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The removal of nutrients from non-point source wastewater by a hybrid bioreactor

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ABSTRACT

The aim of this project was to establish an economical and environmentally benign biotechnology for removing nutrients from non-point source wastewater. The proposal involves a hybrid bioreactor comprised of sequential anaerobic, anoxic and aerobic (A^2/O) processes and an eco-ditch being constructed and applied in a suburban area, Kunming, south-western China, where wastewater was discharged from an industrial park and suburban communities. The results show that the hybrid bioreactor fosters heterotrophic and autotrophic microorganisms. When the hydraulic load is 200 m^3 per day with the running mode in 12 h cycles, the removal efficiencies of the nutrients were 81% for TP, 74% for TDP, 82% for TN, 79% for NO_3-N and 86% for NH_4-N. The improved bacterial community structure and bacterial habitats further implied enhanced water quality and indicates that the easily-deployed, affordable and environmentally-friendly hybrid bioreactor is a promising bio-measure for removing high loadings of nutrients from non-point source wastewater.

1. Introduction

Water pollution arising from diffuse sources is generally defined as non-point source (NPS) pollution (Ongley et al., 2010). There is an increasing contribution of non-point source wastewater as a major source of pollution, as occurs in eutrophication and harmful algal blooms in downstream waters, for both developed and developing countries (Wu et al., 2010a). The run-off from farmland, grassland, forest and road surfaces is the major contributor (Ongley et al., 2010), with nitrogen and phosphorus being the major pollutants in this non-point source wastewater and are the leading causes of degeneration in water quality as well as the degradation of ecosystems (Wu et al., 2010a,b). Therefore, to protect the water quality in downstream surface aquatic ecosystems, it is very important that a benign technology is developed to remove nitrogen and phosphorus simultaneously from non-point source wastewater.

A number of ecological measures have been developed to reduce non-point source pollution (Wu et al., 2010b). They can be summarized into three classes: (1) ecological management based on the model of agricultural non-point source pollution (AGNPS) on a watershed scale (Wang et al., 2008) such as the Best Management Practice (BMPs) issued from the US Environmental Protection Agency (USEPA); (2) integrated ecological solutions, such as hierarchical eco-restoration (Wu et al., 2010a,b) and GIS-based ecological-economic modeling (Lant et al., 2005); and (3) environmental engineering projects such as the use of vegetative strips between the pollution sources and the receiving water bodies (Duchemin and Hogue, 2009) and agricultural drainage ditches (Moore et al., 2010).

The aforementioned measures help to filter non-point source wastewater, to promote sedimentation of the suspended particles and the pollutants bound to them, as well as to restore ecological system function (Duchemin and Hogue, 2009; Wu et al., 2010a,b). These measures are suited to the current socio-economic context in which the adoption of simple and inexpensive agri-environmental practices is advocated, with a view to protecting water quality, say by managing the use of fertilizers in crop production (Duchemin and Hogue, 2009).

Non-point source wastewater originating in urban/suburban areas is due to both natural factors (e.g., rainfall) and human activities (e.g., the wastewater discharges from industrial parks and irrigation systems) (Chen et al., 2008; Martínez et al., 2000). Human activity makes a significant and complex impact on the composition of the non-point source wastewater. For example, heavy metals may be introduced by industrial activities in urban/suburban areas and organic matter may be increased as a result of intensive farming practices. The problem of controlling non-point source wastewater in suburban/urban areas is further complicated by the established infrastructure, such as roads and buildings, and the multiple types of land use in existence (Duchemin and Hogue, 2009). Thus, it is necessary to explore new technologies and/or to integrate current technologies to manage non-point source wastewater in urban/suburban areas.

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The activated sludge method involving the anaerobic–anoxic–aerobic (A^2/O) process (Kassab et al., 2010) has been applied widely to treat high load wastewater and can simultaneously remove organic and inorganic nutrients (e.g., nitrogen and phosphorus) and heavy metals (e.g., chromium and arsenic), because it is highly efficient and environmentally friendly (Samaras et al., 2009; Ying et al., 2010). Photoautotrophic systems such as wetlands and ecological ditches are often used to purify wastewater bearing a low pollutant load because they are inexpensive and environmentally benign. However, the activated sludge method is costly and therefore limits its application in many developing or underdeveloped regions (Wang et al., 2009). The photoautotrophic system typically requires a large area of land to achieve the economic viability of large-scale construction, which is in conflict with the local residents' need for cropland, recreational and industrial land use (Wu et al., 2010b). Thus, there is practical significance in combining the advantages of these two technologies to overcome the limitations in both, for treating "real world" wastewater.

