Supporting Information

Enhancement of Treatment Capacity and Performance of Phytoremediation System by Fed Batch and Periodic Harvesting

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Table S1: Quality of fish farm wastewater

Parameter	Average value	Range
Ammonia, NH_3-N (mg/L)	24.75 ± 1.36	21.60 - 28.10
Nitrate, NO_3^--N (mg/L)	<0.3	<0.3
Nitrite, NO_2^--N (mg/L)	<0.01	<0.01
Phosphate, PO_4^{3-} (mg/L)	2.21 ± 0.11	1.98 - 2.48
TSS (mg/L)	83 ± 4	75 - 95
Turbidity (NTU)	81.20 ± 3.25	71.90 - 86.00
COD (mg/L)	125 ± 2	120 - 130
pH	7.78 ± 0.01	7.74 - 7.80

Table S2: Composition of Hoagland No. 2 medium[1]

Chemicals	Concentration (mg/L)
KNO_3	50.55
$(NH_4)_2.SO_4$	94.34
$MgSO_4.7H_2O$	246.40
KH_2PO_4	27.22
$Ca(NO_3)_2.4H_2O$	153.49
K_2SO_4	243.96
$CaSO_4.2H_2O$	232.36
$MnCl_2.2H_2O$	0.74
H_3BO_3	1.43
$ZnSO_4.7H_2O$	0.11
$CuSO_4.5H_2O$	0.04
$Na_2MoO_4.2H_2O$	0.01
$FeSO_4.7H_2O$	2.51
$Na_2.EDTA.2H_2O$	3.37

