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PURIFICATION OF REFINERY WASTEWATER BY DIFFERENT PERENNIAL GRASSES GROWING IN A FLOATING BED

Hong Li,[1] Hulin Hao,[1] Xiaoe Yang,[1] Lvcheng Xiang,[1] Fengliang Zhao,[1] Hong Jiang,[1] and Zhenli He[2]
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□ *Four species of perennial grasses [*Geophila herbacea O Kumtze (GHK), Lolium perenn *CV. Caddieshack (LCC),* Lolium perenne *Topone (LPT) and* Lolium perenne *L. (LPL)] were used to remove nutrients and pollutants from refinery wastewater, of which the average concentration of total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD_{Mn}), total petroleum hydrocarbons (TPH) were 15.6 mg L^{-1}, 0.6 mg L^{-1}, 142.8 mg L^{-1} and 1720 mg L^{-1}, respectively. These perennial grasses had performed extremely well in removing the nutrients and pollutants and were capable of removing up to 60% of TN and 56% of TP from the refinery wastewater during 35 days treatment period, to which plant uptake contributed only 5%–10% and 10%–20%. The removal rates of COD_{Mn} and TPH were fluctuating within 52% to 67% and 40% to 55%, respectively. The nutritional value of these perennial grasses as animal feeds such as crude protein, crude fat, crude fiber, crude ash and nitrogen-free extraction after growing in refinery wastewater for 35 days were analyzed and the results suggested that these indicators were superior to national animal feed thresholds, with its nitrites and several heavy metals including zinc (Zn), arsenic (As), cadmium (Cd), and lead (Pb) concentration far below the thresholds. We concluded that perennial grass floating-bed system was effective for purifying refinery wastewater during its growing season and harvested perennial grass had great value as animal feeds.*

Keywords: floating bed, nutritional value, perennial grass, phytoremediation, refinery wastewater

INTRODUCTION

Petroleum hydrocarbons from storage of crude oil, spills, wash downs and vessel clean-outs from processing operation often cause contamination

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to water bodies, especially seawater (Chavan and Mukherji, 2008). The effects of oil spill pollution on ecosystem degradation have been widely studied (Duke and Pinzon, 1997; Abuodha and Kairo, 2001) and petroleum hydrocarbon degradation and restoration have also been widely reported (Mohanty and Mukherji, 2008; Mukherji et al., 2004). Physical and chemical methods were the most widely used for the treatment of oil-based wastes, including use of coagulants and coagulant aids (Rebhun et al., 1992), ultra filtration (Elmaleh and Ghaffo, 1996) and combination of coagulation and centrifugation (Tense and Regula, 2000). Although physical collection of the oil was generally the first priority of responders, these methods were inadequate and ineffective (Abu and Ogiji, 1996), and may result in secondary contamination of the environment (Sufferman, 1991).

Bioremediation has emerged as one of the most promising secondary treatment options for oil removal (Zhu et al., 2001). It offers a less ecologically damaging alternative by taking advantage of the oil-degrading microbes (Xia et al., 2006) and by establishing and maintaining the physical, chemical and biological conditions that favor enhanced oil biodegradation rates in the contaminated environment (Zhu et al., 2001). As a result, bioremediation is considered one of the most effective measures to deal with the pollution of natural water bodies because of its high efficiency and low cost (Guo, 2005).

Most research has focused on remediating oil contamination with microbes, which could be cultivated or genetically engineered microorganism. Unfortunately, the majority of microorganisms cannot survive in pure culture conditions, even if some isolated organisms can effectively degrade single pollutant under lab-conditions, they may cease to function when introduced into field conditions with multiple pollutants (Quan et al., 2003; Singer et al., 2005). In addition, the introduced strains may not be able to compete with indigenous microorganisms (Das and Mukherjee, 2007; Mohanty and Mukherji, 2008). During the process of phytoremediation, the interactions between plant roots and microorganisms may help purify wastewater (Calheiros et al., 2009). However, studies demonstrating phytoremediation of organic chemical-rich wastewater are limited and further research is needed to test the efficiency of this remediation system.

Previous studies mostly focused on phytoremediation of eutrophic water and a great quantity of highly efficient plant species have been reported (Table 1). Though bioremediation of wastewater and soil contaminated by petroleum hydrocarbons has been reported, it is usually considered as a secondary treatment option and study on phytoremediation of refinery wastewater is urgently needed. Of importance, and seldom recognized, is the fact that plant root-microbe interactions may enhance the pollutant removal efficiency as well as their different contribution to nutrient removal.

Therefore, the present study was attempted to: (1) investigate the purification efficiency of four species of perennial grasses for refinery wastewater, (2) examine the nutritional value of these grasses as animal feeds after

TABLE 1 Reported plant species with high nitrogen and phosphorus removal efficiency

Variety	Nutrient removal efficiency	Reference
Jussiaen repens L.	TN 63.05%, TP 50.43%	Wu et al., 2007
Eichhornia crassipes	TN 57.73%, TP 53.62%	Wu et al., 2007
Oenanthe javanica (Blume) DC.	TN 89.48%, TP 70.65%	Hu et al., 2008
Herba Ipomoeae Aquaticae	TN 98.22%, TP 85.26%	Hu et al., 2008
Myriophyllum verticillatum	TN 83.86%, TP 91.74%	Hu et al., 2008
Potamogeton maackianus	TN 77.53%, TP 91.71%	Tong et al., 2007
Coix Lachryma-jobi L.	TN 78.32%, TP 73.55%	Gao et al., 2008

TN = total nitrogen, TP = total phosphorus.

harvested, (3) identify one or two species of plants that have the resistance to adverse conditions of hydrocarbon-rich water, (4) evaluate the contribution of plant and microorganism in nutrients removal and (5) provide a model for remediation of refinery wastewater by fodder crops.