Thus, we are proposing an integrated technology – a "hybrid bioreactor" combining the A^2/O approach with an ecological ditch in order to simultaneously remove high load nitrogen and phosphorus from heterogeneous non-point source wastewater and to rejuvenate the ecological (especially microbial) habitat. In the proposed integrated bio-solution, we believe the most important issue is to bring the aquatic ecosystem of the hybrid bioreactor into a sustained self-modulating state. To facilitate application on an industrial scale, five additional considerations need to be taken into account: the hybrid bioreactor should be (i) easily constructed, (ii) simple to operate, (iii) highly efficient, (iv) inexpensive, and (v) the technology should be environmentally benign.

2. Methods

2.1. Hybrid bioreactor

The hybrid bioreactor consisted of the A^2/O process in series with the ecological ditch (eco-ditch) (Fig. 1). There are eight compartments within the hybrid bioreactor: (i) the depositional tank (∼30 m^3) planted with macrophytes (*Canna indica*, *Juncus minimus* and *Cyperus alternifolius* species) with a planting density of 0.5 m × 0.5 m; (ii) an anaerobic tank (∼96 m^3) filled with coarse gravel (diameter 3–10 cm); (iii) an overflow pool (∼4 'm^3) to reduce suspended materials; (iv) a settling tank (∼24 m^3) to further reduce suspended materials; (v) an anoxic fluidized bed (∼72 m^3) which contained biofilm substrates (Industrial Soft Carriers, Wuxi Guozhen Environmental Protection Co. Ltd.) with a density of 0.3 m^3 per m^3 of water; (vi) an aerobic fluidized bed (∼72 m^3) containing suspended biofilm substrates – 'Artificial Aquatic Mats' (Wuhan Zhongke Environmental Engineering Co. Ltd.), also having a density of 0.3 m^3 per m^3 of water; (vii) a clarification tank (∼24 m^3) the function of which was to reduce the suspended materials, and (viii) the eco-ditch with a length of 230 m and average width of 2.5 m (soil wall gradient 45°). A series of 0.04 m^3 nylon tanks, packed with ceramsite adsorbent (Kunming Yuxi Materials Co. Ltd.), was placed on the bottom of the ecological ditch 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 eco-ditch at 0.5 m intervals.

The passage of the wastewater (influent) through the hybrid bioreactor was as follows: the influent entered the deposition pool, the anaerobic tank, and then into the overflow pool. Thereafter, the wastewater was pumped into the settling tank, and then flowed into the anoxic fluidized bed, the aerobic fluidized bed, and the clarification tank. Lastly, the water overflowed into the eco-ditch, after which it was then discharged as the effluent.

2.2. Experimental design

The non-point source wastewater for the hybrid bioreactor was collected from various sources. The effluent from the diffuse wastewater of communities, processing wastewater from an industrial park, and road surface runoff from Liangjia Village, Kunming, in south-western China were combined to form the influent to the hybrid bioreactor. The loading of influent averaged approximately 200 m^{3}day^{-1} on non-rainy days (i.e., 24h rainfall < 10 mm).

To obtain specifically desirable native microorganisms and to facilitate large-scale industrial application, the hybrid bioreactor was inoculated with microorganism aggregates (biofilms). Active sludge (0.6 m^3) from a domestic wastewater treatment plant was loaded into the anoxic and aerobic fluidized beds. The biofilms were cultivated and incubated in the hybrid bioreactor under normal conditions for 15 days before the collection of experimental data commenced. The air temperature ranged from 8 °C to 31 °C over the experimental period with an average temperature of 16 °C and a relative humidity of 42%. A daily average of 200 m^3 of influent was treated by the hybrid bioreactor. The running mode of liquid throughputs involved a 12 h on and 12 h off (down time, the pump being switched off) cycle (12 h/12 h), so that the average inflow in the hybrid bioreactor was approximately 16.7 m^3/h.

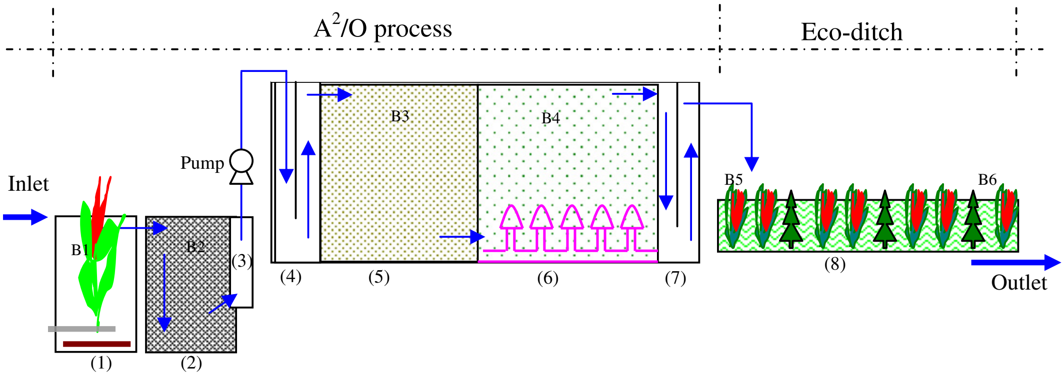

Fig. 1. The schematic of the hybrid bioreactor showing the combined A^2/O process and eco-ditch. The A^2/O process consisted of (1) deposition pool, (2) anaerobic tank filled with gravels, (3) overflow pool, (4) settling tank, (5) anoxic fluidized bed, (6) aerobic fluidized bed, and (7) clarification tank. The eco-ditch (8) is a photoautotrophic system. The arrows indicate the direction of water flow. Water and biofilm sampling sites are indicated as B1–B6.

