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Article

Effectiveness of Domestic Wastewater Treatment Using a Bio-Hedge Water Hyacinth Wetland System

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Abstract: Constructed wetland applications have been limited by a large land requirement and capital investment. This study aimed to improve a shallow pond water hyacinth system by incorporating the advantages of engineered attached microbial growth technique (termed Bio-hedge) for on-site domestic wastewater treatment. A laboratory scale continuous-flow system consists of the mesh type matrix providing an additional biofilm surface area of 54 m^2/m^3. Following one year of experimentation, the process showed more stability and enhanced performance in removing organic matter and nutrients, compared to traditional water hyacinth (by lowering 33%–67% HRT) and facultative (by lowering 92%–96% HRT) ponds. The wastewater exposed plants revealed a relative growth rate of 1.15% per day, and no anatomical deformities were observed. Plant nutrient level averaged 27 ± 1.7 and 44 ± 2.3 mg N/g dry weight, and 5 ± 1.4 & 9±1.2 mg P/g dry weight in roots and shoots, respectively. Microorganisms immobilized on Bio-hedge media (4.06×10^7 cfu/cm^2) and plant roots (3.12×10^4 cfu/cm) were isolated and identified (a total of 23 strains). The capital cost was pre-estimated for 1 m^3/d wastewater at 78 US\$/m^3_{inflow} and 465 US\$/kg $BOD_{5\ removed}$. This process is a suitable ecotechnology due to improved biofilm formation, reduced footprint, energy savings, and increased quality effluent.

Keywords: attached microbial growth; domestic wastewater; on-site treatment; phytoremediation; water hyacinth system

1. Introduction

Municipal wastewater discharge, *i.e.*, sewerage, is one of the most serious threats to the ecosystem. Therefore, the sewage needs to be treated appropriately before the wastewater can be released into the environment [1]. A large number of technologies, such as oxidation ponds or activated sludge processes, have been applied for domestic wastewater treatment but most of these practices are expensive to erect and run [2]. Accordingly, there is a need for a substitute system to overcome these drawbacks and achieve a high elimination rate of pollutants.

In recent years, the application of constructed wetlands (with rooted, emergent and free-floating aquatic plants), and facultative ponds treating domestic sewage have attracted considerable attention because they offer an environmentally sound approach [3–6]. The mechanisms of pollutant removal in constructed wetlands involve an interaction between the bacterial metabolism, plant uptake and accumulation [7]. The impurities are removed in facultative ponds entirely by natural processes involving both algae and bacteria [8]. In that order, vegetation is considered as a dominant feature of constructed wetlands, and acts as an important biotic factor in the treatment process [9]. Among the free-floating species, the water hyacinth (*Eichhornia crassipes*) appears to be a promising candidate for pollutant removal owing to its rapid growth rate and extensive root system [10–12]. The water hyacinth lagoon functions as a horizontal trickling filter, where the submerged roots provide physical support for the bio-film bacterial to growth [9]. Nevertheless, despite the efforts made worldwide, the construction of aquatic systems, particularly the water hyacinth treatment process, has not gained much popularity due to the requirement of a large land area and considerable capital investment [5].

In any conventional water hyacinth system, the water column can be divided into three distinct zones, aerobic, facultative and anaerobic, depending on the oxygen transfer through the floating plants. An excess pond depth (typically ~50–100 cm depth) reduces the oxygen transfer efficiency through the roots and sustains high anaerobic microbial growth [13,14]. The oxygen concentration is likely to be high in the upper part of the lagoon, and begins to decrease further down the water column, approaching almost zero below a 200 mm depth. The higher pond depth can rise to the anaerobic zone; resulting a slow biodegradation process, and cause the emission of foul odors. To counter these disadvantages, a shallow pond water hyacinth system was reported [15,16].

The shallow pond system is an alternative to the conventional water hyacinth process because it has a low water depth (140–150 mm) based on the fully matured plant root submerged (80–130 mm) to avoid the anaerobic zone [16]. This condition ensures the optimal interactions between the wastewater effluent and microbial biomass in the phytoremediation treatment practice. The shallow pond technique is an attempt to minimize these constraints due to the better oxygen diffusion efficiency through the roots and the accumulation of a larger aerobic bacterial population. This is a robust biological process that can be applied to the efficient and reliable elimination of pollutants at a lower hydraulic retention time (HRT) compared to the conventional water hyacinth system, even under environmental stress conditions [15,16]. Despite its adequate performance, further improvements can establish the shallow pond water hyacinth practice as an effective tool for purifying municipal wastewater effluent.

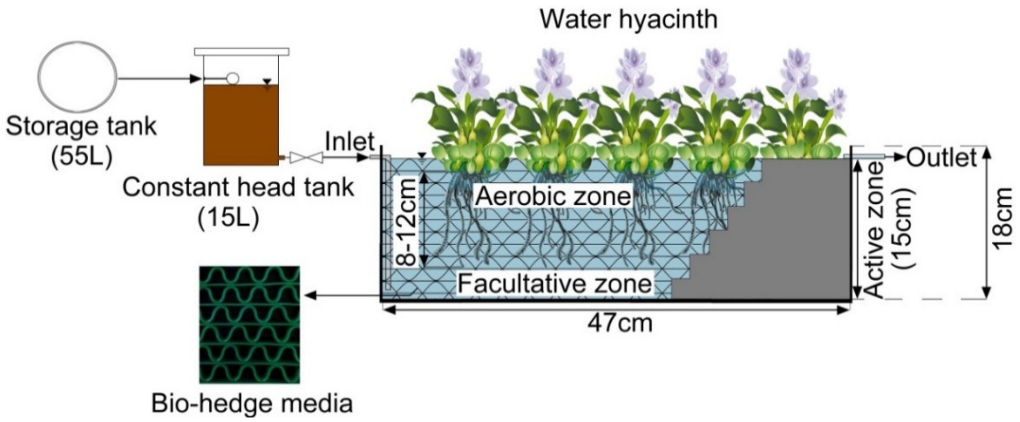
Application of phytoremediation with attached growth-based engineered procedure (using *Phragmites* sp.) has been reported as a new relevant technology in constructed wetlands treating domestic sewage [17,18]. The concept of operation is void of the soil strata used in the root zone systems, and in lieu a support matrix (assembled by the number of vertical PVC pipes) is provided to enrich the microbial population in the form of biofilm within wetland unit. This approach overcomes the limitations of choking, clogging, slow mass transfer, poor root penetration into the multilayer soil column, high area requirement and capital investment owned by soil bed constructed wetlands. A further study also revealed the efficiency of this method by using *Thalia dealbata, Acorus calamus, Zizania latifolia* and *Iris sibirica* for river water treatment [19,20]. Marchand *et al.* [21] effectively applied this technology (combined with a homogeneous mix of gravels and perlite) for the removal of copper ion from synthetic Cu-contaminated wastewaters using *Phragmites australis, Juncus articulates* and *Phalaris arundinacea*. In addition, the vegetated system using *Typha* sp. reported to be a promising solution in domestic wastewater treatment seriously stressed with total dissolved solids (TDS) and Cu metal salt [22]. The integrated anaerobic baffled reactor and phytoremediation process with attached growth (using *Phragmites* sp. and *Typha* sp.), likewise, has been recommended as a novel approach for promoting a sustainable decentralization [23]. As a result, it is recommended that using wetland systems upgraded with the phenomena of engineered attached growth matrix could be considered as a novel scientific advancement in domestic wastewater treatment.