MATERIALS AND METHODS

Materials and Plant Floating-Beds

Four species of perennial grasses *Geophila herbacea* O Kumtze (GHK), *Lolium perenn* cv. ‘Caddieshack’ (LCC), *Lolium perenne* Topone (LPT) and *Lolium perenne* L. (LPL), supplied by Beijing Clover Seed & Turf Co., Beijing, China were artificially germinated. When sprouted, they were transferred to floating beds. Each floating bed was made of polyethylene foam and had a diameter of 77 cm; on the floating bed there were holes with a diameter of 2 mm every 4 mm, through which the roots of plants could elongate into water. The floating beds were covered with peat to act as substrates and were daily irrigated with distilled water to compensate evaporation and transpiration loss. The tested refinery wastewater was provided by Hangzhou Petrochemical Company Limited and the water quality was shown in Table 2.

Experimental Design

The study included five treatments, each with three replicates: (1) water covered with floating bed (CK), (2) water with *Geophila herbacea* O Kumtze (GHK) growing in floating-bed, (3) water with *Lolium perenn* cv.

TABLE 2 Characteristics of the tested refinery wastewater (25 October 2008)

Item	TN	TP	TPH	COD_{Mn}	Zn	Pb	Cd	As
Concentration ($mg.L^{-1}$)	15.60	0.52	1720	142.8	0.30	0.25	0.49	—

—: undetected.

'Caddieshack' (LCC) growing in floating-bed, (4) water with *Lolium perenne* Topone (LPT) growing in floating-bed, (5) water with *Lolium perenne* L. (LPL) growing in a floating-bed.

The experiment was conducted in Zhejiang University during October to December, 2008. The wastewater was filled into 75L plastic containers with a diameter of 80 cm and height of 100 cm. As soon as the plant bud grew to 5 cm long, they were transferred to the experimental pond in Zhejiang University for seedling establishment for 10 days. Plants growing in floating beds with similar growth performance were washed thoroughly with distilled water before they were transferred into the containers mentioned above, covering about 70% of the water surface. The control was designed to cover the container with the same floating bed but without plant. Distilled water was supplied everyday to compensate the water lost by plant uptake and evaporation. The experiment was carried out under natural light and the temperature varied between 0 and 15°C during the experiment.

Sampling and Analytical Procedures

Extensive monitoring of the treatment efficiency was performed by collecting weekly samples from the different treatments. Plants sample were analyzed after they were ground to pass through a 20-mesh (0.84 mm) sieve. Grassquality, total nitrogen (N) and total phosphorus (P) of plant materials were determined according to the methods described in Wang (2004). Zinc (Zn), arsenic (As), cadmium (Cd), and lead (Pb) concentrations in plant and water were determined using inductively coupled plasma mass spectrometer (Agilent, 7500a, Agilent Technologies, Alpharetta, GA, USA) and atomic absorption spectrometry. Total nitrogen (TN), total phosphorus (TP), chemicaloxygendemand (COD_{Mn}) in water was determined according to Vaillant et al. (2003). For the measurement of total petroleumhydrocarbons (TPH), the 0–2 cm surface and 8–10 cm subsurface wastewater were sampled, the TPH was extracted with petroleum ether (PE), and analyzed gravimetrically. The PE extracts were transferred to pre-weighed dishes, the PE evaporated, and the un-evaporated oil remaining in the dishes was weighed to the nearest 0.0001 g (Lin and Mendelssohn, 1998). The TPH concentration was calculated and expressed as mg TPH L^{-1} water.

Nutrients removal by rhizodegradation and absorption into plants was evaluated so as to investigate the contribution of plant in the system, which was calculated according to Körner and Vermaat (1998) with a slight modification and was shown as follows:

$$\text{Contribution Rate} = \{(C_t \times M_2 - Co \times M_1) \div [(C_1 \times V - C_2 \times V) \times 10^{-3}]\}\%$$

Where C_t is nitrogen content in plant at harvest (%), C_o is nitrogen content in plant before treatment (%), M_2 is the plant biomass after experiment (g), M_1 is the plant biomass before experiment (g), C_1 is TN content in the wastewater before treatment (mg/L), V is the volume of water during the experiment (L), and C_2 is TN content in the wastewater after treatment (mg/L).

Nutrient removal ascribed to microorganisms was calculated according to total removal efficiency minus that accomplished through plant absorption. The initial fresh plant weights were measured. Plant biomass was determined from each treatment weekly. When sampling, plants were carefully removed from the floating beds, gently washed with tap water, blotted with absorbing paper and the fresh weights were measured. Dry weight and water contents were obtained by drying at 70°C until a constant mass was reached.

