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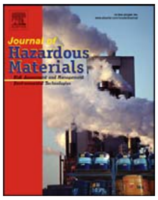

Removal of $UV_{254\,nm}$ matter and nutrients from a photobioreactor-wetland system

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ABSTRACT

The output of organic pollutants and excessive nutrients in intensive agricultural areas has frequently occurred, which easily lead to pollution events such as harmful algal blooms in downstream aquatic ecosystems. A photobioreactor-wetland system was applied to remove $UV_{254\,nm}$ matter and dissolved nutrients discharged from an intensive agricultural area in the Kunming region of western China. The photobioreactor-wetland system was composed of two main components: an autotrophic photobioreactor with replanted macrophytes and a constructed wetland. The results showed that there was a significant correlation between $UV_{245\ nm}$ absorbance and chemical oxygen demand (COD) concentration in the effluent of the agricultural ecosystem. When the hydraulic load of the photobioreactor-wetland system was 500 m^3 day^{-1}, the $UV_{254\,nm}$ absorbance was dramatically reduced, and dissolved nutrients such as TDP, NO_3–N and NH_4–N were effectively removed. The overall average removal efficiencies were as follows in relatively steady-state conditions: $UV_{254\,nm}$ matter (66%), TDP (71%), NO_3–N (75%) and NH_4–N (65%). Simpson's diversity index of zoobenthos indicated that the system could increase the zoobenthic diversity and improve the growth conditions of the zoobenthos habitat. The results also showed that the photobioreactor-wetland system could remove the $UV_{254\,nm}$ matter and dissolved nutrients, providing a promising bio-measure for reducing the risk of pollution event occurrences in downstream surface waters.

1. Introduction

Chemical fertilizers, pesticides, herbicides and plant hormones are often applied in intensive agricultural areas to maintain high yields [1]. These chemicals usually constitute the majority of non-point-source pollutants to the downstream surface aquatic ecosystems when they are carried either by runoff or irrigation effluent [2]. These are some of the most predominant pollution sources causing eutrophication and harmful algal blooms [3]. Furthermore, the migration of some organic contaminants, such as polycyclic aromatic hydrocarbons, into aquatic ecosystems poses a hazardous threat to some beneficial animals such as tadpoles [4,5] and destroys the food web and the balance of the aquatic ecosystem. Thus, the reduction of the inputs of organic contaminants and excessive nutrients into downstream aquatic ecosystems has a practical significance.

Many measures have been proposed to decrease the amount of organic contaminants and nutrients in aquatic ecosystems [6]. These measures include (1) the reduction of the production of potential pollutants (e.g., reducing usage of agrochemicals) [7], (2) the reduction of the migration of pollutants (e.g., improving irrigation management) [8] and (3) the acceleration of the sequestration and degradation of pollutants toward aquatic ecosystems (e.g., buffer zones and wetlands) [9]. Many specific technologies have been developed for the final treatment in the removal of aromatic compounds, and various measures have been introduced, such as the application of soybean peroxidase [10], the utilization of ozone and photocatalytic processes [11], bioremediation via specific aromatic compound-degrading microorganisms [12], and physical sequestration by clays [13] and powdered activated carbon [14].

The aforementioned measures/technologies are useful and have great benefits to downstream environments. However, several new, complex problems have arisen due to the introduction of some 'modern' farming techniques. For example, aromatic compounds have been brought into the soil with the application of some new pesticides, herbicides and phytohormones, such as mecoprop