Therefore, the present study aimed to enhance and examine the efficiency of the shallow pond water hyacinth system by adding a new feature to a treatment unit. In this approach, an attempt was made to incorporate the advantages of a shallow pond and attached growth microbial techniques by introducing Bio-hedge (mesh type structure), which is a support matrix to augment the indigenous microbial population. Although the performance of the water hyacinth treatment systems have been studied elsewhere before; until now, this innovation to our knowledge has not been previously documented. This new system identified to overcome the limitations associated with the traditional water hyacinth and facultative pond technologies, and permits treatment of effluent in the most cost effective method. Moreover, the role of plant, microbial biofilms and evapotranspiration in this phytoremediation system was also determined.

2. Materials and Methods

2.1. Pilot Plant Setup

A bench-scale advanced shallow pond water hyacinth system was configured in the premise of the Department of Environmental Sciences, University of Pune, India. The pilot plant consisted of three major sections; storage tanks, constant head tanks and a wetland unit (Figure 1).



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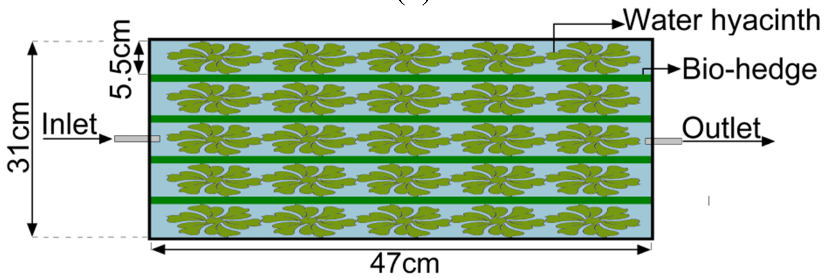


(**b**)

Figure 1. Schematic diagram of the Bio-hedge water hyacinth phytoremediation system: (**a**) cross section view; (**b**) plan view.

A rectangular tank with dimensions of 0.47 m × 0.31 m × 0.18 m (L × W × H) by considering the effective depth of 0.15 m and a 21 L void volume in the presence of vegetation and other internals was used to form an advanced shallow pond system. The unique feature of the system is the presence of plastic mesh type structures to achieve higher biomass concentrations and provide a greater support matrix to microbial growth, which is hereafter termed "Bio-hedge". Bio-hedges with a length equal to the treatment unit and a 0.15 m height were hanging vertically in 5.5 cm distances from one another, parallel to the direction of flow of wastewater. This arrangement helps to effluent flow to pass throughout the wetland unit without any difficulties. The matrix media (Bio-hedges) provided a specific surface area of 54 m^2/m^3 effective volume (56 m^2/m^3 filled volume). The 25 water hyacinth (*Echhornia crassipes*) plants were placed between the Bio-hedges in the reactor at the initial stage of the plantation to give full coverage of 171–172 plants/m^2. The inlet and outlet arrangement was performed according to standard practices. Raw sewage was fed manually into a storage container. Furthermore, the identical configuration of present laboratory scale model was preserved for unplanted condition and used for the control purpose (21.5 L void volume). Alternatively, additional compression was done with our previous study on conventional and shallow pond water hyacinth systems (Table A1) [16,24]. The shallow pond system could possibly consider as a blank reactor (no support matrix material used) since it has almost identical configuration to the Bio-hedge process.

2.2. Planting and Acclimatization

The water hyacinth was collected from a local lake for preliminary planting. The roots of the sampled plants were washed carefully with tap water to remove any adhering dirt. Initially, the plants were exposed separately to a serial dilution of raw sewage in batch mode, starting from a 25% concentration, which was increased gradually to a concentration of 100% over a 10 day period. Subsequently, the Bio-hedge shallow pond system was planted and operated at a hydraulic retention time (HRT) of 22 h. In this stage, the system continued to run for 35 days until the reduction in the percentage of chemical oxygen demand (COD) reached a constant level. This was considered the acclimatization period. Initially, the microbial population was low and gradually bacterial growth was enhanced and their population enriched by attaching to the fully submerged, stationary media (Bio-hedges).

2.3. Operational Procedure

Seasonal variations in temperature (over Pune city, India) might not have a substantial influence on the treatment efficiency in constructed wetland systems, especially under lab-scale conditions, because there is little difference in temperature throughout the year; this detection was observed in previous experiments [16,17]. After the acclimatization period, the experiments were initiated during February 2008–January 2009 (approximately one year) to determine the state of the Bio-hedge shallow pond system in terms of the hydraulic retention time (HRT), treatment performance and plant growth with the intention of organic pollutant elimination considering the effluent discharge norms specified by the local pollution control board (COD < 100 mg/L and biochemical oxygen demand BOD_5 < 30 mg/L). Under steady state conditions, the Bio-hedge system was operated in eight phases between 8:00 pm and 8:00 am the next day HRT with a mean BOD_5 loading of 5.65–13.80 g/d (Table 1).

Table 1. Experimental operating conditions of the Bio-hedge water hyacinth wetland system treating domestic wastewater.

