# Preliminary Design for Phytoremediation of Phosphate From Liquid Fertilizer Waste by Duckweed (*Spirodela* sp.) and Yellow Iris (*Iris pseudacorus* L.) Integrated with Biorefinery-based Processing System to Produce Value-added Bioproducts

Marcelino Putra Perdana*, Rifqi Hakim Dewanto, Muhammad Ihsan Harjanto

Bioengineering Department, School of Life Sciences and Technology, Institut Teknologi Bandung, Jalan Ganesha No 10, Bandung, 40312, Indonesia

**e-mail: marcelinoputra017@students.itb.ac.id*

***Abstract*—Agriculture is one of the biggest of Indonesia's economic sectors, with the area used for agriculture totaling 8.19 million hectares. The usage of agricultural fertilizers is causing significant environmental contamination, with one of the biggest contaminants being excess phosphate. This is because phosphate is one of the most intensely used fertilizers but has the lowest plant absorption rate. Excess phosphate can cause the eutrophication of large bodies of water. One method used to reduce this effect is phytoremediation with *Spirodela* sp. and *Iris pseudacorus* L, which can reduce phosphate concentrations from 29.625 mg/l to 0.2 mg/l. By applying the biorefinery concept, in which plant biomasses are used, *Spirodela* sp. can be utilized to produce duckweed powder with a yield of 20.8% and flavonoids can be extracted from iris plants to produce flavonoid powder with a yield of 20.9%. These byproducts add economic value to the system to generating a gross profit margin of 5.91, thus indicating the profitability of applying the biorefinery concept to phytoremediation.**

***Keywords—phytoremediation; biorefinery; excess phosphate; value-added byproducts***

## I. INTRODUCTION

Agriculture is one of most the most prominent sectors in the Indonesian economy. According to a publication released by the Agricultural Ministry of Indonesia, the land area used for agricultural practices totaled 8.19 million hectares in 2017. This intense activity also requires vast amounts of fertilizer, which is used a source of plant nutrients. Although necessary to use, fertilizer is often applied excessively, which causes nutrients not absorbed by the plants to remain in the soil. These leftover nutrients can contaminate the water and soil, and when transported by rain or water runoff, this contamination can spread to larger bodies of water. One of the intensely used fertilizers contains phosphate, since it is a very effective for plants. However, its level of absorption in plants is relatively low—just 15% of the total amount of phosphates in fertilizer is absorbed. Unabsorbed phosphate is usually transported distances by water runoff and causes eutrophication in lakes and rivers [1].

One of methods for removing this contaminant is phytoremediation, which uses plants to reduce or diminish the effect of contaminants in water and soil. In this process, the factors that determine its feasibility are the contaminant properties, level of contamination, condition of the contaminated area, and the plants used as phytoremediators [2]. *Iris pseudacorus* L. is an acaulescent plant with long leaves and a rhizome root, and reaches 1 meter in height. This plant typically lives in wetlands and other shallowly submerged land. Research shows that its phosphate remediation ability ranges from 25–34.17% [3]. As such, *Iris pseudacorus* L. has good potential for use as a phytoremediator. Duckweed (*Spirodela* sp.) is a plant with a simpler morphology, consisting of a few oval–shaped leaves approximately 5 mm in size. This plant lives in colonies and has a short growing time. Duckweed also has potential for use as a phytoremediator. The authors of one study found that duckweed reduces the total suspended solids, biochemical oxygen demand, chemical oxygen demand, excess nitrate, ammonia, and Cu, Pb, Cd, and Zn contents by 96.3%, 90.6%, 89.0%, 100%, 82.0%, 64.4%, 100%, 100%, 93.6%, and 66.7%, respectively [4].

The construction of wetlands is one application of the phytoremediation concept in which wetland plants or other aquatic plants are used as phytoremediation agents. In this research, we considered the subsurface flow (SSF) type of constructed wetland. This system uses submerged plants as filters. Plants used as phytoremediators can be further processed to reduce waste based on the biorefinery concept in which biological resources are transformed into other products to increase their economic value [5]. In this application, duckweed can be used as a high-protein powder and the iris plant can be utilized for its flavonoid content. Flavonoids can be used as both anti-oxidant and anti-cancer substances. Aside from reducing the degree of phosphate contamination, a phosphate phytoremediation system that incorporates the biorefinery concept increases the economic value of the plants used as phytoremediators.

