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An investigation into the effectiveness of sand media amended with biochar to remove BOD₅, suspended solids and coliforms using wetland mesocosms

P. de Rozari, M. Greenway and A. El Hanandeh

ABSTRACT

Constructed wetland ecotechnologies (CWEs) are a promising solution to effectively treat domestic wastewater in developing countries at low cost. This paper reports the findings of the effectiveness of sand media amended with woody biochar and two plants species (*Melaleuca quinquenervia* and *Cymbopogon citratus*) in removing biological oxygen demand (BOD₅), suspended solids and coliforms. The experimental design consisted of 21 vertical flow (VF) mesocosms. There were seven media treatments using sand amended with varying proportions of biochar. During the first 8 months, the mesocosms were loaded with secondary clarified wastewater (SCW) then septage. The influent had a 4-day hydraulic retention time. Samples were monitored for BOD₅, total suspended solids (TSS), total volatile solids (TVS), total coliforms and faecal coliforms. In the first 8 months, there were no significant performance differences between media treatments in the outflow concentrations of BOD₅, TSS and TVS. The significant differences occurred during the last 3 months; using septage with biochar additions performed better than pure sand. For coliforms, the significant differences occurred after 6 months. In conclusion, the addition of biochar was not effective for SCW. The VF mesocosms system proved to be more effective in removing BOD₅, TSS, TVS and coliforms when septage was loaded into the media.

Key words | biochar, BOD₅, coliforms, mesocosms, suspended solids, vertical flow

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INTRODUCTION

The problems of water pollution have increased significantly in developing countries due to population growth and urbanisation. Domestic wastewater is the main contributor to water pollution since approximately 75% of domestic wastewater is released to the environment without treatment (Kurniadie 2011). This condition leads to transmission of waterborne diseases and accelerated eutrophication of fresh water bodies (Kadlec & Wallace 2009; Konnerup *et al.* 2009).

Constructed wetland ecotechnologies (CWEs) are potentially a good solution to deal with domestic wastewater problems in developing countries, particularly in the tropical climate, such as Indonesia (Kivaisi 2001). These systems utilise natural processes in removing pollutants and have been proved as efficient technologies for wastewater treatment with the advantage of low cost, simple operation, maintenance and implementation. A variety of pollutants can be removed with CWEs through physical,

chemical and biological mechanisms (Kadlec & Wallace 2009). These removal processes occur via microbial degradation, plant uptake, adsorption and filtration (Greenway 2005; Vymazal 2007; Saeed & Sun 2012). Some authors have reported that CWEs have the ability to remove organic matter (80–99%), bacteria (more than 90%), nitrogen (30–80%) and phosphorus (20–70%) from domestic wastewater, depending on the flow regime and type of plant and media (Vymazal 2009; Abou-Elela *et al.* 2013; Mburu *et al.* 2013).

In subsurface flow constructed wetlands, media play an important role in removing wastewater pollutants, they: control either the rate of water infiltration or retention time; filter sediments and particulates; provide sorption surfaces; and provide surface biofilms and nutrient sources for microbes (bacteria, fungi, protozoa, algae) and macrophytes.

Biochar is one option that can be used in the CWE system as media. This material is a form of carbonaceous product obtained by the thermochemical decomposition of

biomass in the absence of oxygen or under oxygen-starved conditions (Hossain et al. 2011; Mukherjee & Zimmerman 2013). Biochar has been extensively utilised in agriculture as this material can change the physical properties of soil such as structure, pore-size distribution, bulk density and texture (Manya 2012). This ability has important implications for increasing soil aeration, improvement of soil nutrient retention and water-holding capacity, as well as enhancement of plant growth (Oguntunde et al. 2008; Manya 2012).

The pore-size distribution of biochar can be classified into micropores (<2 nm), mesopores (2–50 nm) and macropores (50 nm), based on the diameter of the biochar (Lehmann & Joseph 2009). Micropores play an important role in adsorption capacity, whereas macropores become a suitable place for a vast variety of soil microorganisms and root hairs (Lehmann & Joseph 2009). In addition, organic matter attached to the biochar surface and within the macropores can be decomposed by microbes (Joseph et al. 2010). Because of its pore-size distribution, nutrients trapped in the biochar can be absorbed by plants through nutrient-uptake mechanisms (Duku et al. 2011).