Statistical Analysis and Calculations

Statistical analysis of data was performed using SigmaPlot (version 10.0; SigmaPlot, Chicago IL, USA) and was compared with least significance difference (LSD) test at 0.05 level using Statistical Package for Social Science (SPSS, version 16.0; SPSS, Chicago, IL, USA). Data was expressed as the mean ± SE.

RESULTS AND DISCUSSION

Growth Performance and Nutrient Accumulation

All the grasses looked unstressed under the hydrocarbon-rich conditions and sprang up at the beginning but the growth rate decreased later, likely due to the depletion of nutrients. Biomass growth of the four grasses grown in refinery wastewater for 35 days presented in a distinct sequence of LPT>GHK>LPL>LCC, suggesting the great difference in tolerance of wastewater rich in petroleum hydrocarbon among species. Biomass increases, however, should not be counted as a part of the long-term sustainable phosphorus removal capacity (Kadlec and Knight, 1996). The biomass of LPT increased from 55.5g to 94.8 g during the first seven days and increased by 214% by the end, indicating that LPT is predominant species growing in refinery wastewater with TPH concentrations up to 1700 mg L^{-1}. The biomass of other grass species including GHK, LPL, and LCC had a similar trend (Table 3). Among the four species, LCC showed the lowest growth rate, significantly lower than that of LPT and GHK ($P < 0.05$), indicating its poorest adaptation to the oil environment. Zurita et al. (2009) reported that two months after planting, 50% of *Anturium andreanum* died in constructed wetland, indicating that it is impossible for all the plants to

TABLE 3 Biomass (dry weight) changes of four grass species during the experiment (g)

	Sampling date					
Variety	25 Oct. 2008	2 Nov. 2008	9 Nov. 2008	16 Nov. 2008	23 Nov. 2008	30 Nov. 2008
GHK	55.60 ± 1.96a	72.89 ± 0.62b	78.23 ± 1.14b	95.05 ± 1.63b	109.56 ± 2.32b	113.31 ± 3.69b
LCC	56.41 ± 1.31a	67.92 ± 1.54c	69.21 ± 1.66d	80.10 ± 1.47d	80.28 ± 1.32d	94.43 ± 2.38c
LPT	55.55 ± 1.45a	94.84 ± 0.94a	102.51 ± 1.81a	126.46 ± 2.64a	153.28 ± 2.39a	174.29 ± 2.36a
LPL	51.87 ± 1.54a	69.63 ± 1.32c	72.97 ± 1.27c	89.89 ± 1.91c	95.02 ± 1.69c	98.28 ± 1.68c

GHK: *Geophila herbacea* O Kumtze; LCC: *Lolium perenn* cv. 'Caddieshack'; LPT: *Lolium perenne* Topone; LPL: *Lolium perenne* L. Data was expressed as the mean ± SE, $P < 0.05$ was considered statistically significant.

adapt to a aquatic environment. The present study revealed that all grasses survived during the experimentation period and their development varied among species. This was probably due to the fact that perennial grasses possess adaptation mechanisms to water logging conditions.

Nitrogen assimilation refers to a variety of biological processes that convert inorganic nitrogen forms into organic compounds that serve as building blocks for cells and tissues (Vymazal, 2007). Analysis of the plant tissue during the experimental period indicated that plant N content of the perennial grasses consistently increased during the experiment. Plant N concentration in LPT increased by 129%, exceeding each of the other three species. The results in Figure 1a clearly indicates the bioaccumulation of nitrogen in the four cultivars. The increase of TN content in LPT was significantly higher than others, implying LPT is a new variety that has the largest capacity of absorbing and accumulating N from the refinery wastewater. Apparently the

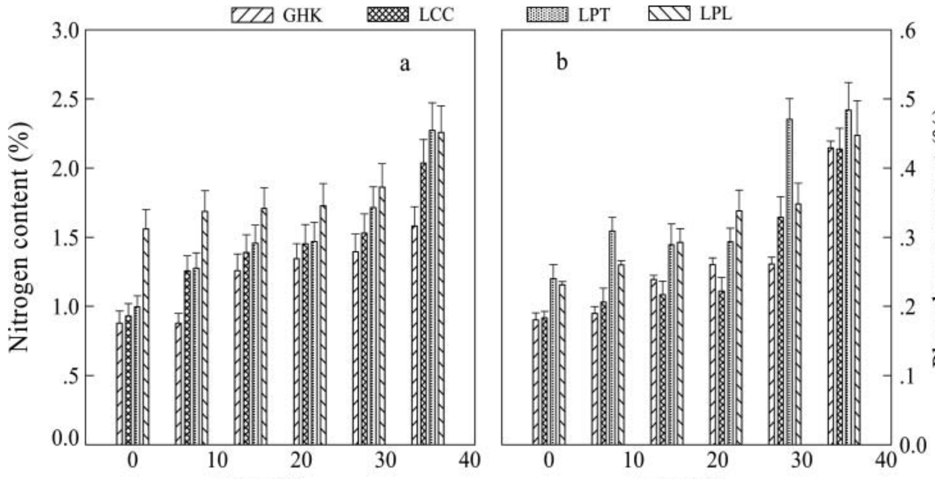

FIGURE 1 Changes of total N (a), total P (b) content in perennial grasses growing in refinery wastewater for 35 days. Species abbreviations are as in Table 3. Data was expressed as the mean ± SE.

higher nitrogen concentration in a plant indicates the stronger ability of the plant to take up nitrogen from water (Xia, 2004).

