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Full Length Research Paper

Treatment performance of small-scale vermifilter for domestic wastewater and its relationship to earthworm growth, reproduction and enzymatic activity

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A vermifilter system packed with quartz sands and ceramsite was studied for domestic wastewater treatment. Results showed that good performance of vermifilter was achieved and removal rates were COD (47.3 – 64.7%), BOD_5 (54.78 – 66.36%), SS (57.18 – 77.90%), TN (7.63 – 14.90%), and NH_4-N (21.01 – 62.31%), respectively. An increase in hydraulic loading led to a decrease in treatment efficiency and adult earthworm abundance. In addition, activities of protease, alkaline phosphatase (ALP), and cellulase in earthworm body dropped, but superoxide dismutase (SOD) and catalase (CAT) increased with the hydraulic loading. Correlation analysis implied that larger earthworm (>0.3 g) abundance might play more positive role on wastewater treatment in vermifilter, compared to smaller worm. Earthworm enzymatic activities had significant correlation with treatment efficiency of COD and BOD_5 by vermifilter. Thus an important relationship exists for earthworm population dynamics and enzymatic activities with COD and BOD_5 removal rates of domestic wastewater by vermifilter.

Key words: Vermifilter, wastewater, earthworm, population dynamics, enzymatic activity, treatment efficiency.

INTRODUCTION

Vermifilter (Lumbrifiltration) was first advocated by the late Professor Jose Toha at the University of Chile in 1992 (Bouché and Qiu, 1998; Aguilera, 2003; Li et al., 2008), which was a low-cost sustainable technology over conventional systems with immense potential for decentralization in rural areas (Taylor et al., 2003; Sinha et al., 2008). It was firstly used to process organically polluted water using earthworms (Li et al., 2008). Introduction of earthworm was a considerable innovation to conventional biofilter of wastewater treatment, and it had created a new method of biological reaction through extending food chains, conversing energy and transferring mass from the biofilm to the earthworm.

Vermifilter had been found to be generally good for swine wastewater treatment (Li et al., 2008), municipal wastewater treatment (Godefroid and Yang, 2005; Xing et al., 2005; Yang and Zhao, 2008; Yang et al., 2008), and domestic wastewater treatment (Taylor et al., 2003; Sinha et al., 2008). However, these studies on vermifilter focused on treatment efficiency of wastewater, and few on earthworm population dynamics and enzymatic activity in the vermifiltration wastewater treatment process.

Wastewater had likely led to an influence on earthworm population dynamics and enzymatic activity, because it contained a complex mixture of contaminants, including nutrients, pathogens and toxic compounds (e.g. endocrine disrupting compounds, Hughes et al., 2007). Hughes et al. (2007, 2008, 2009) had investigated the risk of pH, ammonia/ammonium, and sodium accumulation to earthworm in vermifiltration wastewater treatment. Further, the kinetic model of conventional biofilter was based mostly on organic matter degradation of biofilm (Dorado et al., 2008), but vermifilter has

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Abbreviations: CV, Ceramsite vermifilter; **QV,** quartz-sand vermifilter; **COD,** chemical oxygen demand; **BOD_5,** biochemical oxygen demand; **SS,** suspended solid; **TN,** total nitrogen; **CAT,** catalase; **SOD,** superoxide dismutase; **ALP,** alkaline phosphatase.

an important additional decomposition feature involving earthworms. Thus, research on earthworm population dynamics and enzymatic activity would supply vital data for establishing kinetic model of organic matter degradation in vermifiltration wastewater process.

The aims of the present study are: (1) to evaluate treatment efficiency, earthworm population dynamic and enzymatic activity in different hydraulic loadings of vermifiltration wastewater process, and (2) to analyze correlation between treatment efficiency and earthworm characteristics.

MATERIALS AND METHODS

Vermifilter system

A pilot-scale vermifilter was set up for treating domestic wastewater in a wastewater plant of Shanghai city, China, according to our 6-year studying experience. Figure 1 showed the schematic diagram of vermifilter, with the parameters of vermifilter design outlined in Table 1. Ceramsite vermifilter (CV) was included in the quartz-sand vermifilter (QV) (Figure 1). The influent water was distributed by turning spurt water device. A layer of plastic fibrous filler covered the surface of filter bed. The fibrous filler was used for redistribution of wastewater and was an excellent opaque property for earthworm.