Based on our literature review, no previous studies have reported the use of biochar in CW media for removing suspended solids, biological oxygen demand (BOD) and coliforms from wastewater. Thus, this paper reports findings of the effectiveness of sand media amended with biochar and two plant species (*Melaleuca quinquenervia* and *Cymbopogon citratus*) in removing BOD, suspended solids (total and volatile) and coliforms (total and faecal).

METHODS

Experimental design

Mesocosm scale experiments were conducted at the Loganholme Water Pollution Control Centre, 40 km south of the Brisbane central business district in South East Queensland. The type of CWE system used for this research was vertical subsurface flow (VF). The experimental VF mesocosms used were 240-l plastic containers commonly known as wheelie bins. The dimensions of the wheelie bins are 0.5 m × 0.5 m × 0.98 m. In October 2013, the mesocosms were filled with media to a depth of approximately 65 cm, consisting of a 10 cm layer of gravel (with a diameter of 10–20 mm), which was placed at the bottom in each VF mesocosm, followed by a 55 cm layer of media. In order to ensure that the sand media did not mix with the bottom gravel layer, a plastic filter (1 mm) was placed between the gravel and sand

media (Figure 1). The bottom of each mesocosm was fitted with a drainage port and tap connected with a hose to a height of approximately 5 cm below the height of the media to regulate the retention time. The hose, subsequently, was connected to the 135-l PVC collection chamber to collect the outflow (Figure 1).

There were two sequences types of wastewater used in this experiment: secondary clarified wastewater and septage. During the first 8 months (February–October 2014), the mesocosms were loaded continuously with secondary clarified effluent. In October 2014, septage effluent was loaded intermittently every 2 days. Both types of effluent were obtained from Loganholme Water Pollution Control Centre (Logan City, Queensland). There were three 5,000-l tanks, and each tank distributed effluent to seven media treatment mesocosms. The seven media treatments were based on the percentage of biochar in the sand media (Table 1), and each treatment had three replicates. The biochar (size 0.1–5 mm) was produced from hardwood using fast pyrolysis processes at 500 °C. In addition, coir peat was added to the media to improve moisture retention capacity. The sand, biochar and coir peat were mixed in a cement mixer.

In November 2013, each mesocosm was planted with one melaleuca tree (*Melaleuca quinquenervia*) and one bamboo shoot. Unfortunately, the bamboo plants did not grow well and died off. Therefore, in March 2014, the bamboo plants were replaced with lemongrass (*Cymbopogon citratus*).

The effluent wastewater was distributed to the VF mesocosms using a pump. A 25-mm filter was connected between the tank and pump to prevent solids entering the mesocosms. The pump was a Riva-Flo TF30 pressure pump with a 10-pressure control system (Onga Pty Ltd, Brisbane, Australia). The pump was set up for running 6 hours/day using a timer. The rate of inflow was maintained at approximately 16 l/day or 2.67 l/hour over 6 hours. To control the

Figure 1 | Diagram of mesocosm set-up.

Table 1 | Percentage and characteristics of media in the mesocosm system

Media	Percentage of media (%)			Porosity (%)	% OM	CEC (meq/100 g)
	Sand	Biochar	CP			
S100	100	–	–	41.81 ± 0.13	0.36 ± 0.02	5.73 ± 0.54
SCP	88	–	12	41.38 ± 0.36	0.63 ± 0.08	5.90 ± 0.26
BC5	83	5	12	41.11 ± 1.23	1.28 ± 0.07	6.85 ± 0.54
BC10	78	10	12	39.19 ± 1.22	2.21 ± 0.08	7.55 ± 0.30
BC15	73	15	12	38.07 ± 0.34	3.37 ± 0.11	8.37 ± 0.27
BC20	68	20	12	37.44 ± 0.89	4.52 ± 0.05	9.23 ± 0.35
BC25	63	25	12	37.30 ± 1.27	5.55 ± 0.21	10.21 ± 0.13

OM = Organic matter; CEC = Cation exchange capacity; CP = Coir peat.

inflow rate, adjustable drip irrigations with a maximum flow rate of 4 l/hour were used.