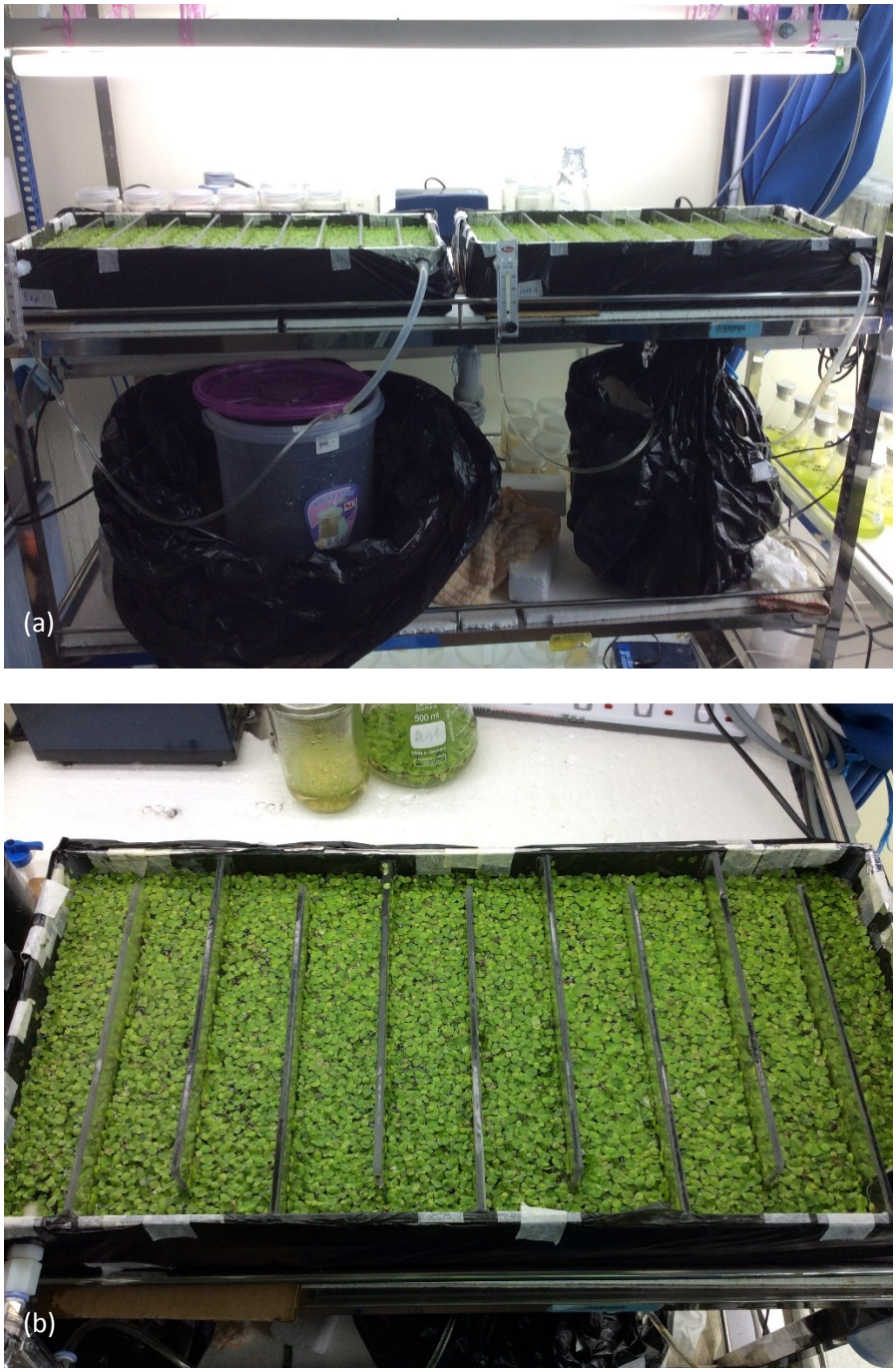

Figure S1: (a) Setup of the raceway pond rig systems in the laboratory with artificial lighting (b) Healthy green fronds of *Spirodela polyrhiza* covering surface of fish farm wastewater in the pond rig. Control system and Fed batch and harvesting system utilized *S. polyrhiza* monoculture system.

Protocol S1: Detailed methods and protocols employed for the water quality assay

Determination of Ammonia, Nitrate and Nitrite

The ammonia was determined by Salicylate Method (Lovibond method 66) using VARIO Am tube test Reagent (VARIO Ammonia Salicylate F5, VARIO Ammonia Cyanurate F5 powder packs and VARIO Am Diluent Reagent High Range reaction tube), with LOVIBOND Maxidirect MD600, Germany. The nitrate was determined by Cadmium Reduction Method (HACH method 8039) using NitraVer®5 Nitrate Reagent Powder Pillows, with HACH DR2800, USA. The nitrite was determined by Diazotization Method (Lovibond method 272) using VARIO Nitri3 F10 powder pack, with MD600 (Tintometer GmbH, Germany).

Determination of phosphate

The phosphate was determined by Ascorbic Acid Method (HACH method 8048) using PhosVer® 3 Phosphate Reagent Powder Pillows, with DR2800 (Hach Company, USA) in accordance to USEPA method 365.2 and Standard Method 4500-P-E for wastewater.

COD Determination

The COD was determined by Dichromate/H_2SO_4 Method (Lovibond method 131) using COD VARIO tube test Reagent, with MD600 (Tintometer GmbH, Germany). This method complies with Standard Methods for the Examination of Water and Wastewater [2].

Determination of Turbidity

The water samples were well-mixed by shaking the centrifuge tube vigorously. A clean cuvette was filled with water sample until the level mark. The cuvette was wiped with lint-free tissue before inserting into measurement cell to measure its turbidity value. The turbidity value

was determined by HI98703 microprocessor turbidity meter (Hanna Instruments, Romania) with range of 0-1000 NTU. The measurement conforms to USEPA Method 180.1 for wastewater and Standard Method 2130B for drinking water.

Determination of TSS

Well-mixed water samples were prepared by shaking the centrifuge tube vigorously. 30ml samples were filtered through a 47mm diameter weighed Whatman™ glass microfiber filters, Grade GF/C (1.2 μm) in filtration apparatus with a mini air pump and the residues retained were dried in an oven at 105^{o}C for 1 hour. The drying process was repeated until constant weight was gained. The increase in weight of filter represents the total suspended solids. This test was performed based on APHA 2540D.