Item	Phase						
	I	II	III	IV	V	VI	VII
Operation period (d)	45	45	45	45	45	45	45
HRT (h)	20	18	16	14	12	10	8
Q (L/d)	25	28	32	36	42	50	63
OLR							
(g BOD_5/d)	5.65 ± 0.50	6.42 ± 0.76	6.85 ± 0.61	7.00 ± 0.55	9.18 ± 1.10	10.78 ± 0.66	13.80 ± 0.98
(gBOD_5/m^2/d) [1]	4.83 ± 0.42	5.50 ± 0.65	5.86 ± 0.52	6.00 ± 0.47	7.85 ± 0.93	9.21 ± 0.57	11.80 ± 0.84
(gBOD_5/m^2/d) [2]	38.70 ± 3.35	44.00 ± 5.21	47.00 ± 4.18	48.00 ± 3.80	62.88 ± 7.50	73.80 ± 4.54	94.50 ± 6.71
(gBOD_5/m^3/d) [3]	217 ± 18.80	247 ± 29.27	264 ± 23.46	269 ± 21.33	353 ± 42.04	414 ± 25.51	531 ± 37.70
(gBOD_5/m^3/d) [4]	269 ± 23.26	306 ± 36.24	326 ± 29.04	333 ± 26.41	437 ± 52.05	513 ± 31.60	657 ± 46.67

Notes: HRT: Hydraulic retention time; Q: flow rate; OLR: Organic loading rate based on the [1] matrix Bio-hedge surface area; [2] pond surface area; [3] total reactor volume; [4] effective (liquid) volume.

The daily flow was calculated by considering the theoretical HRT, t (h), through each phase from the following equation:

$$t = \frac{V}{Q} \quad (1)$$

where V (L) is the void volume of the reactor; and Q (L/h) is the design flow rate. Wastewater flow (L/d) expresses the design wastewater flow over a 24 h period. The organic loading rate (OLR) in g BOD_5/d was approximated by multiplying influent BOD_5 concentration (g/d) by wastewater flow (L/d). The inflow rates were fixed to 25, 28, 32, 36, 42, 50 and 63 L/d for 20, 18, 16, 14, 12, 10 and 8 h HRT, respectively. The system was set to run at each retention time for 45 days, during which, the effluent characteristics with an almost constant percentage reduction were achieved. In addition, the older plants were removed periodically (at every 45 days) and new plants were retained to maintain the initial plant population *i.e.*, 25 sampling vegetation. Age can greatly affect the physiological activity of the plants, particularly its roots. In general, the roots of young plants can display greater ability to absorb impurities and release oxygen than old plants due to the age of the plants and tissues. Therefore, it is important to use healthy young plants for the more efficient removal of contaminants from wastewater [25,26].

2.4. Wastewater Sampling and Analysis

The raw sewage samples were collected from a nearby municipal wastewater treatment plant facility (P.C.M.C., Pune, India) on a daily basis for performance evaluation. The samples were collected from the inlet and outlet of the reactor every 24 h and analyzed for ten water quality parameters (pH, COD, BOD_5, dissolved oxygen (DO), total suspended solids (TSS), total nitrogen (TN), ammonia nitrogen (NH_3-N), total phosphorus (TP), phosphate (PO_4-P) and most probable number (MPN) of coliform bacteria) according to the standard method [27]. The microbial population in the inlet and outlet of the sewage effluents, root surface and on the Bio-hedge surfaces were examined to determine the total viable count (TVC) using the pour plate technique [27]. Bacteria presented on the root surface and the support matrix (Bio-hedge) were merely isolated on nutrient agar medium and identified. The Gram-negative bacterial identification was done using Mini API (BioMerieux, Marcy l'Etoile, France), and for Gram-positive, Bergey's Manual of Determinative Bacteriology [28] was used.

Shoot (above-water plant part) and root tissue samples were oven dried at 60 °C for 24 h, ground, and solubilized (0.5 g) with a tri-acid mixture (conc. HNO_3:H_2SO_4:HCL_4 = 10:1:4) for the analysis of TN and TP concentration [27,29].

The rate of evapotranspiration was measured through the planted Bio-hedge system by using the standard practices of the flow rate differences [30]. Similarly, unplanted identical design configuration was served as a measurement of evaporation from a free water surface for each operating condition (Table 1). The overall characteristic of domestic wastewater during the study period was as follows: Temperature 27.6 ± 1.97 °C, pH 7.38 ± 0.17, COD 418.36 ± 28.50 mg/L, BOD_5 215.42 ± 22.10 mg/L, TSS 164.6 ± 8.30 mg/L, TN 47.15 ± 1.70 mg/L, NH_3-N 33.01 ± 2.53 mg/L, TP 11.43 ± 1.07 mg/L, PO_4-P 7.31 ± 0.6 mg/L, total coliform 1.52×10^7 MPN/100mL, and TVC 3.81×10^6 cfu/mL.

2.5. Determination of Botanical Aspects

The morphological characteristics of vegetation, *i.e.,* the number of leaves, size of the leaves, number of roots, longest root, dry weight, and ash weight, were analyzed using standard practices [31]. Periodically, the same parameters were examined every 10 days over a period of 2 months to determine the changes in the plant morphology. Histochemical studies of the roots of the macrophytes were carried out to assess the impact of domestic sewage [32]. Hand-cut cross sections of the root sample were added to a 50% ethanol solution (v/v) and stained with a safranin solution for 2 h. The sections were washed thoroughly in distilled water for 5 min, transferred to a 95% ethanol solution and counterstained with a light green staining solution for 15 s. The sample was rinsed in alcohol and examined by optical microscopy.

2.6. Statistical Analysis

The data of 365 consecutive days were analyzed to examine the performance of the system. Treatment efficiency was calculated as the percentage of removal for each parameter as follows:

$$\text{Removal efficiency (\%)} = \frac{\text{Ci} - \text{Ce}}{\text{Ci}} \times 100 \quad (2)$$

where Ci and Ce are the influent and effluent concentrations. Organic loading rates, OLR (g/day), were calculated by multiplying the organic concentrations (g/L) and the discharge flow (L/d). A logarithmic trendline as a best-fit curved line was used to express the COD and BOD_5 percentage reduction efficiency, and DO concentration in treated effluent. Regression analysis (Excel, Microsoft, Redmond, DC, USA) was performed to determine the relationship between the inflow and outflow COD and BOD_5 loadings in the Bio-hedge shallow pond water hyacinth system. Relative growth rate of the plants is calculated using the following equation:

$$\text{Relative growth rate (\%)} = \frac{\ln \text{W2} - \ln \text{W1}}{\text{t2} - \text{t1}} \times 100 \quad (3)$$

where W1 and W2 are the dry weight of plants at times t1 and t2.

