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Iron and manganese removal by using manganese ore constructed wetlands in the reclamation of steel wastewater

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ABSTRACT

To reclaim treated steel wastewater as cooling water, manganese ore constructed wetland was proposed in this study for the removal of iron and manganese. In lab-scale wetlands, the performance of manganese ore wetland was found to be more stable and excellent than that of conventional gravel constructed wetland. The iron and manganese concentration in the former was below 0.05 mg/L at hydraulic retention time of 2–5 days when their influent concentrations were in the range of 0.16–2.24 mg/L and 0.11–2.23 mg/L, respectively. Moreover, its removals for COD, turbidity, ammonia nitrogen and total phosphorus were 55%, 90%, 67% and 93%, respectively, superior to the corresponding removals in the gravel wetland (31%, 86%, 58% and 78%, respectively). The good performance of manganese ore was ascribed to the enhanced biological manganese removal with the aid of manganese oxide surface and the smaller size of the medium. The presence of biological manganese oxidation was proven by the facts of good manganese removal in wetlands at chemical unfavorable conditions (such as ORP and pH) and the isolation of manganese oxidizing strains from the wetlands. Similar iron and manganese removal was later observed in a pilot-scale gravel-manganese-ore constructed wetland, even though the manganese ore portion in total volume was reduced from 100% (in the lab-scale) to only 4% (in the pilot-scale) for the sake of cost-saving. The quality of the polished wastewater not only satisfied the requirement for cooling water but also suitable as make-up water for other purposes.

1. Introduction

As an indispensable element for modern society development, steel plays an important role in global economics. Known as a giant water consumer, steel industry is ranked as Top No. 5 in world industrial water consumption rate. To cut down the fresh water consumption rate per unit ton of produced steel, wastewater reclamation is proposed as an effective way to improve water conservation and management in various steel enterprises [1,2]. Among various process waters in steel industry, recirculation of cooling water is a potential major consumer of reclaimed wastewater. To avoid corrosion and scaling problems in vessels and distribution pipe systems, stringent requirements are established for iron and manganese. For example, it says in "The reuse of urban recycling water—Water quality standard for industrial uses" (GB/T19923–2005) that iron and manganese concentration in the reclaimed wastewater should be controlled below 0.3 mg/L and 0.1 mg/L, respectively, when used as make-up water in recirculation cooling water system [3]. However, the wastewater discharged from steel industrial generally carries high concentration of iron and manganese. Hence, polishing treatment targeted at iron and manganese is needed in wastewater reclamation.

The conventional treatment for iron and manganese removal is mainly physical–chemical processes, such as air oxidation, chlorine oxidation and contact oxidation filter [4]. Although chlorine oxidation out-competes air oxidation due to its high efficiency and less sensitivity to soluble silicic acid, chlorine readily reacts with organics in reclaimed wastewater and thereby generates secondary pollutant [5]. This becomes extremely important in wastewater reclamation because of higher organic content of wastewater. In contact oxidation filter, the packed medium not only facilitates chemical oxidation between iron and manganese with oxygen by catalysts coated on its surface, but also filters out the formed iron and manganese oxide [6]. Manganese ore is the most widely used catalytical filtration medium. However, it requests routine permanganate regeneration of the catalytical surface and the resultant high chemical cost makes it less competitive with chlorine oxidation. Recently iron and manganese removal have been widely reported in the studies of biofilters [6–11]. Comparing to chemical oxidation, biological oxidation of iron and manganese is more economic due to the absence of chemical dosing. It was found that the manganese oxide generated by these bacteria would trigger an auto-catalytical

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reaction of manganese with oxygen [12] and thus enabled manganese removal even at unfavorable conditions, such as pH below 8.5 and low DO [11,13]. However, the development of the manganese oxidizing biofilm on general filter medium (such as quartz sand or gravel) took rather long time, normally from 90 days to 120 days [6]. In some studies, inoculation with manganese oxidizing bacteria [12], recirculation of specific culture medium [7,12] or manganese oxide containing filter medium [9] were used to speed up the development of the biofilm. Furthermore, it was found that the biological manganese oxidation seemed to be associated with the presence of certain organics, especially pyruvate [13].

On the other hand, as an advanced treatment wastewater technology, constructed wetland is characterized by low capital and operational cost, easy maintenance, versatility and resistance to load shock [14,15]. It combined the functions of substratum, microbes and macrophytes together to achieve efficient purification of wastewater through a series of physical, chemical and biological reactions such as filtration, adsorption, precipitation, ion exchange, plant uptake and microbial degradation, etc. [16–18]. Therefore constructed wetland is widely applied to the removal of suspended solids, organics, nitrogen and phosphorus [19–21] and also heavy metals removal [22]. Although few studies have been carried out in iron and manganese removal from constructed wetlands, diverse iron-oxidizing bacteria were isolated from the rhizosphere of four different wetland plants [23], which suggested the possibility of iron oxidation in wetlands. As the core component of constructed wetland, substratum not only harbors bacteria which is responsible for the degradation of many pollutants, but also functions as supporter and nutrient source to plants. According to the different surface properties of substratum material, the selection of substratum sometimes can target at the removal of a specific pollutant. For example, gravel is normally the first priority when aiming at the removal of organics [24,25] and nitrogen [25] while shale, limestone, steel slag and oyster shell are used for phosphorus removal [26–28].