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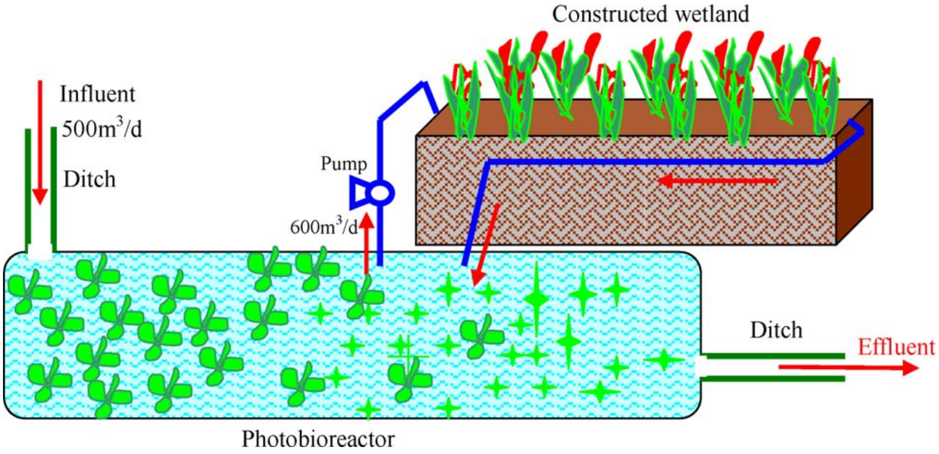

Fig. 1. A schematic model of the photobioreactor-wetland system. The influent load of the photobioreactor-wetland system is 500 m^3 day^{-1} on non-rainy days (rainfall < 10 mm). The load of the wetland is 600 m^3 day^{-1}. The influent of the wetland is from the photobioreactor, which in turn flows into the photobioreactor through the wetland outlet after purification by the wetland, thereby discharging the effluent of the photobioreactor-wetland system.

[2-(2-methyl-4-chlorophenoxy) propionic acid] [15] and auxin [16]. These aromatic compounds can move into the downstream aquatic ecosystems through runoff and irrigation. In addition, the long-term applications of chemical fertilizers have caused the degradation of soil quality and the decline of nutrient-holding capacities [17]. This degradation might lead to excessive dissolved nutrients, such as dissolved nitrogen and phosphorus, flowing easily into downstream aquatic ecosystems, increasing the risk of eutrophication. To reduce the input of these organic contaminants and dissolved nutrients into downstream aquatic ecosystems, these pollutants should be removed by environmentally friendly bio-measures at the downstream catchments of intensive agricultural areas, thus improving the self-purifying capacities of aquatic systems.

We therefore proposed a photobioreactor-wetland system and utilized the technology based on this system at the downstream catchments of an intensive agricultural area in the Kunming region of western China, where the self-purifying capacity had been weakened. To provide an amplified model of the photobioreactor-wetland system at an industrial scale, three additional considerations should be taken into account: (1) the measure should be environmentally friendly, requiring that it not bring any hazardous materials or artificial chemicals into the environment; (2) the habitats of native flora and fauna should be recovered, and then the self-purifying capacity of the recovered ecosystem should be improved, and (3) the construction and operation of the photobioreactor-wetland system should be simple. The capital and operation costs should be affordable.

2. Materials and methods

2.1. Description of the photobioreactor-wetland system

A photobioreactor-wetland system was devised to remove organic matter and dissolved nutrients from upstream areas of surface waters and consisted of two parts (Fig. 1): a photobioreactor and a constructed wetland.

The photobioreactor covered an area of 1800 m^2 (180 m × 10 m) with a mean depth of ~1.7 m and a maximum depth of 2.6 m. Several aquatic plant species reported to have a high capacity for removing organic contaminants, including *Salix rosmarinifolia* L., *Myriophyllum verticillatum* L., *Pistia stratiotes* L., *Hydrilla verticillata* (L. f.) Royle, *Typha latifolia* L., *Zizania latifolia*, were introduced into the photobioreactor from August to October 2006. During the experimental period, about 85–90% of the water area of the photobioreactor was covered by *M. verticillatum* L. and *P. stratiotes* L. A complete list of the introduced aquatic plants is shown in Table 1.

