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Original Article

Iron and Manganese Removal from Wastewater by Constructed Wetlands Planted with *Caladium bicolor*

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Abstract: Constructed wetlands (CWs) have been applied to treat various wastewater types including domestic wastewater, livestock wastewater, industrial wastewater and acid mine drainage with the advantages of low cost, environmentally-friendly technology and high treatment efficiency. Mining wastewater with the high content of heavy metals often causes negative impacts on the ecosystems and human health. In this study, the capacity of using limestone, hydrolyzed rice husk as filter materials and the CWs planted with *Caladium bicolor* to treat iron and manganese in artifical wastewater treatment are evaluated. The wetland model has the size of length x width x height of 50 cm x 15 cm x 20 cm. 10 liters of the synthetic wastewater were used and initial Fe and Mn concentrations varied from 5, 10, 15, 20 and 25 mg/L. The results showed that limestone and hydrolyzed rice husk with the weight ratio of 5 : 2.5 (kg) had good ability to remove Fe and Mn with treatment efficiencies of approximately 99.8% after 144 hours. During a 24-hour retention time, the Fe and Mn concentrations in the wastewater decreased rapidly in CWs and the initial Fe and Mn concentrations affected treatment performance. When the initial Fe and Mn concentrations were below 20 mg/L, treatment efficiencies of Fe and Mn reached about 99% after 144 hours in the CWs and Fe and Mn concentrations met the national regulation QCVN 40: 2011/BTNMT, column B. The study highlights the potential applications of *C. bicolor* in CWs with the use of natural limestone and hydrolyzed rice husk as substrates in treatment of iron and manganese-contaminated wastewater.

Keywords: Constructed wetlands, *Caladium bicolor*, limestone, rice husk, iron, manganese.

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1. Introduction

Heavy metal contamination in the environment is a concerning issue in many countries. Due to the adverse impacts and toxicity of heavy metals on the ecosystems and human health such as disruption of oxidative phosphorylation, DNA, and cellular damage, causing cancer, respiratory diseases and neurological disorders, the treatment of heavy metals from the environment is highly necessary [1]. Many approaches including chemical, physical, and biological methods have been applied to treat heavy metal-contaminated water. Among these methods, biological method has the advantage of having low cost, consuming less energy, and avoiding secondary pollution [2, 3]. Constructed wetland (CW) technology is a biological and eco-friendly method that has been widely applied to treat various types of wastewaters such as domestic wastewater, livestock wastewater, textile dyeing wastewater, and acid mine drainage with good treatment performances [4]. By employing natural processes like sedimentation, adsorption, precipitation, plant uptake, and microbial processes in a controlled manner, CWs have shown effective pollutant removal efficiencies [5, 6].

Wetland plants have been reported to be an important component in pollutant removal in CWs [7]. In this study, *Caladium bicolor* was used as the plant vegetated in the CW with the filtering material containing limestone and hydrolyzed rice husk. *C. bicolor* is a common ornamental plant species in tropical countries, however studies on the use of this plant in CWs for wastewater treatment are still limited. High removal efficiencies of organic matter and nitrate from greywater by the CW planted with *C. bicolor* were shown [8]. Limestone has the main component of carbonate minerals which is often used to increase pH and facilitate the deposition of heavy metals in mining wastewater [9]. Rice husk is an agricultural by-product that have been used as substrate in CWs [10] and as an efficient adsorbent of various toxic heavy metal ions [2, 11]. The study aims to investigate the capacity of the CWs filled with limestone and hydrolyzed rice husk substrates and planted with *C. bicolor* for the treatment of Fe and Mn-contaminated water.

2. Materials and Methods

2.1. Materials

The plant *C. bicolor* used in the study belongs to the *Araceate* family. As one of the herbaceous plants, the plant has arrow shape, green and white spots (Figure 1).

C. bicolor is a common plant in Vietnam and other tropical countries with hot and humid weather conditions. The plant was grown under hydroponic condition and prepared for experiments in the study at Institute of Environmental Technology, Vietnam Academy of Science and Technology (IET - VAST).

Figure 1. *Caladium bicolor* used in this study.

The limestone that was used as filtering material was 2 x 3 cm in size. The limestone was collected in a limestone mine in Quang Ninh province. The limestone was washed before being used in the experiments. The rice husk was collected from a paddy field in Chuong My district, Hanoi. The rice husk was hydrolyzed for

2 months using Sagi-Bio product developed in IET – VAST that was made of beneficial microorganisms including *Bacillus* and *Streptomyces* (microbial density ≥ 10^9 CFU/ml) with the following formula: 300 g of rice husk + 2 liter of tap water + 15 ml of Sagi-Bio + 15 ml of cow dung. The hydrolyzed rice husk would serve as an efficient organic substrate in CWs that helps enhancing treatment performance.

