CAPACITY OF VETIVER GRASS IN TREATMENT OF A MIXTURE OF LABORATORY AND DOMESTIC WASTEWATERS

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ABSTRACT

In this study, laboratory wastewater containing organic matters, heavy metals and aromatic compounds, was treated by vetiver grass (*Vetiveria zizanioides*) as a phytoremediation method to remove the above three groups of pollutants. Sewage effluent, as a source of nutrient supply for plant growth, was firstly fed to two wetland systems: mini horizontal subsurface flow (HSSF) and floating raft (FR) wetlands. Next, laboratory wastewater was added gradually to mix with sewage. Nominal hydraulic retention time in both wetlands are 12 hours. Pollutants removal efficiencies were monitored. Microbial community change corresponding with each stages of sewage only and mixture with laboratory wastewater was also examined. The examined microbial community includes *Nitrogen-fixing* (*N-fixing*) bacteria, *Phosphate-solubilizing* (*P-solubilizing*) microorganism, *Pseudomonas* sp., and *Zoogloea* sp.

In HSSF wetland, base materials (gravel and sand), algae, and vetiver root were in turn investigated for pollutant removal efficiencies. The results reveal that even with the presences of heavy metals and aromatic compounds, vetiver presented reasonable removal efficiencies of about 62%, 68.6%, and 58.3% for BOD, TN, and TP removal, respectively. Base materials showed almost no effect on pollutant removal. Algae was slightly responsible for approximate 6.3%, 16.6%, and 19.7% of BOD, TN, and TP removals, respectively. On the other hand vetiver roots, in term of heavy metals, had an impressive removal efficiencies of 99.2, 95.8, 96.2, and 96.7% of Cr^{+6} (in $K_2Cr_2O_7$), Mn ($MnSO_4$), Fe ($FeSO_4$), and Cu ($CuSO_4$), respectively. For aromatic compounds, the wetland is responsible for 96.8 and almost 100% of correspondingly phenol and benzene removal efficiencies. For microbial aspect, *N-fixing* microorganisms (e.g. *Azospirillum* sp., *Azotobacter* sp.) and *Phosphate-solubilizing* bacteria (*Bacillus* sp.) increased gradually in population during domestic wastewater feeding stage. When laboratory wastewater was added, *N-fixing* and *P-solubilizing* bacteria were quantitatively decreased slightly while population of *Pseudomonas* sp. increased. Besides, *Zoogloea* sp. was also found increasing through out the experiment and keeping a stable growth even during laboratory wastewater adding.

In FR wetland, both algae and vetiver root were also investigated for BOD and aromatic compounds and heavy metals. The outcomes show similar tendencies in treatment and microbial behaviours as in HSSF wetland. Vetiver grass, mainly responsible for organic matters and nutrients removal, presented slightly lower removal efficiencies than those in HSSF wetland. The average values of removal efficiencies are 59%, 63.5%, and 53.0% for BOD, TN, and TP removal, respectively. Algae, also, took minor responsibility for approximate 3.3%, 9.1%, and 8.9% of BOD, TN, and TP removals, respectively. Heavy metals of Cr^{+6} (in $K_2Cr_2O_7$), Mn ($MnSO_4$), Fe ($FeSO_4$), and Cu ($CuSO_4$) were found removing less than in HSSF wetland with average removal efficiencies values of 92.4, 85.1, 91.8, and 91.5%, respectively, by vetiver root. Algae show almost no effect on heavy metals and aromatic removals. The vetiver root likewise plays important role in phenol and benzene removals with values of 91.5 and 96% in efficiency, respectively. *N-fixing* and *P-solubilizing* microorganisms, *Pseudomonas* sp., and *Zoogloea* sp. presented similar responses tendencies to different living condition when domestic and laboratory wastewaters, in turn, were fed.

Keywords: Vetiver; wastewater; hydraulic retention time; phytoremediation; laboratory; microorganism.

1. INTRODUCTION

Among the various wastewater treatment processes, the constructed wetland (CW) could be considered as one of the most "green technology" thanks to its environmental amity, minimised energy consumption, and useful/harmless sub-products [1, 2]. CWs have been conventionally used to treat not only municipal wastewaters since the 1950s [3] but also industrial and agricultural wastewaters, landfill leachate and stormwater runoff [4, 5]. CWs may be classified into two types according to design parameters: horizontal sub-surface flow (HSSF) and floating raft (FR). A comparison in performance between these two types of CWs has still not been summarized.