To purify the water in the photobioreactor, a constructed wetland area of 800 m^2 was built. The construction included subsurface flow and complete effluent percolation through artificial substrates. At a distance of 4.5 m close to the photobioreactor, a wetland was built that was 20-m wide, 40-m long, and 1.5-m deep and was also attached to the photobioreactor. The 20-cm gravel (diameter: 15–25 mm) columns were filled at the bottom with a mixture of clay and sand layers of 60 cm (depth) for plant support. The six macrophytes, *Cyperus alternifolius, Scirpus tabernaemontani,*

Table 1
The species, quantities and sources of young plants used to restore the macrophyte ecosystem.

	Species	Quantities (young plants)	Sources of young plants
1	*Salix rosmarinifolia* L.	600	The aquatic willows (length 30–50 cm, diameter ≈1.5 cm) were collected from an area near the experimental photobioreactor
2	*Myriophyllum verticillatum* L.	18,000	The minor materials were collected from ditches and wetlands near the experimental area
3	*Pistia stratiotes* L.	4000	40,000 were collected from Dianchi Lake, southeast China
4	*Hydrilla verticillata* (L. f.) Royle	12,000	Collected with silt from a cultural area near east of the Caohai Lake
5	*Typha latifolia* L.	5000	From a ditch near the experimental photobioreactor
6	*Zizania latifolia* (Griseb.) Stapf.	3000	From a ditch and wetland near the experimental photobioreactor
7	Other macrophytes[a]	20,000	From a ditch, photobioreactor and wetland near the experimental area

[a] Other macrophytes include *Arundo donax* L. var. 'Versicolor', *Cyperus alternifolius* L., *Iris pseudacorus* L., *Pontederia cordata* L., *Sagittaria pygmaea Miq.*, *Scirpus validus* cv. *Zebrinus*, *Thalia dealbata* Fraser ex Roscoe.

Juncus effuses, Canna indica Linn, Pontederia cordata and *Acorus gramineus Soland*, were planted at a density of 9 rhizomes m^{-2} with the same proportion.

Filter-feeding fish (i.e., inhabiting different water depths) were added to the photobioreactor in order to keep the autotrophic photobioreactor in a self-modulated and self-sustained state. Accordingly, 200 kg of Chub (*Squaliobarbus curriculus*) and 500 kg of Bighead Carp (*Hypophthalmichthys nobilis*) fingerlings with a length of 10–12 cm were introduced into the photobioreactor during the period from August to December 2006.

The wastewater (influent) directly flowed into the photobioreactor from a ditch in the intensive cropland. The hydraulic load of the experimental photobioreactor was 500 m^3 day^{-1} on non-rainy days (rainfall < 10 mm) and the hydraulic retention time (HRT) was about 4.5 days. The inflow load of the wetland was 600 m^3 day^{-1}, and the hydraulic retention time (HRT) was 9.6 h. The wetland inflow was pumped from the photobioreactor while the outflow of the wetland was flowed back into the photobioreactor and sequentially discharged into the downstream ditch (Fig. 1).

2.2. Sampling and analytical methods

Water samples in triplicate (i.e., both influent and effluent) were collected from June 2006 to August 2008. The chemical oxygen demand (COD) in the water samples was measured by the potassium dichromate method. The nitrate nitrogen (NO_3–N), ammonia nitrogen (NH_4–N) and total dissolved phosphorus (TDP) in water were determined using the standard methods of the APHA [18]. The samples for UV_{254} absorbance were filtered before being measured to eliminate the variations in UV absorption, which were caused by suspended particulate matter [19]. In this study, the $UV_{254\,nm}$ matter refers to all matters in the water having absorbance at 254 nm wavelength, including xenobiotics chemicals and natural compounds (e.g., natural humic substances). The dissolved oxygen (DO) and pH levels in water were measured *in situ* by a multi-meter (YSI 52 dissolved oxygen and pH meters).

The sediment samples in triplicate, which were collected from the outlet of the photobioreactor (i.e., the inlet of the downstream ditch of the photobioreactor), were used to investigate the zoobenthos habitats. Each sample consisted of five grabs with surface areas of 50 cm^2 and depths of 10 cm. All fresh sediment samples were sieved through a 4.2-mm mesh to homogenize them. The homogenized samples were sieved through a 1.0-mm mesh to collect the zoobenthos. The material retained on the mesh was preserved in formalin (final concentration in sample: 8%). The intact zoobenthos were identified and counted. Identification procedures were similar to those of previous methods [20].