To support the growth of native microorganisms in the eco-ditch, the highly concentrated sludge in the bottoms of the anoxic and aerobic fluidized beds was directly pumped into the eco-ditch using a strong-pressure sludge-pump during the 12 h 'down time' interval. To avoid the sludge impact on sampling data measured at the start and end of the eco-ditch (B5 and B6 in Fig. 1 respectively), the water samples were collected 10 days after the sludge was pumped into the eco-ditch.

2.3. Samples and analyses

Water samples were collected in triplicate from sites B1 to B6 every week on non-rainy days (i.e., 24 h rainfall < 10 mm) (Fig. 1). To avoid the impact of rain on the data, the samples were collected 3 days after any rainy days (i.e., 24 h rainfall >10 mm). Total phosphorus (TP) and total dissolved phosphorus (TDP) were measured colorimetrically by the persulfate digestion-molybdophosphate reaction method. Total nitrogen (TN) was measured by the persulphate digestion and oxidation-double wavelength (220–275 nm) method. Ammonia-N (NH_4-N) and Nitrate-N (NO_3-N) in water were determined by ion-selective electrode potentiometry with preliminary distillation (APHA-AWWA-WEF, 1998). The dissolved oxygen (DO) and pH levels in water were measured *in situ* by a multi-meter (YSI 52 meter, manufacturer). The arsenic (As) concentration in water was determined by cold vapor atomic fluorescence spectrometry (QM201D, Jiangsu). Chromium (Cr (VI)) concentration was measured using diphenylcarbazide spectrophotometry. These detailed procedures are found in the national standard methods of water and wastewater analyses, China (ChinaEPA, 2002).

After filtration (pore size 0.22 μm) of the water samples, the filtrate residues were observed using optical microscopy. Common microorganisms such as the species of bacteria, diatoms and cyanobacteria, were identified using the national standard guide of microorganisms or common phytoplankton in freshwater (ChinaEPA, 2002).

Biofilm samples were collected in triplicate at random locations from substrates (Coarse gravel, Industrial Soft Carriers, Artificial Aquatic Mats, and Ceramsite) in the anaerobic tank, anoxic and aerobic fluidized beds as well as the eco-ditch. Biofilms were peeled from these substrates using knife generally and kept at 25–30 °C until their moisture contents were reduced to ~85%. The biofilm was then weighed and the total biofilm biomass in the anaerobic tank, anoxic and aerobic fluidized beds was estimated based on the biofilm weight and specific surface area of the sampled substrate.

Before the hybrid bioreactor was constructed, native biofilm samples were peeled from the surfaces of stones at the entrance (A1), middle (A2) and end (A3) of the ditch, corresponding to the locations of deposition pool to eco-ditch. After the hybrid bioreactor was commissioned, the biofilm samples were collected from the substrates along the sampling sites from B1 to B6 (Fig. 1).

The Dice index (Cs) of similarity (LaPara et al., 2002) was used to evaluate the similarity of bacterial community structures based on ERIC-PCR fingerprints. The quantitative use and methodology of the Dice index were reported previously (Miura et al., 2007). Total DNA extraction and purification of biofilms were conducted for the ERIC-PCR analyses. Total DNA was isolated from the biofilm samples following a procedure modified from a previous report (Hill et al., 2002) whereby 1 mL biofilm sample aliquots were cooled in an ice-bath, and the cells harvested by centrifugation at 9000 rpm for 5 min. The extracted DNA was then purified by sequential extraction with Tris-equilibrated phenol, phenol–chloroform–isoamyl alcohol (v/v/v, 25:24:1), and chloroform–isoamyl alcohol (v/v, 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 given in Wei et al. (2004).

Bacterial community "fingerprints" were obtained for the biofilm by using total bacterial DNA as templates for ERIC-PCR. The sequence of the ERIC primers was based on previous work (Li et al., 2006), using E1 (ERIC-PCR): 5′-ATGTAAGCTCCTGGGGATTCAC-3′, and E2 (ERIC-PCR): 5′-AAGTAAGTGACTGGGGTGAGCG-3′. The detailed procedures were as described therein.

SPSS statistical software (version 12.0) was used for analyzing the data, and the level of statistical significance was set at $p < 0.05$. Statistically significant differences between the data were evaluated on the basis of standard deviation determinations and on the analysis of variance method (one way ANOVA).