## II. Materials and Methods

### A. Analysis of Phosphate Removal Efficiency using Phytoremediation

In this research, we analyzed the potential for the phytoremediation of excess phosphate using duckweed, *Spirodela* sp., and the iris plant, *Iris pseudacorus* L. Duckweed was retrieved from Azola Purwodadi and iris plants were obtained from the School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia. This research was conducted at the Instructional Laboratory of the Bioengineering Department, Institut Teknologi Bandung.

Phytoremediation was performed over a time span of two weeks, using *Spirodela* sp. as the phytoremediator in the first week and *Iris pseudacorus* L. in the next. Growth was measured based on the dry and fresh weights. We measured the phosphate concentration in the growth medium and the biomass of the phytoremediating agent using the method developed by Kuttner & Cohen [6].

### B. Design of Process Flow Diagram

Process flow diagrams are used to illustrate a production process and the resulting production units. In this paper, we explain the integration of excess-phosphate phytoremediation with protein supplement production and flavonoid extraction. The process flow diagram used in this research was developed using SuperPro Designer® software.

### C. Mass Balance Analysis

Mass balance analysis is used to estimate the inflow of a substrate and the outflow of products during a process. In this research, we used mass balance analysis to estimate the amount of plant biomass obtained by the phytoremediation activity and their byproducts, which include a protein supplement and flavonoid extracts.

### D. Economic Analysis

We performed an economic analysis to examine the feasibility of this biorefinery-based production system. To do so, we calculated the gross profit margin (GPM) by determining the difference between the product and raw material prices divided by raw material price as shown in Eq. (1).

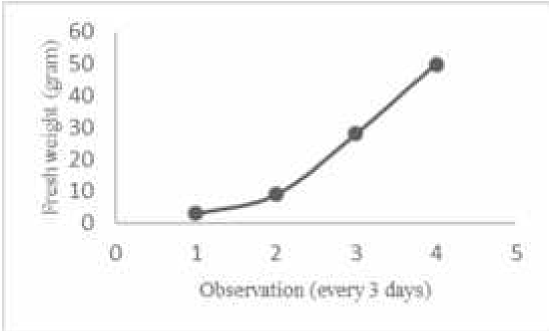
$$GPM = \frac{(product\ price - raw\ material\ price)}{raw\ material\ price} \quad (1)$$

## III. Results and Discussion

### A. Analysis of Phosphate Removal Efficiency using Phytoremediation

We measured the growth of the phytoremediator and the phosphate concentrations in the growth medium and the biomass of the phytoremediator every three days over the two-week study period. Figure 1 shows the dry and fresh weight data obtained for *Spirodela* sp.

a.




b.

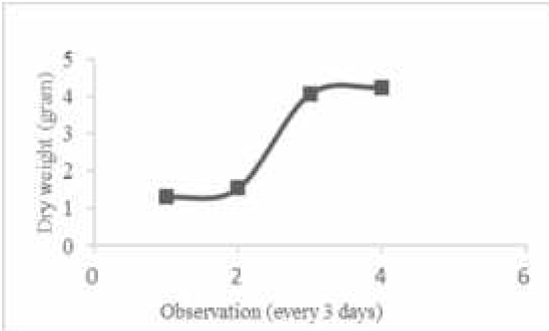
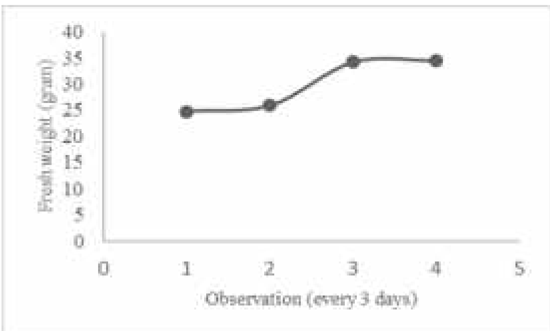



Fig. 1. Growth of *Spirodela* sp. according to its fresh weight (a) and dry weight (b).