Phosphorus uptake by macrophytes in constructed wetlands is usually highest during the beginning of the growing season, before maximum growth rate is attained (Boyd, 1969). On account of the richness of phosphorus in refinery wastewater, the accumulation of P in plant increased all the time during the experiment. The accumulation of phosphorus in the plant tissue varied among the species but the trend of P uptake by the four plant species were similar. The accumulation of P had enhanced 133% than before, while it increased by 133% for GHK and LCC, nearly 100% for LPL and LPT by the end of treatment (Figure 1b), these results indicate that GHK has the greatest ability of taking up and accumulating phosphorus when grown in the refinery wastewater, and therefore is the best candidate for phytoremediation of phosphorus-enriched water. Theoretically, because of the high productivity of GHK, the annual removal amount of phosphorus by this plant could be 31g P $m^{-2}yr^{-1}$ as calculated from Figure 1 and Table 3, whereas discounted by the fact that perennial grasses could not survive in summer. The P standing stocks are much more than those reported elsewhere (Kim and Geary, 2001; Karathanasis et al., 2003; Toet et al., 2005).

Removal Efficiency of Nitrogen and Phosphorus

Reduction of TN in the refinery wastewater could result from plant uptake of NH_4^+–N, volatilization of NH_3, nitrification, entrapment of particulate matter (organic nitrogen) by the extensive root system and settling (Sooknah et al., 2004). However, only a few processes ultimately remove total nitrogen from the wastewater while most processes just convert nitrogen to its various forms. Several studies have demonstrated that microbial denitrification (Gersberg et al., 1986), volatilization and plant uptake (Breen, 1990) are the major mechanisms for nitrogen removal. The original total nitrogen concentration in the tested refinery wastewater varied between 13.6 and 16.5 mg L^{-1} and was reduced to 6.2, 5.9, 5.6 and 4.4 mg L^{-1}, respectively by GHK, LCC, LPL, and LPT,. The TN removal profiles are shown in Figure 2a and the removal efficiency was found to be in the order of LPT (69.5%) > LPL (64.1%) > LCC (62.2%) > GHK (59.1%). In addition, the removal efficiency of wastewater treated with plants was greater than control and the removal efficiency of TN with LPT treatment was 36.4% higher than that of CK. Other three grasses had similar removal trend for TN, which fluctuated within 59.1% and 64.1%. This response was similar to the results reported by Gopal (1999). Generally speaking, the four grasses had good capacity of removing TN from the wastewater. It is interesting to note that the biomass increase is not the most responsible factor for higher TN removal by LPT than other grass species, implying that TN removal may be

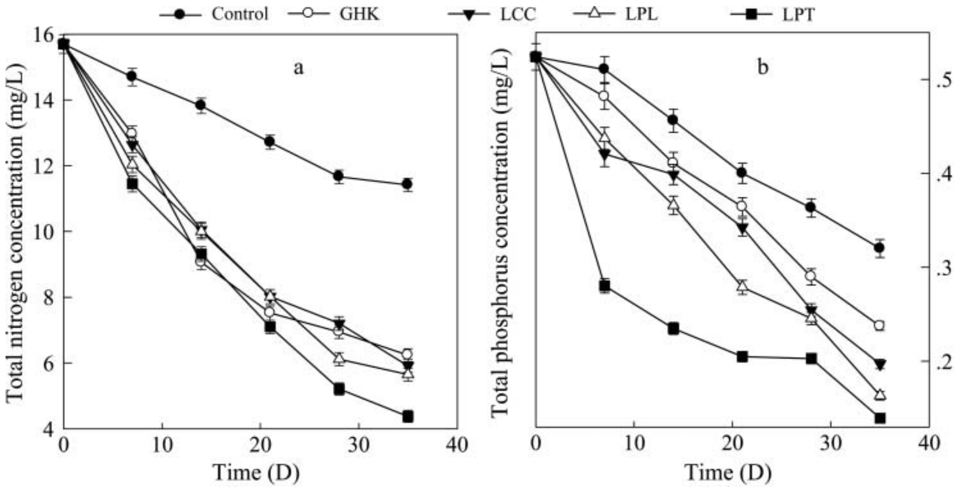

FIGURE 2 Changes of TN (a), TP (b) concentraitons in refinery wastewater with different perennial grasses for 35 days. Species abbreviations are as in Table 3. Data was expressed as the mean ± SE.

also affected by the interaction between plant and N metabolic microorganisms in the wastewater. Since nitrogen removal is primarily dependent on the nitrification/denitrification activity of root-associated bacteria, the presence of aquatic grasses and their associated microbes can vastly enhance the ability of nitrogen reduction levels (Farahbakhshazad and Morrison, 1997; Hu et al., 2008).