Sample collection and analysis

Wastewater samples (inflow and outflow) were collected every 2 weeks for the first 4 months (March–June 2014) and then monthly from August 2014–January 2015. The parameters analysed were BOD₅, total suspended solids (TSS), total volatile solids (TVS), total coliforms (TC) and faecal coliforms (FC). Measurement of TC was carried out for samples from secondary clarified wastewater, meanwhile FC was used to measure the samples from the septage. The analyses were carried out according to *Standard Methods* (American Public Health Association, American Water Works Association, Water Environment Federation 2005). The TSS measurements were conducted based on the procedure number 2540 D (gravimetric, dried at 105 °C) and the TVS tests followed procedure number 2540 E (gravimetric, ignited at 550 °C). The BOD₅ tests (dissolved oxygen (DO) measured using a DO meter YSI 5000, YSI, Yellow Springs, OH, USA) were carried out following procedure number 5210 B. The TC and FC were determined using the 3 M Petrifilm coliform count plate method (3 M Microbiology Products, St Paul, MN, USA), incubated at 37 °C and 44 °C, respectively.

Statistical data analysis

Characterisation of each VF-mesocosm performance was carried out by calculation of the influent and effluent concentration of each parameter for each individual constituent at sampling events. Percentage of removal efficiency (%) was calculated as $\frac{C_{in} - C_{ef}}{C_{in}} \times 100\%$ where C_{in} and C_{ef} are the influent and effluent concentration of each parameter

measured. The parameters were analysed to determine mean and standard deviation. One-way analysis of variance (ANOVA) analyses were applied to compare the mean concentration of water quality parameters among the seven VF-mesocosm treatments. The *post-hoc* tests (Tukey's honest significant difference test) were carried out to determine whether there are significance differences of mean concentration among the treatments. In all cases, the level of significance was $\alpha = 0.05$.

RESULTS AND DISCUSSION

General treatment performance

The performances of the seven mesocosm treatments are presented in Figures 2–5. The concentrations of BOD₅, TSS, TVS and TC in the influents during the sampling period varied and the septage influents were significantly higher than the secondary clarified wastewater ($\alpha < 0.05$). From February to October 2014, secondary clarified wastewater was used. The highest concentrations of BOD₅, TSS and TVS in the influent were 247 mg/L, 51.5 mg/L and 43.5 mg/L, respectively (6 June 2014), while the lowest concentrations were 120 mg/L, 26 mg/L and 18.5 mg/L, respectively (3 May 2014). From mid-October 2014 to January 2015, septage was used. The influent concentrations of BOD₅, TSS and TVS ranged from 383 to 488 mg/L, 240 to 304 mg/L, and 116 to 174 mg/L, respectively.

The effluent concentrations of BOD₅, TSS and TVS from seven treatments using secondary clarified wastewater showed significant reductions ($\alpha < 0.05$), ranging from 9.3 to 28.4 mg/L, 5.7 to 16.2 mg/L, and 3.5 to 11.4 mg/L, respectively. The outflow concentration of BOD₅, TSS and TVS after application with septage were in the range of

Figure 2 | BOD₅ (mg/L) concentrations in the VF mesocosms with seven media treatments. SCW = Secondary clarified wastewater; CL = Continuous loading; STG = Septage; IL = Intermittent loading.

Figure 3 | TSS concentrations (mg/L) in VF mesocosms with seven different media.

35.2–67.0 mg/L, 46.7–109.0 mg/L, and 20.7–49.7 mg/L, respectively. For the performance of coliform in the seven treatments, the percentage of removal ranged from 88 to 98% for TC and 78–99% for FC.