All water and sediment samples were collected 3 days after rainy days (rainfall ≥ 10 mm/d) to avoid the impact of precipitation on the data.

2.3. Data analyses

Simpson's diversity index (*D*) [21] was used to indicate the biodiversity of the photobioreactor habitat. The Simpson's diversity index was used as a community descriptor that represents the probability that two randomly selected individuals in the habitat belong to the same species [21]. When $D < 0.25$, the habitat is extremely polluted; when $0.25 \le D < 0.50$, the habitat is heavily polluted; and when $0.50 \le D < 0.75$, the habitat is moderately polluted. When *D* is between 0.75 and 1.00, the pollution level decreases from slightly polluted to clean [22].

The SPSS statistical software package (version 12.0) was used to analyze the data, and the level of statistical significance was set at $p = 0.05$. Statistically, significant differences between the results were evaluated on the basis of standard deviation determinations and analysis of variance (ANOVA). The linear correlation between $UV_{254\,nm}$ absorbance and COD was analyzed by the Pearson correlation.

3. Results

3.1. Characteristics of influent

Analyses of the influents showed that there was a good linear correlation between $UV_{254\,nm}$ absorbance and COD concentration ($p < 0.05$). The linear equation used was Eq. (1) when the COD concentration was in the range of 11.3 mg L^{-1} to 180.4 mg L^{-1} (Fig. 2). Considering the measurement error of $UV_{254\,nm}$ absorbance caused by suspended matter in water samples, the $UV_{245\,nm}$ absorbance was calculated based on the COD concentration in this study as follows:

$$UV_{254\,nm}\ \text{absorbance} = 0.0033\text{COD} + 0.0755 \qquad (1)$$

3.2. The performance of the photobioreactor-wetland system

During the experiment, the DO in effluent was kept between 6.63 mg L^{-1} and 9.31 mg L^{-1}, and the pH ranged from 7.46 to 8.52. The average DO and pH values of the effluent were about 3.4 mg L^{-1} and 0.2 higher than those in the influent, respectively.

Before the photobioreactor-wetland system began running (from June to December 2006), the removal rate of $UV_{254\,nm}$ matter was low, at 10–24%. However, during the running period of the photobioreactor-wetland system, the $UV_{254\,nm}$ matter removal rates increased with time from 20% to 61% between January and November 2007 (adaptation period) and stabilized within a range of 60–81% between December 2007 and July 2008 (stabilized period) (Fig. 3a). Furthermore, the overall average removal rate during this stabilized period was 66%, higher (by ~48%) than that at the beginning of the experiment to December 2006 (building period) (Fig. 3a). The analysis showed that the average removal rate of $UV_{254\,nm}$ absorbance in the stabilized period was significantly different from that during either the building period or the adaptation period ($p < 0.05$).

The overall average TDP removal rate was 3% before the photobioreactor-wetland system was run. The variation of the TDP removal rate in the adaptation period (i.e., from January to November 2007) was very large, ranging from 30% to 87% (Fig. 3b). The overall average TDP removal rate in the stabilized period (i.e., from December 2007 to July 2008) was significantly (71%) higher than those in the building and adaptation periods by 68% and 13%, respectively ($p < 0.05$).

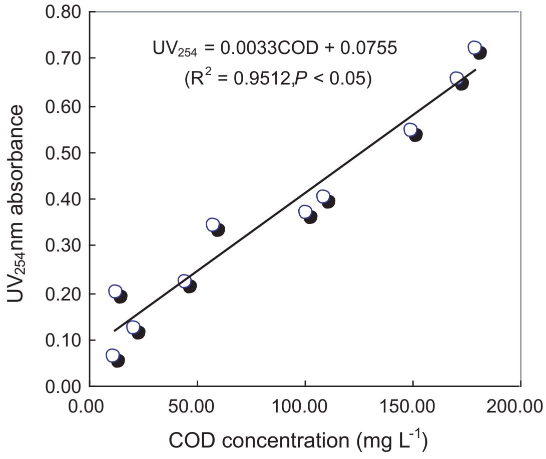

Fig. 2. The correlation between the $UV_{254\,nm}$ absorbance and the COD concentration in the influent ($n = 10$).