3. Results and discussion

3.1. Characteristics of the non-point source wastewater

The physicochemical parameters of the non-point source wastewater are shown in Table 1. The results show high nutrient concentrations including TN (27.20 mg L^{-1}) and TP (2.46 mg L^{-1}) relative to levels designated by the National Water Quality Standard of Water Environments, China (ChinaEPA, 2002). In addition, the water was characterized with high As (113.77 μg L^{-1}) and Cr (VI) (105.32 μg L^{-1}) concentrations. These levels were higher than the baseline values (100 μg L^{-1} for As and 100 μg L^{-1} for Cr (VI)) considered the maximum allowed levels by the National Water Quality Standard of Water Environments, China (ChinaEPA, 2002), indicating that the non-point source wastewater was significantly polluted with As and Cr (VI).

The pH of the influent to the hybrid bioreactor (the B1 sampling site in the deposition pool) varied from 7.2 to 8.4 between April 2007 and May 2008. The DO ranged from 0 to 1.8 mg L^{-1} between April 2007 and May 2008. When the hybrid bioreactor was functioning, the pH in the aerobic fluidized bed and the eco-ditch varied from 6.8 to 8.2 and the DO ranged from 7.2 mg L^{-1} to 9.8 mg L^{-1}.

3.2. Microorganisms in the hybrid bioreactor

The microscopic studies showed the presence of bacteria (e.g., *Methanosarcina* spp., diplobacilli, bacilli, *Brevibacterium* spp. and cocci), *Chladophora* sp., diatoms (e.g., *Cyclostephanos dubius*, *Aulacoseira granulate*, and *Stephanodiscus minutulus*) and cyanobacteria (e.g., *Microsystis aeruginosa* and *Aphanizomenon flos-aquae*) within the hybrid bioreactor. These observations indicate that the hybrid bioreactor fosters the simultaneous culture of heterotrophic and autotrophic microorganisms.

3.3. The removal of phosphorus and nitrogen

The average removal efficiencies for the A^2/O process were 60% (range 50–81%) and 54% (range 44–70%) for TP and TDP, respectively (see Fig. 2a, b). The average removal efficiencies for TP and TDP in the eco-ditch during the experimental period were 56% and 48%, respectively (Fig. 2c, d). The overall average TP and TDP removal efficiencies for the hybrid bioreactor were 81% and 74%, respectively.

Between April 2007 and May 2008, the TP and TDP concentrations in the effluent from the hybrid bioreactor ranged from 0.16 mg L^{-1} to 1.01 mg L^{-1} and from 0.09 mg L^{-1} to 0.42 mg L^{-1}, respectively (Fig. 2e, f). The variations of TP and TDP removal efficiencies in the hybrid bioreactor had ranges from 71% to 95% and from 58% to 90%, respectively with the lower values and major fluctuations occurring in the early months of the trial as the

Table 1
The pre-experimental physicochemical parameters of the non-point source wastewater under investigation ($n = 23$).

	pH	DO (mg L^{-1})	TN (mg L^{-1})	NO_3-N (mg L^{-1})	NH_4-N (mg L^{-1})
Mean ± SD	7.6 ± 0.40	1.40 ± 0.30	27.20 ± 5.64	6.39 ± 2.72	18.16 ± 4.87
	TDP (mg L^{-1})	TP (mg L^{-1})	COD (mg L^{-1})	Cr (VI) (μg L^{-1})	As (μg L^{-1})
Mean ± SD	0.66 ± 0.17	2.46 ± 0.80	146.60 ± 45.49	105.32 ± 5.02	113.77 ± 2.52