A number of plant have been applied for CWs. For wastewaters containing multi-contaminants, the phytoremediation requires different types of plants [6]. According to the report from [7], vetiver grass (*Vetiveria zizanioides*) shows effective treatment of contaminants (e.g. organic matters, nutrients, heavy metals and aromatic compounds), high tolerance to adverse climatic conditions (cold, hot, flood, water shortage, etc.) and low costs of investment and maintenance. For these outstanding characteristics, vetiver grass is a great option for CW.

Results from numerous studies on CWs show great performance in term of domestic wastewater treatment. Different hydraulic retention times (HRT) were applied under various of wastewater strengths [8]. Considerable BOD removal efficiencies of 90.5-91.5% have been reported. Outcomes from the project of treating sewerage effluent at Toogoolawah in South East Queensland, Australia reveals that vetiver wetland could remove 94.6-96.3% of BOD^5 [9]. Vetiver grass presents great performance in removing not only organic compounds but also nutrients. Removals of 86.3-92.8% of total nitrogen (TN) and 83.5-86.3% of total phosphorus (TP) by vetiver wetland have been also presented in the report of [9].

Successful treatments of heavy metals and aromatic compounds have been also reported. A review by [6] affirms that vetiver can tolerate a wide range of heavy metals by accumulating them most in roots. High harvest of vetiver grass has been obtained with residual tailing containing heavy metals from Pb/Zn and Cu mine [10]. A growth of vetiver during an uptake of Cd was also recorded [11]. Capacity of tolerating aromatic compounds of vetiver was examined in a number of studies, e.g. 2,4,6-trinitrotoluene [12], phenol [13], polycyclic aromatic hydrocarbon [14].

According to an investigation of the authors, no wastewater treatment has been built for laboratories in Danang City so far. Treatment of this type of wastewater now becomes imperative for the City. For these reasons, this study aims to assess the removing BOD, nutrients, heavy metal and aromatic compounds from laboratory wastewater treatment by vetiver CW. Additionally, microorganism community was investigated along with the contaminants treatment performance. In order to supply vetiver grass with necessary nutrients, domestic wastewater was added to laboratory effluent feeding to the CW.

2. MATERIALS AND METHODS

2.1. Experimental set up

Two CWs were made by cement in parallel at Danang College of Technology (DCT, The University of Danang, Vietnam): one is a type of HSSF and another is FR. In HSSF type, there were two layers of base materials: gravel of 0.3 m in depth at the bottom and sand of 0.2 m in depth at the top. Firstly, small clumps of vetiver were planted 0.2 m apart. The feed and the outlet were directed to and from the bottom, respectively. In FR type, vetiver were set in holding cups on floating so that their roots sank totally in water (Figure 1b). Small clumps of vetiver were planted in cups 0.2 m apart. Hydraulic retention time (HRT) in both CWs were controlled as long as 12 hours.

Domestic wastewater (DW) was firstly diluted with tap water with the ratio of 1:1 and fed to CWs for 8 weeks. Then laboratory wastewater (LW) was added with the ratio of DW:LW = 1:1. The qualities of DW, LW and the mixture of these two type of wastewaters are displayed in Table 1.

Table 1. Quality analysis of DW and LW.

Parameter	Dimension	Value		
		DW	LW	Mixture
pH	-	6.2	5.5	6.0 ± 0.2
BOD	$mg.L^{-1}$	420	15	220 ± 12
TN	$mg.L^{-1}$	65	34	55 ± 3
TP	$mg.L^{-1}$	10	12	11 ± 2
Cr^{+6}	$mg.L^{-1}$	ND	9.5	4.5 ± 0.4
Fe^{2+}	$mg.L^{-1}$	ND	38.5	19.8 ± 0.3
Mn^{2+}	$mg.L^{-1}$	ND	47.0	24.2 ± 0.6
Cu^{2+}	$mg.L^{-1}$	ND	35.1	17.6 ± 0.7
Benzene	$mg.L^{-1}$	ND	4.3	2.3 ± 0.4
Phenol	$mg.L^{-1}$	ND	7.8	3.8 ± 0.2