From the data shown in Fig. 1, we determined that the specific growth rate of *Spirodela* sp. was 1.1329 grams day$^{-1}$. There was no indication of a deceleration in the growth of *Spirodela* sp., therefore it can be concluded that growing *Spirodela* sp. in excessive phosphate conditions does not cause an inhibitory effect.

a.



b.

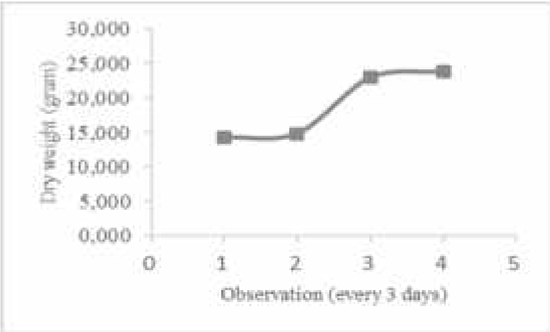
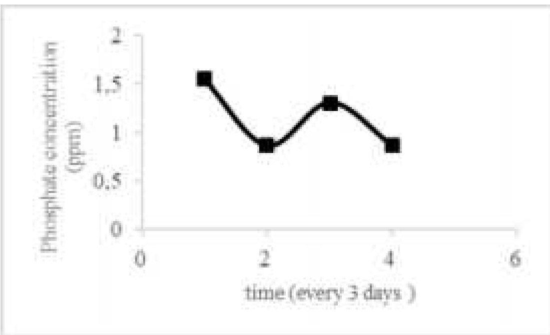



Fig. 2. Growth of *Iris pseudacorus* L. according to its fresh weight (a) and dry weight (b).

We investigated the growth of *Iris pseudacorus* L. in the same fashion. From the data shown in Fig. 2, we can see that growing *I. pseudacorus* in an excess-phosphate medium yielded a specific growth rate of 3.6832 grams $day^{-1}$. Thus, growing *I. pseudacorus* in an excess-phosphate condition generated no inhibitory effect.

Next, we determined the phosphate concentration in the growth medium using the spectrophotometry method, with ammonium molybdate and $SnCl_2$ as reagents. The addition of these reagents promotes a reaction by the phosphate ion to create an ammonium phosphomolybdate complex. This complex is then reduced by $SnCl_2$, and takes on a distinct shade of blue from the molybdenum, which serves as an indicator of the amount of phosphate in the mixture and can be analyzed by spectrophotometry to obtain its degree of absorbance. By creating a standard curve, the concentration in the mixture can be obtained [7]. Figure 3 shows the data used to determine the absorbance in this assay.

a.




b.

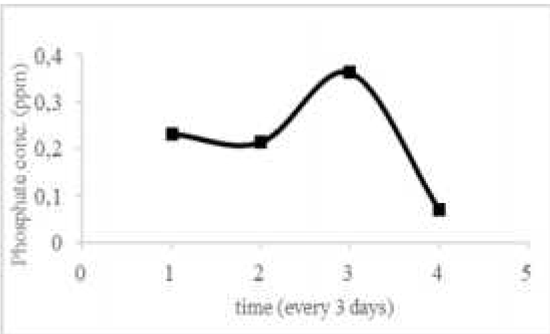



Fig. 3. Reduction of phosphate concentration in growth medium of *Spirodela* sp. (a) and *Iris pseudacorus* L. (b).

As depicted in Fig. 3, there were considerable reductions in the phosphate concentrations in both the growth media of *I. pseudacorus* L. and *Spirodela* sp. The reduction of phosphate in *Spirodela* sp. occurrred at a rate of 0.1602 ppm $day^{-1}$ and in *I. pseudacorus* L. at 0.034 ppm $day^{-1}$.

Figure 4 shows a diagram of the proposed constructed wetland used for phytoremediation with *Spirodela* sp. and *Iris pseudacorus* L.

