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PHYTOTOXICITY OF COFFEE WASTEWATER TO WATER HYACINTH AS PRIOR STEP TO PHYTOTREATMENT ASSESSMENT: INFLUENCE OF CONCENTRATION AND AMOUNT OF PLANT BIOMASS

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Abstract

Phytoremediation is an alternative technology for treating domestic and industrial wastewater. Coffee wastewater contains high value of biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solid (TSS) and turbidity. The wastewaters also possess a distinctive dark brown colour with acidic medium characteristic. This research was amended to determine the capability of water hyacinth (*Eichhornia crassipes*) to treat coffee wastewater. This phytotoxicity test comprised of plant analysis, coffee wastewater characterization, and removal of pollutant parameters under various amount of initial number of plants and coffee wastewater concentration. The result of wastewater characterization showed high COD, TSS, NH₃-N, colour and acidic pH. Phytotoxicity test indicated that all plants grow in 10% of coffee wastewater and in 30%, almost all the plants died at day 5. The best removal efficiency reaching up to 93.8% for COD, 90.2% for TSS, 95.0% for NH₃-N, and 45.4% for colour at 28 days of exposure confirming that Phyto-treatment of coffee wastewater using water hyacinth is very reliable, especially for polishing the effluent before discharge into water bodies.

Key words: aquatic plant, toxicity, *Eichhornia crassipes*, phytoremediation, wastewater treatment

Received: September, 2020; Revised final: January, 2021; Accepted: March, 2021; Published in final edited form: September, 2021

1. Introduction

The wastewater created from coffee industry contains high concentration of organic compound like pectin, proteins and sugars (Ijanu et al., 2020; Sengupta et al., 2020). In general, coffee wastewater having a characteristic of high biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solid (TSS), turbidity, dark colour and acidic pH (Said et al., 2020). If not treated properly, this kind of wastewater will harm the environment during discharge into the water bodies. Aquatic environment will be the most affected ecology by the

discharge of this wastewater without proper handling (Al-Ajalín et al., 2020; Yahya et al., 2020).

Several treatments had already been applied to treat coffee wastewater, including physical and chemical treatments (Pérez et al., 2007). Due to high concentration of pollutants, the effluent of these processes mostly still do not fulfil the effluent standard set by the government (Said et al., 2021). A technology to polish the effluent before final discharge to water bodies is highly needed and biological treatment could be the better option (Sheikh and Bramhecha, 2018). Biological treatment is currently a popular alternative due its ability in performing high

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removal efficiency of pollutants without leaving any harmful by-products (Imron et al., 2020). Phyto-treatment, one type of biological treatment, is one of the choices in polishing wastewater with high organic pollutants content (Abdullah et al., 2020; Imron et al., 2019, 2020; Purwanti et al., 2019).

Water hyacinth (*Eichhornia crassipes*) is one type of floating aquatic plants which are proven to have good capability in removing pollutants (Cossu et al., 2001; Nash et al., 2019; Said et al., 2020; Shardendu et al., 2012; Sooknah and Wilkie, 2004). Tien et al. (2018) reported the feasibility of *E. crassipes* in treating high wastewater containing ammoniacal nitrogen. Water hyacinth also showed the capability of performing pH neutralization and reduction of BOD, COD, total nitrogen, nitrate, ammonium and phosphate around 90% from aquaculture wastewater (Akinbile and Yusoff, 2012). Shardendu et al. (2012) also signified the capability of water hyacinth in performing luxury uptake of phosphorus from their environment. The combination of *Phragmites karka* and *E. crassipes* had exhibited the capability of removing TSS, colour and COD from coffee processing wastewater up to 94%, 79% and 95%, respectively (Said et al., 2020).

Based on the previous research results, there is no doubt that water hyacinth is a prospective treatment agent to be used in Phyto-treatment. The capability of this plant alone in treating coffee wastewater is currently still not much explored yet. Hence, this research was aimed to analyse the phytotoxicity of coffee wastewater to water hyacinth, to determine the treatable concentration according to pollutant to plant biomass ratio and to analyse the removal efficiency of some parameters in coffee wastewater using water hyacinth. The presented results might highlight the potential of polishing coffee wastewater or any wastewater with similar characteristics using this plant.

2. Materials and methods

2.1. Experimental set-up

Coffee wastewater was collected from a local

coffee factory in Penang, Malaysia and characterized for pH, COD, TSS, NH₃-N and colour. It was used throughout the whole research period. This phytotoxicity test was conducted to determine the concentration of coffee wastewater and the amount of plant biomass to be used in the subsequent main stage (Cleuvers and Ratte, 2002; Purwanti et al., 2019; Titah et al., 2014).