The synthetic wastewater was made by dissolving $FeSO_4.7H_2O$ and $MnSO_4.H_2O$ in tap water to achieve the desired concentrations of Fe and Mn in experiments. The sulfuric acid was added to adjust the pH of wastewater at 4 to mimic the mining wastewater.

2.2. Methods

2.2.1. Evaluating the Ability to Treat Fe, Mn of Limestone and Hydrolyzed Rice Husk

This experiment was implemented to evaluate the ability to treat Fe and Mn of limestone and hydrolyzed rice husk at different ratios at laboratory scale. The experimental models were presented in Table 1. All formulas in this experiment were conducted without plants.

10 L of the artificial wastewater with Fe and Mn concentration of 10 mg/L was added to each bucket with the corresponding experimental formula (Table 1). Sampling was conducted at the intervals of 0 h, 24 h, 48 h, 72 h, 96 h, 120 h and 144 h. Each experiment was repeated three times.

Table 1. The experimental models for testing the ability to treat Fe and Mn of limestone and hydrolyzed rice husk

Experimental models	Limestone (kg)	Hydrolyzed rice husk (kg)
TN1	5.0	0
TN2	5.0	0.5
TN3	5.0	1.0
TN4	5.0	1.5
TN5	5.0	2.0
TN6	5.0	2.5
TN7	0	2.5

2.2.2. Evaluating the Ability of Constructed Wetlands Planted with C. bicolor to Treat Fe, Mn

This experiment was conducted to evaluate the treatment efficiency of Fe and Mn in wastewater of the wetland system planted with *C. bicolor* over time at laboratory scale. The schematic diagram of the experimental wetland model is shown in Figure 2. The CW system was comprised of limestone and hydrolyzed rice husk as substrates and the plant *C. bicolor*. The CW has 3 layers: the bottom layer is limestone with 5 cm thickness, the second layer is hydrolyzed rice husk with 5 cm thickness, and the top layer is limestone with 5 cm thickness. The *C. bicolor* was planted in the system with the initial 6 plant clusters. The total volume of the artificial wastewater was 10 liters. The added Fe and Mn concentration in the wastewater was 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L with corresponding wetland model symbols as following CW1, CW2, CW3, CW4 and CW5. The Fe and Mn concentrations were set based on the range of these metals in wastewater collected from some coal mines in Thai Nguyen and Quang Ninh province [12]. The sampling was conducted at the intervals of 0 h, 24 h, 48 h, 72 h, 96 h, 120 h and 144 h. Each experiment was repeated three times.

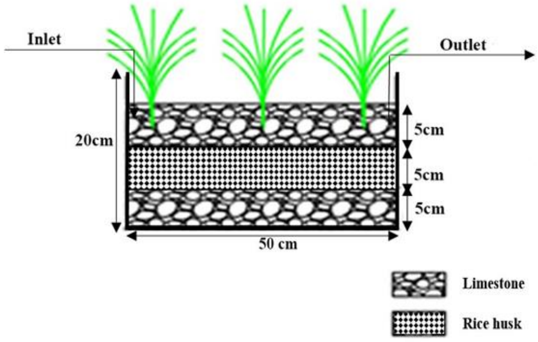

Figure 2. The schematic diagram of the experimental wetland model.

2.2.3. Sample Treatment and Analysis

pH was measured by the pH meter (pH 320 WTW, Germany). Water samples were filtered through a 0.45 μm Whatman disposable capsule filter prior to elemental determination. Fe and Mn concentrations were measured by using the spectrophotometer UV-VIS 2450 (Shimazu, Japan). The standard reference solution (Merck, Germany) was used for evaluation of analytical accuracy.

The national technical regulation on industrial wastewater QCVN 40: 2011/BTNMT, column B was used as the regulation comparison of results in all experiments in this study.

The treatment efficiencies of Fe and Mn were calculated by the difference of concentrations between the inlet and outlet. The formula for calculating the treatment efficiency is presented as following:

$H = (C_{in} - C_{out})/C_{in} \times 100$ (%)

Where:

H is the treatment efficiency (%);

C_{in} is the inlet concentration of the pollutant (mg/L);

C_{out} is the outlet concentration of the pollutant (mg/L).