In this study, a manganese ore constructed wetland is proposed to polish the treated steel wastewater. The objective is to examine its efficacy for iron and manganese removal in steel wastewater reclamation. Owing to its high adsorption capacity of iron and manganese, manganese ore may be a favorable medium for fast development of iron and manganese biofilm. Through the comparison with the performance of a conventional constructed wetland using gravel as substratum, the impact of substratum in wetland on iron and manganese removal were investigated. The lab-scale results were then verified in a pilot-scale manganese ore wetland during one-year operation.

2. Materials and methods

2.1. Configuration of lab-scale constructed wetlands

Fig. 1 shows a schematic diagram of lab-scale manganese ore constructed wetland and gravel constructed wetland. Each of these two vertical flow constructed wetland was confined inside a PVC tank, which is 1 m long, 0.6 m wide and 0.8 m high. The substratum layer is 0.6 m deep, composed of either 6–8 mm manganese ore or 5–15 mm gravel without any soil on the surface. Reeds were planted in a population density of 50 pieces per square meter. Wastewater was distributed evenly over the wetland surface and treated effluent was collected through the under-drain manifold. The wastewater flowrate was manipulated by a constant flow peristaltic pump (BT100-100M, Longer, China). The feed of wastewater continued for 24 h.

The test started in March of 2006 at hydraulic retention time (HRT) of 5 days. After continuous operation for 2 weeks, fresh reed roots, 30 cm long and having more than 3 buds, were transplanted to the constructed wetlands at depth of 5–10 cm. When sprouts appeared in April and grew healthily, the monitoring on influent and effluent then started.

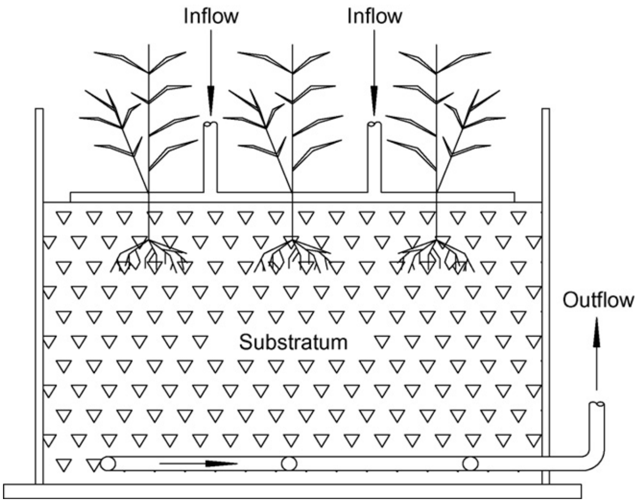

Fig. 1. Experimental set-up of lab-sale constructed wetland.

2.2. Configuration of pilot-scale constructed wetland

To further qualify the results of lab-scale test, a pilot-scale constructed wetland was built as shown in Fig. 2, the constructed wetland was semi-underground with effective volume of 18 m^3. The dimension was 13 m long, 2.3 m wide and 0.9 m deep. The 0.6 m deep substratum layer was mainly composed of gravel (5–15 mm) with only 0.5 m long of manganese ore (8–16 mm) zone, which was about 4% of the total substratum volume. In order to ensure good hydraulic conditions and avoid short circuit along the flow path, round aggregates (50–100 mm) were packed in the inlet and outlet zone to evenly distribute or collect wastewater. To facilitate the monitoring of dissolved oxygen (DO), redox potential (ORP) and other parameters inside the wetland, two pieces of perforated pipes were installed beneath the substratum layer so that liquid could be drained directly from wetland at 1/3 and 2/3 of the flowpath. For easy transplantation of vegetation, 15 cm of soil was laid above the substratum. After being fed with the wastewater for two months, system effluent quality stabilized and then routine sampling and analysis of influent and effluent started. The treatment performance was evaluated at increasing hydraulic load.

2.3. Characteristics of feed wastewater

The incoming wastewater is mixed treated waste streams discharged from steel and iron making, cold rolling, hot rolling, steel pipe processing and process water treatment plant. Its characteristics were summarized in Table 1. Although the treated effluent quality conformed to “Integrated wastewater discharge standard” (GB 8978–1996) [29], it was still far below the requirement of national standard of reclaimed water quality (GB/T 19923–2005) [3], regarding turbidity, ammonia nitrogen, iron and manganese.

2.4. Analytical parameters and procedures

2.4.1. Analytical reagents

All reagents used in analysis were obtained from Shanghai Chemical Reagent Company (Shanghai, China) and conformed to the purity requirements of analytic grade.

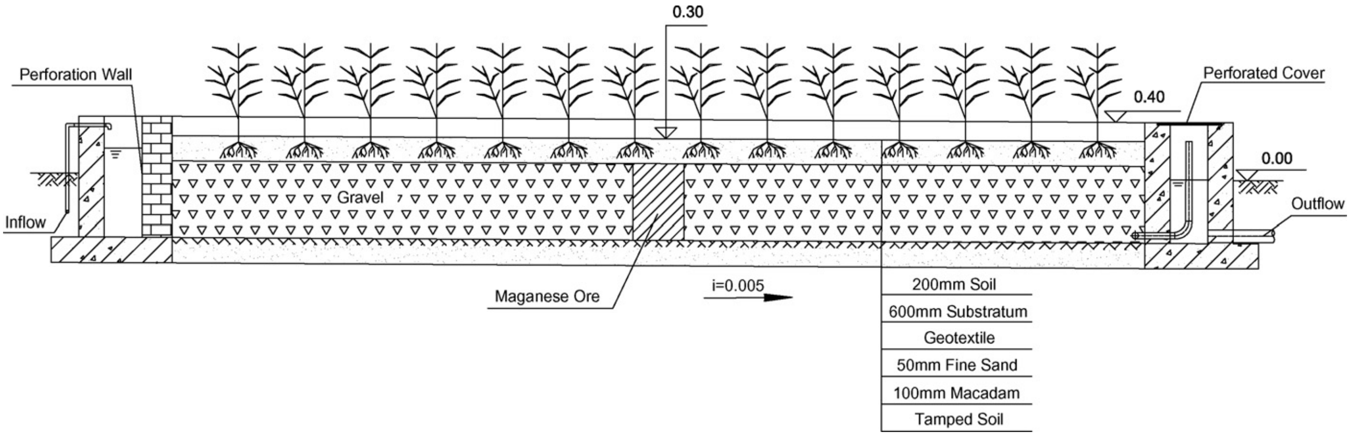

Fig. 2. Schematic diagram of pilot-sale constructed wetland.