The influence of initial plant biomass was assessed by exposing different number of plants (*Eichhornia crassipes*) to different concentration of coffee wastewater. Table 1 shows the reactor set-up on this study. The number of plants used in this research were 8, 10, and 12 plants with each plant had an average of 30-40 g for the initial wet weight. The coffee wastewater concentrations used in this study were 10%, 20%, and 30% (v/v diluted with tap water), with another tank filled with tap water was used as control reactor (Fig. 1).

All variables were identified initially. This test was conducted using clear glass tank (0.28 m × 0.19 m × 0.46 m). The total exposure period in this study was 28 days with parameter analyses conducted on day 0, 5, 7, 14, 21, and 28. Physical observation was also monitored to analyse the effect of the coffee wastewater concentration to the visual appearance of water hyacinth.

Plant growth in terms of wet weight was obtained at days 0 and 28, while plant response towards coffee wastewater was evaluated based on relative growth rate (RGR) using Eq. (1) (Al-Baldawi et al., 2020; Ismail et al., 2020). The benefit from this calculation is to get a better understanding of the ability of this plant to survive while Phyto-treating this contaminant.

$$RGR = \frac{\ln W_{28} - \ln W_0}{days} \tag{1}$$

where: W_0 and W_{28} are the wet weight of plant at 0 and 28 days of exposure towards coffee effluent. Calculation of RGR was conducted using wet weight to avoid plant destruction during the process since number of plants was one of the factors to be assessed in this study.

Table 1. Experimental set-up for phytotoxicity of water hyacinth in coffee wastewater

Plant numbers (wet weight)	Coffee wastewater concentration			
	0%	10%	20%	30%
8 plants				
10 plants				
12 plants				

2.3. Analysis of coffee wastewater quality

2.3.1. pH measurement

The pH was analysed by a pH meter (Metrohm, Model 827, Malaysia). The pH was measured by dipping the electrode into a container containing the coffee wastewater sample.

2.3.2. Measurement of chemical oxygen demand (COD)

The measurement of COD was conducted by using a spectrophotometer (HACH DR 3900, USA) following the Standard Method (APHA, 2017). A 2 mL of distilled water (blank) and 2 mL of wastewater samples were poured into the vials containing COD reagent. Both of the mixture was shaken in order to accelerate the reaction and then placed into the COD reactor (HACH DR 200, USA). COD reactor was preheated until it achieved a temperature of 150 °C for 2 hours. After 2 hours, COD reagent was allowed to cool down until the temperature drops to room temperature.

The wastewater sample were then tested by using wavelength 420 nm spectrophotometer with the prepared blank as the reading standard. In addition, the ratio of initial COD content to wet weight of plants in coffee wastewater was determined using Eqs. (2) and (3).

$$\frac{COD}{WW} \left(\frac{mg}{g} \right) = \frac{COD_i}{WW} \quad (2)$$

$$COD_i (mg) = [COD] \times V \quad (3)$$

where: COD_i is initial COD content (mg), WW is wet weight of plant (g), $[COD]$ is initial COD concentration (mg/L) and V is volume of the sample (L).

2.3.3. Measurement of total suspended solid (TSS)

A 0.45 µm filter paper was previously heated at temperature 105°C for an hour and then allowed to cool in the desiccator for 15 min to remove water content. The initial weight of the filter paper was measured after drying. A 20 mL of wastewater sample was filtered on the paper by using a vacuum pump. The filter paper that contained suspended solid was heated in the oven for an hour at temperature of 105°C. The weight of the filter paper with suspended solid was then measured again. The total suspended solids present in the sample was calculated by using the Eq. (4) (Ye and Li, 2009).

$$TSS \left(\frac{mg}{L} \right) = \frac{(W_F - W_0)}{V} \quad (4)$$

where: W_F is final weight of plant (mg), W_0 is initial weight of plant (mg), and V is volume of the sample (L).

2.3.4. Measurement of ammoniacal nitrogen

The concentration of ammoniacal nitrogen (NH_3-N) in the coffee wastewater sample was measured using 425 nm wavelength spectrophotometer (HACH DR 3900, USA). A 25 mL of distilled water (blank) and 25 mL of wastewater sample was filled in vials. A 1 mL of Nessler reagent, three drops of mineral stabilizer and three drops of dispersing polyvinyl reagent was mixed into both of the vials. Measurement was conducted on the HACH spectrophotometer with prepared blank as the standard reading (Sooknah and Wilkie, 2004).

2.3.5. Colour measurement

Colour of the wastewater was measured using spectrophotometer (HACH DR 3900, USA) at 455 wavelengths. Distilled water was used as a blank during colour measurement (Said et al., 2021).