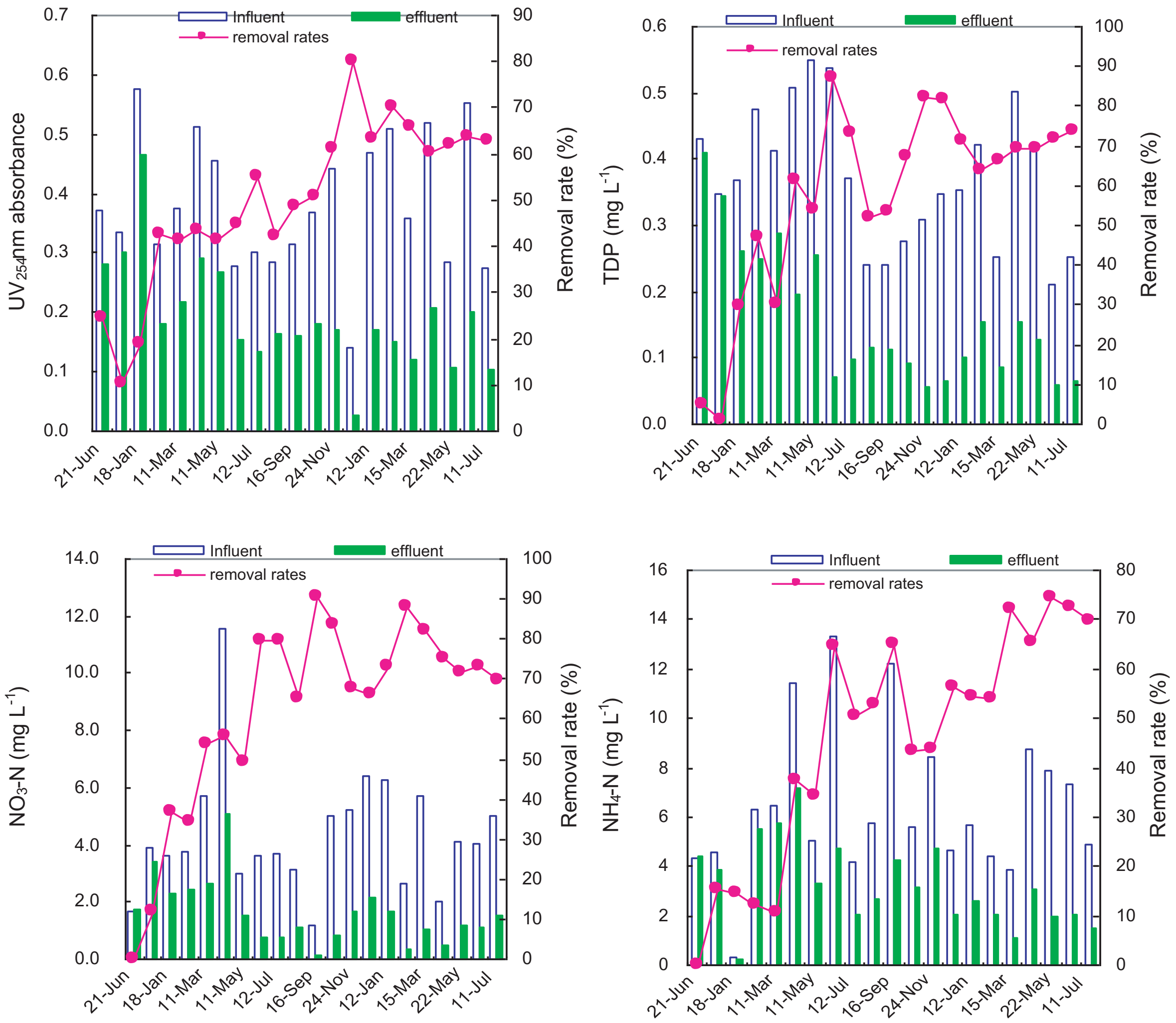

Fig. 3. The removal efficiency of the photobioreactor-wetland system for treating $UV_{254\,nm}$ matter, TDP, NO_3–N and NH_4–N. The values from June to December 2006 were control values. The period from January to November 2007 was the adaptation period of the photobioreactor-wetland system. The photobioreactor-wetland system was operated under steady-state conditions after December 2007.

The overall average NO_3–N removal rates ranged from 0% to 12% before the photobioreactor-wetland system began running (Fig. 3c). The overall average NO_3–N increased from 37% to 91% between January and September 2007, followed by a rapid decrease from 91% to 68% between September and November 2007. During this adaptation period of the photobioreactor-wetland system, the overall average NO_3–N removal rate was 63% (Fig. 3c). It is worth noting that the removal rate remarkably increased from 65% to 88% between December 2007 and February 2008 and then rapidly decreased from 81% to 70% between March and July 2008. During this stabilized period, the overall average NO_3–N removal rate was 75% (Fig. 3c). There were significant differences in the overall average NO_3–N removal rates among the building, adaptation and stabilized periods of the photobioreactor-wetland system ($p < 0.05$).

The overall average NH_4–N removal rate was 8% during the period when the photobioreactor-wetland system was running, while the overall average NH_4–N removal rates were 39% and 65% during the adaptation and stabilized periods of the photobioreactor-wetland system operation, respectively (Fig. 3d). Analysis showed that the overall removal rate in the stabilized period of the photobioreactor-wetland system was significantly