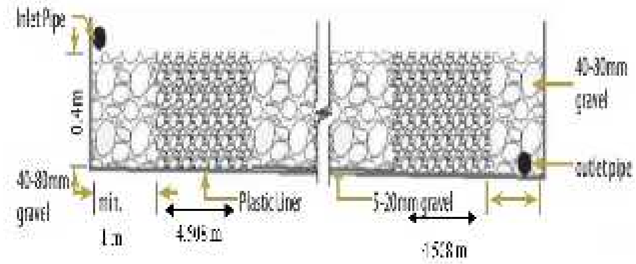



Fig. 4. Schematic diagram of the constructed wetland.

The constructed wetland was designed to utilize a horizontal subsurface flow (HSF) system. This system design has several important parameters, including the depth of the wetlands, the wetland area, and the hydraulic residence time. The depth of the HSF wetland was set at 40 cm and the porosity of the substrate at 40%. Using as a basis an average daily influent wastewater flow rate (Q) of 40 $m^3$/day, the size of the constructed wetland can be determined using Eq. (2) [8]:

$$A_h = \frac{Q_d(\ln C_i - \ln C_e)}{K}, \quad (2)$$

where $A_h$ is the surface area of the wetland ($m^2$), $Q_d$ is the average daily flow rate of wastewater ($m^3$/day), $C_i$ is the influent phosphate concentration (mg/l), $C_e$ is the effluent phosphate concentration (mg/l), and K is a first-order rate constant (m/day). We assumed the average daily influent phosphate concentration ($C_i$) to be 29,625 mg/l [9] and our objective was to reduce the effluent phosphate concentration ($C_e$) up to 0.2 mg/l by the use of the phytoremediation method. K can be determined using Eqs. (3) and (4) as follows:

$$K = K_T dn \quad (3)$$

$$K_T = K_{20}\,(1.06)^{(T-20)}, \quad (4)$$

where $K_{20}$ is the first-order rate constant at 20 ºC ($day^{-1}$) = 1.1 $day^{-1}$, T is the operational temperature of the system (25 ºC), d is the depth of the water column (m), and n is the porosity of the substrate medium (expressed as a fraction). Given that the average value of d is 0.4 m and that of n is 40% or 0.4, as recommended by UN-Habitat [10], the value of KT was determined to be 0.236 m/day. Thus, the $K_T$ value was used to obtain the surface area of the wetland ($A_h$), which was 847.128 $m^2$.

Stagnant pools in a wetland can be caused by a poor flow pattern, which can lead to scum accumulation and mosquito breeding. A solution to this problem is to design multiple flow paths, so that the system is broken down into units that are easier to maintain. In general, an optimum length-to-width ratio in a constructed wetland is 5:1 [11]. Figure 5 shows the design and dimensions of a constructed wetland.

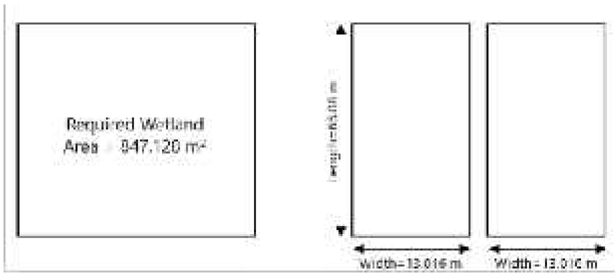



Fig. 5. Design criteria of the constructed wetland system.

The hydraulic residence time is the average time that the wastewater influent remains in the wetland system, which is directly correlated with the effectiveness of contaminant removal in the wetland system [12, 13]. The hydraulic residence time (t) can be determined using Eq. (5):

$$t = \frac{Lwnd}{Q_A}, \quad (5)$$

where L is the length of the wetland system parallel to the flow direction (m) = 65.08 m and w is the width of the wetland system (m) = 13.016 m. Given that the value of L is 65.08 m and w is 13.016 m, the hydraulic residence time (t) was calculated to be 3.389 days for each path.

## B. *Process Flow Diagram*

Figure 6 shows a diagram of the process in which the duckweed biomass is washed and dewatered to reduce the possibility of contamination, and is then heated at a certain temperature until dry [14]. Then, the dried duckweed is ground and milled to a certain size to obtain a powdered form of duckweed. This product can be used by food industries as a high-protein supplement. With respect to *I. pseudacorus*, after being washed and dewatered, it is dried at room temperature (23–34 °C) and then ground to a powder. The flavonoid content is then extracted from this powder by submerging it in 75% ethanol for 24 hours. The solid waste of this extraction is then separated from the ethanol and used as organic compost and the liquid extract of *I. pseudacorus* is freeze-dried and powdered, then stored at −4 °C for preservation until use [15].