Experimental design

The vermifilter began operation February 28th 2006. Initially, the filter system had undergone a biofilm culturing stage of 40 days. The water load was 4.8 $m^3 \cdot m^{-2} \cdot d^{-1}$ in the phase. After 40 days, earthworm (*Eisenia foetida*) was added evenly in the first filter bed with an initial density of ca. 21 000 ind·m^{-2}, and the total earthworm biomass was ca. 30.3 kg in the whole vermifilter. *E. foetida* was chosen because it was widely used in vermifiltration (Taylor et al., 2003) and had been shown to process organic wastes with the greatest efficiency (Edwards and Bater, 1992). After earthworm inoculation period of 20 days, vermifilter system was taken up to the steady operation.

During May 1st to August 31st 2006, four working conditions (W_1, W_2, W_3, and W_4) were used to treat the wastewater from the Quyang wastewater plant in Shanghai, China. Each working condition was operated for 30 - 31days. Hydraulic loading of the four working conditions in $m^3 \cdot m^{-2} \cdot d^{-1}$ was W_1 = 2.4, W_2 = 4.8, W_3 = 6.0, and W_4 = 6.7. The characteristics of influent water in the four working conditions were outlined in Table 2.

Water sampling and analysis

Influent and effluent samples were collected weekly for chemical oxygen demand (COD), five-day carbonaceous biochemical oxygen demand (BOD_5), suspended solid (SS), total nitrogen (TN) and ammonium (NH_4-N) analysis. COD was measured by a COD analyzer (NOVA 60, Merck, Germany). BOD_5 was measured using a WTW oxitop IS 12 BOD analyzer. SS, TN, and NH_4-N were analyzed according to the American Public Health Authority (1995). All samples were analyzed in triplicate and the results were averaged during a working condition.

Earthworm sampling and analysis

Earthworms were sampled monthly from the first filter bed of vermifilter. Four sampling points were set up evenly in the filter, and 100 ml of sampling was taken in every point for analyzing earthworm numbers (adults, hatchlings and cocoons) and clitellated development. Earthworms and cocoons were separated from the samples by hand sorting, after which they were counted, examined for clitellated development and weighed after washing with water and drying them by paper towels (Garg et al., 2005). The worms were weighed without voiding their gut content. Corrections for gut content were not applied to any data in this study. The results from four sampling were averaged.

Meanwhile, earthworm enzymatic activities were analyzed monthly. Protein activity was determined according to the method of Lowry et al. (Bradford, 1976). Alkaline phophatase (ALP) activity was measured as described by Li Sui-yan (Li and Li, 2004). Cellulase activity was assayed according to the method of Zhang Dean (Zhang et al., 1991). Superoxide dismutase (SOD) activity was estimated by the pyorgallol auto-oxidation method (325 nm) (Yu et al., 2005). Catalase (CAT) activity was assayed according to the method of Saint-Denis (Saint-Denis et al., 1998). All the samples were analyzed in triplicate and the results were averaged.

Correlation analysis

The data were analyzed using SPSS 13.0 and Origin 7.5. Correlation analysis was used to find out the relationship between treatment efficiencies and earthworm characteristics.

RESULTS

Treatment efficiency

Figure 2 showed the removal rates of COD, BOD_5, SS, TN, and NH_4-N in the two kinds of vermifilter. With the increase in hydraulic loadings from 2.4 to 6.7 $m^3 \cdot m^{-2} \cdot d^{-1}$, the removal rates of COD (57.55 to 47.26%), BOD_5 (60.89 to 54.78%), SS (77.9 to 62.06%), TN (11.9 to 9.82%) and NH_4-N (62.31 to 21.01%) in quartz sands vermifilter all decreased. Removal efficiency of COD by ceramsite vermifilter were significantly higher than quartz-sand vermifilter (T-Test, P= 0.03 and 0.004). However, other removal efficiency had no significant difference between two kinds of vermifilter (T-Test, P > 0.05).