Determination of pH

pH measurement was performed using Hanna Edge® pH meter HI-2020, USA. The water sample was continuously stirred with pH probe until the pH reading stabilised.

$$Pollutant\ removal\ efficiency(\%) = \frac{C_0 - C_t}{C_0} \times 100\%$$

(Equation S1)

where C_0 represents initial pollutant concentration and C_t is the pollutant concentration on day t of experiment

For control system,

$$Total\ pollutant\ removed\ (mg) = (C_0 - C_{16}) \times V_0$$

(Equation S2)

For fed batch and harvesting system,

$$Total\ pollutant\ removed(mg) = (C_0 - C_4) \times V_0 + (C_{4,i} - C_8) \times V_{4,i} + (C_{8,i} - C_{12}) \times V_{8,i} + (C_{12,i} - C_{16}) \times V_{12,i}$$

(Equation S3)

where C_0, C_4, C_8, C_{12} and C_{16} represent pollutant concentration at day 0, 4, 8, 12 and 16 respectively, $C_{4,i}$, $C_{8,i}$ and $C_{12,i}$ show the pollutant concentration after replenished with fresh wastewater at day 4, 8 and 12, V_0 represents wastewater volume at day 0 while $V_{4,i}$ $V_{8,i}$ and $V_{12,i}$ are wastewater volume after replenished with fresh wastewater at day 4, 8 and 12.

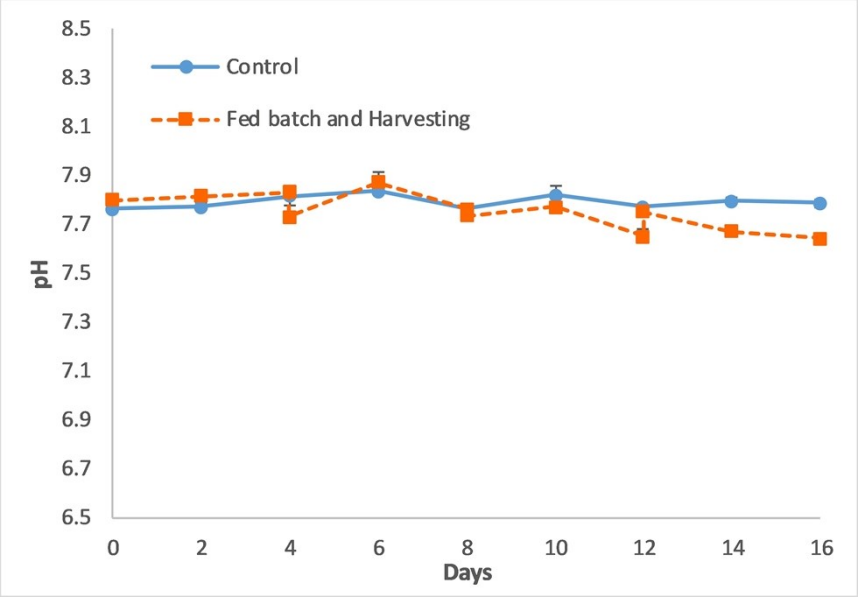

Figure S2: pH profile of fish farm wastewater in *S. polyrhiza* monoculture systems with fed batch and periodic harvesting, and without fed batch and periodic harvesting (control) during 16 days of treatment study.

Table S3: Summary of treatment capacity and performance for control system and fed batch and harvesting system.

	Control system	**Fed batch and harvesting system**
Volume of wastewater treated/processed/discharged	12 L	30 L
Ammonia (Total removed/ removal efficiency/ meet 5 ppm limit?)	298.20 mg 83% yes	756.60 mg 81% yes
Nitrate (% of samples exceeding 10 ppm)	0	18
Nitrite (% of samples exceeding 1 ppm)	0	64
Phosphate (Total removed/ removal efficiency/ meet 5 ppm limit?)	21.60 mg 68% yes	74.88 mg 76% yes
TSS (Total removed/ removal efficiency/ meet 50 ppm limit?)	840 mg 61% yes	4365 mg 87% yes
Turbidity (% of decrement compared to initial value)	74 (first 4 days)	93 (average percent in the end of all terms)
COD (Total removed/ removal efficiency/ meet 80 ppm limit?)	1176 mg 65% yes	5466 mg 75% yes

The removal efficiency shown for control system is first 4 days' efficiency while for fed batch and harvesting system, its removal efficiency is shown by the average efficiency in the end of all terms. As for determining whether the level meeting their standard limit, first 4 days' level is used for control system while mean level in the end of all terms is employed for fed batch and harvesting system.

References

1. Hoagland, D. R.; Arnon, D. I., The water-culture method for growing plants without soil. *Circular. California Agricultural Experiment Station* **1950,** *347*, (2nd edit).
2. APHA; AWWA; WEF, *Standard methods for the examination of water and wastewater*. American Public Health Association: Washington (DC), 1992.