(ND: Not Detected)

2.2. Analyses

Removal efficiencies of BOD, nutrients (TN and TP), heavy metals and aromatic compounds were examined. Additionally, microbiological community's behaviour was also assessed along with the removal performance. The removal performance is possibly attributed to vetiver roots, microalgae and base materials (sand and gravel). At the beginning, only removal performance of base materials was assessed without vetiver planted. During the operations of CWs, a separate CW of 5 litre was modelled according to the HSSF wetland and also fed with DW and LW at the same condition as that applied for HSSF wetland. The analyses of BOD, TN, TP, heavy metals and aromatic compounds were carried out according to [15]. The qualification and quantification of microbial communities in CWs were carried out by standard plate count method that the protocols can be found in [16].

Each analysis was triplicated to obtain standard deviations. The data, which are used in bellow Figures, are average values.

3. RESULTS AND DISCUSSION

3.1. Removal performance

The whole experiment could be divided to two stages: (1) only DW and (2) additional LW feedings. Removal performances of contaminants were attributed to three components: base material (in HSSF configuration), microalgae and vetiver roots. The removal efficiency of CW is assessed by total removal efficiencies of the components.

3.1.1. Removals of BOD and nutrients of vetiver

For HSSF configuration, the BOD removal efficiency developed continuously from 12.7 to 33.6% over Stage 1 (Figure 1a). At week 9, when LW started being fed, the removal performance decreased down to 26.8%. Nonetheless, the removal increased afterwards and reached the values of 59.2-62.0% in the weeks of 12-15. P removal performance shows similar tendencies as that of BOD. In stage 1, removal efficiencies of N and P increased from 1.5 to 41.4% and 3.0 to 45.0 %, respectively. Due to heavy metals and aromatic compounds, the performance of N removal was slowed down at 54.0-54.5% for two weeks. The P treatment efficiencies were slightly declined down to 39.1% at week 10 and recovered up to 68.6% at week 15. Base materials (gravel and sand) almost showed no effect on BOD and nutrient treatments. Algae was slightly responsible for approximate removal efficiencies of 6.3%, 16.6%, and 19.7% for BOD, TN, and TP, respectively, when the system obtained steady state.

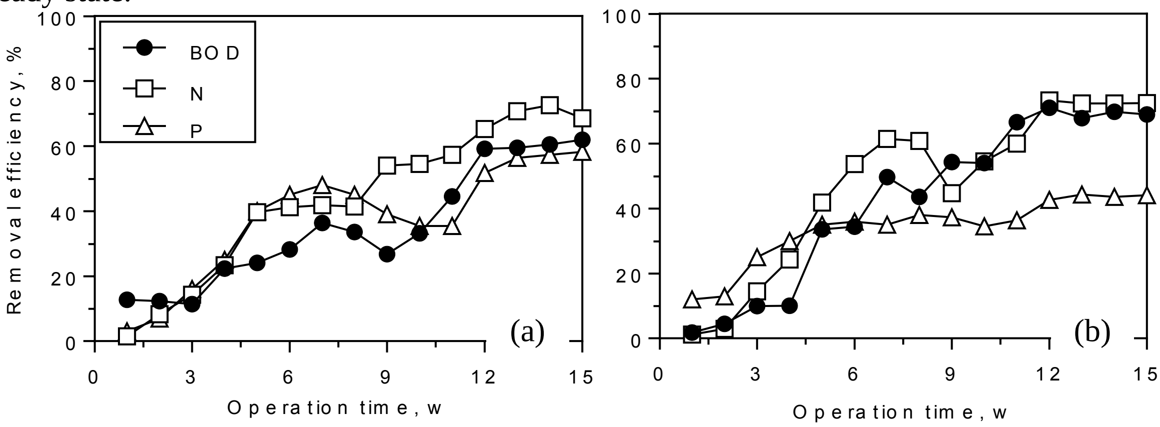


Figure 1. Removal efficiencies of BOD, N and P by vetiver in (a) HSSF and (b) FR wetlands.