3. Results and Discussion

3.1. Effectiveness of Using Limestone and Rice Husk to Remove Fe and Mn in Wastewater

The changes in Fe and Mn concentrations over the experimental time (0, 24, 38, 72, 96, 120 and 144 h) in different systems TN1, TN2, TN3, TN4, TN5, TN6 and TN7 using limestone and (Table 1) were evaluated. The changes in Fe and Mn concentrations in the seven experimental systems are shown in Figure 3 and Figure 4, respectively. With the initial Fe and Mn concentration of 10 mg/L in the wastewater, Fe and Mn concentrations gradually decreased over the experimental time period. The Fe concentrations after 72 h were below 5 mg/L and met the national regulation on industrial wastewater QCVN 40: 2011/BTNMT, column B in all experiment systems. Meanwhile, after 72 h the Mn concentrations were below 1 mg/L and met the regulation QCVN 40: 2011/BTNMT, column B in the treatment systems TN4, TN5 and TN6. After 144 h, the Mn concentrations met the regulation QCVN 40: 2011/BTNMT, column B in TN3, TN4, TN5, TN6 and TN7. After 144 h, the Fe and Mn concentrations were lowest in TN6 (0.02 mg/L). Treatment efficiencies of Fe and Mn after 144 h were highest in TN6 (about 99.8%). Furthermore, it is worth noting that the Fe and Mn treatment performance of the system TN7 (containing only rice husk) was much higher than that in the system TN1 (containing only limestone). After 144 h, the Fe and Mn concentrations decreased by about 93% in TN7 and 75% in TN1. The initial pH in all experimental models was 4, at the end of the experiment, the pH value of the outlet in TN7 (without limestone) was 6.7, while the pH values were in the range of 7.1 – 7.3 in all the other experimental models (TN1 – TN6).

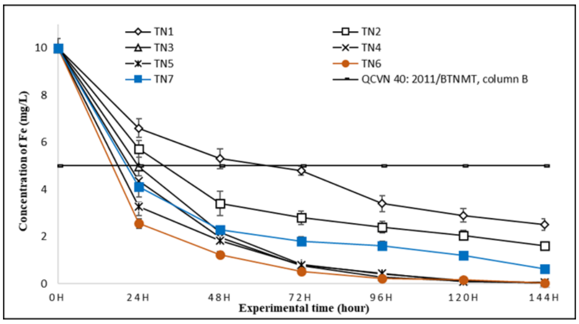

Figure 3. The changes in Fe concentrations in the experimental systems using limestone and rice husk with different ratios.

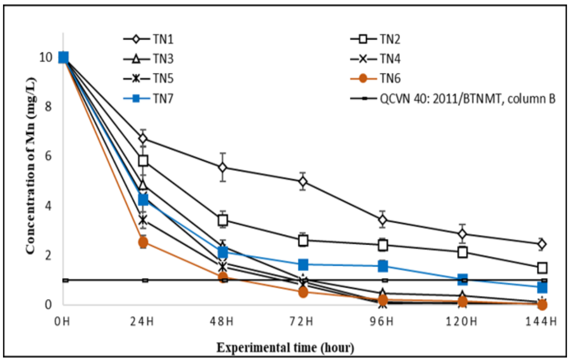

Figure 4. The changes in Mn concentrations in the experimental systems using limestone and rice husk with different ratios.

In general, the results showed that limestone and hydrolyzed rice husk efficiently removed Fe and Mn in the wastewater and the treatment performance varied depending on the ratios of the materials used in the treatment systems. The highest Fe and Mn treatment performance was found in the experimental system TN6 which contained 5 kg of limestone and the highest amount of hydrolyzed rice husk (2.5 kg) among the systems. Good removal efficiencies of Fe and Mn in other systems containing the mixture of limestone and hydrolyzed rice husk were also recorded (> 84% removal after 144 h). Specially, the promising results on Fe and Mn removal was obtained even in the system TN7 which contained 2.5 kg of hydrolyzed rice husk and no limestone. The capacity of limestone in heavy metals removal has been well-documented in previous studies [9, 13]. The main constituents of limestone are carbonate minerals. Under acidic environment the carbonate minerals can be dissolved to produce the anions OH^- and CO_3^{2-} which can precipitate metal cations; therefore heavy metals can be removed from wastewater [14]. The precipitation of metal carbonates has been reported to be the main removal mechanism of toxic heavy metal ions by natural limestones [13]. Rice husk is a common agricultural waste which contains major components like cellulose (32.35%), hemicellulose (21.62%) and lignin (21.55%) [11]. The presence of various functional groups such as carboxyl, hydroxyl and amidogen in rice husk makes the adsorption processes possible [11]. The hydrolysis of rice husk could provide organic substrates like glucose, pentoses, amino acids that are favourable for microbial degradation processes. Dissimilatory sulfate reduction is the reduction of sulfate coupled with oxidation of organic compounds to produce sulfide [15]. The process is catalyzed by sulfate reducing bacteria such as *Desulfovibrio, Desulfomicrobium, Desulfobacterium* [15]. The production of sulfide from microbial sulfate reduction is important for metal removal due to the metal sulfides precipitation mechanism [16]. Sulfate reducing bacteria use sulfate as the electron acceptor to oxidize hydrogen or organic compounds and generate sulfide that can deposit with metal ions [15]. It has been demonstrated that rice husk and sugarcane bagasse based activated carbon efficiently removed Fe and Mn from groundwater with efficiencies of over 81% [17]. The results of this study were comparable to other prior studies which showed that the removal efficiencies of Fe and Mn from synthetic landfill leachate by limestone achieved 94 – 99% and 69 – 86%, respectively [18]; furthermore, over 90% of Fe was removed from acid mine drainage by using rice husk and the sulfate reducing bacteria *Desulfotomaculum nigrificans* [2].