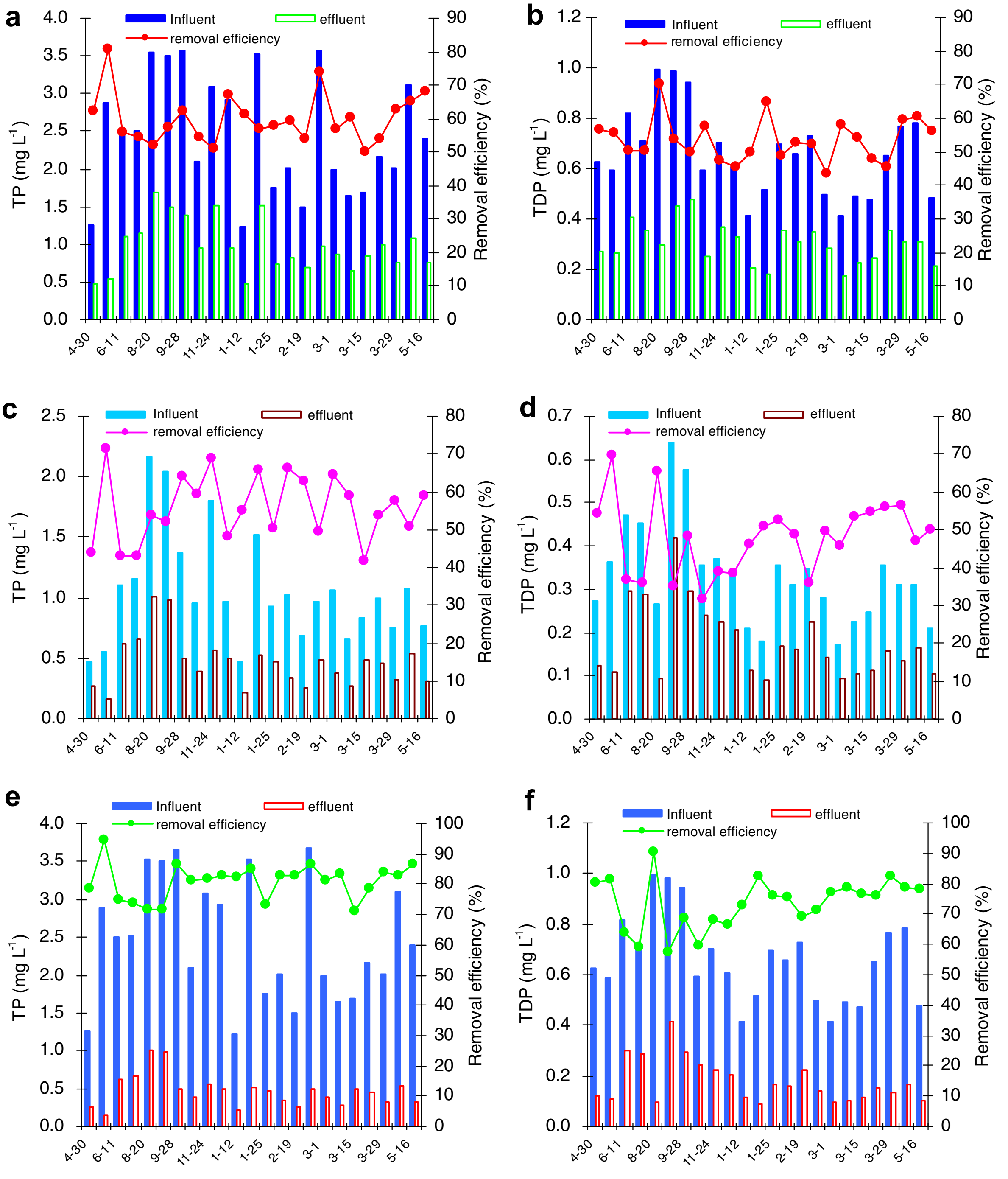

Fig. 2. The removal efficiencies of the A^2/O process (a and b), eco-ditch (c and d), and the hybrid bioreactor (e and f) for total phosphorus (TP) and total dissolved phosphorus (TDP).

systems stabilize (Fig. 2e, f). Overall, the results indicate that the hybrid bioreactor is effective at removing TP and TDP from non-point source wastewater.

The average TN removal efficiency in the A^2/O process was 63% and in the eco-ditch was 52%. For the influent, the TN content was dominated by NH_4-N with the proportion of NH_4-N to TN ranging from 51% to 85%. The average NO_3-N and NH_4-N removal efficiencies in the A^2/O process were 56% and 69%, respectively whilst their removal efficiencies in the eco-ditch were, 53% and 58%, respectively (Table 2).

The overall average removal efficiencies for the hybrid bioreactor were TN (82%), NO_3-N (79%) and NH_4-N (86%). These were steady during the experimental period, with variations in TN, NO_3-N and NH_4-N removal efficiencies ranging from 72% to 89%, 71% to 86%, and 81% to 94%, respectively (Fig. 3), indicating that the hybrid bioreactor also effectively removes TN, NO_3-N and NH_4-N from non-point source wastewater.

Biological measures such as the A^2/O, active sludge processes and membrane bioreactor are preferred measures for purifying wastewater with high nutrient loads because they are highly efficient, cost effective and environmentally friendly (Ahn et al., 2003; Wang et al., 2009). However, the common problem is that when the wastewater contains metals (e.g., As and Cr (VI)) toxic to microorganisms (such as *Gammarus pulex* L.), the nutrient removal efficiency by biological means was negatively affected (Lin et al., 2010; Schaller et al., 2010). In this study, the combination of the A^2/O process and eco-ditch has successfully resolved this problem. This indicates that the hybrid bioreactor is highly effective and has significant practical potential for simultaneously removing nitrogen and phosphorus from such non-point source wastewater.

The average proportions of TP and TDP removed in the A^2/O process compared with the overall removal of TP and TDP were about 70% and 68%, respectively. Similarly, the average proportions of TN, NO_3-N and NH_4-N removed in the A^2/O process compared with their total removal amounts were 76%, 67% and 87%, respectively. These results demonstrate that the nutrients (i.e., TP, TDP, TN, NO_3-N and NH_4-N) are more effectively removed in the A^2/O process than in the eco-ditch.

There are at least two explanations for this. The first is that the concentrations of P- and N- nutrients in the influent to the A^2/O process were higher than those in the influent to the eco-ditch.