2.4.2. Analytical parameters

Grad samples of influent and effluent were collected and analyzed routinely. Some parameters are analyzed on site by instruments, such as dissolved oxygen (LDO HQ10, Hach, USA), pH–ORP and temperature (PHB-2, Shanghai, China), and turbidity (2100P, Hach, USA). According to “Standard Methods for the Examination of Water and Wastewater” (APHA) [30], COD (5220 B) total phosphorus (4500 P B,E), ferrous iron and total iron (3500 Fe B) were analyzed (with their method code shown in bracket). Total iron was determined by phenanthroline method with the addition of hydroxylamine hydrochloride. Ammonia nitrogen was analyzed by Nessler method (GB 7479-87) and divalent manganese by periodate method (GB 11906-89) according to China national methods. Samples were filtered through 0.45 μm membrane before manganese analysis. To evaluate the fouling potential of the wetland effluent on reverse osmosis desalination system, Slit Density Index (SDI) of effluent was analyzed by the SDI test skid (Millipore, USA) according to the protocol (ASTM D4189-95).

2.4.3. Microbiological analysis

When system stabilized, a handful of manganese ore and gravel were sampled from lab-scale wetlands and added into 100 mL of sterilized water. After being thoroughly shaken on a vortexer (XW-80A, Haimen, China) for 20 min, 1 mL of aliquot was sampled and diluted 10 times before being seeded on sterilized liquid JFM culture medium, which was composed of ammonium iron(III) citrate 10.0 g/L, $MnSO_4{\cdot}H_2O$ 2.0 g/L, K_2HPO_4 0.5 g/L, $MgSO_4$ 0.5 g/L, NaCl 0.1 g/L, $NaNO_3$ 0.5 g/L and $CaCl_2$ 0.5 g/L. Then it was cultivated at 27 °C in constant-temperature incubator for 10 days. After the cultivation, the culture medium was examined. Colonies on the surface and sediments on the bottom was carefully picked up with sterilized inoculating loop and streaked on sterilized PYCM solid agar plate (composed of peptone 0.8 g/L, yeast extract 0.2 g/L, $MnSO_4{\cdot}H_2O$ 0.2 g/L, K_2HPO_4 0.1 g/L, $MgSO_4{\cdot}7H_2O$ 0.2 g/L, $NaNO_3$ 0.5 g/L, $CaCl_2$ 0.1 g/L and $(NH_4)_2CO_3$ 0.14 g/L, pH 6.8–7.0). Then the agar plate was cultivated at 27 °C for another 10 days. Then brown colonies developed on the plates were picked up and made into a wet mount specimen. After stained with 2% potassium ferrocyanide and 1% hydrochloric acid, the wet mount was examined under microscope for the presence of iron sediment [31].

The colonies were picked up and streaked on a Petri dish for isolation. This step was repeated until single strain was obtained. Then the obtained strain was cultivated in solid PYCM slant. With the aid of Gram stain, the morphology of the isolate was observed under microscope [32] and compared with the descriptions in Bergey’s Manual of Determinative Bacteriology [33] for identification.

To verify the manganese oxidization ability of the isolated strains, manganese removal batch test was carried out as follows. Colonies on PYCM slant were picked up to make cell suspension in sterilized water. The batch test medium was prepared by 1 g/L of glucose, 0.1 g/L of K_2HPO_4, 0.2 g/L of $NaNO_3$ and 0.1 g/L of $(NH_4)_2CO_3$. Prior to autoclave sterilization, manganese stock solution was added into the tset medium to achieve manganese concentration of 0.6 mg/L and 2.0 mg/L, respectively. It was found in the study that divalent manganese concentration in the cultivation medium did not change significantly after the autoclave. Four millilitre of the cell suspension was added into 250 mL of sterilized test medium and divalent manganese concentration was monitored during the cultivation in a shake flask (100 rpm) at 27 °C. The control tests were conducted by replacing inoculum with 4 mL of sterilized water.

3. Results and discussion

3.1. Pollutant removal in lab-scale constructed wetlands

The effectiveness of wetland as a wastewater polishing treatment was firstly investigated in lab-scale constructed wetlands.

Table 1
Characteristics of feed wastewater: pH, turbidity (NTU) and concentration (mg/L) of DO, COD, ammonia nitrogen, total phosphorus, total iron, manganese, ferrous iron. n.a. stands for “not available”.

Items	Feed wastewater		Reclaimed water quality as cooling water (GB/T 19923–2005)
	Range	Mean	
pH	7.3–9.7	8.1	6.5–9.0
DO (mg/L)	3.5–8.3	6.9	n.a.
Turbidity (NTU)	3.2–83.4	12.6	5
COD_{Cr} (mg/L)	7.8–17.8	11.8	60
Ammonia nitrogen(mg/L as N)	0.2–2.6	0.8	1
Total phosphorus (mg/L as P)	0.1–0.55	0.2	1
Total iron (mg/L as Fe)	0.16–3.0	0.6	0.3
Manganese (mg/L as Mn)	0.1–2.2	0.4	0.1
Ferrous iron (mg/L as Fe)	0.04–0.6	0.1	n.a.