2.4. Statistical analysis

Statistical analysis was performed using SPSS Statistics version 21 (IBM, USA). Normality test was performed using Kolmogorov-Smirnov Test and normally distributed data then analysed further using One-Way analysis of variance (ANOVA) (Kurniawan and Imron, 2019a). ANOVA was conducted to analyse the correlation between factors (initial concentrations and number of plants) to the response (parameter removal). Significant correlation between factor and response then analysed further using Tukey HSD test to obtain the significant difference between the obtained results (Kurniawan and Imron, 2019b). All conclusions were drawn using p -value with $p < 0.05$ indicates the significant difference between result.

3. Results and discussion

3.1. Characteristics of coffee wastewater

The original and several wastewater concentrations used in this study (10%, 20% and 30%) physico-chemical characterization of the coffee wastewater were listed in Table 2. It can clearly be seen that the measured parameters were high above the permissible effluent standard set by the Environmental Quality Act 1974 (Department of Environment Malaysia, 2020). The coffee wastewater was rich of organic compounds, having high turbidity, acidic pH, and high colour viscosity as also stated by Said et al. (2020).

3.2. Physical observation and analysis of plant growth

Table 3 and Fig. 1 represent the physical observation and plant growth response of water hyacinth when exposed to coffee wastewater, respectively. It can be seen that the exposure of 30% coffee wastewater concentration had a negative effect on the water hyacinth. All plants were observed dead with weight reduction at the end of the exposure period.

Table 2. Initial characteristics of coffee wastewater

Parameter	Unit	Concentration				Effluent Standard*	
		Original	10%	20%	30%	A	B
pH	-	3.192	5.13	4.08	3.82	6-9	6-9
COD	mg/L	17,900	1,600	2,400	3,600	80	200
TSS	mg/L	150	43	115	120	20	100
NH ₃ -N	mg/L	600	61	66	130	10	20
Colour	ADMI	4,300	850	1,900	2,610	100	200

*Department of Environment Malaysia (2020)

Table 3. Visual observation of plants during treatment period

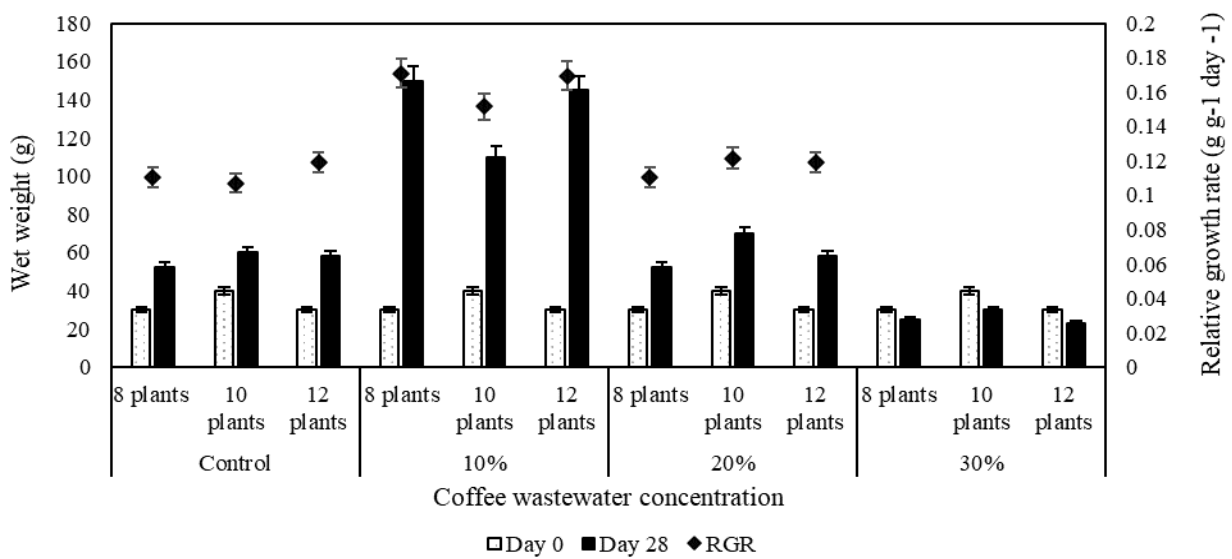
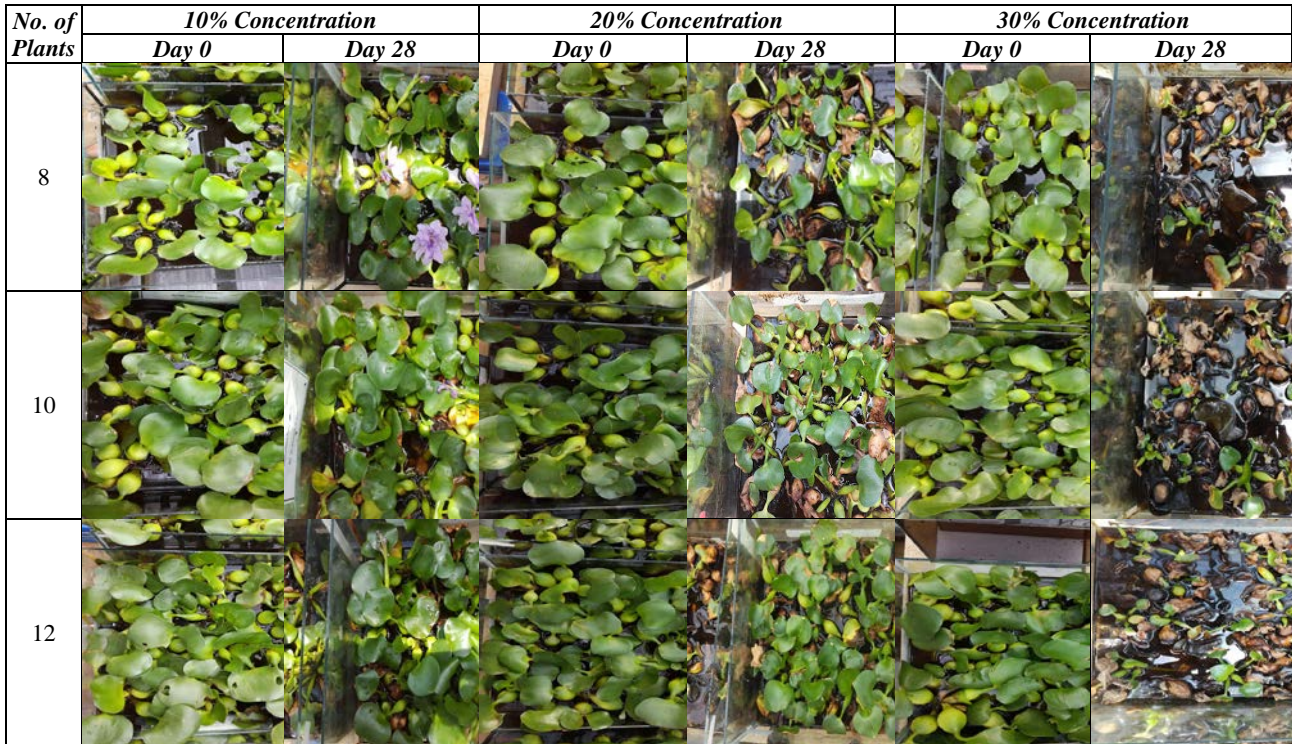


