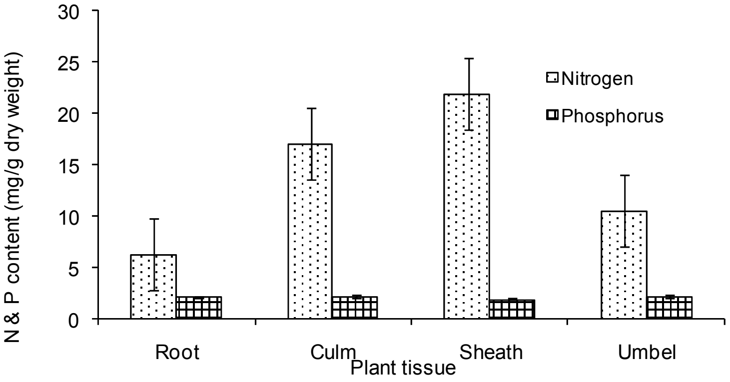
**Figure 4** Influent and effluent values of COD over a period of 15 weeks



### 3.2 Nutrient uptake by C. papyrus

Nitrogen and phosphorus content (measured as mg/g dry weight) in different parts of *C. papyrus* tissues (roots, sheath, culm and umbel) is shown in Figure 5. One-way ANOVA showed that nitrogen content levels in different plant tissues was significantly different ($p = 0.009$). Tukey's multiple comparisons showed that nitrogen content in *C. papyrus* tissue followed the trend sheath > root = culm = umbel. Although phosphorus content was found to be highest in the culm of *C. papyrus* (2.24 mg/g dry weight), one-way ANOVA did not show that it was significantly higher than in the other plant tissues ($p = 0.78$).

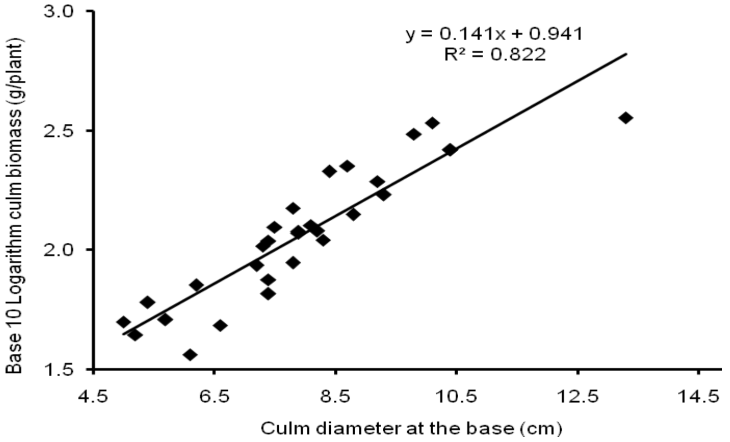
**Figure 5** Nitrogen and phosphorus content in *C. papyrus*



### 3.3 Productivity of C. papyrus

The productivity of *C. papyrus* in the HSSF-CW system is presented in Figure 6. There was significant correlation ($p = 0.000$) between culm biomass (g/plant) and culm diameter at base (cm). The regression coefficient was 94% for the dependent variable (culm biomass).

**Figure 6** Culm biomass (g/plant) as a function of *C. papyrus* culm diameter at base (cm)



## 4 Discussion

The treatment system in this study comprised of integrated anaerobic and aerobic/anoxic SBR and HSSF-CW. Abattoir wastewater contains very high nutrient and organic matter (Ruiz et al., 1997; Massé and Masse, 2000; Fuchs et al., 2003; Mittal, 2006; Li et al., 2008) as well as high loads of bacteria and parasites (Mittal, 2004). As such treatment of abattoir wastewater requires a sequence of treatment processes each with a particular purpose. In the current study, as an integral part, constructed wetland was used for tertiary treatment of abattoir wastewater pretreated in anaerobic and aerobic/anoxic SBR. In constructed wetlands, plants improve wastewater treatment by regulating wastewater flow, filtration, adsorption and absorption, precipitation and sedimentation of organics and suspended solids (Finlayson and Chick, 1983; Tanner and Sukias, 1995; Vymazal, 2007). Macrophytes provide attachment sites for microorganisms on submerged rhizomes, roots and shoots and stimulate microbial activity in the rhizosphere by release of organic carbon, sugars and amino acid exudates (Coleman et al., 2001). Furthermore, plants translocate oxygen from aerial parts to roots, thus facilitating biodegradation of organic matter by aerobic microorganisms.