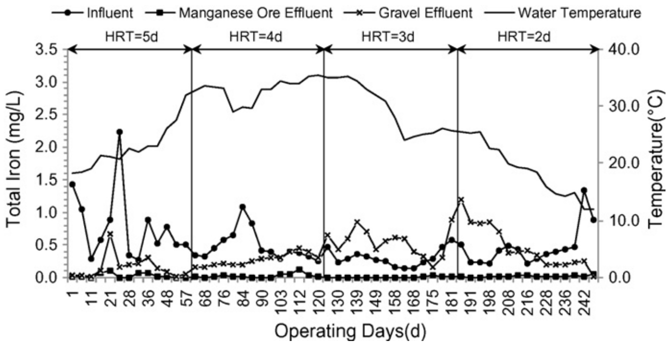

Fig. 3. Total iron concentration in influent and effluents from greensand and gravel constructed wetlands, respectively and water temperature at various HRT.

3.1.1. Removal of organics, turbidity, ammonia nitrogen and total phosphorus

Table 2 summarized the removal of COD, turbidity, ammonia nitrogen and total phosphorus of both wetlands under various hydraulic retention time. It can be seen that wetlands treatment significantly improved the treated wastewater quality. Moreover, manganese ore wetland exhibited a much better performance than gravel wetland. This difference may be ascribed to the different size of manganese ore and gravel. Because the manganese ore used in this study had a much smaller size than the gravels, they would provide a smaller pore size and large specific surface area for biofilm development. The former may explain the better removal of turbidity (especially at high hydraulic loading rate) and the latter could be a possible reason to higher removal efficiency of organics and ammonia. According to the observations of Seo et al. [34], the phosphorus adsorption capacity would also be increased with the decreasing size of medium and the presence of iron in the system. Owing to its adsorption ability of iron, manganese ore could maintain a high iron concentration inside the system so as to facilitate the phosphorus removal.

Regarding to the influence of hydraulic retention time, both wetlands showed similar trend. Except that effluent turbidity increased, removal efficiencies of other pollutants did not deteriorate as hydraulic loading increased. Moreover, nitrification improved significantly as operation continued, showing a gradual accumulation of nitrifying biofilm inside the system. Even though the water temperature dropped to 12°C when retention time was reduced to 2 days, the manganese ore wetland still achieved 91% of ammonia oxidation. This showed that the wetlands application was a suitable polishing treatment under the local climate.

3.1.2. Removal of iron and manganese

Figs. 3 and 4 show the removal of iron and manganese in both constructed wetlands. It can be seen that the influent concentration

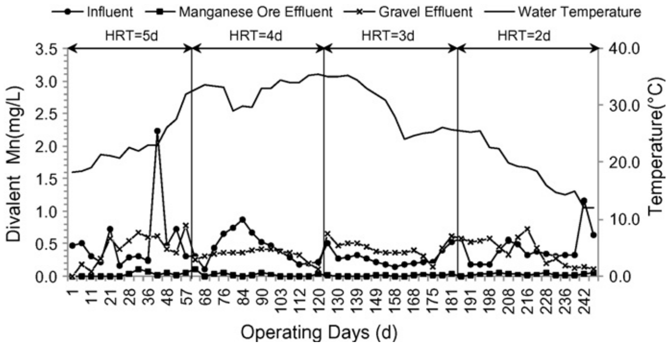

Fig. 4. Manganese concentration in influent and effluents from greensand and gravel constructed wetlands, respectively and water temperature at various HRT.

Table 2
Removal efficiency of COD, turbidity, ammonia nitrogen and total phosphorus in manganese ore and gravel constructed wetlands at various HRT. All values were reported as mean ± standard deviation.

Substratum	HRT (d)	Temp. (°C)	COD			Turbidity			Ammonia nitrogen			TP		
			Influent (mg/L)	Effluent (mg/L)	Removal (%)	Influent (NTU)	Effluent (NTU)	Removal (%)	Influent (mg/L)	Effluent (mg/L)	Removal (%)	Influent (mg/L)	Effluent (mg/L)	Removal (%)
Manganese ore	5	18–35	12.64 ± 2.12	5.85 ± 2.20	54.0	12.41 ± 8.05	0.50 ± 0.47	94.6	1.00 ± 0.63	0.25 ± 0.29	76.1	0.21 ± 0.06	0.02 ± 0.01	90.9
	4	29–35	11.77 ± 2.03	6.19 ± 1.66	45.4	13.45 ± 7.96	0.78 ± 0.17	92.8	0.51 ± 0.14	0.13 ± 0.08	76.5	0.18 ± 0.04	0.01 ± 0.01	95.2
	3	22–28	10.96 ± 2.53	5.35 ± 1.86	49.7	6.17 ± 2.41	0.83 ± 0.18	85.4	0.43 ± 0.28	0.06 ± 0.06	79.4	0.14 ± 0.05	0.01 ± 0.01	100.0
	2	12–21	10.67 ± 1.35	5.26 ± 1.26	50.6	9.23 ± 8.80	1.02 ± 0.22	82.8	0.79 ± 0.27	0.06 ± 0.07	91.1	0.17 ± 0.03	0.00 ± 0.00	100.0
Gravel	5	18–35	12.64 ± 2.12	9.98 ± 2.26	20.3	12.41 ± 8.05	0.73 ± 0.62	91.6	1.00 ± 0.63	0.33 ± 0.29	54.0	0.21 ± 0.06	0.08 ± 0.03	60.6
	4	29–35	11.77 ± 2.03	8.50 ± 1.59	26.9	13.45 ± 7.96	2.07 ± 0.17	79.6	0.51 ± 0.14	0.23 ± 0.10	51.1	0.18 ± 0.04	0.05 ± 0.01	68.9
	3	22–28	10.96 ± 2.53	7.28 ± 2.25	33.4	6.17 ± 2.41	3.17 ± 1.64	46.6	0.43 ± 0.28	0.15 ± 0.07	53.6	0.14 ± 0.05	0.02 ± 0.01	86.0
	2	12–21	10.67 ± 1.35	7.53 ± 1.46	29.5	9.23 ± 8.80	1.85 ± 1.09	63.8	0.79 ± 0.27	0.19 ± 0.11	73.8	0.17 ± 0.03	0.01 ± 0.01	94.7