Fig. 1. Wet weight and relative growth rate (RGR) of water hyacinth in coffee wastewater (RGR for 30% concentration were undefined due to negative values of weight difference).

Asterisk symbol (*) indicates the significantly higher RGR between wastewater concentrations

In 20% concentration of coffee wastewater, 6 out of 8 plants, 6 out of 10 plants, and 7 out of 12 plants were withered. Even though the plants were withered, these survived plants showed an increase in weight on day 28. For 10% concentration of coffee wastewater, the plants showed the highest increase in growth and even showing good metabolism activity as flower bloom on reactor filled with eight plants. No plant death was recorded in 10% coffee wastewater concentration.

These results indicating that plants cannot tolerate the concentration of 20% and 30% of coffee wastewater, due to the toxicity given by the higher concentration of pollutants inside the wastewater (Chandanshive et al., 2017; Purwanti et al., 2020). The 10% concentration with 1800 mg/L COD showed the most feasible wastewater concentration to be treated by this plant ($p < 0.05$). Finding from this research was higher at 2.1 fold than a Phyto-treatment of high organic effluent from sago mill using water hyacinth, with the highest COD concentration that the plant

could tolerate and survive was 820 mg/L (Nash et al., 2019).

3.3. pH variations

The observed pH varied significantly among different treatments (Fig. 2). The pH increased gradually until day 28 specially in 10% coffee wastewater, starting from 5.13 to 6.4. For 20% concentration, the pH was increased from 4.08 to 6.12 and for 30% concentration, there is no significant change observed on pH ($p \geq 0.05$). Measured pH on 10% and 20% concentration was significantly higher as compared to the 30% concentration ($p < 0.05$), while there is no significant difference for the variation of plant biomass ($p \geq 0.05$).

These results confirm the potential of water hyacinth in performing pH stabilization of wastewater (Akinbile and Yusoff, 2012), indicated by all final pH on 10% and 20% wastewater concentration was measured as neutral.