BOD₅

Figure 2 shows BOD₅ removal in the VF mesocosms with seven media treatments: secondary clarified wastewater (February–October 2014) and septage (November 2014–January 2015). Inflow concentrations varied between 120 and 247 mg/L for secondary clarified wastewater and from 418

to 488 mg/L for septage. The septage had double inflow BOD₅ concentrations, indicating higher concentrations of organic matter.

BOD₅: secondary effluent (March–October 2014)

BOD₅ removal efficiencies in the seven types of VF mesocosms ranged from 87 to 93%, with BOD₅ in the outflow measuring between 9.3 and 28.4 mg/L, as shown in Figure 2. Statistical analysis of outflow concentrations revealed that the mean values of the seven types of media using secondary clarified wastewater (February–October 2014) were not

Figure 4 | TVS concentrations (mg/L) in VF mesocosms with seven different media.

Figure 5 | Mean log population of TCs (CFU/100 mL; April–October 2014) and FCs (November 2014–January 2015) in mesocosm media treatments.

statistically different. The results show that the mean outflows of BOD₅ on seven treatments from secondary clarified wastewater were lower than domestic wastewater discharge to surface water guidelines, according to Indonesian Ministry of Environment Decree No. 5/2014 (2014).

BOD₅: septage (November 2014–January 2015)

The outflow of BOD₅ from septage ranged from 35 to 37 mg/L. BOD₅ removal efficiencies were in the range of 86–91%. Although the outflow BOD₅ concentrations were relatively higher compared to the outflow from secondary clarified effluents, the mean concentrations of BOD₅ outflow loaded with septage met the admissible standard for

discharge in surface water, according to Indonesian Ministry of Environment Decree No. 5/2014 (2014). One-way ANOVA testing shows that there were significant differences of outflow BOD₅ concentrations among the seven types of media when loaded with septage. This indicated that the addition of 10, 15, 20 and 25% of biochar into the sand media significantly reduced the outflow BOD₅ concentrations. Table 2 shows the matrix of significant differences among the treatments.

The BOD₅ removal efficiencies obtained in this study are similar to those reported by Abou-Elela *et al.* (2013), who reported 92.9% and 93.6% removal by using VF constructed wetlands with a depth of 0.85 m planted with *Canna* and *Phragmites australis*. Neralla *et al.* (2000) also

Table 2 | Significant differences of BOD₅ among the treatments ($\alpha < 0.05$)

	S100	SCP	BC5	BC10	BC15	BC20	BC25
S100	–	–	–	x	x	x	x
SCP	–	–	–	x	x	x	x
BC5	–	–	–	–	–	–	x

x: significant difference ($\alpha < 0.05$).

–: no significant difference.

reported an 80–90% reduction in BOD₅ over a 2-day retention time using VF planted with ornamental plants. The process of BOD₅ removal is influenced by the combination of physical and microbial mechanisms (Trang *et al.* 2010). In this process, oxygen plays a crucial role in removing organic matter via aerobic degradation. In VF systems, oxygen can be supplied from atmospheric diffusion, convection, and the plant rhizosphere (Saeed & Sun 2012). A significant reduction of BOD₅ in outflow when loaded with septage could be caused by the presence of oxygen in the media as the septage was loaded intermittently.

A high removal efficiency of BOD₅ can be attained at a low hydraulic loading rate (HLR) (Bolton & Greenway 1999). In our research, the HLR applied was low (0.064 m/day), allowing enough contact time between wastewater and media. The addition of biochar in the sand media may have assisted in decreasing the outflow BOD₅ concentrations when the septage was loaded into the mesocosms. This condition was supported by the presence of oxygen in the media. Therefore, the macropores of biochar might have provided a suitable place for microorganisms and root hairs to grow. The presence of plants is an important factor in organic matter removal. CWEs provide better performance when macrophytes are established (Li *et al.* 2008). However, plant growth appeared to be the same in all media.