Results of the present study revealed that the removal of turbidity stood at about 76%, comparably higher than that observed by Finlayson and Chick (1983) who achieved 58–67% in a similar set up of HSSF-CW treating abattoir wastewater. The difference in turbidity removal is possibly related to the differences in plant root and rhizome biomass established in the wetland systems. Indeed, findings of Finlayson and Chick (1983), showed differences in removal of turbidity among the experimental plants.

The average COD value in the influent and effluent of the SBR system were 8,576.00±861 mg/L and 262.1±16.7 mg/L, respectively. Furthermore, the HSSF-CW reduced the COD to 103.9±14.4 mg/L. This translated to COD removal efficiency in the SBR and the HSSF-CW systems of 97% and 60%, respectively. The integrated system therefore, achieved an overall COD removal of about 99%. The reduction in COD in the HSSF-CW was similar to removal (56–60%) achieved by Kaseva (2004) from two HSSF-CW set ups planted with *Phragmites mauritianus* and *Typha latifolia*, respectively treating domestic wastewater in Tanzania. This removal, however is lower than 70% efficiency reported by Okurut et al. (1999) in a HSSF-CW treating pre-settled municipal wastewater. The COD removal in this study is higher than that recorded by Gutierrez-Sarabia et al. (2004), (89%) from an integrated system treating abattoir wastewater comprising of sedimentation tank, anaerobic lagoon and HSSF-CW.

Ammonium-nitrogen ($NH_4$-N) removal in the HSSF-CW (47%) was higher compared to 20% recorded by Okurut et al. (1999) in a similar set up treating domestic wastewater, but lower than findings of Sekiranda and Kiwanuka (1998) who reported removal of >90% in laterite gravel rooted mesocosms planted with *Phragmites mauritianus* treating domestic wastewater. The removal of TN in the HSSF-CW was 46%, comparatively lower than 85–96% reported by Soroko (2007) in hybrid vertical flow and HSSF-CW system treating abattoir wastewater. However, the overall removal by the current integrated anaerobic and aerobic SBR and HSSF-CW system was up to 88%. This overall TN removal was higher than what Gutierrez-Sarabia et al. (2004) achieved (80%) using a settling tank, anaerobic lagoon and HSSF-CW to treat abattoir wastewater.

The TN in the SBR system was reduced from an average value of 200.7±38.7 mg/L to 44.6±4 mg/L. Furthermore, the HSSF-CW reduced the TN to 23.9±4.2 mg/L.

This translated to TN removal efficiency in the SBR system of 78%, with the integrated system achieving an overall TN removal of about 88%. The removal of nitrogen in wastewater is largely dependent on the removal of components particularly $NH_4$-N, $NO_2$-N and $NO_3$-N, which take place in wetland systems mainly via the nitrification-denitrification route, microbial/plant uptake and anaerobic ammonium oxidation (ANAMMOX). Nitrification takes place near the surface of HSSF-CW and in the rhizosphere, while denitrification occurs under anoxic conditions, in the deeper parts of the wetland (Gersberg et al., 1986; Gale et al., 1993). The overall nitrogen removal is usually limited by nitrification, a slower rate reaction, compared to denitrification (Busnardo et al., 1992). Because of insufficient oxygen supply, nitrogen removal in HSSF-CW is relatively low in comparison to vertical subsurface flow systems which achieve higher nitrification because of aeration of top layer of gravel (Merlin et al., 2002; Kantawanichkul et al., 2009). Nitrogen may be lost from wetlands by ANAMMOX, where nitrite-nitrogen ($NO_2$-N) and ammonium-nitrogen ($NH_4$-N) are anaerobically converted to nitrogen gas using nitrate as an electron acceptor (Jetten et al., 1999). Further, microorganisms and macrophytes uptake and assimilate nitrogen in the form of ammonium and nitrate ions. Macrophytes in constructed wetlands take up considerable amount of nitrogen in their tissues (up to 200–2500 $kg.ha^{-1}.yr^{-1}$, Brix, 1997). High concentrations of nitrogen were also found in papyrus tissue by van Dam et al. (2007). This is probably attributable to high productivity of papyrus, requiring high nitrogen concentrations. Assimilation of nitrogen by macrophytes however is not a definite removal path due to plant senescence, necessitating regular plant harvesting to ultimately achieve nitrogen removal.

