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A multi-level bioreactor to remove organic matter and metals, together with its associated bacterial diversity

Yonghong Wu [a,b,*], Zhengyi Hu [a,b], Philip G. Kerr [c], Linzhang Yang [a,**]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Sciences, Chinese Academy of Sciences, 71, East Beijing Rd, Nanjing 210008, Jiangsu, People's Republic of China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China
[c] School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW 2678, Australia

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ABSTRACT

The purpose of this study was to treat complex wastewater consisting of domestic wastewater, tobacco processing and building materials washings. The proposed multi-level bioreactor consists of a biopond–biofilter, anoxic/aerobic (A/O) fluidized beds and a photoautotrophic system. The results show that when the hydraulic load of the bioreactor was 200m^3/d, it successfully and simultaneously removed the organic matter and metals. When the bioreactor was in a relatively steady-state condition, the overall average organic matter and metals removal efficiencies are as follows, COD (89%), UV_{245} nm-matter (91%), Cu (78%), Zn (79%) and Fe (84%). The growth conditions of the native bacterial habitat were improved, which resulted from the increase of the in bacterial diversity under the rejuvenated conditions induced by the bioreactor. The results demonstrate that the multi-level bioreactor, without a sludge treatment system, can remove heterogeneous organic matter and metals from wastewater.

1. Introduction

Diverse measures are used for treating domestic wastewater. For example, aerobic technologies, based on activated sludge processes are widely applied in the treatment of domestic wastewater by dint of their high efficiency, the possibility of nutrient removal and the high operational flexibility (Gavrilescu and Macoveanu, 1999; Jechalke et al., 2010). Anaerobic (pre-)treatment of domestic wastewater serves as a viable and cost-effective alternative due to its relatively low construction and operational costs, operational simplicity, low production of sludge, production of energy in the form of biogas and applicability on both small and large scales (Lettinga, 1995).

In addition, measures including adsorption, bioaccumulation, chemical precipitation, ion-exchange, electroflotation, membrane separation, reverse osmosis, electrodialysis, and solvent extraction have been developed to remove metals from wastewater (Namasivayam and Ranganathan, 1995; Peng et al., 2008). Among the technologies, those based on adsorption are applied widely due to the attractive operation costs and environmental safety (Wan Ngah and Hanafiah, 2008). For example, zinc can be adsorbed by the microbial biofilms that are present in soil, sediments and natural waters (Toner et al., 2005).

The aforementioned measures have shown obvious benefits in treating domestic wastewater and removing metals. However, the industrial wastewater discharged tends to be heterogeneous (ChinaEPA, 2002; Kumar et al., 2008). Thus the development of a multi-function wastewater treatment system has practical significance for simultaneously removing the diverse array of contaminants from wastewater. It is well known that biological measures are an attractive option in wastewater treatment. Unfortunately, some metals such as Cu and Zn that accumulate in biofilm have negative effects on biofilm development and microbial aggregation (Duong et al., 2010; Fang et al., 2002). Thus, the negative impacts of these metals on biofilm formation need to be considered (Fang et al., 2002). Accordingly, there is a priority to develop biologically-based wastewater treatment systems that can handle toxic metal removal.

UV of wavelength 254 nm is a useful tool to determine the presence of organic components within a wastewater sample (Potter and Wimsatt, 2005). Strong correlations exist between UV_{254} absorption and organic carbon, color, and other disinfection by-products (Potter and Wimsatt, 2005; Wu et al., 2005). Considering the complex composition of the wastewater treated in this study, the removal of UV_{254} nm-matter was also investigated.

* Corresponding author at: State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Sciences, Chinese Academy of Sciences, 71, East Beijing Rd, Nanjing 210008, Jiangsu, People's Republic of China. Tel.: +86 25 8688 1591; fax: +86 25 8688 1000.
** Corresponding author. Tel.: +86 25 8688 1591; fax: +86 25 8688 1000.
E-mail addresses: yhwu@issas.ac.cn, hongyongwu83@126.com (Y. Wu), Lzyang@issas.ac.cn (L. Yang).