3. Results and Discussion

3.1. Process Performance

The COD and BOD_5 concentration of the treated effluent was less than 100 and 30 mg/L at HRT levels above 14 h in the Bio-hedge system, respectively (Figure 2a), and exceeded the permissible limit at below 14 h HRT. Therefore, a HRT of 14 h is believed to achieve satisfactory organic removal performance under the given operating conditions. The mean COD and BOD_5 reduction were 80% and 86% at 14 h HRT. Regression analysis showed a reasonable linear correlation ($r^2 \geq 0.98$ and $r^2 \geq 0.95$, respectively) between the inflow and outflow COD and BOD_5 loadings (Figure 2b). The value of 14 h HRT is shorter than previous finding in conventional (reasonable HRT estimated to be 43 h) and shallow pond (reasonable HRT estimated to be 21 h) water hyacinth systems, which achieved 76% and 81% of COD and 87% and 91% of BOD_5 removal, respectively under the same environmental conditions (Figure 2a) [16,24]. Beyond these HRTs (43 and 21 h), the COD and BOD_5 concentrations reached above 100 and 30 mg/L, respectively. The Bio-hedge technique is also more efficient than the reported literature on the non-aerated and aerated water hyacinth systems (batch mode) with 36 h retention to accomplish a respective 48% and 82% COD and 50% and 83% BOD_5 reduction [10]. Moreover, the treatment efficiency was found to be higher in the Bio-hedge wetland compared to the values reported in the literature on the facultative ponds either as primary (49% COD and 68% BOD_5 reduction at 15 d HRT) or secondary (62% COD and 79% BOD_5 reduction at 7 d HRT) treatment unit [4]. The high performance of the system could be strongly explained by the type of the plant, the desirable rate of the oxygen transfer efficiency through the roots, and the high microbial accumulation responsible for organic degradation on the surface of the Bio-hedges.

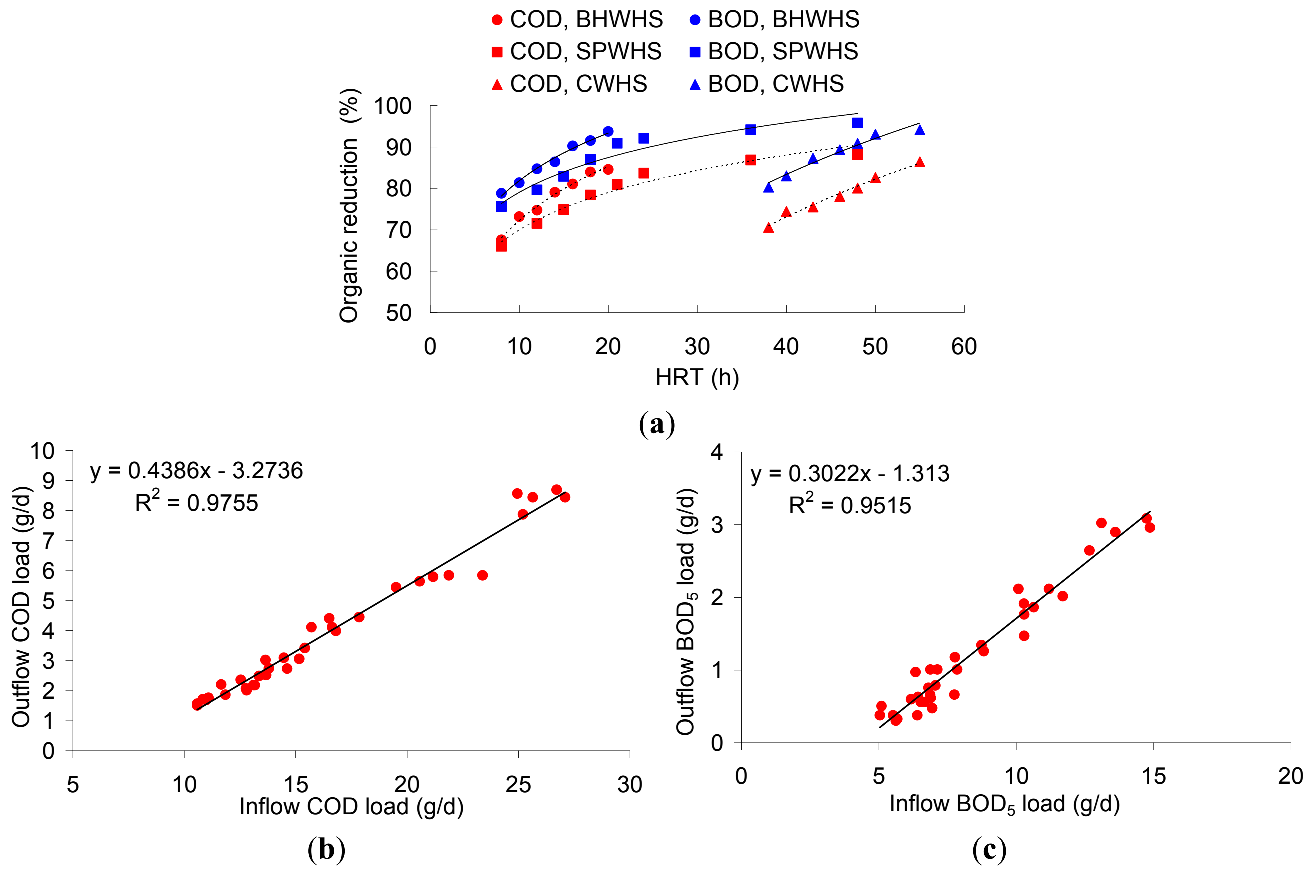


Figure 2. (**a**) Mean reduction of the COD and BOD_5 values in the water hyacinth treatment systems (n = 5) (COD value represents the mean of daily sample of 10 consecutive days merely in SPWHS); Relationship between the inflow and outflow COD (**b**) and BOD_5 (**c**) loadings under steady-state operation in the Bio-hedge system. BHWHS: bio-hedge water hyacinth system, SPWHS: shallow pond water hyacinth system [16]; CWHS: conventional water hyacinth system [24].