3.2. The Capacity of Constructed Wetlands Planted with C. Bicolor in Fe and Mn Removal

The wastewater containing Fe and Mn with different concentrations of 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L was fed into CW systems planted with *C. bicolor* with the use of limestone and hydrolyzed rice husk as substrates. The results of Fe and Mn concentrations over experimental time (24, 48, 72, 96, 120 and 144 h) are shown in Table 2 and Table 3, respectively. Removal efficiencies of Fe and Mn in the wetland models are shown in Figure 5 and Figure 6, respectively.

The results showed that the wetland models efficiently treated Fe and Mn and the removal efficiencies gradually decreased when the initial Fe and Mn concentrations increased in the wastewater. The pH values after the treatment by CWs were in the range 7.2 – 7.5. The highest removal efficiencies of Fe (99%) and Mn (98.8%) were recorded in CW1 with the initial concentration of Fe and Mn was 5 mg/L after 144 h. In CW2 with the initial Fe and Mn concentrations increased to 10 mg/L, the removal rate of Fe and Mn was about 99.5% after 144 h. When the initial concentrations of Fe and Mn was 15 mg/L (in CW3), the removal efficiencies of Fe and Mn were still high, reaching about 91.1% and 88.4%, respectively after 144 h. However, when the initial Fe and Mn concentrations increased to 20 mg/L (in CW4), the removal efficiencies of Fe and Mn rapidly decreased to 59.4% and 62.4% and when the initial concentrations increased to 25 mg/L (in CW5), the removal efficiencies of Fe and Mn were only 39.5% and 35.8%, respectively after 144 h. After 24 h, Fe concentrations in CW1 and CW2 (1.52±0.26 and 4.28±0.56 mg/L, respectively) met the national regulation QCVN 40: 2011/BTNMT, column B. After 96 h, Fe concentrations in CW3 (4.16±0.42 mg/L) and met the regulation QCVN 40: 2011/BTNMT, column B. With regards to Mn, after 24 h, Mn concentrations decreased but still exceeded the national regulation QCVN 40: 2011/BTNMT, column B in all the five CWs. After 48 h, Mn concentration in CW1 (0.93±0.13 mg/L) met the national regulation QCVN 40: 2011/BTNMT, column B and after 72 h, Mn concentration in CW2 (0.68±0.07 mg/L) met the regulation QCVN 40: 2011/BTNMT, column B.

Table 2. Concentrations of Fe in the wetland models during the experiment (mg/L)

Wetlands	Experimental time (hour)						
	0 h	24 h	48 h	72 h	96 h	120 h	144 h
CW1	5	1.52±0.26	0.82±0.08	0.5±0.07	0.38±0.04	0.06±0.01	0.05±0.008
CW2	10	4.28±0.56	2.17±0.22	0.85±0.17	0.52±0.05	0.11±0.02	0.05±0.006
CW3	15	9.23±0.37	7.46±0.75	6.58±0.33	4.16±0.42	2.13±0.23	1.33±0.19
CW4	20	14.2±1.42	13.88±1.39	11.12±1.1	9.61±1.15	8.84±0.89	8.12±0.8
CW5	25	21.4±1.07	19.22±1.15	18.31±0.55	17.37±1.21	15.82±1.6	15.12±0.76
QCVN 40:2011/BTNM, column B	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Table 3. Concentrations of Mn in the wetland models during the experiment (mg/L)