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To facilitate large-scale industrial applications, three additional considerations need to be addressed: (1) that the process does not leave potentially hazardous compounds or artificial materials in the environment; (2) that any sludge discharged should be 'self-digesting', thus no sludge discharge facility needs to be built and (3) that the biodiversity such as the native bacterial community in the contaminated ecosystem should be recoverable where feasible. With these factors in mind, we have proposed a multi-level bioreactor that will simultaneously remove organic matter and heavy metals including Cu, Fe and Zn from heterogeneous wastewater.

2. Methods

2.1. Description of the multi-level bioreactor

The multi-level bioreactor involved three connected assemblages: a biopond–biofilter, anoxic/aerobic (A/O) fluidized beds and a photoautotrophic system. The biopond–biofilter assemblage involved three parts as follows: (1) a biological pond (biopond), (2) an anaerobic biological filter (biofilter) filled with gravels, and (3) an overflow pool. The A/O fluidized beds included: (4) a settling tank, (5) an anoxic fluidized bed, (6) an aerobic fluidized bed, and (7) a clarification tank. (8) The final assemblage was the photoautotrophic system.

The influent entered the biopond (1) (capacity of approximately 30 m^3) comprising macrophytes (*Canna indica*, *Juncus minimus* and *Cyperus alternifolius*) with a planting density of 0.5 m × 0.5 m), and then flowed into the anaerobic biofilter (2) (capacity of 96 m^3) that was filled with coarse, mixed gravels (diameter 3–10 cm).

The wastewater from the overflow pool (3) of the anaerobic filter was pumped into the settling tank (4) (24 m^3), which then flowed sequentially into the anoxic fluidized bed (5) (72 m^3), aerobic fluidized bed (6) (72 m^3), and clarification tank (7) (24 m^3). The biofilm substrates in the anoxic fluidized bed were Industrial Soft Carriers (Wuxi Guozhen Environmental Protection Co., Ltd.), with a placement density of 0.3 m^3 per m^3 water. The suspended biofilm substrates in the aerobic fluidized bed were Artificial Aquatic Mats (Wuhan Zhongke Environmental Engineering Co., Ltd.), also placed at a density of 0.3m^3 per m^3 water.

Finally, the water overflowed into a photoautotrophic system (8), and subsequently discharged from the multi-level bioreactor. The photoautotrophic system was built as an ecological ditch with a total length 230 m and average width of 2.5 m (soil wall gradient 45°). Nylon tanks (114, each 0.04 m^3), containing ceramsite adsorbent (Kunming Yuxi Materials Co., Ltd.), were placed on the bottom of the ecological trunk channel at 2.0-m intervals for the adsorption of pollutants from the wastewater. Macrophytes, including *Scirpus tabernaemontani*, *C. indica*, *Zizania latifolia*, *J. minimus*, *C. alternifolius*, *Zantedeschia aethiopica*, and *Acorus calamus*, were planted along the walls of the ecological ditches at 0.5-m intervals.

2.2. Characterization of wastewater

The influent to the multi-level bioreactor was a combination of effluent from the domestic wastewater of Liangjia Village, Kunming City, South-western China; the washing wastewater from plastic bags used to process tobacco leaves; and the building material washings from nine small companies. The quantity of domestic wastewater flowing to the multi-level bioreactor was around 120 m^3/d and the quantity of the processing wastewater was about 80 m^3/d. The pre-experimental values of selected physicochemical parameters of the wastewater were as follows, pH (7.6 ± 0.40), DO (1.4 ± 0.30 mg L^{-1}), UV absorbance at 254 nm (0.7 ± 0.04), COD (146.6 ± 45.49 mg L^{-1}), BOD (70.6 ± 25.20 mg L^{-1}), Cu (604.7 ± 8.30 μg L^{-1}), Zn (377.9 ± 18.02 μg L^{-1}) and Fe (384.1 ± 12.26 μg L^{-1}).

2.3. Experimental design

2.3.1. Optimization of running modes

To immobilize robust native microorganisms and form native biofilms on the substrates, 0.6 m^3 of active sludge from a domestic wastewater treatment plant was placed in both the anoxic fluidized bed and the aerobic fluidized bed. The biofilms in the multi-level bioreactor were cultured and incubated under natural conditions.