## C. *Mass Balance Analysis*

As the basis for calculation in this analysis, we used a 40 cm x 40 cm area of one yellow iris, the weight of which was 100 g [16], and a duckweed density of 1 kg/m$^3$. About 40,000 liters of wastewater will flow through phytoremediation system 1. At this rate, the duckweed biomass will increase to about 423 kg, assuming that the growth rate of all the duckweed is similar. The duckweed biomass is then washed and dewatered, dried, and powdered, resulting in 88.27 kg of dry duckweed powder. The wastewater from system 1 is then processed again in system 2 that contains *Iris pseudacorus* L. The iris biomass will increase to about 275 kg and then be washed and dried, and its flavonoid content extracted using 423 liters of methanol 75% to obtain 170.5 kg of flavonoid extract. The solid waste from this extraction can be used as organic compost, with a total amount of 123.2 kg. The flavonoid extract is then dried and maltodextrin added to

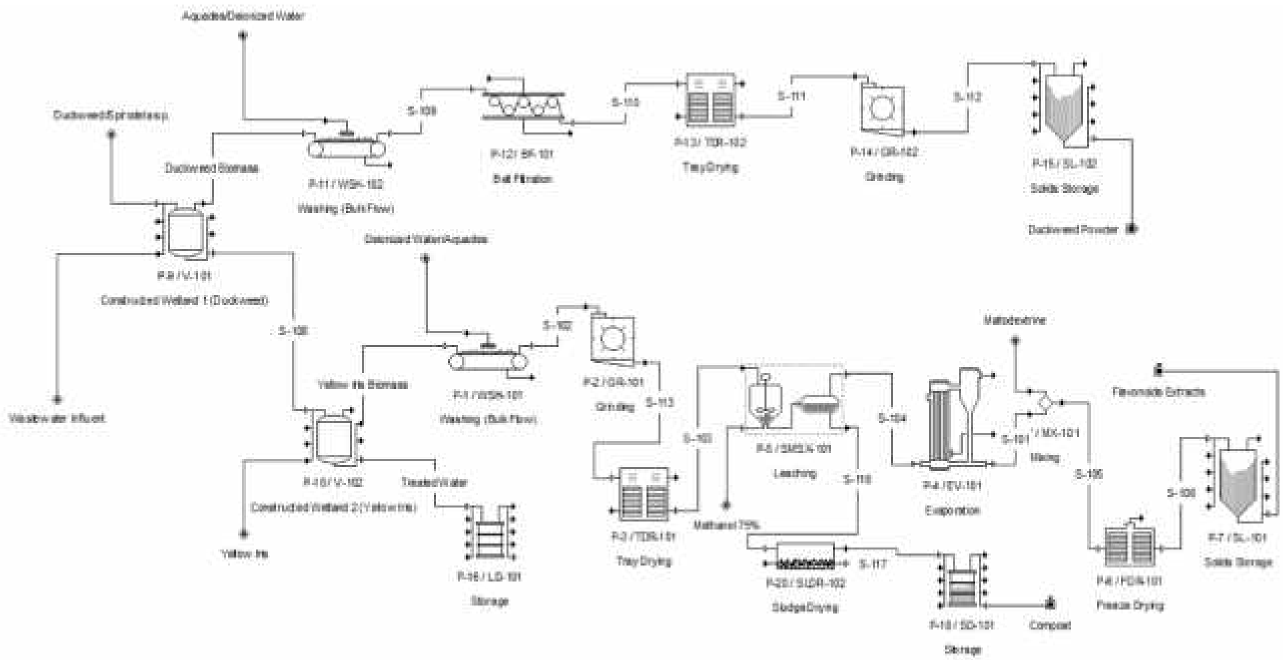


Fig. 6. Process flow diagram.

yield a powdered form in the amount of 57.6 kg. Figure 7shows a diagram of the overall mass balance analysis procedure.