Wetlands provide an environment for the inter-conversion of all forms of phosphorus. Soluble reactive phosphorus is taken up by plants and converted to tissue phosphorus or it may be sorbed into sediments. Organic structural phosphorus may be released as soluble phosphorus if the organic matrix is oxidized (Vymazal, 2007). In the present study, total P in the tested water was high and was not completely removed by the perennial grass floating bed system, but the average total P removal in the planted system was higher than that of CK. Despite the relatively good performance of GHK for P removal, LPT may have advantage over GHK in the earlier adaptation to aquatic culture and faster reach to the TP removal peak. Monitoring of the nutrient removal efficiency during the experiment revealed that the TP concentration decreased to 0.3mg L^{-1} after seven days treated with LPT and then maintained at a stable level. TP removal by GHK, LCC, and LPL was steadily increased with time during the experiment, though lower than that of LPT (Figure 2b). This may be due to the fact that these species need longer time to establish a stable plant-microorganism system. The removal of TP with LPT treatment was high in the early period but remained steady later. In general, the TP removal of the four grass species decreased in the order: LPT (72.3%) > LPL (68.5%) > LCC (63.1%) > GHK (55.7%). Phosphorus transformations during wastewater treatment include adsorption, desorption, precipitation, dissolution, plant and microbial uptake, fragmentation, leaching, mineralization, sedimentation and burial, and the major phosphorus removal processes are sorption, precipitation, plant uptake (with subsequent harvest) in constructed wetlands (Vymazal, 2007). The introduction of perennial grasses alleviated water turbulence, thus enhancing phosphorus precipitation on one hand and take up soluble phosphorus on the other hand, especially under field conditions. However, the transformation and absorption are affected by many factors. Sharma et al. (1996) discovered that low temperatures caused a decrease in plant and microbial activity, and an increase in water viscosity, thus resulting in relatively lower P uptake and transformation. In addition, the higher TP removal rate by LPT may be also due to its greater cold-resistantcapability.

Removal Efficiency of TPH and COD_{Mn}

Total petroleum hydrocarbon (TPH) is one of the major pollutants in refinery wastewater. Microorganisms play a leading role during the purification process, which consumed petroleum hydrocarbons and organic matter as their nutrients and carbon source for growthandreproduction. TPH and COD_{Mn} concentration of the tested refinery wastewater was 1720 mg L^{-1} and 142.8 mg L^{-1} and both of them steadily decreased when the four perennial grasses were introduced into the system. Efficiencies of the plants floating bed system in terms of percentage removal are presented in Figure 3. During the 35 d of treatment, control pots without plants cannot be neglected because of microbial influence. At 35 days after the treatment, concentrations of TPH in the phytoremediation treatment were significantly lower

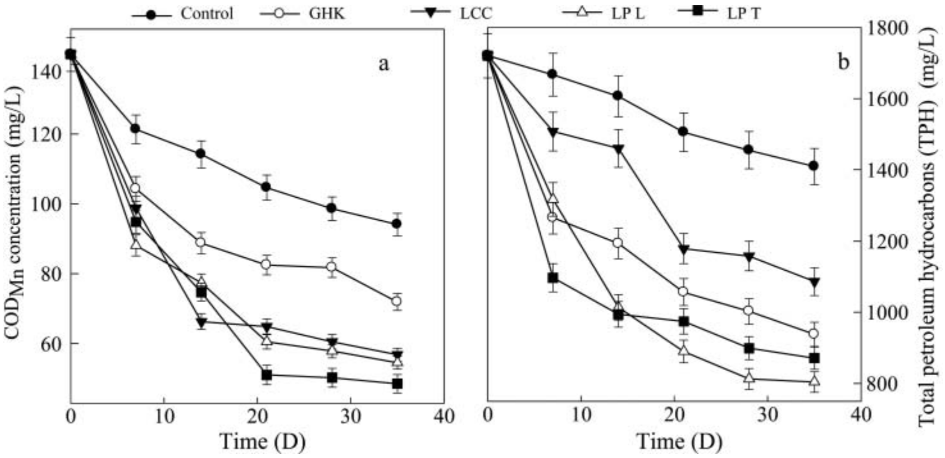

FIGURE 3 Decrease of COD_{Mn} (a) and TPH (b) concentration in refinery wastewater treated with different species of perennial grasses for 35 days. Species abbreviations are as in Table 3. Data was expressed as the mean ± SE.

than that of CK. The COD_{Mn} decreased significantly during the growth period of plants because the plant root mates were fully developed and the filtration capacity of the roots of suspended solids increased as well as the absorption of dissolved nutrients. During the first 21 days, the concentrations of these two pollutants declined steadily, and LPT had the highest COD_{Mn}, reduction of 66% while GHK had the lowest total reduction of 52%. Gloger et al. (1995) compared the COD removal rate of hydroponic tanks that had lettuce plants with aeration and that without plants in treating fish wastewater, they reported 54% higher COD removal rate for the lettuce-growing tanks. Compared with CK, the COD removal rate of LPT was 35% higher. A major part of the degradation of COD in the wastewater is attributed to microbial activities, which may establish a symbiotic relationship with the plants, which provide a large surface area for microbial attachment. However, Ghaly et al. (2005) believed that plants were able to remove all the COD in the wastewater during the growth period, and 32.6%–85.9% of the COD reduction was caused by the released substances. In terms of TPH reduction, LPL performed better than others; TPH concentration was reduced from 1720 mg L^{-1} to 974 mg L^{-1} in the first 21 days and further to 871 mg L^{-1} in the end (Figure 3b). The decline of removal efficiency at later stage (from day 21 to day 35) may be due to the low temperature that decreased plant growth rate and microbial activities. Percentage removal of COD_{Mn} in the wastewater further demonstrated the enhancement of phytoremediation by the four perennial grasses on oil degradation. The removal efficiency of COD_{Mn} by the four species of grasses decreased in the order: LPT (66.0%) > LPL (62.6%) > LCC (62.2%) > GHK (52.2%), whereas the removal efficiency of TPH by the four grass species changed in the order: LPL (55.6%) > LPT (51. 9%) > GHK (48.2%) > LCC (40.0%). The maximum removal of TPH was obtained with LPL at 35 days of treatment and was much higher than that with LCC.