To evaluate the effectiveness of the daily treatment of 200 m^3 of complex wastewater, four running modes were investigated. The first mode of liquid throughputs was for 2 h at 2-hourly intervals (2 h on, 2 h off – 2 h/2 h); the second every 4 h (4 h/4 h); the third mode every 6 h (6 h/6 h) and finally at 12 h (12 h/12 h). Each had the same liquid throughput of 0.28 m^3/min with the throughput for 12 h each day. The air temperature ranged from 15 to 28 °C (average temperature = 18 °C) during the experimental period. To maintain independence for each of the running modes, water samples were not collected until after the bioreactor had run for 6 d in each mode.

2.3.2. Regular running

To obtain native microorganisms and facilitate large-scale industrial application, the biofilms were cultivated and incubated in the manner described above. The natural air temperature ranged from 8 to 31 °C over the experimental period with an average temperature of 16 °C and a relative humidity of 42%. To ensure the system was in a relatively steady state, the regular running experiment commenced only after the multi-level bioreactor had run for 8 months.

To support the growth of native microbes in the autotrophic bioreactor, the sludge liquid in the bottoms of the anoxic and the aerobic fluidized beds was pumped directly into the photoautotrophic system using a strong-pressure sludge-pump during the no-throughput intervals. To avoid the sludge impact on data, the water samples were collected after the sludge had been discharged for 10 d.

2.4. Samples and analyses

Water and biofilm sampling sites were located at biopond (B1), anaerobic biofilter (B2), anoxic fluidized bed (B3), aerobic fluidized bed (B4), the inlet (B5) and the outlet (B6) of the photoautotrophic system. Water samples were collected in triplicate. Chemical oxygen demand (COD) of the water samples were measured by the potassium dichromate method (APHA-AWWA-WEF, 1998). The dissolved oxygen (DO) and pH levels in water were measured *in situ* by a meter (YSI 52 dissolved oxygen and pH meters). Biological oxygen demand (BOD) for 5 d was calculated as the difference between DO level at the beginning and the DO level at the end of 5 d (APHA-AWWA-WEF, 1998). Samples for UV_{254} nm absorbance were filtered before being measured in order to reduce the UV absorption caused by particulate matter (Wu et al., 2005).

The concentrations of copper (Cu) in water were measured using graphite furnace atomic absorption spectrometry (AA-7001, Beijing). The concentrations of zinc (Zn) and iron (Fe) in water were determined using flame atomic absorption spectrometry (AA-7001, Beijing). These procedures are described in the National Standard Methods of Water and Wastewater Analyses, China (ChinaEPA, 2002).

Ten biofilm samples (in triplicate) were collected at random locations from substrates in the anaerobic biofilter, anoxic and aerobic fluidized beds and then kept at 25–30 °C until their moisture contents were about 85%. The biofilms were then weighed and the total biofilm mass in the anaerobic biofilter, anoxic and aerobic fluidized beds was estimated based on the biofilm weight sampled and the surface area of substrates. Based on ERIC-PCR fingerprints

in June 2007 and May 2008, the Shannon diversity index (Eichner et al., 1999) was used to evaluate the bacterial community diversity in the biofilm at each of the sampling sites (B1 to B6).

The use of the Shannon diversity index to quantify the bacterial diversity is referred to in a previous report (Miura et al., 2007). Total DNA extraction and purification of the biofilm samples was conducted for the ERIC-PCR analyses. DNA was isolated from the biofilm samples following a procedure modified from Hill et al. (2002). Biofilm sample aliquots (1 mL) were thawed in an ice-bath, and the cells were harvested of their DNA by centrifugation for 5 min, and then purified by sequential extraction with Tris–equilibrated phenol, phenol–chloroform–isoamyl alcohol (25:24:1), and chloroform isoamyl alcohol (24:1) followed by precipitation with two volumes of ethanol. DNA was collected by centrifugation, air-dried and dissolved in 50 μL sterile TE buffer. The detailed procedures are described in the paper by Wei et al. (2004).

Community fingerprints were obtained for bacteria in the biofilm by using total bacterial DNA as templates for ERIC-PCR. The sequence of the ERIC primers and the detailed procedures are described in previous work (Li et al., 2006),E1 (ERIC-PCR): 5′-ATGTAAGCTCCTGGGGATTCAC-3′,E2 (ERIC-PCR): 5′-AAGTAAGTGACTGGGGTGAGCG-3′.