Table 2
The concentrations of total nitrogen (TN), nitrate (NO_3-N) and ammonia (NH_4-N) in influent and effluent and their removal efficiencies in A^2/O process and eco-ditch of the hybrid bioreactor from April 2007 to May 2008, respectively ($n = 23$).

		Influent (mg L^{-1})			Effluent (mg L^{-1})			Removal efficiencies (%)
		Max.	Min.	Means ± SD	Max.	Min.	Means ± SD	
A^2/O process	TN	36.62	16.16	27.2 ± 5.64	14.45	5.37	10.2 ± 2.61	62.7 ± 7.20
	NO_3-N	11.79	2.04	6.4 ± 2.72	5.74	0.67	2.9 ± 1.14	55.8 ± 7.11
	NH_4-N	29.99	11.76	18.2 ± 4.88	10.87	2.02	5.7 ± 1.91	69.3 ± 7.80
Eco-ditch	TN	14.41	5.36	10.1 ± 2.61	6.84	2.53	4.7 ± 1.12	52.2 ± 7.94
	NO_3-N	5.70	0.66	2.8 ± 1.14	2.12	0.28	1.3 ± 0.49	53.4 ± 4.76
	NH_4-N	10.84	2.01	5.5 ± 2.06	4.44	1.02	2.4 ± 0.96	58.1 ± 7.67

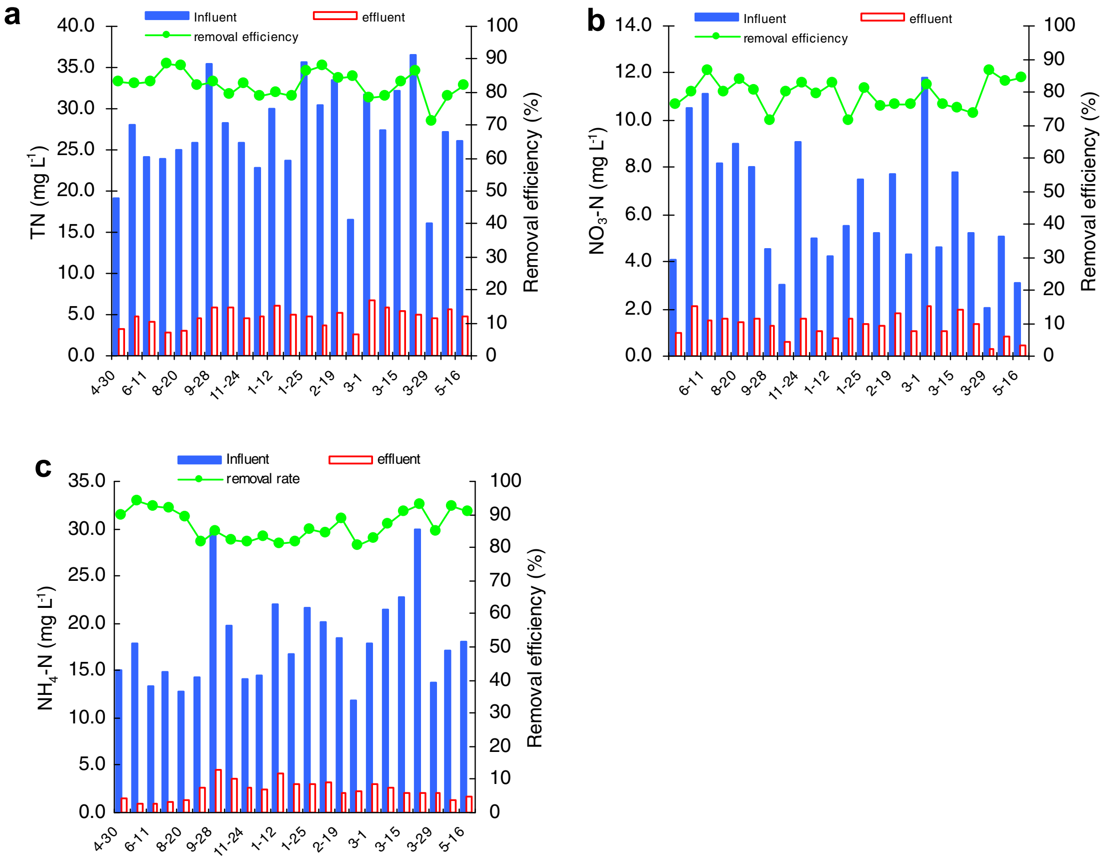

Fig. 3. The removal efficiencies of the hybrid bioreactor for (a) total nitrogen (TN), (b) nitrate (NO_3-N) and (c) ammonia (NH_4-N) from April 2007 to May 2008.