Figure 3 shows the profiles of the dissolved oxygen concentration at the given operating HRTs. The mean estimated dissolved oxygen inside the Bio-hedge reactor was 3.22 ± 0.15 mg/L O_2 at 14 h HRT. Oxygen diffusion from the atmosphere through the water's surface is a relatively inefficient process that seems to be much smaller in the water hyacinth pond because dense plant cover reduces surface gas-exchange. In contrast, vegetation plays an important role in transferring atmospheric oxygen down to their root system through an internal gas space (known as aerenchyma), and release a fraction of this oxygen (30%–40%) into the rhizosphere for aerobic microbial activity [33,34]. Root oxygen release rate from free floating plants has been reported to be 0.25–9.6 $g/m^2/day$ [35]. The results suggest that the present system has a high oxygen rich zone, facilitating growth of the aerobic microorganisms. This is comparable with previous study of phytoremediation of domestic wastewater using a shallow pond water hyacinth system (stated 4.48 ± 0.1 mg/L O_2 at a HRT of 21 h) [16]. The oxygen content in the shallow ponds was also found to be higher compared to the results of an earlier study of a conventional water hyacinth system; 2.54 ± 0.24 mg/L O_2 was obtained within the conventional cell at a HRT of 43 h [24]. This can be a proven in describing the effect of a depth water on oxygen transfer capacity.

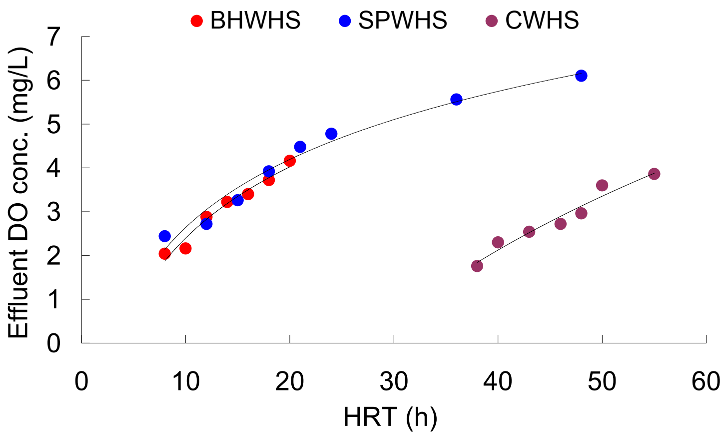


Figure 3. Mean DO values of the process effluent (*n* = 5).

The treated effluent of the Bio-hedge system at 14 h HRT was found to have a mean reduction of 73% ± 2.23% TSS, 77% ± 1.60% TN, 72% ± 1.84% NH_3-N, 45% ± 2.05% TP, 39% ± 1.76% PO_4-P, 94% ± 1.2% MPN, and 96% ± 0.75% TVC as compared to the inlet (Table 2). As presented in Table 2, the efficiency of the system was potentially greater than the previously reported conventional and shallow pond water hyacinth systems by 67% & 70% TSS, 69% & 74% NH_3-N, 31% & 41% PO_4-P, 91% & 96% MPN, and 92% & 98% TVC reduction at 43 & 21 h HRT, respectively [16,24]. Similarly, the Bio-hedge system has shown higher treatment efficiency than the primary facultative and the secondary facultative ponds reported in the literature, which achieved 52% & 67% TSS, 14% & 11% NH_3-N, and 5% & 21% TP reduction at 15 & 7 d HRT, respectively [4].

It is reported that particles can be removed in the water hyacinths treatment systems through filtration and sedimentation [36]. However, according to Kim *et al.* [37], the removal efficiency mostly depends on the retention time in wetland systems. As suspended solids pass through the plant roots (similarly to filtration process), they can be trapped, accumulate, and eventually settle under the force of gravity or become metabolized by microorganisms, while particulate matter sinks to the bottom. In the Bio-hedge system, the support matrix and plant roots act simultaneously to support the growth of biomass and retain solid particles continually into the biological process. The efficiency of nutrient (N, P) removal can be explained adequately by the plant uptake and nitrification/denitrification route. The Bio-hedge technique with an enriched microbial population and adequate oxygen transfer efficiency into the lagoon can provide a relatively higher nitrification rate over a retention time of 14 h. Phosphorus was the least efficiently removed pollutant in various water hyacinth treatment practices, even though greater P removal can be achieved by incorporating divalent cation materials as a result of the ligand exchange reaction. In addition, nutrient diminution can be accomplished by the frequent harvesting of vegetation.

Table 2. Summary and comparative evaluation of the treated effluent characteristics of water hyacinth treatment systems under satisfactory operating condition.

Parameters	BHWHS (14 h HRT)		SPWHS (21 h HRT)		CWHS (43 h HRT)	
	Outlet	Reduction at Outlet (%)	Outlet	Reduction at Outlet (%)	Outlet	Reduction at Outlet (%)
Temperature (°C)	27.20 ± 0.45	-	25.20 ± 0.84	-	24.20 ± 0.83	-
pH	7.25 ± 0.14	-	7.42 ± 0.12	-	7.20 ± 0.09	-
DO(mg/L)	3.22 ± 0.15	-	4.48 ± 0.08	-	2.54 ± 0.24	-
COD (mg/L)	85.20 ± 6.76	79.08 ± 1.48	81.70 ± 4.83	80.93	88.72 ± 4.80	75.53
BOD_5(mg/L)	26.40 ± 3.05	86.42 ± 1.67	20.20 ± 0.84	90.90	27.00 ± 1.60	87.26
TSS (mg/L)	44.40 ± 2.30	73.02 ± 2.23	45.00 ± 1.58	70.31	46.80 ± 1.92	67.40
TN (mg/L)	11.03 ± 0.60	76.61 ± 1.60	-	-	-	-
NH_3-N (mg/L)	9.08 ± 0.57	72.48 ± 1.84	8.14 ± 0.73	74.19	9.27 ± 0.50	69.27
TP (mg/L)	6.31 ± 0.47	44.84 ± 2.05	-	-	-	-
PO_4-P (mg/L)	4.48 ± 0.32	38.69 ± 1.76	4.59 ± 0.27	41.23	4.72 ± 0.24	30.80
MPN/100mL	9.00×10^5	94.08 ± 1.20	1.98×10^5	96.45	3.54×10^5	91.19
TVC (cfu/mL)	1.60×10^5	95.80 ± 0.75	3.04×10^5	98.20	2.64×10^5	92.35

Notes: All values are the mean of daily sample of 5 consecutive days, while the COD value represents the mean of 10 consecutive days merely in the shallow pond water hyacinth system; BHWHS: Bio-hedge water hyacinth system; SPWHS: Shallow pond water hyacinth system [16]; CWHS: Conventional water hyacinth system [24].

These results suggest that the performance of water hyacinth-based constructed wetland depends on the response of the system to the flow velocity and nutrient level. The conventional and shallow pond water hyacinth process as well as facultative pond systems can account for the resulting poor removal of organic compounds, and therefore, a longer HRT is required for a more effective wastewater treatment compared to the Bio-hedge practice. On the other hand, they require disinfection before being discharged into the receiving water bodies or else the effluent can be soil infiltrated without applying disinfectant agents.