Data analysis was performed using SPSS statistical software (version 15.0), with the level of statistical significance set at $p < 0.05$. Statistically significant differences between the results were evaluated on the basis of standard deviation determinations and on the analysis of variance method (one way ANOVA). Non-parametric correlations between the removal efficiencies of COD and of UV_{254} nm-matter; and between total biofilm mass and the removal efficiencies of metals (Cu, Zn and Fe) were analyzed by Kendall's tau-b.

3. Results and discussion

3.1. The optimization of bioreactor running mode

To optimize the performance for removing COD and UV_{254} nm-matter, the multi-level bioreactor was operated in each of the four different running modes. Fig. 1 shows that the COD and UV_{254} nm-matter removal efficiencies in the different running modes tended to increase and stabilize. The removal efficiencies of COD and UV_{254} nm-matter significantly increased from 66% to 83% and from 45% to 72% when the interval run-time increased from 2 h to 12 h, respectively. Thus the 12 h/12 h running mode was chosen to be used in the regular running of the bioreactor.

3.2. Removal of COD and UV_{254} nm-matter

When the running mode of the multi-level bioreactor was 12 h/12 h, each of the performances of the biopond–biofilter, A/O fluidized beds, and the photoautotrophic system were investigated. The results show that the average removal efficiencies of COD and UV_{254} nm-matter from April 30 2007 to May 16 2008, for the biopond–biofilter and A/O fluidized beds were 70% and 75%, respectively (Fig. 2a, b). The highest and lowest removal efficiencies for COD and UV_{254} nm-matter occurred on the same days, May 16, 2008 and July 12, 2007, respectively (Fig. 2a, b). In addition, the variations in COD and UV_{254} nm-matter concentrations of both influent and effluent with time were very similar, implying that there may be associations between the removals of both COD and UV_{254} nm-matter.

The average removal efficiencies of COD and UV_{254} nm-matter from April 2007 to May 2008 for the photoautotrophic system were 58% and 52%, respectively (Fig. 2c, d). The removal efficiencies of the photoautotrophic system from April to September 2007 were ~14% and ~12% lower for COD and UV_{254} nm-matter than those from October 2007 to May 2008, respectively (Fig. 2c, d). This implies that although the multi-level bioreactor had run for eight months before the regular running experiment, the open nature of the photoautotrophic system has been affected by environmental factors such as air temperature and macrophyte biomass. In this case, it is likely the growing macrophytes are the main reason for the observed lag in removal efficiencies. Before the macrophytes achieved maximum biomass, the removal efficiencies of COD and UV_{254} nm-matter generally increased with time. In addition, the organisms in the photoautotrophic system (including macrophytes and native microorganisms) required an adaptation period before attaining their optimal eco-functions (Somova et al., 2005). Moreover, the variations in the COD and UV_{245} nm-matter concentrations of influent and effluent of the photoautotrophic system as well as their removal efficiencies showed very similar trends (Fig. 2c, d).

In the B1, B4 and B6 sampling sites, the changes in UV_{254} nm-matter values closely reflected the changes in COD (Fig. 2). The goodness-of-fit correlation ($p < 0.05$) between the overall COD and UV_{254} nm-matter removal efficiencies suggests strongly that the compounds comprising the UV_{254} nm-matter in the influent and effluent were principally (or derived from) the compounds contributing to the COD and are most likely polyphenolic in nature (Balcıoğlu and Ötker, 2003). The calculated relationship was [UV_{245} nm-matter removal efficiency] = 0.6615 × [COD removal efficiency] + 30.35 ($n = 23$, $R^2 = 0.89$).