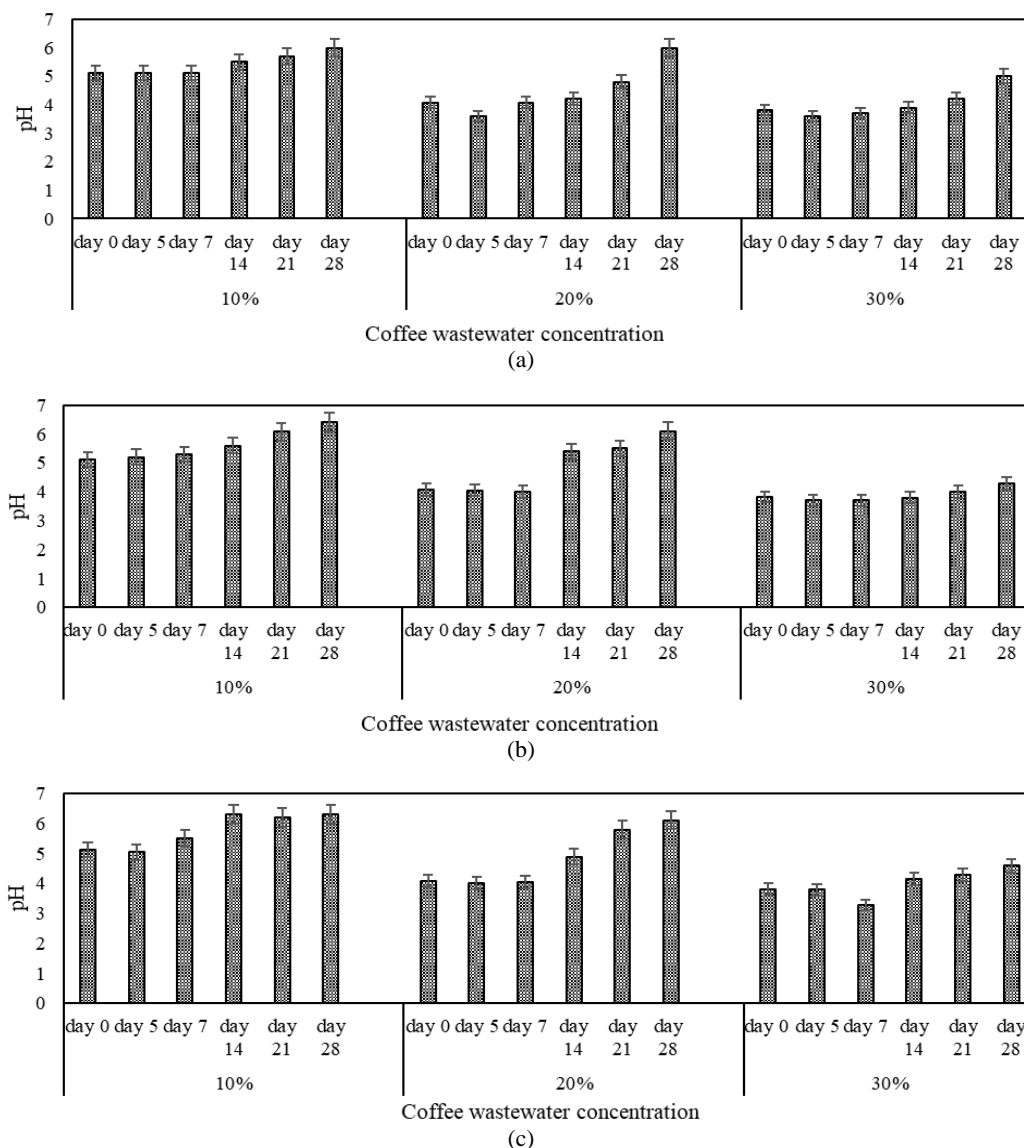


Fig. 2. pH of wastewater for (a) 8 plants (b) 10 plants (c) 12 plants systems. Asterisk symbol (*) indicates the significantly higher pH between wastewater concentrations

Acidic pH of coffee wastewater was observed due to the organic acid content inside wastewater (measured as COD) (Xu et al., 2020). Removal of these compounds from wastewater may affect (increase) the pH of effluent after treatment. The obtained result was in accordance with (Xu et al., 2020), which stated that floating macrophytes can uptake ionic compounds and organic materials thus contributes to the neutralization of pH.

3.4. COD variations and COD to plant ratio

Fig. 3 shows the concentration and removal efficiency of COD from coffee wastewater after exposed to water hyacinth. Most of the reactors has been experiencing decrease and increase of COD

throughout the research period. The increase of COD concentration was subjected to the death and decomposition of plants inside reactors (Žaltauskaitė et al., 2014). The optimum removal efficiency in 10% coffee wastewater was 93.8% at day 28 for 8 plants. In 20% concentration of coffee wastewater, the optimum removal efficiency was 64.8% at day 7 for 10 plants after that the efficiency dropped to 27.5%. For the 30% concentration, the highest removal was 44.1% then decreased until 0% due to the death of the plants towards the end of exposure period. There is no significant difference in the variations of plant biomass to the removal of COD ($p \geq 0.05$), while 10% of wastewater concentration showed significantly higher COD removal than other concentrations ($p < 0.05$).