different from that in either the building or adaptation period ($p < 0.05$). The fluctuations of NH_4–N removal rates in the adaptation period were very large (ranging from 10% to 72%), while the changes of the NH_4–N in the stabilized period were relatively steady (ranging from 54% to 75%).

The capital cost of the photobioreactor-wetland system was estimated based on the local price level, which ranged from 100 to 120 US dollars per cubic meter of water, and the operation cost was about 0.015 US dollars per cubic water.

3.3. *Response of zoobenthos to habitat conditions*

The species composition and biomass of the zoobenthos were examined in August 2006. Only 15 species of zoobenthos in 12 families were found at the inlet of the downstream ditch of the photobioreactor (Table 2). The average density and biomass of the zoobenthos were 960 individuals m^{-2} and 245 g m^{-2}, respectively. The species composition and biomass of zoobenthos were also examined in August 2008. The average density and biomass of zoobenthos were 735 individuals m^{-2} and 214 g m^{-2}, respectively. *Radix auricularia*, *Stratiomys* sp. and *Sphaerodema* sp. were not found in August 2008. However, five species of zoobenthos, *Procladius choreus*, *Parakiefferella* sp., *Tanytarus* sp., *Procladius* sp., and *Cipangopaludina dianchiensis* appeared for the first time (Table 2).

To assess the effect of our technology on sediment conditions and water quality, Simpson's diversity indices of zoobenthos were calculated. Fig. 4 shows that the pollution levels of zoobenthos habitats decreased between August 2006 and August 2008. In addition,

Table 2
The species of zoobenthos identified in the outlet of the photobioreactor during August 2006 and 2008.