Fig. 3 shows that the overall average removal efficiencies of COD and UV_{254} nm-matter, from April 2007 to May 2008, were 87% and 88%, respectively. The average removal efficiencies of COD and UV_{254} nm-matter during from April to September 2007 were lower by 7% and 5% than those from October 2007 to May 2008 ($p > 0.05$) (Fig. 3). This result implied that although factors such as the growing macrophytes obviously affected the removal efficiencies of COD and UV_{254} nm-matter in the photoautotrophic system, such environmental factors did not significantly affect the overall removal efficiency of COD and UV_{254} nm-matter in

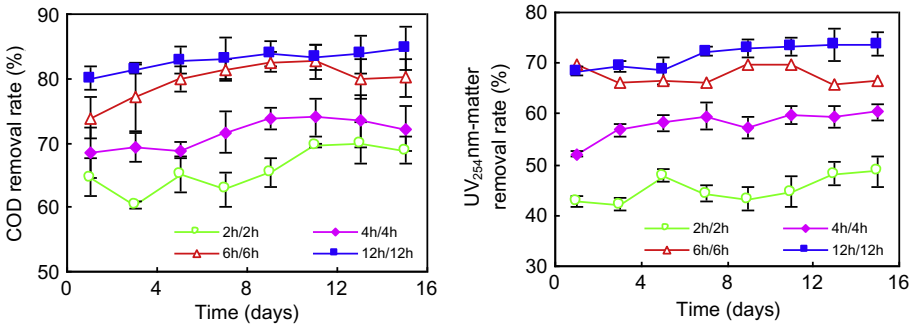

Fig. 1. The removal efficiencies of COD and UV_{254} nm-matter in different running modes.

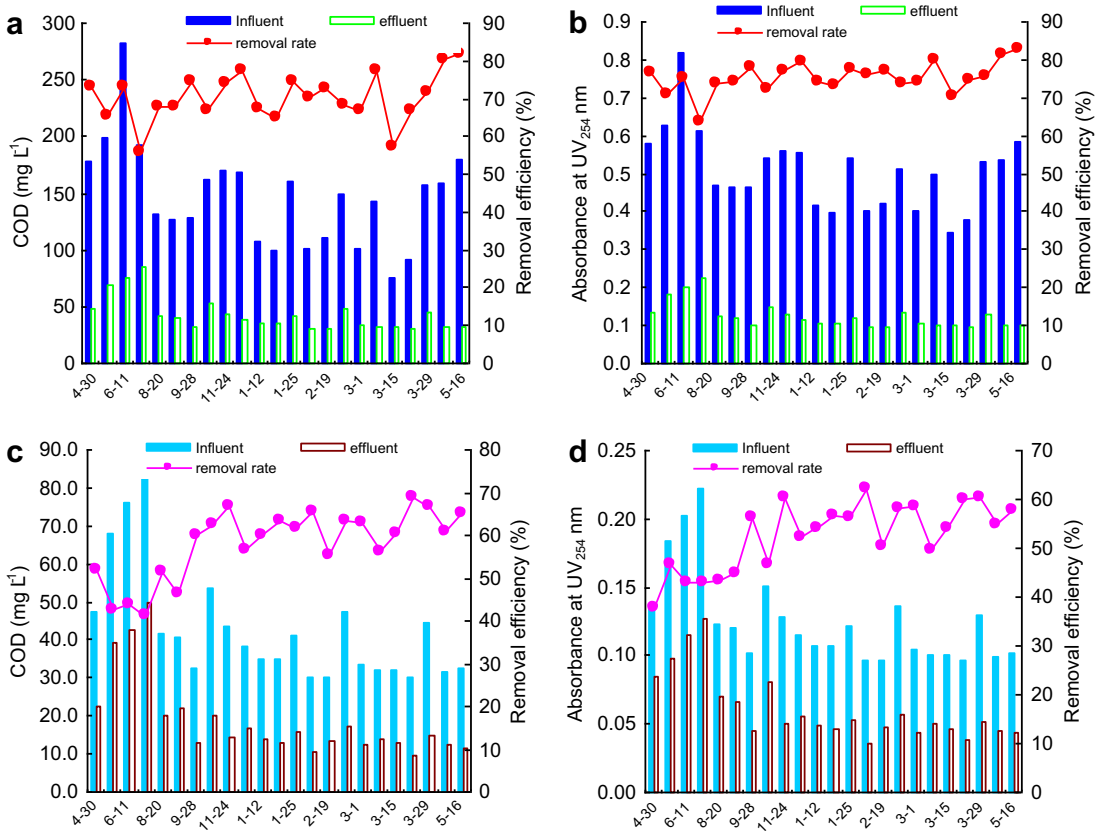

Fig. 2. The influent and effluent concentrations and the removal efficiencies of COD and UV_{254}nm-matter treated by the biopond–biofilter and A/O fluidized beds (a and b), and photoautotrophic system (c and d) from April 2007 to May 2008. The influent and effluent of the biopond–biofilter and A/O fluidized beds (a and b) were sampled from B1 and B4 sampling sites, respectively. The influent and effluent of the photoautotrophic system (c and d) were sampled from B5 and B6 sampling sites, respectively.