TSS and TVS

The mean concentrations of TSS and TVS in the influent can be distinguished into secondary clarified wastewater (February–October 2014) and septage (November 2014–January 2015). For secondary clarified wastewater, the inflow of TSS mean concentrations were in the range of 26–52 mg/L (Figure 3). The inflow concentrations of TSS loaded with septage were five times higher than the secondary clarified wastewater, ranging from 240 to 331 mg/L.

Figure 4 shows that the concentrations of TVS in the influent loaded with secondary clarified wastewater ranged from 16.5 to 43.5 mg/L. For septage, the inflow

concentrations were in the range of 116–183 mg/L. Compared to secondary clarified wastewater, the septage had approximately four times higher TVS concentrations.

TSS and TVS: secondary effluent (March–October 2014)

The mean outflow of TSS from secondary effluent ranged from 5.7 to 16.2 mg/L. The percentage of TSS removal varied considerably, ranging from 56.9 to 88.9%. This result was in the range obtained by Karathanasis *et al.* (2003) with the removal efficiency of TSS ranging from 46 to 90%. Figure 3 shows that the mean inflow and outflow of TSS in seven different treatments from secondary clarified wastewater were still lower than domestic wastewater discharge to surface water guidelines according to Indonesian Ministry of Environment Decree No. 5/2014 (2014). There were no statistically significant differences of outflow TSS concentration among the treatments using secondary clarified wastewater.

The concentrations of TVS in the effluents from seven media treatments ranged from 3.5 to 11.4 mg/L. The removal efficiency of TVS ranged from 45.5 to 90.1%. Statistical analysis showed that the concentrations of TVS outflow among seven treatments were not significantly different.

TSS and TVS: septage (November 2014–January 2015)

The mean outflows of TSS and TVS loaded with septage were in the range of 46.7–109.0 mg/L and 20.7–49.7 mg/L, respectively. The removal efficiency of TSS ranged from 61 to 83%, while for TVS, it ranged from 58 to 84%. Figures 3 and 4 also show that the outflow concentrations of TSS and TVS from the septage were mostly still higher than the inflow concentration of secondary clarified wastewater. The outflow TSS of sand media (S100) loaded with septage was slightly higher than domestic wastewater discharge to surface water guidelines according to Indonesian Ministry of Environment Decree No. 5/2014 (2014) (Figure 3).

One-way ANOVA testing shows that significant differences of TSS and TVS outflow concentrations among treatments occurred when media were loaded with septage. This indicates that biochar added to the media significantly reduced TSS and TVS removal when the septage was loaded. The differences of TSS occurred between sand media (S100 and SCP) and media with a 15, 20 and 25% addition of biochar; for TVS outflows, the significant differences took place between sand media (S100 and SCP) and the media amended with biochar 25% (BC25). Table 3 illustrates the matrix of significant differences of TSS and TVS among the treatments.

Table 3 | Significant differences of TSS and TVS among the treatments ($\alpha < 0.05$)

	S100	SCP	BC5	BC10	BC15	BC20	BC25
S100	–	–	–	–	x	x	x +
SCP	–	–	–	–	–	x	x +

x: significant difference of TSS ($\alpha < 0.05$).+: significant difference of TVS ($\alpha < 0.05$).

–: no significant differences.

Suspended solids in CWEs are primarily removed by physical processes: filtration, sedimentation and interception (Kadlec & Wallace 2009; Abou-Elela *et al.* 2013). There are many factors influencing the process of TSS removal in CWE systems. Surface area and the presence of plants are important factors (Kadlec & Wallace 2009). In our research, the addition of biochar to CWE media was expected to increase the surface area of the media, leading to increased effectiveness of TSS removal processes. However, there were no significant differences in TSS removal among the seven media treatments using secondary clarified wastewater. Significant differences occurred when the media were loaded with septage. The addition of biochar provided a better performance in removing TSS and TVS when septage effluents were used. There was high positive correlation between TSS and TVS ($r^2 = 0.966$), indicating that most suspended materials were organic matter. This finding is in agreement with the results reported by Neralla *et al.* (2000).