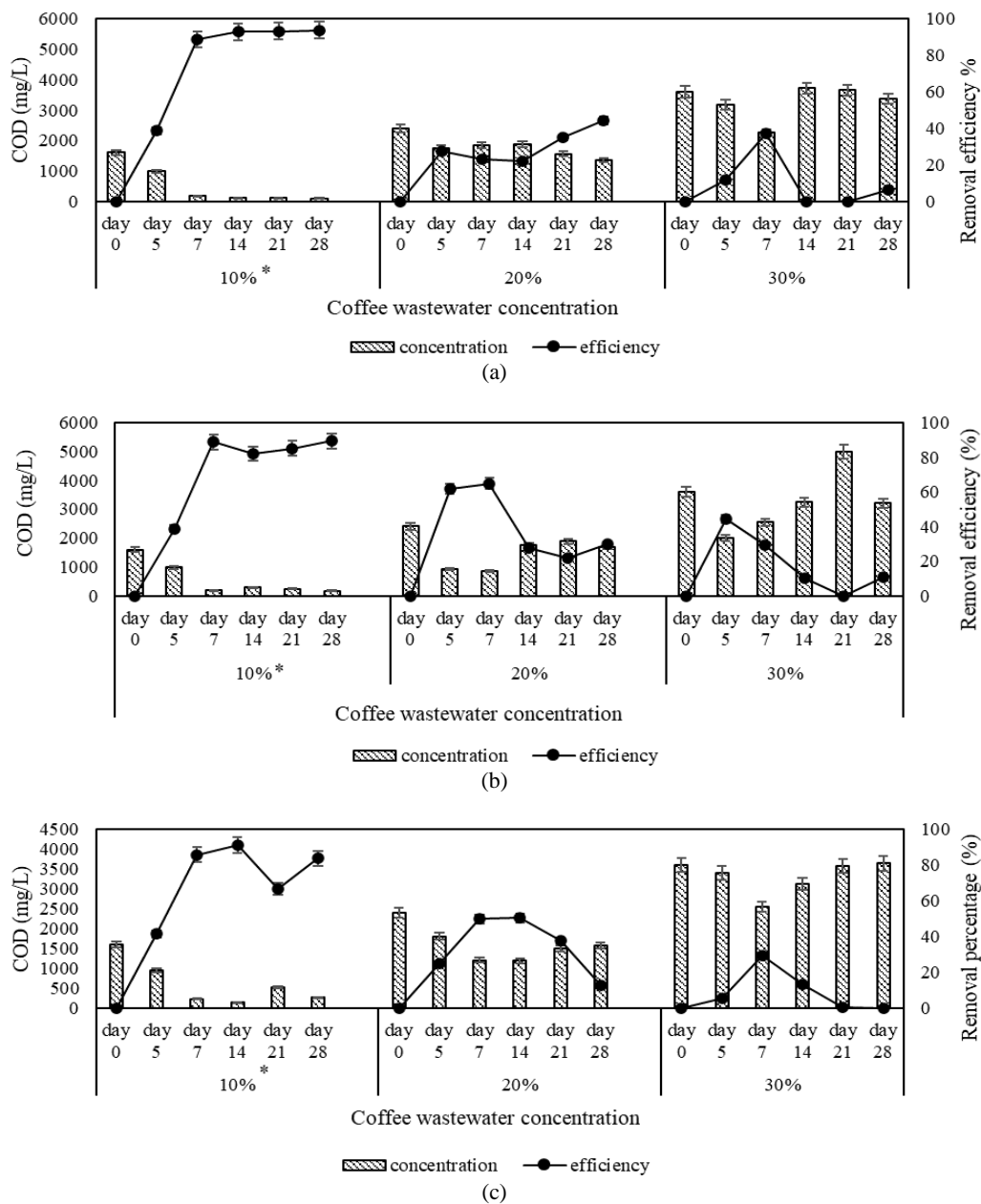


Fig. 3. COD in wastewater for (a) 8 plants (b) 10 plants (c) 12 plants systems. Asterisk symbol (*) indicates the significantly higher COD removal between wastewater concentrations.