of iron and manganese fluctuated greatly over the operating days, with total iron varying from 0.16 mg/L to 2.24 mg/L and manganese from 0.11 mg/L to 2.23 mg/L. Regardless of the fluctuation of influent concentration and water temperature and the variation of hydraulic retention time (HRT), manganese ore constructed wetland showed excellent and stable removal of iron and manganese, with effluent iron and manganese concentration always below 0.05 mg/L and average removal efficiency of 95%. Contrast to this, gravel wetland seemed to perform poorly, showing average removal efficiency of only 60% and 46% for iron and manganese, respectively.

Over the operating days, both effluent pH and influent pH varied mainly from 7.0 to 8.5. Comparing to influent pH, there was slight pH drop (about 0.3–1 unit) in the effluent. As shown in Fig. 5, effluent dissolved oxygen (DO) level at most time was above 1 mg/L. This indicated that both pH and DO were favorable for chemical oxidation of ferrous iron. Since ferrous iron was a small portion in total iron of the feed wastewater (as shown in Table 1), iron oxidation was not an issue as long as the colloidal ferric iron hydroxide was retained in the wetlands. Due to the high activation energy, chemical oxidation of manganese is extremely difficult under pH of 8.5 [9,13,12] and generally requires pH above 9.0–9.5 [6,10]. Moreover, Fig. 5 showed a remarkable DO drop in the effluents after day 90. This may resulted from biological degradation of organics and nitrification by a fully developed biofilm inside the wetland, which makes the conditions even more unfavorable for chemical oxidation of manganese. However, biological manganese oxidation is likely under such unfavorable conditions. Because the activation energy barrier in manganese oxidation can be biologically overcomed, biological manganese oxidation was widely observed in either nature environment and water treatment system such as sand filters and biofilters when pH was about 5–6 or DO as low as 1 mg/L [6–11].

In previous studies, an auto-catalytic mechanism of biological manganese oxidation was proposed as follows [6,12,13,35,36]. Manganese oxidizing bacteria can derive energy for their growth from the oxidation of divalent manganese and deposit manganese oxide around their cells. These manganese oxide deposit in turn catalytically facilitates the adsorption of divalent manganese and even oxidation with oxygen (when oxygen is abundant). As manganese oxidizing bacteria continues to convert the adsorbed divalent manganese to manganese oxide, manganese adsorption from the wastewater continues without reaching the equilibrium. It was found that the ripening of manganese oxidizing biofilm will take 90–120 days or even longer on sand, activated carbon or gravels. But this period can be shortened when using manganese sand [6] or manganese oxide-coated sand [9], because both exhibited initial adsorption of manganese. In this study, the manganese ore wetland achieved excellent manganese removal within 30 days of startup (data not shown in figures above). This strongly suggested the important role of manganese ore in manganese removal inside the constructed wetland.

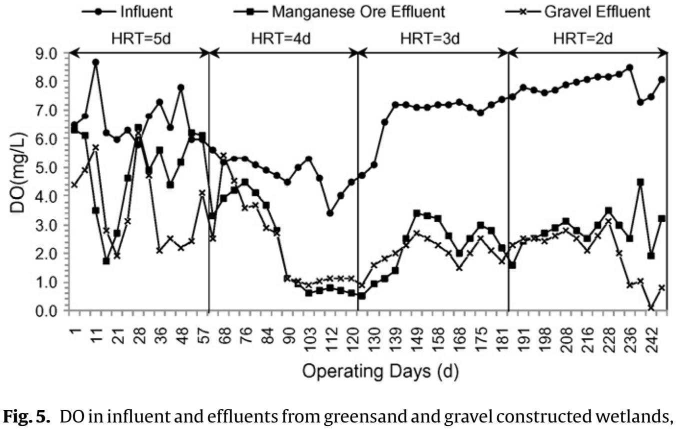

Fig. 5. DO in influent and effluents from greensand and gravel constructed wetlands, respectively.

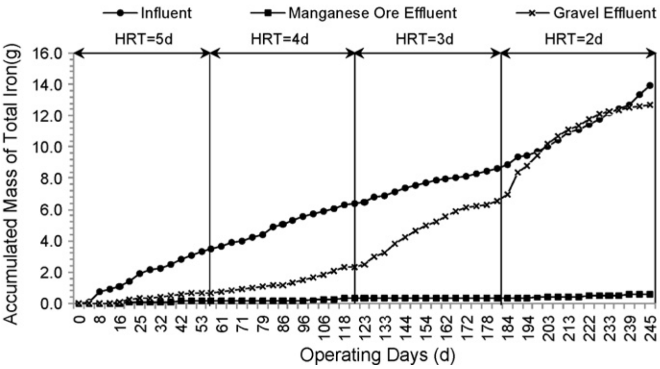

Fig. 6. Accumulated mass of total iron in influent and effluent.