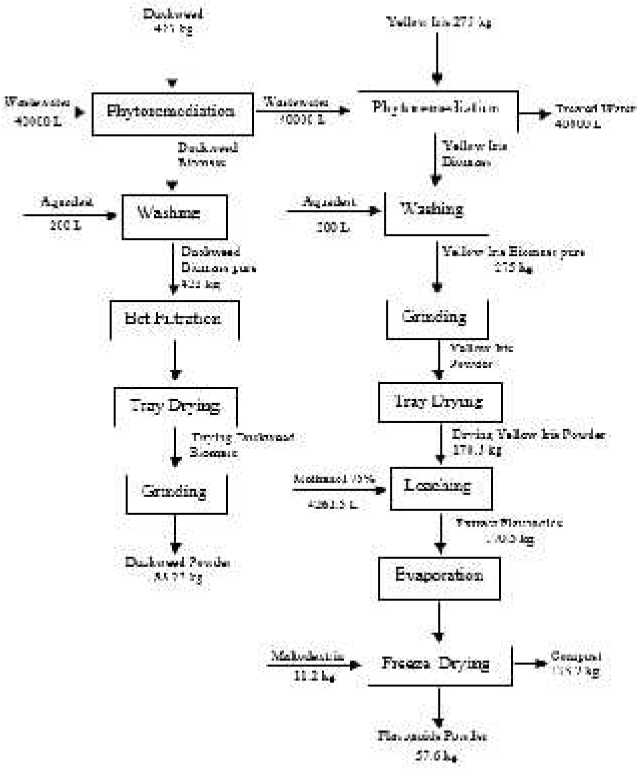



Fig. 7. Mass balance analysis diagram.

### D. Economic Analysis

Using the estimated amounts of products and materials from the mass balance analysis, it is possible to perform an economic analysis to obtain an overview of the financial aspects of this production system. This economic analysis was conducted by calculating the resulting GPM, as shown in Table I.

TABLE I. GPM ANALYSIS

| Raw Materials | | | |
|---|---|---|---|
| ***Materials*** | ***Amount (kg)*** | ***Price (Rupiah per kg)*** | ***Total Price (Rupiah)*** |
| Aquadest | 500 | 1,000 | 500,000 |
| Yellow iris | 275 | 20,000 | 5,500,000 |
| Duckweed | 423 | 10,000 | 4,230,000 |
| Methanol 75% | 337 | 11,000 | 371,3600 |
| Maltodextrine | 11.2 | 13,000 | 145,600 |
| TOTAL | | | 14,089,500 |
| **Products** | | | |
| ***Materials*** | ***Amount (kg)*** | ***Price (Rupiah per kg)*** | ***Total Price (Rupiah)*** |
| Treated water | 40,000 | 750 | 30,000,000 |
| Duckweed powder | 88.27 | 455,790 | 40,232,583 |
| Flavonoids powder | 57.6 | 468,870 | 27,006,912 |
| Compost | 123.2 | 2,000 | 246,400 |
| TOTAL | | | 97,485,895 |
| GPM | | | 5.919 |

This calculation yielded a GPM of 5.919, which means that this activity is deemed to be profitable.

## IV. CONCLUSIONS

In this research, we developed an excess-phosphate phytoremediation system that integrates the use of *Spirodela* sp. and *Iris pseudacorus* L. based on a biorefinery concept to reduce 29.625 mg/l phosphate to 0.2 mg/l in 40000 liters of work volume, thereby obtaining water of acceptable quality according to Indonesian standards. In addition, this system can produce 88.27 kg of duckweed powder for use as a protein supplement and 57.6 kg of flavonoid powder extract from *Iris pseudacorus* L., as well as 123.2 kg of organic compost per batch. These products are value-added byproducts that increase the economic value of this system, yielding a 5.92 GPM. These research results show that the application of the biorefinery concept can not only reduce the negative impact of excess-phosphate waste, but also provide economic value, thus reinforcing the sustainability of this system. One significant aspect of this research is its demonstration of the feasibility of performing environmental remediation while also generating economic benefits, thereby contradicting popular beliefs that remediation practices are costly and unprofitable. This finding encourages our research team as well as other prospective parties to conduct further more extensive research and applications of this technology, as this research represents only a preliminary step toward understanding the potential of this system.