Earthworm population dynamics

Figure 3 compared earthworm abundance (adults, clitellated individuals, hatchling and cocoons) in two kinds of vermifilter. In quartz-sand vermifilter, with the hydraulic loading, adult and clitellated earthworm abundance decreased, from 16.55×10^3 to 6.8×10^3 individual(ind.) · m^{-2}, and 6.1×10^3 to 1.7×10^3 ind. · m^{-2}, respectively. However, earthworm hatchling and cocoon abundance increased, from 0.33×10^3 to 4.36×10^3 ind. · m^{-2} and 1.2×10^3 to 3.3×10^3·ind. · m^{-2}, respectively. Ceramisite vermifilter had similar variation to quartz-sand vermifilter.

Figure 4 showed the weight distribution of earthworm in two kinds of vermifilter. Proportion of less than 0.2 g earthworm increased gradually with the operation time. The less the earthworm's weight was, the higher the

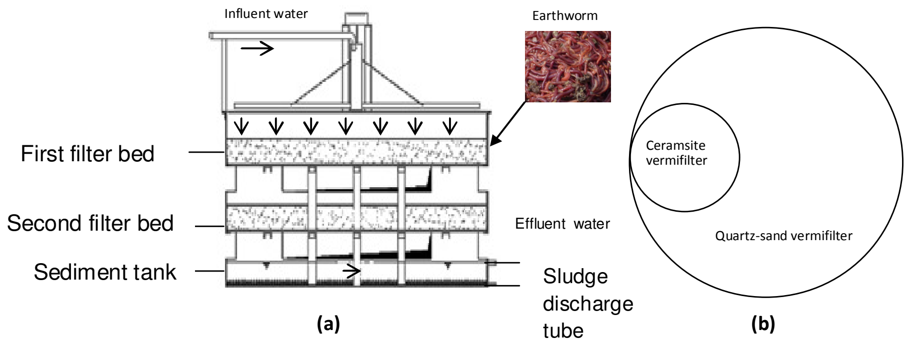

Figure 1. Schematic diagram of vermifilter. a) front view; b) top view (Ceramsite vermifilter was included in quartz-sand one).

Table 1. Parameters of vermifilter design.

Physical property	Vermifilter Ceramsite	Quartz-sand
Filter area (m^2)	2	8.7
Filter total height (m)	1.83	1.83
First filter media	Ceramsite	Quartz sand
First filter media diameter (mm)	3.00-5.00	1.40-2.36
First filter bed height (m)	0.2	0.2
Second filter media	Quartz sand	Quartz sand
Second filter media diameter (mm)	1.40-1.65	1.40-1.65
Second filter bed height (m)	0.1	0.1

Table 2. Characteristics of the influent water for four working conditions of both vermifilters.

Parameter		Working conditions			
		W_1	W_2	W_3	W_4
Temperature (℃)	Range	22.5 - 24.5	25 - 29	27 - 32	29.5 – 34
	Mean	23.50	27.25	29.20	30.25
pH	Range	7.27 - 7.79	7.47 - 7.90	7.45 - 7.97	7.59 - 7.89
	Mean	7.52	7.68	7.70	7.73
COD $mg \cdot L^{-1}$	Range	63.82 - 99.80	48.83 - 103.79	43.65 - 89.84	42.47 - 75.75
	Mean	78.3	69.13	59.41	59
BOD_5 $mg \cdot L^{-1}$	Range	31 - 44	30 - 42	14 - 42	29 – 38
	Mean	38.4	31.6	29.6	34.4
SS $mg \cdot L^{-1}$	Range	20.8 - 47.6	14.6 - 40.5	13.8 - 37.2	14.8 - 45.4
	Mean	37.44	26.58	24.46	26.08
TN $mg \cdot L^{-1}$	Range	24.87 - 31	24.66 - 27.85	18.98 - 31.8	16.45 - 28.49
	Mean	28.53	26.31	26.03	24.63
NH_4-N $mg \cdot L^{-1}$	Range	5.94 - 27.12	19.62 - 29.16	8.29 - 21.68	14.57 - 21.70
	Mean	18.84	25.37	15.21	17.96