A large number of studies have indicated the potential of phytoremediation for reducing the concentrations of petroleum hydrocarbons (Reilley et al., 1996; Liste and Alexander, 2000; Harvey et al., 2002; White et al., 2006). Oil-contaminated soil and water may affect plants by retarding seed germination, decreasing plant height, photosynthetic rate and biomass, or causing complete mortality (Lin and Mendelssohn 2009). In the present study, when plants were introduced, TPH removal was much more significant than control, suggesting that rhizo-degradation prevailed during phytoremediation (Escalante-Espinosa et al., 2005) and the improvement of the TPH removal might be due to exudates and oxygen input from these perennial grasses roots. Hutchinson et al. (2001) reported that 49% TPH removal from weathered contaminated soil was achieved with Bermuda grass and Tall fescue with an initial concentration of 48.8g of TPH kg^{-1} of dry soil after 180 days of culture. In the present study, 55% of TPH removal occurred in 35 days of treatment through phytoremediation in aquaticenvironment, the difference in TPH reduction is most likely due to the differences of plant roots and microorganisms interaction. In addition, the enhancement of oil degradation might involve a number of other mechanisms besides plant uptake (Lin and Mendelssohn, 2009).

The present study showed that the utilization of perennial grasses to remediate oil contaminated water was feasible with an adequate purification-efficiency and there existed significant differences in the removal efficiency of TN, TP, TPH and COD_{Mn} among species. Refinery wastewater contained higher concentration of organic material and the four species of grasses showed different capacity to remove them. More marked decreases in TN and TP as well as COD_{Mn} were achieved with LPT treatment as compared with others, indicating that LPT is the best candidate among the four grass species for remediating petroleum hydrocarbons contaminated wastewater, though LPL is equally effective in removing TPH as LPT (Figure 4).

During the purification of refinery wastewater, a stable plant-microbe system played a vital role in the removal of nutrients and pollutants. Plant uptake leads to the removal of most of nutrients, which is essentialfor plant growth. However, plant uptake did not contribute much to the removal of organic pollutant and most of the organic pollutants were degraded by microbes, either indigenousorplant-inducedmicroorganisms. Besides, the high concentrations of N and P in the wastewater promoted plant growth whereas organic pollutants provided carbonsource for enhancing microbial activity, particularly in the rooting zone of the plants, thus resulted in the enhanced degradation of hydrocarbons. Chavan and Mukherji (2008) reported that different N: P ratio in wastewater had a significant effect on TPH and COD

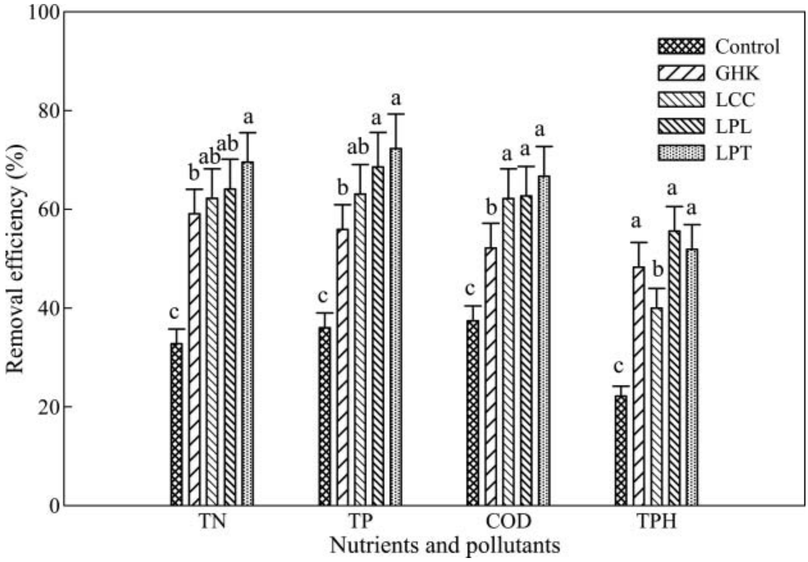

FIGURE 4 Nutrients and pollutant removal capacity of the four perennial grasses from refinery wastewater. Species abbreviations are as in Table 3. Data was expressed as the mean ± SE. $P < 0.05$ was considered statistically significant.

removal efficiencies in the plant-microbe system, which indicated that nutrients may also influence organic material removal efficiency.