In some studies, recirculation of culture medium containing favorable carbon source (especially pyruvate or glucose) were applied to stimulate the growth of manganese oxidizing bacteria in biofilters [12,13]. Hallberg and Johnson even found that manganese was not removed in the biofilter when pyruvate was omitted from the feed water [13]. However, in the wetlands of this study, the only available organic carbon was residual slow-biodegradable organic, because the feed wastewater was treated by chemical coagulation and biological treatment before being discharged to the wetlands. Biological manganese removal observed in other water treatment plants indicated that manganese oxidizing bacteria can utilize dissolved organics in groundwater and river water as carbon source [6,9]. The discrepancy suggested that manganese oxidizing bacteria may have versatile metabolism pathway. Hence, the target cultivation medium, used by some researchers [12,13], would be favorable for the quick development of certain manganese oxidizing species but may not be an indispensable condition for biological manganese removal. Another possible explanation would be the cooperation of various microorganisms in fully developed diverse microbial community inside wetlands or biofilters. For instance, pyruvate may be generated from certain microbial metabolism during the degradation of organics.

Even though the operating conditions and the growth of plants were almost the same in manganese ore and gravel wetland, their iron and manganese removal performance was distinctly different. The gravel wetland exhibited iron removal since the beginning while manganese removal was observed since day 64. As the test continued, the removal of iron and manganese deteriorated coincidently and sometimes the effluent even carried higher concentration of iron and manganese than the influent. The accumulated input and output of iron and manganese (plotted in Figs. 6 and 7) showed an acceptable mass balance in the wetland. This implied that the excess iron and manganese in the effluent came from the wetland itself. The gravel wetland gradually released all its adsorbed iron and manganese since day 123, which seemed to coincide with increasing effluent turbidity and the increasing hydraulic load. As mentioned above, iron can be easily oxidized and removed in the wetland if the iron oxide particles can be retained. Because the gravels used in the wetland of this study had a much bigger size than manganese ore and filtration medium used in other studies of biofilters, gravel wetland would perform poorly regarding particle removal in a vertical flow regime. Therefore ferric oxide or iron hydroxide colloidal, which were previously retained in the voids of gravel, were very likely to be flushed out of wetland at an increasing hydraulic load, resulting in the release of total iron. It was found in simultaneous removal of iron and manganese in the previous research that chemically formed iron hydroxide (FeOOH)

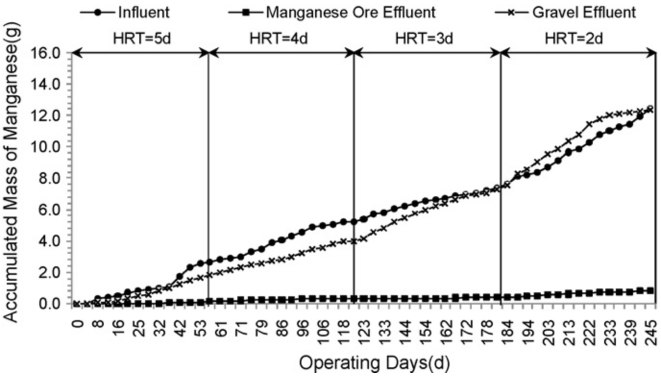

Fig. 7. Accumulated mass of manganese in influent and effluent.

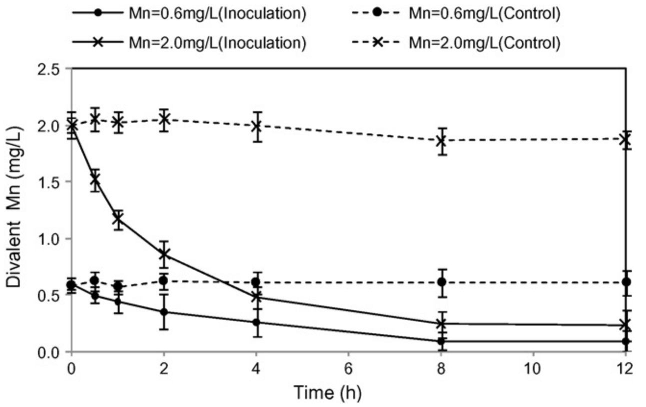

Fig. 8. Manganese oxidation in batch test with cell suspension of isolated strains.

also showed adsorption affinity to manganese [31]. Since gravel did not have manganese oxide surface as manganese ore, it would lose its adsorption ability of manganese as a consequence of iron release mentioned above. This may explain the simultaneous deterioration of iron and manganese removal in gravel wetland.

3.2. Identification of iron and manganese oxidizing strains in wetlands

To support the assumption of biological manganese removal above, gravels and manganese ore were taken respectively from the lab-scale wetlands for microbiological analysis. During the sampling, black-brownish slurry was observed at pore size of the wetlands. After being cultivated in JFM medium for 10 days, a brown-black and metal-shining layer formed on the surface of the culture medium, together with some black sediment on the bottom. This implied the presence of iron and manganese bacteria. After another 10-day cultivation on PYCM solid culture medium, brown colonies appeared. The dispersed colonies changed from initially yellow-brownish to blue-green after being stained with 2% potassium ferrocyanide and 1% hydrochloric acid. This obviously indicated the presence of iron deposit inside the colonies.