Coliforms

Figure 5 shows coliform populations (log units) in the VF mesocosms with seven media treatments: TC loaded with secondary clarified wastewater (April–October 2014) and FC loaded with septage (November 2014–January 2015). Mean of TC counts from the inflow in the secondary clarified wastewater (April–October 2014) ranged between 4.54 and 4.66 log units, while the mean of FC counts from the inflow loaded with septage ranged from 4.30 to 4.44 log units.

Total coliforms: secondary effluent (March–October 2014)

The mean of TC counts from the effluents ranged from 2.94 to 3.70 log units. Compared to TCs in the influent, the TCs in the effluent were reduced significantly. Based on the one-way ANOVA analysis on each sampling period, there was no significant difference for April 2 and May 2014. However, there were significant differences of TC outflow for September and October 2014 with 15, 20 and 25% biochar being significantly

higher. Figure 5 shows that the mean of TC counts loaded with secondary clarified wastewater met the admissible standard for water quality guidelines with the provision for irrigation, cultivation of fresh-water fish and livestock, according to Government of Indonesia Regulation No. 82/2001.

Total coliforms: septage (March–October 2014)

The mean of FCs loaded with septage from the outflow ranged from 1.53 to 3.69 log units. Figure 5 shows that only sand media amended with 15, 20 and 25% of biochar met the admissible standard for water quality guidelines with the provision for irrigation, cultivation of fresh-water fish and livestock according to Government of Indonesia Regulation No. 82/2001. Statistical analysis reveals that the FC counts among the treatments reduced significantly, indicating that the addition of biochar (5, 10, 15, 20 and 25%) in the sand media influenced the performance of treatments in reducing FCs. Table 4 shows the matrix of significant differences of FCs among treatments.

TC and FC were removed in the CW system primarily through filtering in the porous media (Gikas & Tsihrintzis 2012). In this study, the addition of biochar to sand media in the first 6 months did not influence the removal of TCs. However, after 6 months, there were significant differences in TC reductions among the treatments. The significant reduction also occurred in FC counts loaded with septage. In VF systems, the reduction of TCs can be improved by the following condition: (1) longer hydraulic retention time, (2) finer bed materials, (3) warmer water temperature and (4) shallower bed depth (Kadlec & Wallace 2009). The presence and maturity of plants in CW systems also provide an advantage in coliform reduction due to the effects on system hydraulics or the existence of antibiotic exudates in the rhizosphere (Vacca *et al.* 2005).

CONCLUSION

In this study, the performance of VF-CWEs on the seven media treatments has been evaluated based on the

Table 4 | Significant differences of FCs among the treatments ($\alpha < 0.05$)

	S100	SCP	BC5	BC10	BC15	BC20	BC25
S100	–	–	x	x	x	x	x
SCP	–	–	x	x	x	x	x
BC5	–	–	–	–	x	x	x

x: significant difference ($\alpha < 0.05$).

–: no significant differences.

percentage of biochar. The results revealed a successful performance of seven types of VF mesocosms in reducing BOD₅, TSS, TVS and TCs at a 4-day hydraulic retention time. The removal efficiencies of BOD₅, TSS and TVS loaded with secondary clarified wastewater were 87–93%, 57–89% and 46–90%, respectively. In addition, the removal efficiency of BOD₅, TSS and TSS loaded with septage ranged from 86 to 91%, 61 to 89% and 58 to 84%, respectively. TCs were reduced significantly from 0.94 to 1.70 log units, while FCs were reduced from 0.66 to 2.91 log units.

During the first 8 months of this study, secondary clarified wastewater was used. Although, a trend has been observed suggesting that higher biochar content led to better performance, there were no statistically significant differences between media treatments in the outflow concentrations of BOD₅, TSS and TVS. Nevertheless, when raw septage was loaded, the trend observed earlier became more prominent and resulted in statistically significant improvement in performance, with biochar additions performing better than pure sand. Furthermore, the results suggest that biochar benefits are more prominent at higher BOD₅, TSS and TVS loads into the media, making the extra cost incurred from the addition of biochar economically justifiable. Media amended with biochar showed significantly better total and FCs removal performance after system maturation.

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