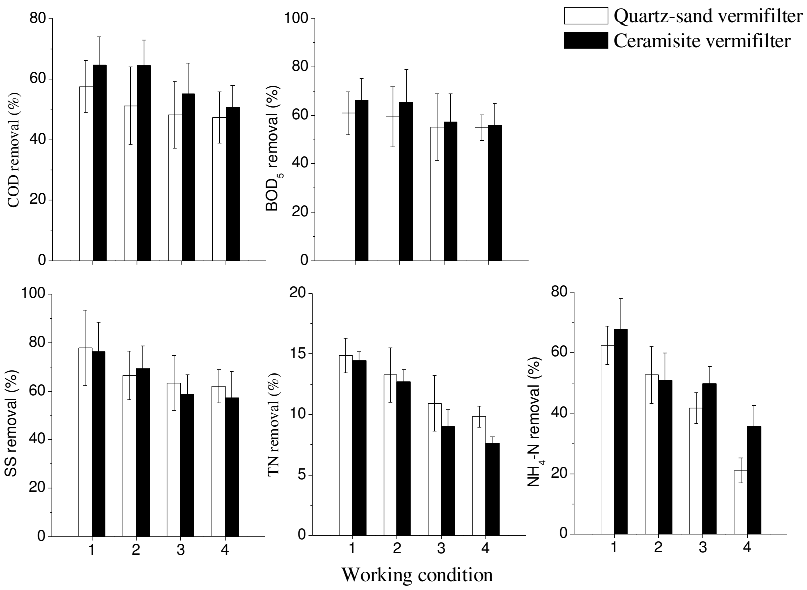

Figure 2. Removal rates of wastewater in four working conditions of both vermifilters. Values are means, bars are S.E., and n = 4.

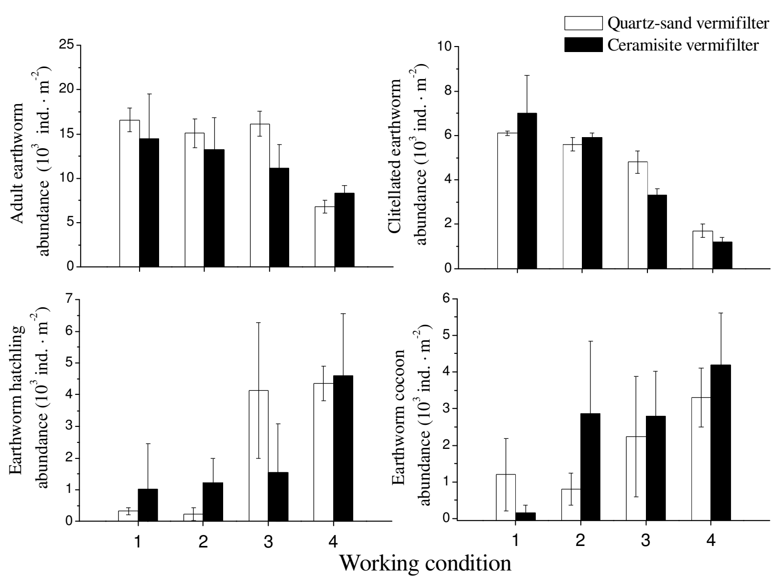

Figure 3. Earthworm abundance in four working conditions of both vermifilters. Values are means, bars are S.E., and *n*=4.

Proportion was. The proportion of less than 0.1 g earthworm increased by 2.64 times, while 0.1 – 0.2 g earthworm proportion rose by only 0.55 times in quartz-sand vermifilter. However, percentage of more than 0.3 g earthworm decreased from 28.35 to 3.21% with the operation time in quartz-sand vermifilter. There was a marked drop in 0.2 – 0.3 g earthworm percentage when the hydraulic loading was more than 6 $m^3 \cdot m^{-2} \cdot d^{-1}$. Ceramisite vermifilter had a similar variation to quartz-sand vermifilter in earthworm weight distribution.

Earthworm enzymatic activity

Figures 5 and 6 showed variations of earthworm enzymatic activities, including protease, ALP, cellulase, SOD and CAT in the two kinds of vermifilter. In the quartz-sand vermifilter, protein declined from 66.3 to 45.66 mg g^{-1} dw (dried weight) earthworm, and ALP activity from 74.62 to 19.75 U · mg^{-1} protein with hydraulic loading. In addition, cellulase had the maximum in the W_2 condition (100.2 U · mg^{-1} protein), and the lowest in the W_4