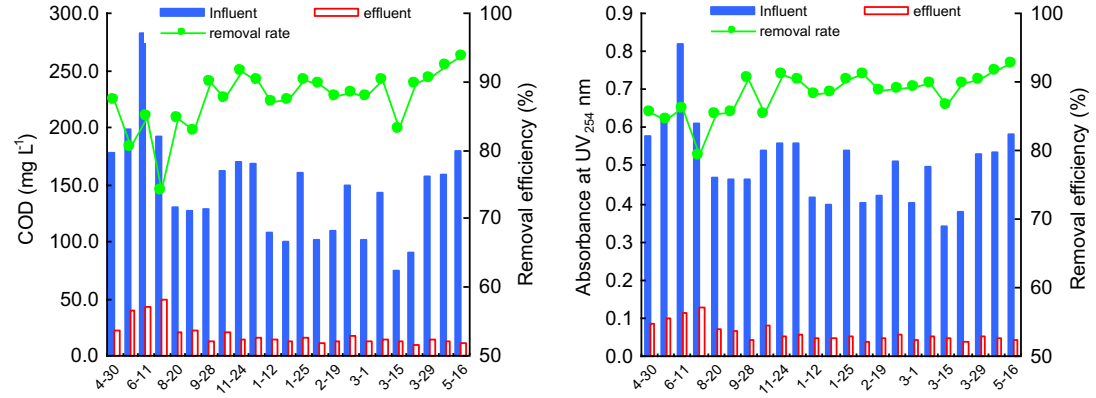

Fig. 3. The influent and effluent concentrations and the removal efficiencies of COD and UV_{254} nm-matter treated by the multi-level bioreactor from April 2007 to May 2008. The influent and effluent were sampled from B1 and B6 sampling sites, respectively.

the multi-level bioreactor because the photoautotrophic system is not the main contributor to their removal. Approximately 80% of the removed COD and around 85% of the removed UV_{254} nm-matter was attributed to the biopond–biofilter and A/O fluidized beds (Fig. 2 and Fig. 3). This is due to the wastewater being settled in the anaerobic biofilter and anoxic fluidized bed for ~24h. These anaerobic and anoxic conditions accelerated the biodegradation of the organic matter in the wastewater (Kapdan and Oztekin, 2006), which resulted in the reduction of the COD- and UV_{254} nm-matter in the effluent.

3.3. Simultaneous removal of metals

The influent wastewater suffered heavy metal pollution. The annual average influent metal concentrations were Cu = 668.6 μg L^{-1}, Zn = 358.0 μg L^{-1} and Fe = 623.8 μg L^{-1}. The average removal efficiencies were Cu (77%), Zn (78%) and Fe (82%) from June 2007 to May 2008 (Fig. 4a, b, c). The variation in removal efficiencies of Cu, Zn and Fe were very similar in different seasons, which implied that these metals might be subjected to a common factor, thereby leading to their removal.