3.2. Role of Plant

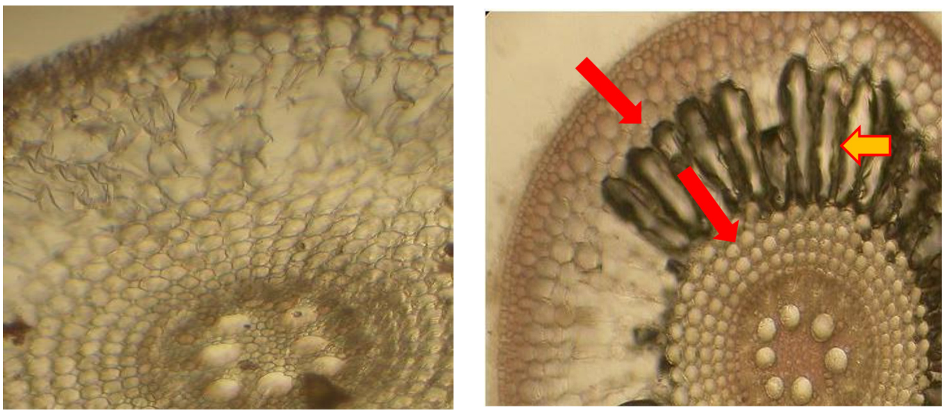
Macrophytes have a key function in relation to wastewater purification by provisioning a surface area for attached microorganisms, pollutant uptake, enhancing filtration, and releasing oxygen; however, the role of the vegetation still required quantification [38]. In this study, the comparison between the planted and unplanted systems (Table 1: Phase IV) has shown obvious difference in organic matter removal efficiencies indicating the positive role of water hyacinth in the process of phytoremediation. The planted Bio-hedge system proved to be more efficient (60%–70%) in removal of organic matters (79.08% ± 1.48% COD, 86.42% ± 1.67% BOD_5 (see Table 2) than unplanted unit (25.48% ± 3.51% COD, 33.30% ± 4.42% BOD_5). The mean COD and BOD_5 concentration at the outlet of the control reactor (unplanted unit) were 316 ± 20 mg/L and 142 ± 14 mg/L, respectively. This can be explained by the fact that in the absence of the rhizomes of the plants, the micro-sites occupied by the wetland unit become anaerobic; lead to a lower rate of impurities elimination.

The study on morphological aspects of water hyacinth clearly indicated that the domestic wastewater had no detrimental effects on the plant morphology, and despite the absorbed nutrient, the yield of the plants was increased (Table 3). This could verify the results of previous studies [16,24]. The relative growth rate (RGR) of plants was estimated to be 1.15% per day in treating domestic wastewater.

Table 3. Morphological characteristics of the plant at initial setup and after 10 days operation ($n = 5$).

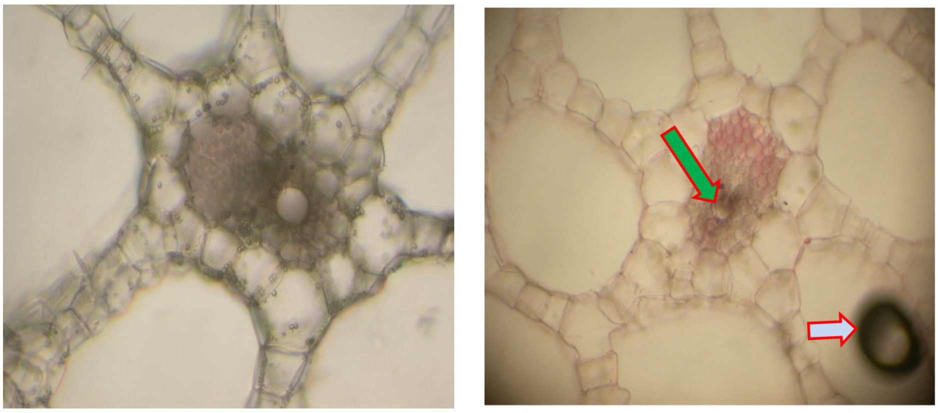
Parameter	Initial	After 10 Days
Number of leaves	7.80 ± 1.92	10.40 ± 1.36
Size of leaves (cm^2)	17.00 ± 3.36	20.24 ± 3.41
Number of roots	47.40 ± 10.14	92.20 ± 9.23
Longest root (cm)	9.66 ± 1.24	12.04 ± 1.31
Dry weight (g/plant)	0.90 ± 0.13	1.01 ± 0.11
Ash weight (g/plant)	0.07 ± 0.006	0.15 ± 0.005

Similarly, the histochemical studies also showed no anatomical damage on plants caused by wastewater exposure (Figure 4). A cross section of wastewater exposed root indicates presence dark patches due to absorbed impurities (Figure 4b). This result suggested that water hyacinth absorbs impurities through the epidermal regions and vascular bundles of the roots. Pollutants are translocated from the epidermis to the vascular bundles and further transported upwards to the plant. Cross section of water hyacinth petiole showing a bulbous portion (made of sclerenchymal cells and reduced vascular bundles) and large empty areas (walled by cells) that contain air to keep the plant afloat (Figure 4c,d). The pollutants conducted into the leaves via vascular bundles of the petiole (Figure 4d). In addition, aquatic plants can adapt morphologically and anatomically to growth in a water-saturated substrate through an internal gas space (called aerenchyma) [39].



(**a**) (**b**)

Figure 4. *Cont.*



(**c**) (**d**)

Figure 4. Cross sectioning of the root (**a**,**b**) and petiole (**c**,**d**) of water hyacinth before (**a**,**c**) and after treatment (**b**,**d**) of domestic wastewater (50× magnification). ⇨ Presence of the sewage; ➡ Translocation of impurities from the epidermis into vascular bundle and further upward to the plant (**b**); ➡ Translocation of impurities through the vascular bundles of the petiole (**d**); ⇨ Air bubble in the air cavities of the petiole.