Accordingly, the higher the contaminant concentration in the influent, the greater is its removal from the load (assuming first-order exponential removal processes). The second is that the combination of the deposition pool, anaerobic tank, and anoxic and aerobic fluidized beds is more capable of removing nutrients compared with the eco-ditch. Many studies have shown that the removal efficiencies of the A^2/O process based on biofilm (such as the combination of anaerobic tank and anoxic and aerobic fluidized beds) are greater than those of the eco-ditch under similar hydraulic and nutrient loadings (Ding et al., 2006; Martínez et al., 2000; Nootong and Shieh, 2008).

The overall average ratios of NH_4-N to TN in influent between April 2007 and May 2008 were not significantly different ($p > 0.05$) whilst their proportions in effluent significantly decreased from 68% to 49% ($p < 0.05$). Likewise, the overall average ratios of NO_3-N to TN in influent between April 2007 and May 2008 were not significantly different whilst their proportions in effluent significantly increased from 24% to 30% ($p < 0.05$) (Fig. 3).

The average oxygen level in the influent increased from anaerobic (0 mg L^{-1}) to aerobic (7.2–9.8 mg L^{-1}) levels along the water flow path. This change in the DO level accelerates the nitrification process, oxidizing more NH_4-N into nitrate or nitrite (Nootong and Shieh, 2008). This process led to a significant decrease in the proportion of NH_4-N to TN from 68% to 50% along the sampling sites from B1 to B6.

3.4. The contribution of biofilm to nutrient removal

After the sludge from the A^2/O process was pumped into the eco-ditch, a new layer of high concentration liquid sludge (with sediments) formed immediately, but disappeared within one week. The volume of this liquid discharged into the eco-ditch (based on the pump flux and time) was determined twice during the experimental period, in July 2007 (about 22 m^3) and in March 2008 (43 m^3).

It has been reported that the eco-ditch system is able to treat and minimize sludge (Uggetti et al., 2010; Wang et al., 2010). The direct discharge of high concentration liquid sludge into the eco-ditch of the hybrid bioreactor supplies the nutrients and habitat for native microbes and macrophytes. This process keeps the eco-ditch in a self-maintaining state. Most importantly, the input of sludge from the A^2/O process to the eco-ditch avoids having to build specific facilities for sludge treatment. This process saves on both capital and operational costs.

Biofilm is a basic element in bio-treatment technologies such as the A^2/O process and its biomass correlates directly with the removal efficiency of pollutants (Ding et al., 2006; Martínez et al., 2000). The biofilm mass in the A^2/O process tanks of the hybrid bioreactor was calculated to be between 152 ± 7.6 and 157 ± 20.4 kg in 2007 and from 184 ± 18.4 to 193 ± 28.9 kg in 2008. The biofilm biomass in the eco-ditch was kept between 60 ± 14.3 and 65 ± 15.4 kg in 2007 and from 85 ± 20.7 to 91 ± 22.5 kg in 2008 (Fig. 4a).

On two occasions during the study period, the biofilm biomass in the A^2/O process was compared with that in the eco-ditch. The majority of nutrients (about 60–92%) were removed in the A^2/O process of the hybrid bioreactor and the distributions of biofilm biomass and nutrient removal in the A^2/O process appear to be associated. For example, the biofilm mass tends to concentrate (via biosorption, digestion, precipitation and bioaccumulation) relatively more nitrogen and phosphorus. Indeed, analyses showed that there were significant relationships between the removal loads of nutrients (i.e., nitrogen and phosphorus) and the biofilm biomass in A^2/O process. Their relationships are as follows: [N load removed (kg)] = 0.311 × [Biofilm mass (kg)] − 33.68 ($n = 6$, $R^2 = 0.928$, $p < 0.05$); [P load removed (kg)] = 0.02 × [Biofilm mass (kg)] − 1.708 ($n = 6$, $R^2 = 0.607$, $p < 0.05$). Moreover, the average proportions of nutrients removed by the eco-ditch decreased with time from June 2007 to May 2008 despite the flourishing growth of the macrophytes. Therefore, it was surmised that the biofilm in the hybrid bioreactor was the main contributor to nutrient removal.

Due to the porous structure of biofilms (Wimpenny and Colasanti, 1997), the pollutants can be adsorbed to or detached from the active sites on the biofilm surface. This allows the nutrients being freely transported into the biofilms where they are transformed or sequestered (Scinto and Reddy, 2003). Indeed, biofilm is an important means for removal of pollutants (such as excessive nitrogen and phosphorus) that can adversely affect food webs (Aouad et al., 2006; Hill and Larsen, 2005), as well as bioconcentrating inorganic and organic nutrients from the surrounding water (Haack and Warren, 2003; Quintelas et al., 2009). Therefore, it is concluded that the nutrient removal from non-point source wastewater is primarily due to the adsorption and assimilation onto/into the biofilms.