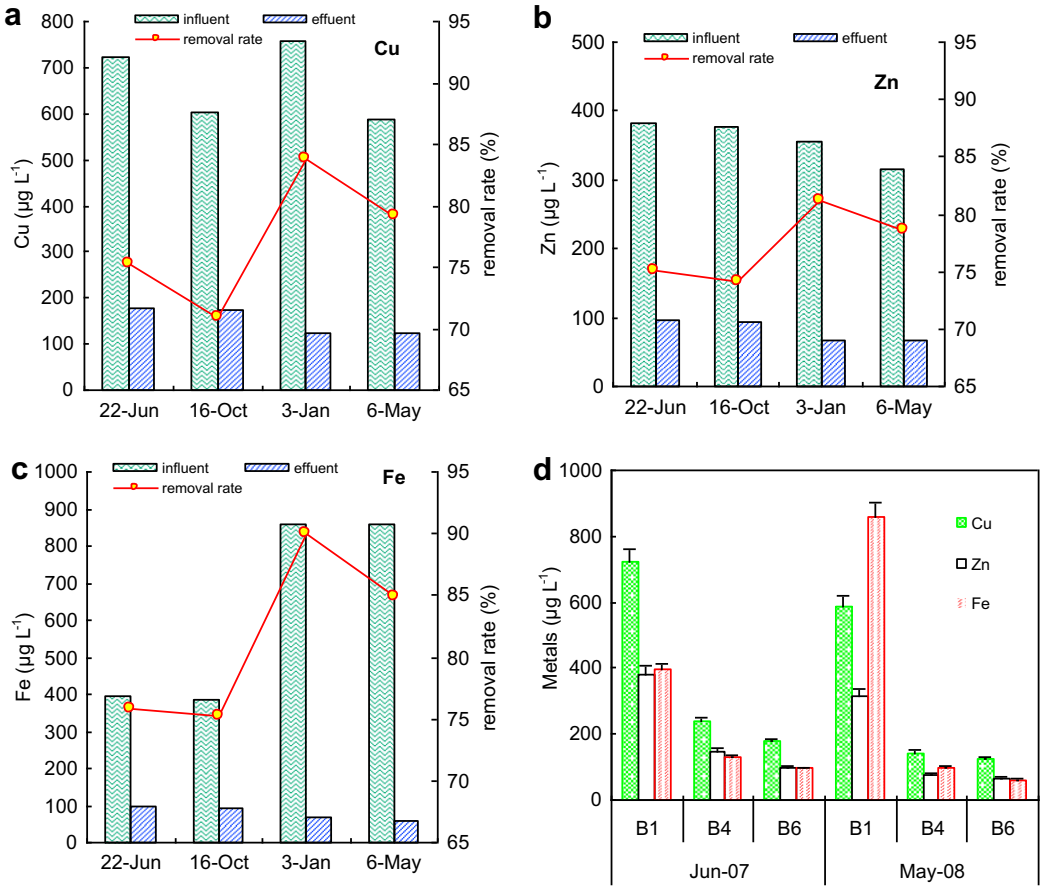

Fig. 4. The influent and effluent concentrations and removal efficiencies of Cu (a), Zn (b) and Fe (c) at June & October of 2007 and January & May of 2008, and (d) The concentrations of metals including Cu, Zn and Fe in the influent of the multi-level bioreactor (B1), the effluent of aerobic fluidized bed (B4) and the outlet of the photoautotrophic system (B6).

The metal concentrations from sampling sites at different stages of the treatment process (B1 to B6) were determined in June 2007 and May 2008. Fig. 4d shows that the metal (Cu, Zn and Fe) concentrations had predominantly decreased from sampling sites B1–B4 at both sampling times, while their concentrations did not markedly decrease during the latter stages of the treatment process (B4–B6). This suggested that most of the metals determined (Cu, Zn and Fe) in the influent, were about 81–96% removed by the biopond–biofilter and A/O fluidized beds.

Although the concentrations of pollutants in influent varied largely: COD (75–282 mg L^{-1}), UV_{254} nm-matter (0.34–0.82), Cu (588–759 μg L^{-1}), Zn (316–383 μg L^{-1}) and Fe (384–860 μg L^{-1}), the removal efficiencies of these pollutants was maintained at relatively high levels. In addition, the multi-level bioreactor ran under relatively varied environmental conditions such as the air temperature with a range from 8 to 31 °C. These implied that it is feasible that the multi-level bioreactor is applicable in-service under different environmental conditions.