For FR CW, the BOD removal efficiency increased in range of 1.8-43.6% in Stage 1 (Figure 1b). When LW was fed at week 8, the removal performance kept consistent at around 54.0% for two weeks and then continue rising up to about 70% from week 12. N and P removal efficiencies varied similarly as BOD with values in the range of 1.2-42.3% and 12.0-38.0%, respectively, in Stage 1. In Stage 2, N removal remained increasing, while P removal was reduced down to 35.5% with LW addition. Then, N and P removals efficiencies rose up again to 63.5 and 44.1% at the end of Stage 2, respectively. Algae, also, took minor responsibility for approximate removal efficiencies of 3.3%, 9.1%, and 8.9% of BOD, TN, and TP, respectively, when the system obtained steady state.

In 8 weeks with DW only, BOD removals efficiencies of both CWs developed over time. The LW, with heavy metals and aromatic compounds, put some impacts on organics treatment performance. Nevertheless, the vetiver roots recovered quickly its treatment capacity after approximately 2 weeks. Similar tendencies of P treatments in HSSF and N treatment in FR CWs have been observed. The additions of heavy metals and aromatic compounds just affected slightly vetiver grass on N removal in HSSF and P removal in FR. It is interesting that when the LW was fed and the operations of CWs obtained steady state, BOD and nutrient removal performances were improved, compared to those as CWs were fed with only DW.

3.1.2. Removals of heavy metals and aromatic compounds of vetiver

In HSSF wetland, removal efficiencies of heavy metals increased quickly, following the shape of standard-growth-curve-like. The efficiency values are in the ranges of 8.9-99.2%, 14.9-96.7%, 18.2-96.7%, and 11.9-97.3% for Cr, Mn, Fe, and Cu, respectively (Figure 2a). Base materials (gravel and sand) almost showed no effect on heavy metals and aromatic compounds treatments. In FR wetland, the data of heavy metal treatments present efficiency slightly lower than those in HSSF wetland with ranges values of 2.2-92.4%, 6.6-86.4%, 8.6-92.3, and 5.7-92.0% for Cr, Mn, Fe, and Cu, respectively (Figure 2b).

For aromatic compounds, the HSSF wetland was responsible for 96.8 and almost 100% of phenol and benzene removed, respectively. Whilst, values of removal efficiencies of 91.5 and 96.0% for phenol and benzene, respectively, were recorded.

The removal performances took place over three phases: lag, exponential, and stationary, in turn corresponding to acclimatization of vetiver grass in absorbing the metals, development of absorption capacity, and saturation in heavy metals absorption efficiency. It took long duration of 5 weeks for CWs to obtain steady state. HSSF presented a slightly higher heavy metals removal efficiency than FR wetlands.

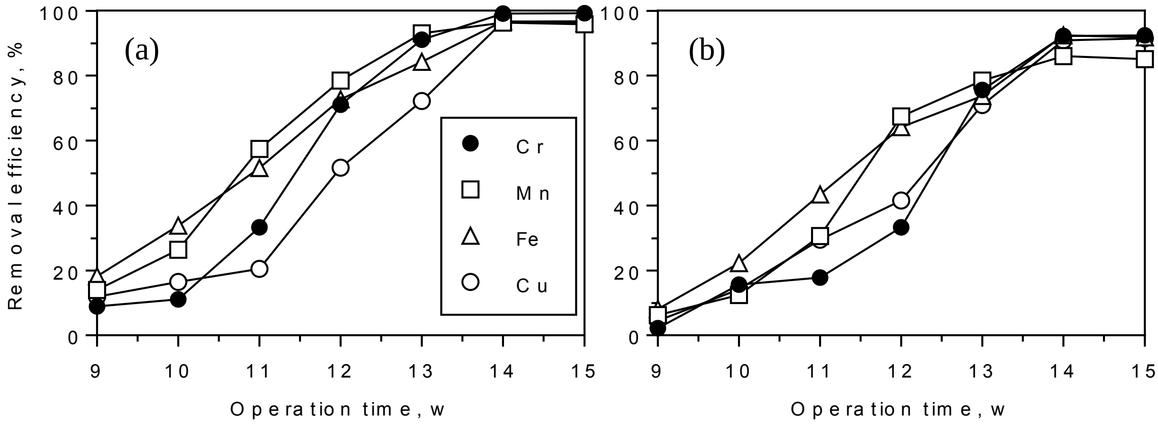


Figure 2. Removal efficiencies of (a) HSSF and (b) FR wetlands in heavy metals.