The relevant amounts of nutrients contained in plant tissues are shown in Figure 5. Plants used for domestic wastewater treatment are subjected to have 78% and 82% higher TN concentrations, and 84% and 87% higher TP concentrations in their roots and shoots, respectively, compared to those under natural levels of nutrients (control plants). This could be explained by the fact that the plant species tend to accumulate more nutrients than are needed for growth when they exposed to nutrient-rich effluent. The plants are also allocating more nutrients in shoots than roots (by 38% for TN and 44% for TP in the excremental plants, and 27% for TN and 32% for TP in the control plants) which can probably be related to the maintenance of protein synthesis, electron transference in photosynthesis, and respiration processes. This indicated that the roots of the plants are mainly involved in the transportation. On the other hand, it found that plants tissues contained, on average 4–5 and 6–7 times more nitrogen than phosphorus in excremental and control plants, respectively, which can probably due to nitrogen standing stock (capacity to store N) of the plant [40].

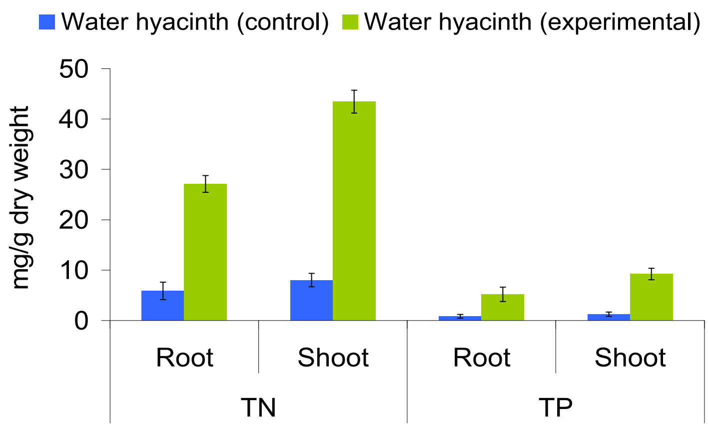


Figure 5. Nutrients (N & P) content in plant tissues before and after treatment by the Bio-hedge system (n = 5).

3.3. Role of Biofilms

The support matrix (Bio-hedges), immersed leaves, and plant roots create a unique environment for the attachment of the many microorganisms that are responsible for the degradation of pollutants. The design process is an improved phytoremediation system (using Bio-hedges) to enrich attached microbial populations that are dominated by aerobic respiration, particularly due to an efficient oxygen diffusion (caused by low water depth) and its supply to microsites largely by plant roots. Because aerobic bacteria grow much faster than anaerobic bacteria, they are greatly more practical and convenient for treatment performance. In present wetland unit, there is a reduction of the TVC level at the outlet, and biological mass is getting attached to the surface of the Bio-hedges. This could be phenomena in the initial stage of operation. At steady state condition, there will be uniform growth on the Bio-hedge surfaces, and biomass will slough and come out with the flowing water. Yet, a sufficient population of microflora is consistently preserved in the wetland unit to degrade the pollutant in the wastewater.

Bio-hedges provide evidence of considerable stability of the microbial population corresponding to the respective environmental condition of 4.06×10^7 cfu/cm^2. The amount of biofilm formed on the surface of the Bio-hedge was estimated to be 7 to 8 g/L. In addition to the support matrix (Bio-hedges), the root surface of the plants was found to be responsible for 3.12×10^4 cfu/cm of the bacterial consortia. Through nutrient agar medium, 15 bacterial strains (*Achromobacter xylosoxidans*, *Acinetobacter baumannii*, *Acinetobacter haemolyticus*, *Acinetobacter lowffii*, *Aeromonas sobria*, *Alcaligenes faecalis*, *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Moraxella lacunata*, *Pantoea* sp., *Pseudomonas stutzeri*, *Salmonella* sp., *Shigella* sp.) were isolated from the surface of the support matrix (Bio-hedges), and and 8 strains (*Acinetobacter lowffii*, *Aeromonas sobria*, *Bacillus circulans*, *Bacillus subtilis*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Pontoea* sp., *Salmonella* sp.) from the plant root surface. There is a possibility of other microbial populations, which could not be detected in a nutrient agar medium. The result indicated that the most of the bacteria are predominantly gram negative; this may be related to their ability to efficiently utilize growth substrates available in the rhizosphere and to cope with polluted environments due to the presence of detoxifying enzymes [41]. Accordingly, the root of vegetation was estimated to support a bacterial population of 2.72×10^4 cfu/cm and 3.95×10^4 cfu/cm, respectively, as used in the conventional and shallow pond water hyacinth systems [16,24]. The shallow pond technique contrary to conventional water hyacinth process provides a desirable condition for a superior aerobic microbial population responsible for organic degradation as a result of low water depth. Yet, the immersed leaves, and roots of the wetland plant merely create a site for biofilm growth in these two systems. This is suggested that the novel Bio-hedge technology facilitates a high population of microflora in the water hyacinth treatment system resulted in greater rate of impurities elimination (Table 2).

3.4. Role of Evapotranspiration and Evaporation

The evapotranspiration plays an additional important role by increasing the hydraulic retention time in wetland treatment systems. It is positively related to the impurity absorption, volatile compound emission into the atmosphere, and water purification capability index of plants [42]. The mean evapotranspiration rate of planted Bio-hedge system was evaluated to be 42.41 ± 5.70 mm/d (Table 4). Accordingly, the mean evaporation rate from unplanted Bio-hedge system was 2.60 ± 0.62 mm/d. This result suggest that evapotranspiration from a dense cover of water hyacinth could be 14–20 times greater than evaporation from equivalent unplanted area. The reason may be explained as that water hyacinth has a large surface area which contributes to a high rate of evapotranspiration. Moreover, there was little difference in the level of evapotranspiration, and evaporation between the given operating times that could likely due to small variations in temperature throughout the operation (Table 4). The same phenomenon is comparable to the conventional and shallow pond water hyacinth systems: an evapotranspiration rate of 58.38 ± 3.78 & 37 ± 1 mm/d in planted units, and an evaporation rate of 2.11 ± 0.70 & 1.94 ± 0.66 mm/d in unplanted units were observed at 43 (influent temperature: 25.40 ± 1.14 °C) & 21 (influent temperature: 26.80 ± 0.84 °C) h HRT, respectively [16,24]. There is no obvious difference in the relative evapotranspiration, and evaporation levels flanked by treatment systems. This can be explained by the almost similar surface area of the wetlands, plant population, plant size, and climatic conditions under which they have been operated. In fact, a dense stand of wetland plants is given a reason for high evapotranspiration rates [43]. It should be recognized that the evapotranspiration rate between different plant species can vary greatly. Therefore, evapotranspiration may also be an important selection criterion for plant species to use in a constructed wetland systems.

Table 4. Mean of influent temperature, evapotranspiration from planted unit, and evaporation from unplanted unit of a Bio-hedge system at different retention times (*n* = 5).