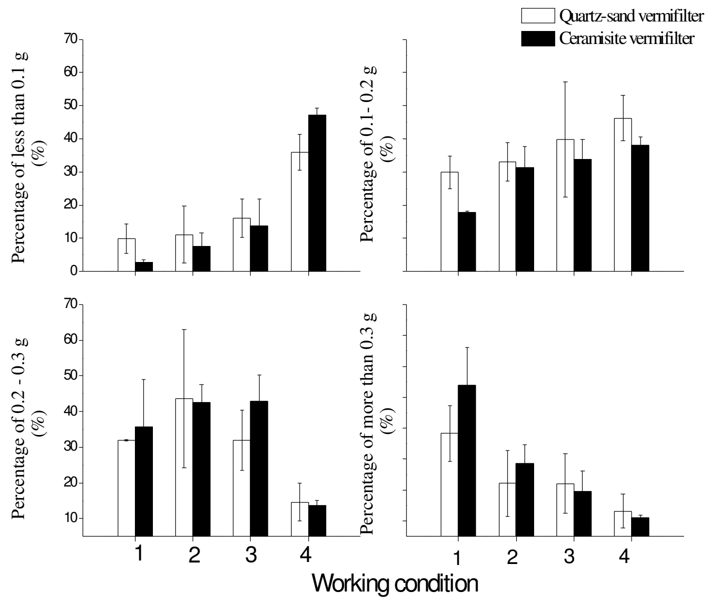

Figure 4. Earthworm percentage of different weights in four working conditions of both vermifilters. Values are means, bars are S.E., and n = 4.

condition (4.83 U · mg^{-1} protein). However, SOD and CAT activity increased from 47.35 to 59.74 U · mg^{-1} protein, and 3.50 to 7.45 U · mg^{-1} protein with hydraulic loading. Furthermore, cellulase and SOD activity were significantly different in both kinds of vermifilter (T-Test, P= 0.04 and 0.02), but there were no significant differences for protease, ALP and CAT activities (T-Test, P > 0.05).

Relationship between treatment efficiencies and earthworm characteristics

Table 3 showed correlation between treatment efficiencies and earthworm characteristics in the two kinds of vermifilter. Clitellated earthworm abundance had significant positive correlation with treatment efficiencies of BOD_5 (r= 0.779, P < 0.05), SS (r= 0.782, P < 0.05), TN (r= 0.834, P < 0.05) and NH_4-N (r= 0.828, P < 0.05). Proportion of 0.1 – 0.2 g earthworm correlated negatively with treatment efficiencies of COD (r= -0.853, P < 0.01), BOD_5 (r= -0.846, P < 0.01), SS (r= -0.716, P < 0.05) and NH_4-N (r= -0.915, P < 0.01). Proportion of more than 0.3 g earthworm displayed positive correlation with treatment efficiency of COD (r= 0.781, P < 0.05), BOD_5 (r= 0.772, P < 0.05), SS (r= 0.895, P < 0.01), and NH_4-N (r= 0.751, P < 0.05).

Treatment efficiencies of COD and BOD_5 correlated significantly with protein (r= 0.807, P < 0.05 and r= 0.837, P < 0.01); AKP activity (r= 0.892, P < 0.01 and r= 0.898, P < 0.01); cellulase (r= 0.711, P < 0.05 and r= 0.853, P < 0.05); SOD activity (r= -0.883, P < 0.01 and r= -0.835, P < 0.01); CAT activity (r= -0.780, P < 0.05 and r= -0.791, P < 0.05). CAT activity negatively correlated with the removal efficiencies of SS (r= 0.941, P < 0.01) and TN (r= 0.814, P < 0.05) by vermifilter.

DISCUSSION

Treatment efficiency

At present, there is an abundance of ecological and decentralized wastewater treatment technology, such as constructed wetland (Babatunde et al., 2008; Zhang et al., 2009), stabilization pond (Garcia et al., 2000; Heubeck et al., 2007), and land treatment (Li et al., 2005). These technologies had good treatment effect, but they were restrictively applied on wastewater treatment due to large occupied area. Our results showed that vermifilter could achieve good performance; the results were close to or even better than those of conventional decentralized wastewater treatments (Zhang et al., 2009). Further, the hydraulic loading of our vermifilter could reach 2.4 - 6.7 $m^3 \cdot m^{-2} \cdot d^{-1}$. However, the conventional ecological wastewater treatment, such as constructed wetland, was usually less than 1.55 $m^3 \cdot m^{-2} \cdot d^{-1}$ (Zhang et al., 2009). Higher hydraulic loading indicated more processing capacity of wastewater. Therefore, we considered that vermifilter would have desirable application due to less land area, compared to other ecological and decentralized waster treatments.