## ACKNOWLEDGMENT

Greatest appreciation is expressed to Mr. Taufikurrahman, lecturer of the Phytoremediation course, who made this research possible, and to the Professional Development Division of HMRH ITB for their help in the realization of this research.

## REFERENCES

[1] Interstate Technology and Regulatory Council (ITRC), *Phytotechnology*: *technical and regulatory guidance and decision trees*, Revised, Phyto-3, Washington, DC, 2009.

[2] U.S.EPA, “In situ treatment technologies for contaminated soil: engineering forum issue paper,” U.S.EPA, Paper EPA 542-F-06-013, Nov. 2006.

[3] Zhang Xiao-bin, Liu Peng, Yang Yue-suo, and Chen Wen-ren, “Phytoremediation of urban wastewater by model wetlands with ornamental hydrophytes,” *Journal of Environmental Sciences*, vol. 19, no. 8, (2007): pp. 902-909.

[4] J. V. Pancho and M. Soerjani, *Aquatic weeds of southeast Asia*: *a systematic account of common Southeast Asian aquatic weeds*. Los Baños: University of the Philippines, 1978.

[5] H. Chen, *Lignocellulose biorefinery engineering: principles and applications*. Cambridge: Woodhead Publishing, 2015.

[6] T. Kuttner and H. R. Cohen, “Micro colorimetric studies: I. A molybdic Acid, stannous chloride reagent. The micro estimation of phosphate and calcium in pus, plasma, and spinal fluid,” *J. Biol. Chem.*, vol. 75, (1927): pp. 517-531.

[7] M. D. Wichman, “Fate and toxicity of volatile organic chemicals in a poplar plot,” M. S. thesis, Univ. Iowa, Iowa City, 1990.

[8] S. C. Reed, R. W. Crites, and E. J. Middlebrooks, *Natural systems for waste management and treatment*, 2nd ed. New York: McGraw-Hill, 1995.

[9] C. A. Stefhany, M. Sutisna, and K. Pharmawati, “Fitoremediasi phospat dengan menggunakan tumbuhan eceng gondok (*Eichhornia crassipes*) pada limbah cair industri kecil pencucian pakaian (laundry),” *Jurnal Reka Lingkungan*, vol. 1, no. 1, (2013): pp. 1-11.

[10] *Constructed Wetlands Manual*, UN-Habitat Water for Asian Cities Programme, Kathmandu, Nepal, 2008, pp. 18-24.

[11] G. Siracusa and A. D. La Rosa, “Design of a constructed wetland for wastewater treatment in a Sicilian town and environmental evaluation using the emergy analysis,” *Ecological Modelling*, vol. 197, no. 3-4, (2006): pp. 490-497.

[12] M. Piñeyro, J. Cabrera, F. Quintans, M. Tejera, and G. Chalar, “Effects of hydraulic residence time in experimental constructed wetlands on wastewater treatment of a fish factory,” *Pan-American Journal of Aquatic Sciences*, vol. 11, no. 2, (2016): pp. 93-102.

[13] R. M. Conn and F. R. Fiedler, “Increasing hydraulic residence time in constructed stormwater treatment wetlands with designed bottom topography,” *Water Environment Research*, vol. 78, no. 13, (2006): pp. 2514-2523.

[14] C. D. Collins, “Strategies for minimizing environmental contaminants,” *Trends in Plant Sci.*, vol. 4, no. 2, (1999): pp. 45.

[15] K. J. Appenroth, K. S. Sree, V. Böhm, S. Hammann, W. Vetter, M. Leiterer, and G. Jahreis, “Nutritional value of duckweeds (Lemnaceae) as human food,” *Food Chem.*, vol. 217, (2007): pp. 266-273.

[16] C. Caldelas, J. L. Araus, A. Febrero, and J. Bort, “Accumulation and toxic effects of chromium and zinc in *Iris pseudacorus* L.,” *Acta Physiologiae Plantarum*, vol. 34, no. 3, (2012): pp. 1217-1228.