Number	Zoobenthos species in August 2006	Zoobenthos species in August 2008
1	*Glossiphonia complanata*	*Glossiphonia complanata*
2	*Lumbriculus variegates*	*Lumbriculus variegatus*
3	*Cipangopaludina ventricosa*	*Cipangopaludina ventricosa*
4	*Ampullaria gigas spix*	*Ampullaria gigas spix*
5	*Margarya monody*	*Margarya monodi*
6	*Bellamya aeruginosa*	*Bellamya aeruginosa*
7	*Bellamya purificata*	*Bellamya purificata*
8	*Radix auricularia*	/
9	*Radix swinhoei*	*Radix swinhoei*
10	*Radix ovate*	*Radix ovata*
11	*Galba pervia*	*Galba pervia*
12	*Einfeldia* sp.	*Einfeldia* sp.
13	*Tokumagayuswika akamusi*	*Tokumagayuswika akamusi*
14	*Stratiomys* sp.	/
15	*Sphaerodema* sp.	/
16	/	*Procladius choreus*
17	/	*Parakiefferella* sp.
18	/	*Tanytarus* sp.
19	/	*Procladius* sp.
20	/	*Cipangopaludina dianchiensis*

the results of the Simpson diversity indices showed that the habitats of zoobenthos in the inlet of the downstream ditch of the photobioreactor improved from moderately polluted to slightly polluted.

4. Discussion

$UV_{254\,nm}$ absorbance has been used to monitor industrial wastewater effluents and organic pollution indicators such as lignin, tannin, humic substances, and carious aromatic compounds [23]. In this study, the influent was heterogeneous wastewater, which was combined with irrigation water, chemical fertilizers, pesticides, herbicides and phytohormones. In some cases, when the $UV_{254\,nm}$ represented the aromatic content of wastewater, the changes in the COD and aromatic content in the same wastewater samples were very similar [24,25]. The significant correlation between the $UV_{245\,nm}$ absorbance and COD concentration implies that the $UV_{254\,nm}$ matter in this study might have originated primarily from aromatic compounds.

Aromatic compounds are a class of organic compounds that includes benzene ring molecular structures such as phenol, acetophenone and isopropylbenzene [26]. In addition to the natural origin of these compounds, anthropogenic input such as excessive uses of various agrochemicals, such as pesticides, herbicides and phytohormones in agricultural fields, has contributed most to the introduction of aromatic compounds to environments. These aromatic compounds pose potential risks to humans and ecosystems [27]. Thus, it is important to study the removal of these compounds in agricultural areas, especially in the upstream areas of surface waters.

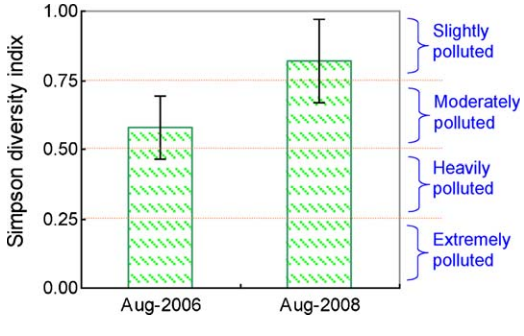

Fig. 4. The assessment of the habitats of zoobenthos by the Simpson biodiversity index in the outlet of the photobioreactor.

The proposed photobioreactor-wetland system showed a promising potential in the removal of aromatic compounds for the dramatic reduction of $UV_{254\,nm}$ absorbance in the stabilized period (Fig. 3a). In addition, the system exhibited some advantages in the removal of aromatic compounds. For instance, the removal of aromatic compounds in this system is a bio-process that brings either a minimum amount or no amount of potentially hazardous materials into environments. Because it is inexpensive, effective and easily deployed, the photobioreactor-wetland system can be deployed to remove aromatic compounds in undeveloped regions such as rural areas in developing countries.

The removal rate of TDP increased with the duration of the adaptation period of the photobioreactor-wetland system due to the bio-accumulation (including uptake, assimilation and biosorption). TDP is a preferential form of phosphorus-nutrients in organisms. The depletion of TDP in water increased with the prosperity of native organisms such as macrophytes and biofilms [28]. When the biomass of macrophytes and biofilms increased at a stable level, the demand of P-nutrients for these organisms was kept at a specific level. For this reason, the TDP removal rates in the stabilized period of the photobioreactor-wetland system were kept at a relatively steady state.