Our observations showed that hydraulic loadings could affect the removal rates of COD, BOD_5, SS, TN, and NH_4-

Table 3. Pearson correlation coefficients of treatment efficiencies and earthworm characteristics in vermifilter[a].

Correlations		Removal efficiency (%)				
		COD	BOD_5	SS	TN	NH_4-N
Earthworm population dynamics	Adult earthworm (10^3 ind. · m^{-2})	0.367	0.386	0.675	0.722*	0.622
	Clitellated earthworm (10^3 ind. · m^{-2})	0.690	0.779*	0.782*	0.834*	0.828*
	Earthworm hatchling (10^3 ind. · m^{-2})	-0.651	-0.648	-0.678	-0.712*	-0.712*
	Earthworm cocoon (10^3 ind. · m^{-2})	-0.410	-0.457	-0.848*	-0.696	-0.617
	Earthworm (<0.1 g) (%)	-0.671	-0.685	-0.704	-0.766*	-0.787*
	Earthworm (0.1- 0.2 g) (%)	-0.853**	-0.846**	-0.716*	-0.410	-0.915**
	Earthworm (0.2- 0.3 g) (%)	0.531	0.554	0.345	0.656	0.757*
	Earthworm (>0.3 g) (%)	0.781*	0.772*	0.895**	0.517	0.751*
Earthworm enzymatic activities	Protein (mg protein · g^{-1} earthworm)	0.807*	0.837**	0.535	0.952**	0.423
	ALP activity (U · mg^{-1} protein)	0.892**	0.898**	0.570	0.549	0.531
	Cellulase activity (U · mg^{-1} protein)	0.711*	0.853*	0.393	0.667	0.674
	SOD activity (U · mg^{-1} protein)	-0.883**	-0.835**	-0.371	-0.331	-0.805*
	CAT activity (U · mg^{-1} protein)	-0.780*	-0.791*	-0.941**	-0.814*	-0.653

[a]n = 8; * $P < 0.05$; ** $P < 0.01$.

N (Figure 2). We proposed the following reasons: (1) increasing hydraulic loadings means shortening hydraulic retention time, so organic substrates are not fully degraded before discharged from the vermifilter; (2) increasing hydraulic loadings leads to stronger scour for media surfaces, which was also responsible for the decrease in treatment efficiencies of vermifilter (Liu et al., 2008).

Earthworm characteristics

With vermifilter operation, adult and clitellated earthworm abundance decreased, but the densities of hatchling and cocoon increased (Figure 3). The increase of earthworm hatchling and cocoon indicated that earthworm could breed and incubate in vermifilter very well, and were suitable to the vermifilter environment. The weight distribution of earthworm also showed that the proportion of the smaller individuals rose with operation time (Figure 4). However, the decrease in the proportion of adult and bigger individuals may be due to two reasons. It could be the result of normal metabolic process: the adults produced cocoons, then the cocoons became juveniles, and the adults died. It could also be related to the increase of hydraulic loading which increased humidity and scouring of vermifilter, which was not beneficial for earthworm growth.

Digestive enzymes existed in the body of earthworm, such as protease, alkaline phosphates, and cellulase. These enzymes had an important relationship with the N and P cycle, and the turnover of carbon (Alef et al., 1995; Paul and Clark, 1996; Chapin et al., 2002; Schimel and Bennet, 2004; Aira et al., 2007). Another kind of earthworm enzymes were the antioxidant enzymes, such as SOD and CAT, which had been often used as biomarkers of environmental stress (Song et al., 2009). These enzymes could protect cells against adverse effects of reactive oxygen species. An increase in the activities of these enzymes indicated deterioration in environmental condition. In our study, the activities of digestive enzyme dropped, and that of antioxidant enzyme rose with the increase of hydraulic loading (Figures 5 and 6). This indicated that the increase of hydraulic loading was not beneficial for the digestion and growth of earthworm, as revealed in the data of earthworm abundances.