Two strains, BG-1 and BG-2, were isolated from these colonies and their morphological traits were summarized in Table 3. According to Bergey's Manual of Determinative Bacteriology [33], they showed morphological similarity to sheathed bacteria, especially *Leptothrix* sp. and *Sphaerotilus* sp. They are facultative autotrophical–heterotrophical sheathed bacteria and iron oxidizing species widely observed in groundwater, swamps, ponds and sediments, soils, wells and even water distribution systems. *Leptothrix* sp. is able to oxidize both iron and manganese [10].

Batch test was conducted to verify the manganese oxidizing ability of the isolated strains. As shown in Fig. 8, Mn^{2+} oxidation in control tests was negligible comparing to Mn^{2+} oxidation in the medium with cell suspension, regardless of the initial manganese concentration. This obviously indicated that isolated strains were able to biologically oxidize manganese at neutral pH and aerobic condition and biological oxidation prevailed in manganese removal.

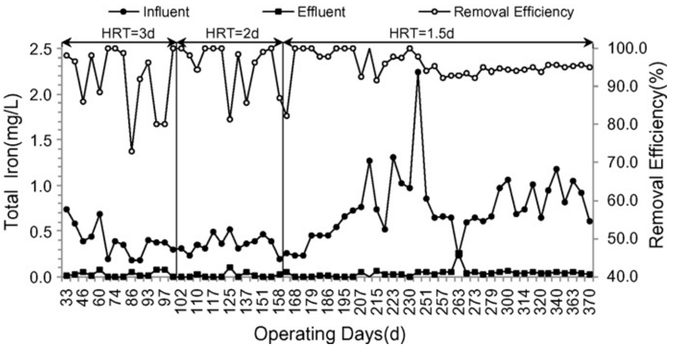

Fig. 9. Iron removal in pilot-scale constructed wetland at various HRT.

3.3. Performance of pilot-scale manganese ore constructed wetland

The polishing treatment of same treated wastewater was further tested in a pilot-scale constructed wetland. Different from the lab-scale manganese ore wetland, only 4% of total volume of the pilot wetland was made of manganese ore and the rest was gravel. The flow regime was changed to submerged horizontal flow, which benefited particle removal in the gravel bed. Because in the lab-scale test, the reduction of HRT from 5 days to 2 days showed little impact on iron and manganese removal, HRT was further reduced to 1.5 days in pilot test.

As shown in Figs. 9 and 10, the removal efficiencies of total iron and divalent manganese in the pilot-scale wetland were 94% and 81%, respectively, regardless of shortened HRT. The effluent total iron was constantly below 0.05 mg/L over the operation. Comparing to purely gravel wetland (lab-scale), the pilot-scale wetland exhibited better manganese removal. Although effluent manganese fluctuated in the initial period, it eventually remained constantly below 0.1 mg/L after 117 days' continuous operation. Moreover no manganese and iron release was observed from this gravel-manganese-ore wetland during the following 253-day operation. The iron and manganese removal efficiency remained satisfactory even when HRT was reduced to 1.5 days. This indicated that a horizontal flow gravel-manganese-ore con-

Table 3
Morphology of colonies and strain after Gram stain.

Code	Colonies	Strains
BG-1	Red-brownish, 1 cm in diameter, protuberant, smooth surface, clear edge and readily picked up from the culture medium	Gram negative, short rod shaped with a clear sheath
BG-2	Brown-blackish 1–2 cm in diameter, smooth surface, irregular edge and protuberant in the center	Gram negative, cocci, 2 or more than are encapsulated inside a sheath and mobile

Table 4
Removal of COD, turbidity, ammonia and total phosphorus in pilot-scale wetland under various HRT. All values were reported as mean ± standard deviation.

HRT (d)	COD			Turbidity			Ammonia nitrogen			TP		
	Influent (mg/L)	Effluent (mg/L)	Removal (%)	Influent (NTU)	Effluent (NTU)	Removal (%)	Influent (mg/L)	Effluent (mg/L)	Removal (%)	Influent (mg/L)	Effluent (mg/L)	Removal (%)
3	10.58 ± 2.12	5.90 ± 1.85	43.7	7.61 ± 3.67	0.70 ± 0.17	89.3	0.61 ± 0.35	0.15 ± 0.17	77.5	0.16 ± 0.03	0.06 ± 0.02	59.8
2	10.44 ± 1.25	5.75 ± 1.45	44.9	7.11 ± 3.58	0.83 ± 0.31	86.5	0.81 ± 0.60	0.12 ± 0.10	76.7	0.14 ± 0.04	0.04 ± 0.02	73.0
1.5	11.49 ± 2.03	5.90 ± 1.25	48.1	10.47 ± 4.21	0.88 ± 0.31	90.4	0.86 ± 0.21	0.12 ± 0.05	85.3	0.17 ± 0.03	0.06 ± 0.02	66.2

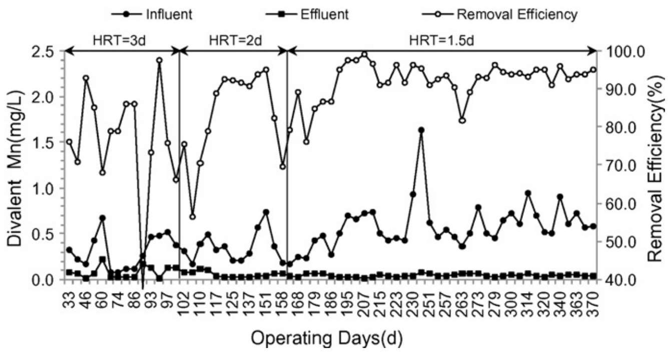

Fig. 10. Manganese removal in pilot-scale constructed wetland at various HRT.

structed wetland was feasible and efficient in iron and manganese removal.