The present study recorded 74% and 63% orthophosphate (*o*-$PO_4$-P) and TP removal, respectively compared to 68–79%, reported by Finlayson and Chick (1983) for both parameters. Orthophosphate may have been removed through uptake by plants and microorganisms, adsorbed and precipitated in substrates, or it may have been leached and buried in the substratum (Vohla et al., 2007). Depending on several factors, for example wastewater strength, plant health and growth rate, plants can assimilate phosphorus up to a range of 6-150 $kg\ ha^{-1}.yr^{-1}$ (Brix, 1997).

With regard to faecal coliform removal, a total (100%) removal was recorded from the current HSSF-CW. The performance was efficient compared to reduction by 2 log units reported by Okurut et al. (1999) and by order five log units from an integrated system of sedimentation tank, anaerobic lagoon and HSSF-CW treating abattoir wastewater reported by Gutierrez-Sarabia et al. (2004). The removal of faecal coliform in constructed wetlands is related to the retention time of water in the wetland (Okurut et al., 1999), the establishment of biological and chemical mechanisms aided by plant roots and gravel, such as filtration, sedimentation, adsorption, natural die-offs and predation by protozoa (Werker, 2007).

The establishment and growth of macrophytes determine the extent to which plants contribute to removal of pollutants in wastewater in constructed wetlands. The rate of dry weight increment is the most suitable measure to determine how well a given plant grows in a wetland (Rutter, 1955). However, periodic harvesting to determine rate of dry weight increment is destructive. Measurement of radial girth of plants is an alternative non-destructive method to determine rate of dry weight increase. The type of plant used in the constructed wetland also influences treatment efficiency. *C. papyrus* has been considered a preferable plant in treating wastewater because of high biomass and productivity, reaching 48–143 tonnes/ha/yr (Thompson et al., 1979). In addition, papyrus is a C4 plant; therefore it concentrates carbon dioxide near carboxylation site, allowing it to achieve lower carbon dioxide compensation point and minimal photorespiration, and ultimately higher net photosynthesis (Ehleringer and Monson, 1993). Furthermore, papyrus has high efficiency for utilisation of water, nitrogen and light radiation (Mnaya et al., 2007).

## 5 Conclusions and recommendations

In this study, high removals for nitrogen, phosphorus and organic matter were achieved. Further, there was excellent (100%) removal of faecal coliforms, suggesting that a combination of factors, including, gravel substrate, allelopathy, predation and natural die-offs influenced its removal in the wetland. Turbidity level in the effluent (29.2 NTU) met the Uganda national discharge limit (300 NTU). The levels of TN (23.9 mg/l), TP (31.6 mg/l) did not meet the Uganda national discharge limits for both variables (10 mg/l). However, the removal of both TN and TP in the HSSF-CW system could be improved by optimising the removal of both variables in the secondary step, using the anaerobic aerobic/anoxic SBR. Currently, follow up experiments are being developed to improve treatment efficiency to meet national effluent discharge standards for all variables. This study recommends the use of HSSF-CW system for final polishing of secondarily treated abattoir wastewaters prior to discharge to the environment. The integrated system offers possibilities for recycling treated effluent for selected abattoir operations such as use for washing paved pen surfaces and cultivation of vegetables under hydroponic (soilless) conditions.

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