These results indicate that the higher COD concentration, the lower the removal it achieves (Mahajan et al., 2019). As also mentioned by Ali et al. (2020), higher pollutant concentration caused the death of floating plants used in phytoremediation. The death biomass also contributed to the COD concentration, thus increasing the COD value throughout the research period (Žaltauskaitė et al., 2014). *Eichhornia crassipes* was proven to be able to remove COD from wastewater with the efficiency ranged from 40 to 70% (Priya and Selvan, 2017). COD removal was occurred via Rhizo-degradation mechanisms, in which bacteria in rhizosphere area performed the degradation of organic materials (Jehawi et al., 2020), while plants providing good environment for bacteria to live in by secreting exudates (also known as Phyto-stimulation) (Hawrot-Paw et al., 2019). Plants can also uptake organic materials (and its intermediate compounds) then utilize further in their metabolism (known as phytoextraction and phytodegradation) (Li et al., 2016, Tangahu et al., 2019). In addition, it can be seen as the COD to wet weight of plants ratio decreased (Table 4), the plant growth increased (Fig. 1). Increasing plant growth will lead to high COD removal. The lowest COD to plant ratio was achieved at 10% coffee wastewater concentration, i.e., 2.000 to 2.667 mg COD/g. Under this ratio, all plants survived throughout the exposure period with the highest removal of COD (93.8%). In contrast, within 4.500 to 6.000 mg COD/g, most of the plants cannot survive at the end of the treatment period and lead to 0 to 44.1% of COD removal. A proper selection of COD to wet weight of plants ratio is a great importance to remove COD effectively. Therefore, the ratio value suggested for future treatment of coffee wastewater using water hyacinth is within 2.000 to 2.667 mg COD/g.

3.3. TSS variations

Fig. 4 show the results of TSS analysis on each sampling day. Most of the reactors showed a reducing trend during the experimental time for all plants systems. The 30% concentration of coffee wastewater with 12 plants showed an increase of TSS after 5 days of treatment due to the additional suspended solid caused by the death plants. The highest TSS removal of 93.02% was obtained on 10% coffee wastewater concentration planted with 8 water hyacinths. The TSS removal was not solely affected by the increasing of concentration and the greater number of plants also not showing the increasing of TSS removal. Among all wastewater concentration, 10% showed significantly higher TSS removal as compared to other concentrations ($p < 0.05$). The 8 plants initial biomass also showed a significantly higher TSS removal as compared to other initial biomasses ($p < 0.05$). TSS removal inside this system is considered to be occurred from the physical precipitation and Rhizo-filtration mechanisms (Elias et al., 2014). TSS removal performance by *E. crassipes* was also

highlighted by Priya and Selvan (2017) during the treatment of textile effluent wastewater with removal efficiency up to 90%.

3.4. $\text{NH}_3\text{-N}$ variations

Fig. 5 shows the results of $\text{NH}_3\text{-N}$ analysis on each sampling day during the research period. Most of the reactors exhibited an increasing trend of removal until day 21, then stagnant and even decreasing until the day 28. Some reactors (the 20% and 30% concentration planted with 10 plants) showed a high decrease of removal on day 14, this result might be subjected to the release of N from the death plants biomass inside the reactor (Aczel, 2019). For 10% concentration the $\text{NH}_3\text{-N}$ was reduced greatly up to 95% on 10 plants system. These final concentration from this treatment set up at day 28 already fulfil the standard A and B of the Environmental Quality Act 1974. For 20% concentration, the highest removal at day 28 was obtained on 12 plants system with 87.9% $\text{NH}_3\text{-N}$ removal efficiency. The highest removal of 80% was obtained on 30% coffee wastewater concentration with 10 water hyacinths planted on the system. The concentration of 10% showed significantly higher ammoniacal nitrogen removal as compared to other concentrations ($p < 0.05$), while there is no significant difference between the initial plant biomass ($p \geq 0.05$). Ammonium was undertaken by plants as nutrient source, also degraded by bacteria in the rhizosphere area, resulting in the removal of this compound from wastewater (Kadir et al., 2020; Sooknah and Wilkie, 2004). These results showed that the water hyacinth was capable in removing ammonium from coffee wastewater with reliable removal efficiency (Ijanu et al., 2020).

3.5. Colour variations

Fig. 6 shows the results of colour analysis on each sampling day during the experiment. The best removal efficiency in this experiment was 45.4% obtained on 20% of coffee wastewater in 8 plants system with colour decreased from 1900 to 1033 ADMI. The 20% coffee wastewater with 10 plants system also showed a noticeable removal of 36.3%. The rest of the reactors showed low final colour removal efficiency. This obtained result indicated that the addition of plants inside the system did not correlated with the colour removal of coffee wastewater ($p \geq 0.05$). The 10% concentration might also be clear enough to be treated while the 30% concentration might be too dense thus creating a toxic effect to plants. The removal of colour by plants can be obtained via several mechanisms including Phyto-stabilization, Rhizo-degradation, Rhizo-filtration, and Phyto-degradation (Abdullah et al., 2020; Almaamary et al., 2019; Tangahu et al., 2011). In accordance with the obtained result, Priya and Selvan (2017) highlighted the superior performance of *E. crassipes* in treating colour in textile wastewater with removal efficiency up to 99.5%.