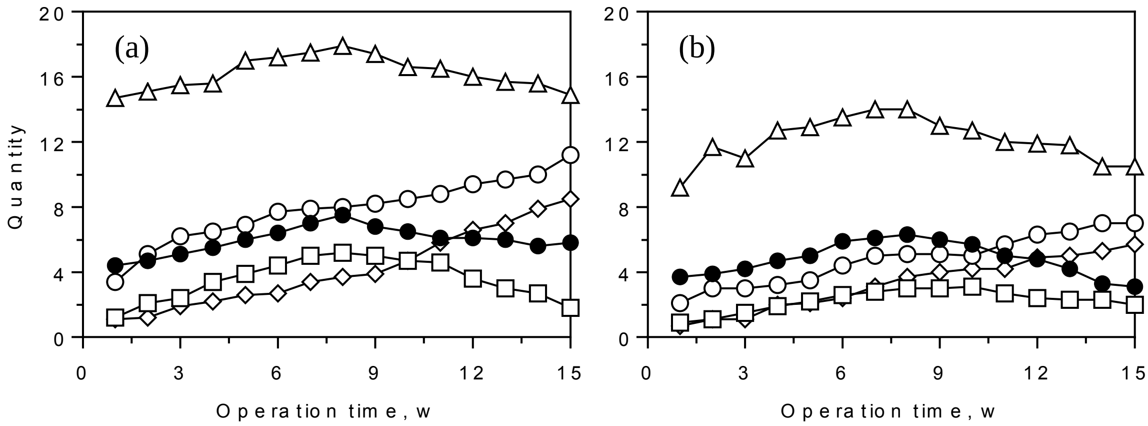
Heavy metals treatment by vetiver has been reported in numerous studies [6, 10]. Positive effect of heavy metals have been reported and can be explained by several ways in [11] that metal ions may serve as activators of enzyme(s) or change the plant hormones leading to grass growth and development. A number of studies on removing aromatic compounds of vetiver have been carried out [12, 13, 17].

3.2. Microbial behaviours in wetland body

N-fixing microorganisms show increase and decrease trends in population before and after adding LW. Quantity of *P-solubilizing* microorganisms stayed almost consistent while those of other microbial groups grew up throughout the experiment, in both HSSF and FR CWs (Figure 3).

The growths of *Azospirillum*, *Azotobacter* and *Bacillus* species were affected by components of LW. With the presences of these matters, population of these three species were declined. Nonetheless, the contaminants in LW have no effect on the growths of *Pseudomonas* and *Zoogloea* species.

Ability in absorbing aromatic compounds of *Pseudomonas* and *Zoogloea* species has been reported in a number of studies [18]. The continuous growths of *Pseudomonas* and *Zoogloea* species led to high treatment performance of aromatic compounds.



● *Azospirillum* sp. (x 10^4 CFU/100 mL); □ *Azotobacter* sp. (x 10^4 CFU/100 mL);
Δ *Bacillus* sp. (x 10^4 CFU/100 mL); ○ *Pseudomonas* sp. (x 10^8 CFU/100 mL);
◊ *Zoogloea* sp. (x 10^7 CFU/100 mL).

Figure 3. Behaviours of microbial species in (a) HSSF and (b) FR wetlands under different operation conditions.

4. CONCLUSIONS

Several conclusions are able to withdrawn from the outcomes of this experiment:

- Heavy metals and aromatic compounds just affect slightly on N and P removals of vetiver grass in HSSF and FR wetland accordingly. This effect was significant for P and N removals in HSSF and FR CW, respectively.
- By adding LW (containing heavy metals and aromatic compounds) and the operations of CWs reaching steady state, BOD and nutrient removal performances are improved, compared to the performance as CW is fed with only DW;
- The growths of *Azospirillum*, *Azotobacter* and *Bacillus* species in HSSF and FR CWs are affected by contaminants in LW, which are displayed in reduction of population. Nonetheless, the contaminants in LW have no negative effect on the growths of *Pseudomonas* and *Zoogloea* species;
- HSSF presented a marginally higher removal capacity of heavy metals than FR wetlands;

The results reveal capacity of vetiver grass in treating heavy metals and aromatic compounds of laboratory wastewater in mixture with domestic wastewater. The outcomes also allow to apply a larger scale for treating DW and LW from DCT and other laboratories in Danang city.

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