During the pilot test of the constructed wetland, pH and ORP of the effluent and liquids inside the wetland were monitored and plotted in a pH–Eh (redox potential) diagram provided by Mouchet, which clearly identified the boundary of chemical and biological iron and manganese oxidation [10]. From this diagram (Fig. 11), it can be seen that all monitored ORP was positive (above 300 mV), indicating a dominant oxidative condition. Most monitored pH and ORP were below the line of Mn^{2+}/Mn_2O_3 but in the range of biological manganese oxidation. This implied that from thermodynamic point of view, manganese would be oxidized in the wetland biologically rather than chemically. The observed pH and ORP range showed consistency with the findings mentioned above. For example, the monitored values of OPR and pH indicated a favorable growth environment for neutralphilic aerobic manganese oxidizing species. The isolated strains exhibited strong oxidizing ability of manganese under neutral pH and aerobic condition. In addition, most ORP observations were high enough to initiate spontaneously chemical oxidation of iron. This agreed with the good iron removal in wetlands when turbidity removal was satisfactory.

It was found that about 50% of total iron and manganese removal was achieved within the initial 1/3 of flow path of wetland. Meanwhile DO concentration dropped from 7–9 mg/L to about 1 mg/L as organic degradation, nitrification and iron oxidation occurred. This implied that the remaining 40% of iron and manganese removal was achieved when DO was below 1 mg/L. It was very likely inside constructed wetlands due to the following two reasons. Firstly, vascular plants can provide aerobic habitat in an otherwise anaerobic environment through transferring oxygen from stems to the roots and released it into the rhizosphere [23]. This can alleviate the oxygen competition between organic degradation, nitrifica-

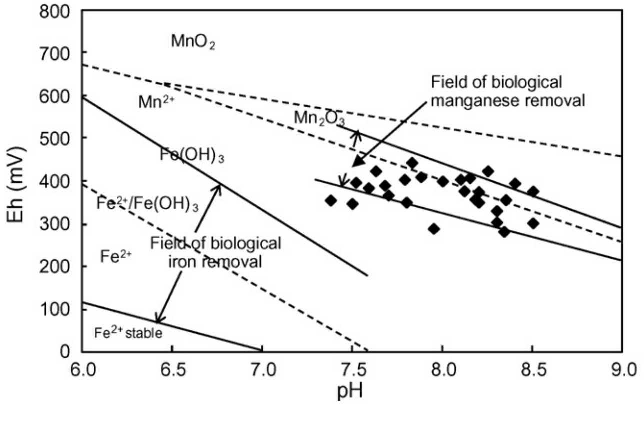

Fig. 11. pH–Eh diagram of pilot constructed wetland.

tion, chemical iron oxidation and biological manganese oxidation. Secondly, versatile metabolism of iron and manganese oxidizing bacteria may enable the manganese oxidation under microaerobic conditions [37]. From the roots of wetland plant, substantial numbers of iron oxidizing bacteria were isolated, in which some isolated species were able to grow at either aerobic or anoxic condition.

As shown in Table 4, the removal of other pollutants in pilot-scale wetland was also satisfactory, showing less influence of scale-up. After the polishing treatment with constructed wetland, the effluent quality further improved in COD, ammonia nitrogen, turbidity, total iron and manganese, and satisfied the requirements for cooling water quality (as listed in Table 1).

Because the treated effluent still carried high concentration of total dissolved salts, the effluent from wetland needs to be desalinized through reverse osmosis, if it is reclaimed as process water. In the operation of reverse osmosis, Silt Density Index (SDI) is used to assess the quality of feed water. As a rule of thumb, all membrane manufactures recommended a maximum SDI of 5 for RO operation in wastewater reclamation. Otherwise the membrane will be severely fouled and resulted in frequent chemical cleaning, which not only interrupts the operation but also impairs the water recovery. Among 18 observations during the operation (data not shown), the observed effluent SDI from pilot wetland was all below 5, regardless of the changes of HRT and water temperature. This indicated that the polished wastewater can be considered as RO feed when the wastewater is reclaimed as process water in the future. This provides more options for the reclamation of this steel wastewater.

4. Conclusions

In this study, manganese ore constructed wetland was proven to be a feasible and cost-efficient treatment technology for wastewater reclamation. Comparing to single gravel wetland, manganese ore wetland showed better removal for all target pollutants (COD, turbidity, ammonia nitrogen and total phosphorus), especially for iron and manganese. The effluent concentrations of both species were constantly below 0.1 mg/L under unfavorable conditions for chemical oxidation. This clearly demonstrated the contribution of manganese ore. With iron and manganese culture medium, two strains were isolated from the wetlands. These isolates not only showed morphological similarity to sheathed iron manganese oxidizing bacteria, but also exhibited prominent manganese oxidation ability in batch test. These evidence, together with the in situ ORP observations in pilot constructed wetlands, strongly supported the assumption of biological manganese oxidation in the constructed wetlands. To save the construction cost, a gravel-manganese-ore wetland was built instead of a complete manganese ore wetland. The monitoring data showed that such a cost-saving measure did not sacrifice the treatment performance in long term but improved the cost-efficiency and applicability of wetland. In addition, throughout the test, the performance of manganese ore constructed wetland showed little dependence on hydraulic loading rate. As a result, HRT of manganese ore wetland was reduced from 5 days to 1.5 days.

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