HRT (h)	Influent Temperature (°C)	Evapotranspiration (mm/d)	Evaporation (mm/d)
8	25.20 ± 0.84	35.48 ± 1.23	1.92 ± 0.75
10	25.60 ± 0.55	35.07 ± 0.75	2.19 ± 0.57
12	26.80 ± 0.84	41.23 ± 1.40	2.05 ± 0.48
14	27.20 ± 0.40	42.20 ± 1.146	2.33 ± 0.61
16	28.40 ± 0.90	45.34 ± 0.57	3.01 ± 0.78
18	29.80 ± 1.10	49.86 ± 1.23	3.30 ± 0.57
20	30.20 ± 0.84	47.67 ± 1.04	3.42 ± 0.68

3.5. Pre-Estimation of Capital Cost

Financial analysis is a valuable tool for examining sustainable technology [44]. A pre-estimation of economic analysis has been done for 1m^3/day of sewage inflow based on the results generated by bench scale trials (Table 5). The result of this analysis suggested that the application of the Bio-hedge process could lead to 66% less land space than the conventional system and 32% less land space than the shallow pond wetland water hyacinth systems; making the phytoremediation process more sustainable. The capital investment of Bio-hedge, shallow pond and conventional water hyacinth systems for an estimated capacity of 1 m^3/day sewage is USremove78, US$115 and US$231, respectively. In addition, there would be a cost associated with the matrix of the Bio-hedges, which depends on the type of material and market forces. The evaluation showed that the Bio-hedge technique is also a more economically viable treatment process in terms of capital cost compared to the conventional wetland systems and facultative ponds reported by the Economic Social Commission for

Western Asia (ESCWA) [45]. The purpose of this pre-estimation was merely to verify the advantages of the Bio-hedge treatment practice. The HRT of 14 h in bench scale Bio-hedge system may not translate as readily to full scale. Bench scale tests often give higher efficiencies than pilot and field experiments due to scale and edge effects. A detailed cost analysis of this technology needs to be performed in subsequent pilot and field experiments.

Table 5. Pre-estimated capital cost of the water hyacinth wetland system under satisfactory operating condition (Q = 1 m^3/d, COD < 100 mg/L and BOD_5 < 30 mg/L).

Item	BHWHS (14 h HRT)	SPWHS (21 h HRT)	CWHS (43 h HRT)
Volume (m^3/m^3_{inflow})	0.61	0.90	1.80
Area (m^2/m^3_{inflow})	4.06 (×0.15 depth)	6.21 (×0.145 depth)	3.83 (×0.47 depth)
Cost			
US$/$m^3_{inflow}$	78	115	231
US$/kg $BOD_{5\ removed}$	465	565	1247

Notes: BHWHS: bio-hedge water hyacinth system; SPWHS: shallow pond water hyacinth system; CWHS: conventional water hyacinth system.

According to the literature reports [8,46], water hyacinth and facultative ponds are reported with relative ease to construct, simple to design, low maintenance, inexpensive operation, and habitat value. They can also be used for wastewaters with higher levels of suspended solids. On the other hand, the conventional type of water hyacinth treatment system as well as facultative pond have lower removal rates of contaminants per unit volume, therefore they require more land space, and are expensive to construct in terms of capital cost. Odors and insects are a problem due to the free water surface and low treatment efficiency. Consistent with the findings from our previous research [16], however, shallow pond system has a higher removal rate of pollutant as compared to others, and prevents odors because of oxygen-rich environment, but there is still concerns regarding the large land area, capital investment, and insect-related problem. In that order, the Bio-hedge process is thought to have several advantages over types of water hyacinth and facultative ponds. In the Bio-hedge system, high pollutant elimination can be accomplished by simple and inexpensive means. This exceptional process offers a prominent cell mass concentration and microbial activity in treating wastewater. The specific arrangement of the Bio-hedge technique hampers the odor and insect-related problems due to effective transfer of oxygen through the root zone and the short retention time of 14 h. As a result, the performance of the Bio-hedge system can provide quantitative advantage given relatively smaller footprints and lower capital cost.

4. Conclusions

The study highlights the performance of an improved water hyacinth phytoremediation system coupled with attached microbial growth in a domestic wastewater treatment. The main features of the system are a low effective depth of 150 mm, highly dense plant population and the presence of plastic mesh type structures, called Bio-hedge. The Bio-hedges provide an efficient surface area for the microbial population to adhere and grow. The system also provides better oxygen transfer efficiency to sustain high levels of aerobic microflora for the degradation of many pollutants, particularly in tropical regions. The Bio-hedge technique has low space requirements, low capital investment and most importantly, a high degradation of organic pollutants, which was capable of achieving COD and BOD_5 removal as high as 79% and 86%, respectively, at a HRT of 14 h. Regression analysis revealed a correlation between the inflow and outflow for the COD and BOD_5 loadings. A high accumulation of nitrogen and phosphorus in the plant, complimented with high productivity and no anatomical changes, also indicated that water hyacinth could be a feasible macrophyte in the phytoremediation process. The system eliminates all the disadvantages of the conventional water hyacinth and facultative pond systems, *i.e.*, odor and insect related problems, slow biodegradation rate, and so forth. Similarly, it can be a great deal of promise in overcoming the possible limitations associated with shallow pond water hyacinth technique. Therefore, a new Bio-hedge water hyacinth approach can be an ideal practice to promote low cost green technology for organic pollutant degradation and impurity elimination, but more detailed pilot scale and field studies will be needed.

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Author Contributions

The overall implementation of this study including experimental design, data analysis and manuscript preparation were done by Alireza Valipour, Venkatraman Kalyan Raman and Young-Ho Ahn. Alireza Valipour and Young-Ho Ahn critically reviewed the article. All authors read and approved the final manuscript.

Appendix

Table A1. Experimental plant details of conventional and shallow pond water hyacinth treatment systems [16,24].

Features	**Details**	
	CWHS	**SPWHS**
Shape	Rectangular	Cylindrical
Area of the pond (m^2)	0.111 (0.37 m × 0.30 m)	0.140 (0.422 m diameter)
Height of the pond (m)	0.5	0.17
Effective depth (m)	0.47	0.145
Void Volume (L)	52	20
Density of plants	22 samplings (198–199 plant/m^2)	27 sampling (192–193 plant/m^2)

Note: CWHS: Conventional water hyacinth system; SPWHS: Shallow pond water hyacinth system.

Conflicts of Interest

The authors declare no conflict of interest.

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