Contribution of Plant Uptake vs. Plant-Microbe Interaction to TN and TP Removal

It is commonly considered that nitrogen removal in constructed wetland system is caused by plantuptake and nitrification/denitrification by microorganisms (Brix, 1993; Sakadevan and Bavor, 1998; Sundberg et al., 2007). Since both processes of nitrification-denitrification and plant uptake are the main removal mechanisms for N due to their high porosity, plant growth was assessed throughout the laboratory experiments to evaluate the contribution of plant biomass to remediation efficiency in this study. The results indicate that plant uptake contributed a small part to TN, TP removal from wastewater. For instance, plant uptake by GHK, LCC, LPT, and LPL accounted for only 2.8%, 4.5%, 6.1%, and 3.3% of the total N removal after 35 days treatment, respectively. The majority of TN was also removed through microbe-plant interactions, which accountfor56.2%, 57.7%, 63.4%, and 60.9% with the treatment of GHK, LCC, LPT, and LPL, respectively (Figure 5). Apparently, the removal of TN under plant treatment was mainly accomplished by plant-microbe interactions, which were enhanced by the growing plants. In the control treatment, the nitrogen removal was accomplished by sedimentation and nitrification-denitrification. Stottmeister et al. (2003) reported that the uptake of nitrogen into the plant biomass is of minor importance from a technical viewpoint since harvesting the aboveground

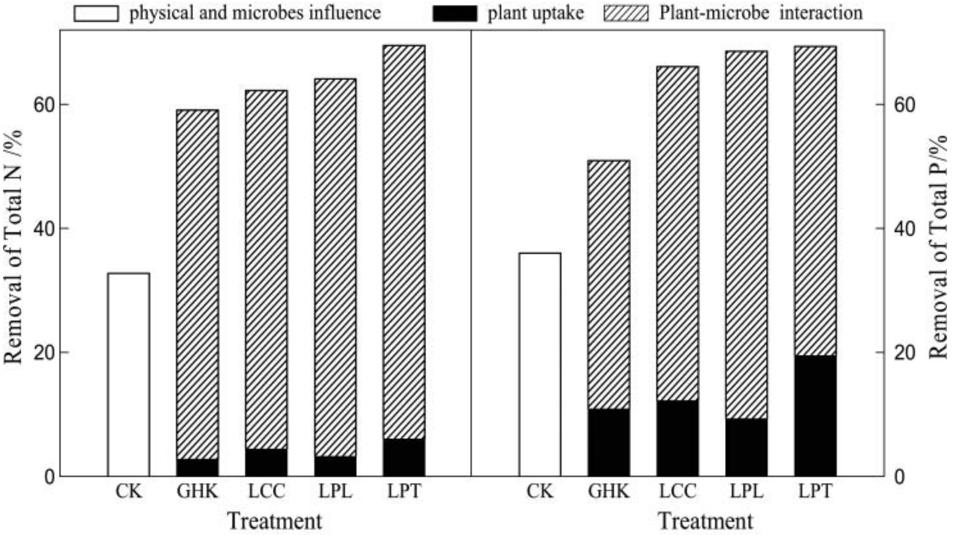

FIGURE 5 Contribution of plant uptake and plant-microbe interaction to TN, TP removal. Species abbreviations are as in Table 3.

biomass removes only 5–10% of the nitrogen, most of total N was removed through microbial nitrification–denitrification. Similar observation was also reported by (Czepiel et al., 1995).

The major role of plants is probably to act as "ecological engineers" (Tanner, 2001), through the release of oxygen and other compounds from the plant roots. Microbial processes are vitally important for the proper functioning of constructed wetlands. However, questions remain concerning what role plants play in the treatment of wastewater (Tanner, 2001; Edwards et al., 2006). In constructed wetlands or plant floating bed systems, the major role in the transformation and mineralization of nutrients is played not by plants but by microorganisms (Stottmeister et al., 2003). When the microbial activities of the entire wetland ecological system are enhanced, TP removal rate will be improved. Similar to nitrogen, the amount of P removed via harvesting of perennial grass is low but important, ranked in the order of LPT (19.6%) > LCC (12.3%) > GHK (10.9%) > LPL (9.3%). The total P removal by LPL treatment was nearly equal to that of LPT though uptake by LPL did not contribute much to total P removal, indicating that microbial activity, enhanced by LPL, played a more prominent role than LPT. LPT took up more P but its total P removal was close to LPL, indicating less efficiency of the plant-microbe system for nutrient removal. Also, plants provide carbon compounds through plant litter and root exudates. The amount of these carbon inputs is related to plant growth (Lu et al., 2002; Jones et al., 2004). In addition, systems with floating plants may achieve higher removal of nitrogen and phosphorus via harvesting due to multiple harvesting schedules and it is important to develop an efficient harvesting frequency in order to keep perennial grasses at the optimum growth stage to ensure optimum nutrient and pollutant removal. The results from this study support the contention that plants, to a great extent, indirectly affect the functioning of plant floating bed systems.