Wetlands	Experimental time (hour)						
	0 h	24 h	48 h	72 h	96 h	120 h	144 h
CW1	5	1.41±0.19	0.93±0.13	0.41±0.05	0.22±0.04	0.04±0.01	0.06±0.01
CW2	10	4.68±0.47	2.51±0.35	0.68±0.07	0.42±0.06	0.21±0.03	0.05±0.01
CW3	15	9.73±0.98	8.21±0.98	7.38±0.74	5.06±0.71	2.65±0.42	1.74±0.17
CW4	20	15.12±1.5	14.18±0.71	11.36±1.17	9.25±0.92	8.67±0.87	7.52±0.75
CW5	25	21.43±0.86	19.22±0.38	19.02±1.14	17.67±1.77	15.91±0.48	16.04±1.61
QCVN 40:2011/BTNMT, column B	1.0	1.0	1.0	1.0	1.0	1.0	1.0

The good removal efficiencies of Fe and Mn in CW1 and CW2 in this study are correlated well to other studies which found that about 86 - 98% of Fe and 76.8 - 94% of Mn concentrations were removed in CWs planted with *Phragmites australis* treating acid mine drainage [19, 20]. In addition to the capacity of limestone and rice husk materials in heavy metals removal, the role of plants in CWs in treatment of mining water has been demonstrated [21]. The common plants that have been used in CWs in treatment of acid mine drainage are *Phragmites australis* and *Typha latifolia* [22]. *C. bicolor* has been effectively utilized in CWs treating domestic wastewater, however the use of this plant species in CWs in heavy metals removal has not yet been investigated [8]. It has been documented that plants provide surfaces and organic substrates for microorganisms to grow and therefore enhancing pollutants removal in CWs [5, 21]. Plants could also store heavy metals in the below ground tissues and only little metals translocation into shoots [22]. It has been found that about 0.16±0.04 mg/g of Mn and 16.29±4.15 mg/g of Fe were accumulated in plant roots grew in heavy metals-contaminated soil [22]. In another study, it has been found that about 3.92 mg/g and 0.465 mg/g of Mn was accumulated in the plant roots and stems of *Phragmites australis* [23]. In addition to plant uptake, it has been reported that the transformation of metals in CWs involved abiotic processes like filtration, precipitation, and biological activities in the root zones [19]. The combination effects of limestone, hydrolyzed rice husk and wetland plants in CWs contributed to the efficient treatment of the heavy metals Fe and Mn in the present study.

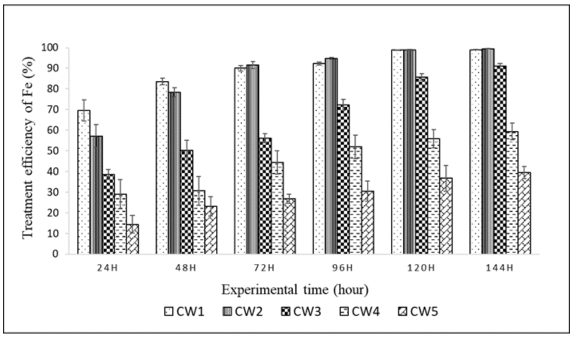

Figure 5. Removal efficiencies of Fe in the wetland models during the experiment.

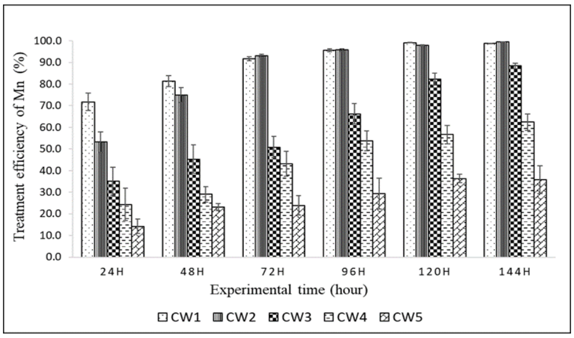

Figure 6. Removal efficiencies of Mn in the wetland models during the experiment.

4. Conclusion

The CWs planted with *C. bicolor* with the use of limestone and rice husk effectively treated Fe and Mn in the synthetic wastewater. The unplanted treatment system with the optimum ratio 5 kg of limestone and 2.5 kg of hydrolyzed rice husk showed high removal efficiencies of Fe and Mn after 144 h (99.8%). When the initial Fe and Mn concentration was 10 mg/L, the treatment efficiencies of Fe and Mn by the CWs reached 99% and 98.8%, respectively after 144 h and both Fe and Mn concentrations after treatment met the national regulation QCVN 40: 2011/BTNMT, column B. As a result, the study demonstrates the potential of CWs planted with *C. bicolor* using limestone and hydrolyzed rice husk as substrates in treating Fe and Mn in wastewater.

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