3.5. The response of bacteria to water quality improvement

To evaluate the response of bacterial communities to the experimental conditions, the Dice index of similarity of ERIC-PCR

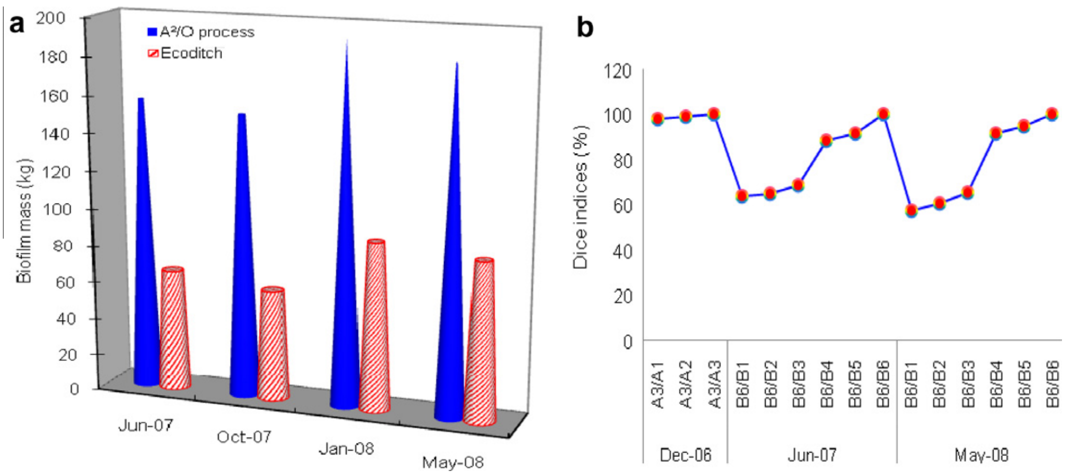

Fig. 4. (a) The biofilm biomasses (25–30 °C, moisture ~85%) in the A^2/O process and eco-ditch of the hybrid bioreactor (means ± SD, kg) ($n = 15$), and (b) Dice index of similarity (%) of ERIC-PCR fingerprints for biofilm bacterial communities.

fingerprints for biofilm bacteria was investigated before and after the hybrid bioreactor experiment was conducted. Before the construction of the engineering project, the non-point source influent directly entered into a ditch (subsequently becoming the location of the deposition pool to eco-ditch in the experiment). The Dice indices in sampling sites A1, A2, and A3 were all above 98%, indicating that the bacterial community structures in these three sampling sites were very similar. This result shows that the non-point source wastewater was 'biologically homogeneous' before the hybrid bioreactor was commissioned.

During the running of the hybrid bioreactor there were obvious differences in the bacterial community structures between the anaerobic–anoxic treatment phases (deposition pool, anaerobic tank and anoxic fluidized bed) and the aerobic treatment phases (aerobic fluidized bed and eco-ditch). The Dice indices of similarity for the bacterial communities between the sampling sites B1–B3 (anaerobic and anoxic phase) and B4–B6 (aerobic phase) ranged from only 58%–73%, suggesting that the hybrid bioreactor had a large impact on the composition of the bacterial community.

The Dice indices of similarity for the bacterial communities along the sampling sites from B1 to B6 increased from 64% to 100% in June 2007 and from 58% to 100% in May 2008. In addition, the rates of index increase in June 2007 were very similar to those in May 2008 (Fig. 4b). Furthermore, the results observed with microscope showed that the microorganism (i.e., bacteria) density in the water in B4–B6 sampling sites (anoxic phase) was markedly higher than that in the B1–B3 sampling sites (anaerobic and anoxic phase). These findings implied that the bacterial habitats improved along the sampling sites.

This optimization of the bacterial community structure was due to the sequential improvement of water quality with the sampling site distance and time. Indeed, previous studies have shown that the bacterial community structure changes in parallel with the environmental factors, such as nutrient load, DO, pH and the presence of heavy metals (Ciric et al., 2010; Nyyssonen et al., 2009). In this study, the oxygen content significantly increased in the aerobic fluidized beds and eco-ditch, which explains the different microbial communities in these processes. Moreover, the nutrient concentrations decreased with the increasing distance along the sampling site, another reason that may explain the improved bacterial community structure. In turn, the more robust bacteria enhance the efficiency of removal of contaminants such as excessive nitrogen and phosphorus. In many cases, the efficiency of removal of contaminants (such as nitrate) is directly associated with bacterial community structure and activities (Rajakumar et al., 2008).

4. Conclusion

The employment of the environmentally benign hybrid bioreactor simultaneously supports the culture of both heterotrophic and autotrophic microorganisms, and efficiently removes TN, NO_3-N, NH_4-N, TP and TDP. The sludge from the A^2/O process of the hybrid bioreactor discharged into the eco-ditch, in turn maintained the eco-ditch in a self-sustaining state. The application of the hybrid bioreactor improved the bacterial habitat, in turn confirming the improvement in water quality. This study provides a promising, highly-effective and easily-deployed bio-measure to remove high-load nutrients from non-point source wastewater on an industrial scale as well as enhancing bacterial habitats.

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