Nutritional Value of Perennial Grasses Grown in Refinery Wastewater as Animal Feeds

With the flourishing of phytoremediation technology, the disposal of plant residues is under extensive studies. Studies were performed to utilize biomass of aquatic plants produced in phytoremediation as a source of energy (Chankya et al., 1993; Moorhead and Nordstedt, 1993). According to the national standard of raw feed material and using alfalfa powder quality thresholds (GB13089-89) as comparison, the essential parameters of these four species of grasses as feeds were analyzed. The quality data of these grasses growing in refinery wastewater for 35 days were shown in Table 4. The protein content of the plant materials were in the range of 15.9% (GHK) to 20.2% (LPL) on dry weight basis, which is higher than the limits for a typical protein concentration (18%) except GHK. Compared with other

TABLE 4 The nutritional value of perennial grasses as feeds after growing in refinery wastewater for 35 days

	Perennial grasses				
GHK	LCC	LPL	LPT	GB	
Crude protein (%)	15.88 ± 0.76	18.73 ± 0.98	20.22 ± 5.69	20.11 ± 6.87	≥18.0 (Top grade)
Crude fat (%)	8.420 ± 0.29	7.460 ± 0.51	10.19 ± 6.36	10.63 ± 3.45	*
Crude fiber (%)	11.19 ± 0.39	12.20 ± 0.78	10.32 ± 4.96	16.42 ± 5.79	≤25.0 (Top grade)
Crude ash (%)	13.56 ± 0.67	11.53 ± 0.59	10.70 ± 6.37	10.12 ± 4.62	≤12.5 (Top grade)
N-free extraction (%)	50.94 ± 3.69	50.09 ± 4.98	48.59 ± 3.29	42.74 ± 9.93	*
Ca (%)	0.97 ± 0.02	1.35 ± 0.04	1.17 ± 0.05	2.320 ± 0.06	*
Zn (mg/kg)	0.06 ± 0.01	0.06 ± 0.00	0.07 ± 0.00	0.04 ± 0.00	*
As (mg/kg)	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	≤2.0 (GB/T13079)
Cd (mg/kg)	0.03 ± 0.00	—	—	0.01 ± 0.00	≤0.5 (GB/T13082)
Pb (mg/kg)	0.19 ± 0.00	0.15 ± 0.00	0.13 ± 0.00	0.09 ± 0.00	≤5.0 (GB/T 13080)
Nitrite (mg/kg)	0.15 ± 0.00	0.29 ± 0.00	0.42 ± 0.00	0.16 ± 0.00	≤15 (GB/T 13085)

Species abbreviations are as in Table 3. Data was expressed as the mean ± SE.
—: undetected, *: no requirement.

three grass species, LPL had higher crude protein content (20.2%) and lower crude fiber and crude ash contents. LPT had the highest crude fat and crude fiber content among the four grass species. The crude fiber contents in the plants ranged from 11.2% (GHK) to 16.4% (LPT) on dry weight basis, which is lower than the limits for typical fat content (25%). The crude ash contents varied slightly among the four species and were lower than the recommended level of 12.5% except GHK (13.6%). The difference of nitrogen-freeextractioninplants was remarkable, with the highest of 50.9% for GHK and the lowest of 42.7% for LPT, respectively (Table 4).

In view of the refinery wastewater, the perennial grass may accumulate nitrite and heavy metals, which influence domestic animal health and subsequently human health. Therefore, the concentrations of nitrite and heavy metals in the plants were analyzed. Nitrite content in the perennial grasses was much lower than the national thresholds and the concentrations of Zn, Cd and Pb were considerable in the refinery wastewater, but the contents of these elements in the plants were far lower than critical level (Table 4). These results indicate that the utilization of perennial grasses to remediate oil contaminated wastewater and use of plant biomass as animal feeds are feasible and thus providing a promising approach for sustainable remediation of wastewater. Similarly, Ghaly et al. (2005) examined five plants and found three of them (rye, barley and oat) have the ability to reduce the pollution potential of aquaculture wastewater and have potential use as fish feed. The results are more encouraging than those obtained in recently published work on utilization of plants residue as a source of energy (Singhal and Rai, 2003) in the convenience and low-cost.

CONCLUSION

Phytoremediation as a way of enhancing total petroleum hydrocarbons (TPH) degradation in refinery wastewater showed great potential. The present study demonstrated that concentration of residual TPH remaining in refinery wastewater in which four cultivars of perennial grass was planted were significantly lower than that without plants, indicating that the floating bed phytoremediation system is effective for rapid removal of nutrients and pollutants from refinery wastewater by fodder crops. However, the removal efficiency differed substantially between species. Of the four species of grasses tested in this study, LPT presented the best result for ecological rehabilitation of refinery wastewater, and it had the highest survival rate, the largest biomass, and the best phytoremediation efficiency, as well as a higher percentage removal of TN, TP and COD_{Mn} were achieved with LPT treatment. Phytoremediation with LPL was more effective for the degradation of TPH than others although it also effectively reduced TN, TP and COD_{Mn}. Plant uptake madeonlyasmallcontribution to the removal of TN, TP and microbe-plant interactions likely played the leading role. Moreover, the perennial grass floating beds may remarkably improve the activities of microbes in the wastewater. Not only do they have the ability to reduce the pollution potential of refinery wastewater, the four perennial grasses used in this study also have the potential to use as animal feeds. The crude protein, crude fiber and crude ash contents of the perennial grasses are as good as or better than the national feed thresholds and the contents of nitrite, Zn, As, Cd, and Pb were far below the critical levels for animal feeds. These results indicate that the plant residues generated from the phytoremediation system could be safely and beneficially reused as the raw material for animal feeds.

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