3.4. Characteristics of biofilm and sediments

The calculated total biofilm masses (25–30 °C, moisture ~85%) in the anaerobic biofilter, anoxic and aerobic fluidized beds were 156.8 ± 20.4 (June 2007), 152.9 ± 7.6 (October 2007), 193.2 ± 28.9 (January 2008), and 184.2 ± 18.4 kg (May 2008). The variation patterns in the biofilm masses and the metals removal efficiencies in the different seasons were very similar, decreasing from June to October, and rapidly rising from October to January, and then decreasing again from January to May. The correlations between the biofilm mass and the metals (Cu, Zn and Fe) removal efficiencies in the different seasons were significant ($p < 0.05$). This implied that the metal removals were associated with biofilms in the biopond biofilter and A/O fluidized beds. In most cases, the metals such as Cu (Lee et al., 2008; Meylan et al., 2003), Fe (Quintelas et al., 2009), and Zn (Meylan et al., 2003) were removed by the biofilm adsorption.

The behavior of the bioreactor in relation to the microbial community structure (Xia et al., 2008), and the bacterial diversity was investigated. The Shannon diversity indices increased along the sampling sites from B1 to B6 at both sampling times (June 2007 and May 2008). The Shannon diversity indices at the sampling sites markedly increased from 1.3 to 1.8 (B3), from 1.5 to 2.1 (B4), from 1.7 to 2.7 (B5) and from 1.9 to 2.9 (B6) between June 2007 and May 2008, respectively. Also, by pair-wise comparisons, the Shannon diversity index of these four sampling sites at May 2008 was significantly higher than that at June 2007 ($p < 0.05$).

A change in bacterial community diversity is likely to be related to environmental conditions (LaPara et al., 2002). A previous report showed that heavy organic contamination resulted in the dramatic decrease in community diversity (Li et al., 2007). In this study, with the increased running time and location of sampling sites, as the pollution level decreased the bacterial diversity index increased. When compared with other studies, the bacterial communities in our multi-level bioreactor were very diverse. For example, the Shannon diversity index in the membrane bioreactor (MBR) and the combination of MBR and Hybrid MBR treated wastewater of similar loadings were on average 0.82 and 1.3–1.6 during a 100-day operation (Miura et al., 2007; Stamper et al., 2003). This is less

than half of our community diversity. This is because the anaerobic, anoxic and aerobic conditions concurrently existed in the multi-level bioreactor and these conditions simultaneously fostered development and growth of different microorganisms (i.e., bacteria). It is also likely that the direct discharge of sludge (sediments) into the photoautotrophic system supplied the nutrients and habitats for a wider range of microbes, thus enhancing the diversity in comparison with the aforementioned treatment systems.

For the multi-level bioreactor, no sludge discharge system was deemed necessary. The discharged sludge was treated simply by exposure to the photoautotrophic 'bioreactor'. This avoids having to manage the key issue in current wastewater treatment systems based on A/O fluidized beds, namely the high capital and operational costs of sludge treatment facilities. In general, sludge treatment systems involve high costs, ranging from 20% to 60% of the total operating cost of wastewater treatment plants (Uggetti et al., 2010). The capital cost of the multi-level bioreactor was estimated based on the local price level, which ranged from 120 to 150 US dollars per cubic meter of water capacity, and the operation cost was about 1.4 US cents per cubic meter of water treated.

The high concentration sludge (sediments) liquid discharged into the photoautotrophic bioreactor was estimated twice during the experimental period, at about 22 and 43 m^3 (based on the pump flux and time) in July 2007 and March 2008, respectively. It was found that the new dark settled sludge (sediments) layer in the photoautotrophic system had disappeared within 1 week. In the photoautotrophic system, the aerobic conditions of the sludge layer promoted the growth of aerobic microorganisms and ultimately improved sludge mineralization (Nielsen, 2005). Moreover, the treatment of sludge by the photoautrophic bioreactor might allow the wastes to be converted into a by-product such as an organic fertilizer or soil conditioner suitable for native microbes and macrophytes (Uggetti et al., 2010).

4. Conclusion

The results show that the proposed multi-level bioreactor affords a practical solution for simultaneously removing organic matter, expressed by high removal efficiencies of COD and UV_{254} nm-matter, and heavy metals, *viz*. Cu, Zn and Fe. The bacterial communities were diversified in the multi-level bioreactor with their diversity indices being strongly determined by the output water quality. The photoautotrophic system 'ingests' the sludge discharged from A/O fluidized beds, which in turn enhances the multi-level bioreactor in a self-modulating and self-sustaining way.

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