Relationship between treatment efficiency and earthworm characteristics

Earthworm played a critical role on the vermicompost (Domínguez and Edwards, 2004). This was because earthworm could improve activity of microorganism and stabilization of organic matter (Arancon et al., 2004, 2005, 2006; Aira et al., 2007; Ravikumar et al., 2008; Pramanik et al., 2009). In our study, abundance and enzymatic activities of earthworm had significant correlation with treatment efficiency of vermifilter (Table 3). We supposed that the treatment efficiency was not influenced only by earthworm abundance, but also by

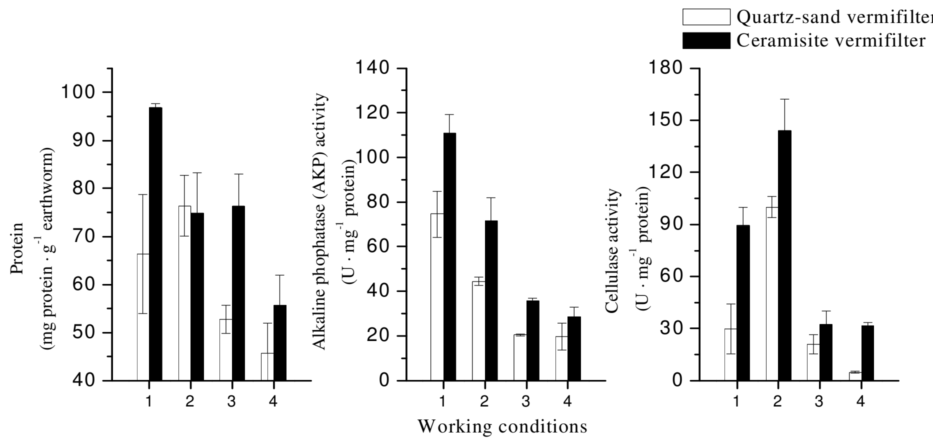

Figure 5. Earthworm digestive enzymatic activities in four working conditions of both vermifilters. Values are means, bars are S.E., and n = 3.

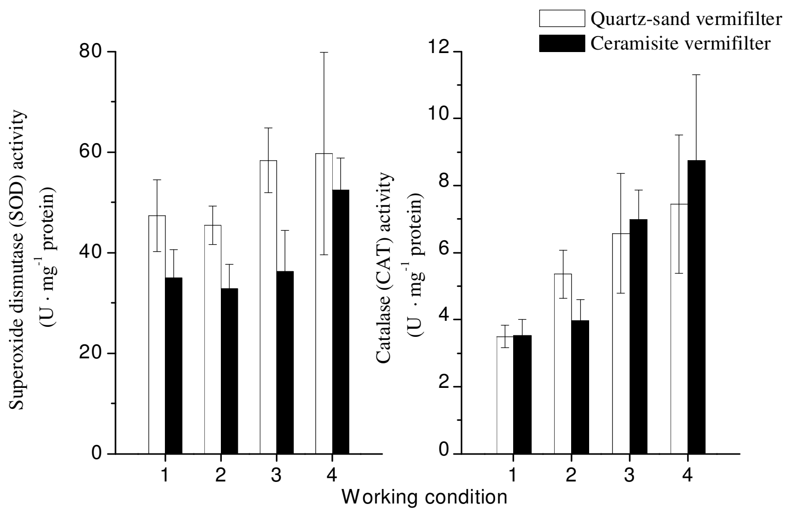

Figure 6. Earthworm antioxidase activities in four working conditions of both vermifilters. Values are means, bars are S.E., and n = 3.

earthworm growth state. In order to keep the vermifilter in good treatment efficiency, therefore, we need to maintain sufficient density of earthworm in vermifilter. More importantly, good condition is necessary for earthworm growth in suitable hydraulic loading.

The proportion of more than 0.3 g earthworm correlated significantly with removal rates of COD, BOD_5, SS, and NH_4-N, which indicated that the bigger earthworm might have a more important role on wastewater treatment of vermifilter, compared to the smaller one. Thus, although hydraulic loading had little influence on earthworm reproduction and increase of juveniles, the decrease in adults and larger earthworms contributed to the drop in treatment efficiency of vermifilter.

In our study, we found that CAT activity had a significant correlation with most of the indicators tested except NH_4-N reduction and the proportion of 0.2 – 0.3 g earthworm. Thus, in comparison to other characteristics of earthworm, CAT activity of earthworm should be a good indicator for the treatment efficiency and earthworm characteristics.

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