The TDP removal rates rapidly decreased during the adaptation period between June and November 2007. This decrease might be attributed to the slowed growth of plants and the accelerating mineralization of dead plant tissues. The term "extractable biogenic phosphorus" has been proposed [29] because algae- and bacteria-produced inorganic phosphorus such as polyphosphate [30] are also included in this pool. Similarly, the lower the anoxic level, the larger the rate of decrease of the organic phosphorus content [19]. This means that the anoxic conditions at the bottom of the constructed wetland have converted the available organic phosphorus into inorganic phosphorus. This conversion might have occurred because of the transformation of the pioneer bacterial community caused by the photobioreactor-wetland system [31].

One of the reasons for the increased removal rates of NO_3–N during the adaptation period of the photobioreactor-wetland system is that the NO_3–N was extracted by the growing macrophytes. Multiple regression analysis indicated that NO_3–N and DO concentrations were significant predictors of denitrification rate; the increased DO concentration in water can accelerate the process of denitrification–nitrification [32]. This suggests that the removal of NO_3–N was associated with the increased DO content in water during the operation of the photobioreactor-wetland system.

Previous reports showed that the rein habitation of aquatic plants (i.e., submerged macrophytes and phytoplankton) can have a positive impact on pH in water during periods of high productivity [33]. When pH levels become greater than 7, ammonium (NH_4^+) can be spontaneously converted into ammonia gas (NH_3), which allows it to diffuse from the vicinity of the air–water interface [34]. Considering that the NH_4–N removal rate increased with time, it is likely that most ammonia losses in the photobioreactor-wetland system are due to the uptake and assimilation of macrophytes and phytoplankton as well as to the irreversible diffusion of gaseous ammonia from the water. Indeed, both macrophyte and phytoplankton uptake are integral to the treatment within the photobioreactor [35]. The decreases of NH_4–N removal rates during winter (3–18 °C, from November 2007 to February 2008) revealed that the NH_4–N removal rate decreased with the decline in temperature. This finding is consistent with previous studies showing that the rate of ammonia removal decreased with the decrease in temperature in biological treatment systems [36].

When the photobioreactor-wetland system was in operation, the organic debris became fine [37] due to disturbance by fish and the cluster of macrophytes [38], which leads to the debris not reaching the bottom as freely as it did previously. As a result, the water quality improved with the decrease of $UV_{254\,nm}$ matter (aromatic compounds) and nutrients. The emergence of five new species in the inlet of the upstream ditch of the photobioreactor (Table 2) provided further evidence of water quality improvement because these species only thrive in clean water. Subsequently, several species of zoobenthos whose food consisted of macro-organic debris faced famine and died. In addition, the species of zoobenthos that had disappeared in the early part of the experiment reappeared when the water became clear [22,37]. This is the reason that the total density and biomass of zoobenthos in the outlet of the photobioreactor decreased. In addition, the fish (i.e., older filter-feeding fish) ate some of the zoobenthic animals [39] at the same time that the less-dominant species died out. The replanted macrophytes became the habitat for zoobenthos and zooplankton, which led the dominant species to recover and grow [31].

5. Conclusion

This study showed that the influent could be characterized using the $UV_{254\,nm}$ matter. There was a significant correlation between the $UV_{254\,nm}$ absorbance and COD concentration in the influent, implying that the $UV_{254\,nm}$ matter might originate mainly from aromatic compounds. The proposed photobioreactor-wetland system was implemented on a pilot scale for two years and proved to be highly effective in removing $UV_{254\,nm}$ matter and dissolved nutrients such as TDP, NO_3–N and NH_4–H. The increasing Simpson biodiversity indices of zoobenthos suggested that the photobioreactor-wetland system could provide a suitable habitat for zoobenthos due to the reduction of both organic contaminants and nutrients. This system is simple in terms of its construction, operation and maintenance; therefore, it is suitable for use in rural areas to prevent the risk of pollution event occurrences such as eutrophication and harmful algal blooms in downstream surface waters.

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