Table 4. Ratio of initial COD content in coffee wastewater to the fresh wet weight of water hyacinth

Concentration of coffee wastewater	10%			20%			30%		
COD concentration (mg/L)	1,600			2,400			3,600		
Volume of coffee wastewater (L)	20			20			20		
Initial COD content (mg)	80			120			180		
No. of plants	8	10	12	8	10	12	8	10	12
Initial fresh wet weight of water hyacinth (g)	30	40	30	30	40	30	30	40	30
Ratio of COD to wet weight (mg/g)	2.667	2.000	2.667	4.000	3.000	4.000	6.000	4.500	6.000

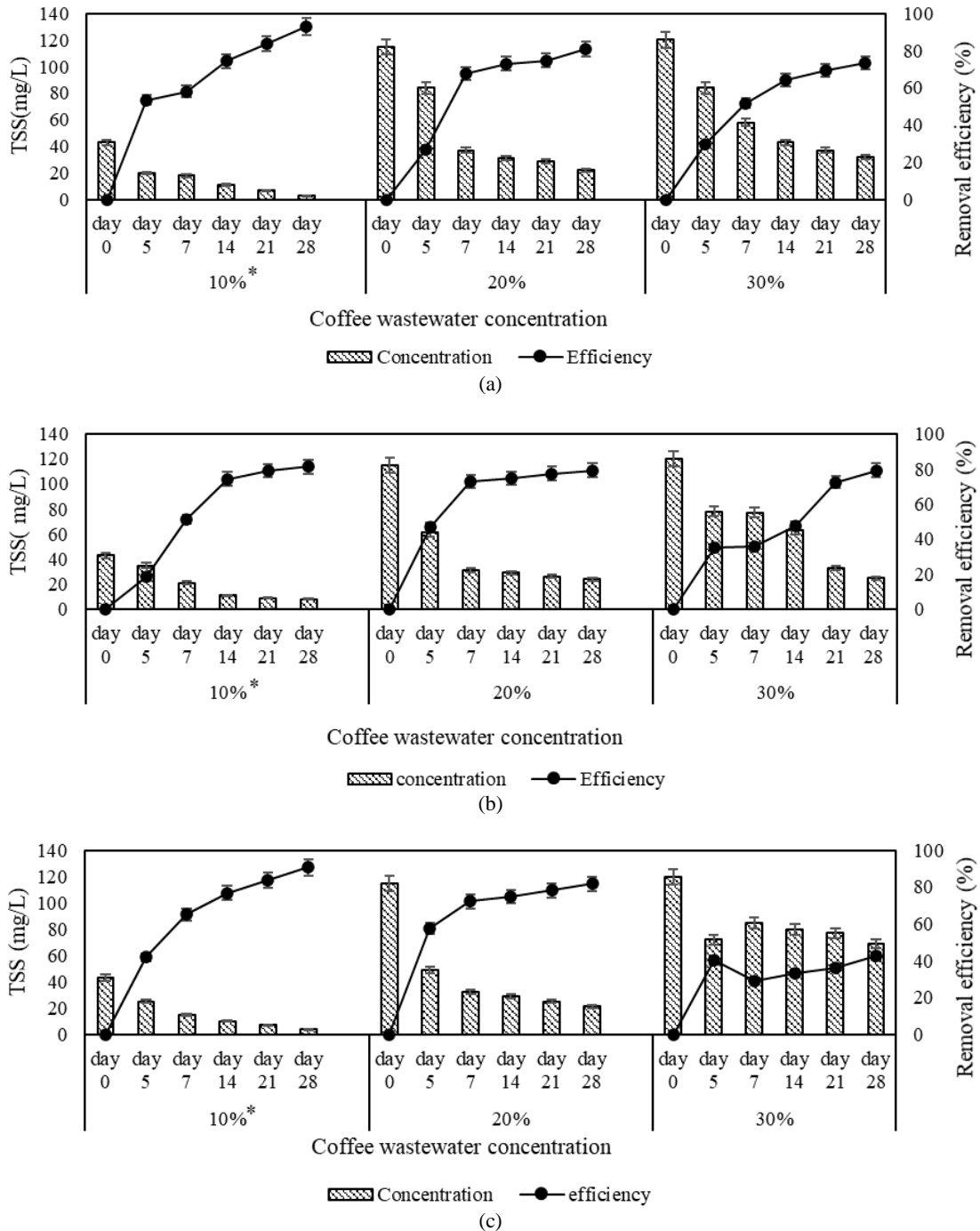


Fig. 4. TSS in wastewater for (a) 8 plants (b) 10 plants (c) 12 plants systems. Asterisk symbol (*) indicates the significantly higher TSS removal between wastewater concentrations. Octothorpe symbol (#) indicates the significantly higher TSS removal between initial plant biomasses

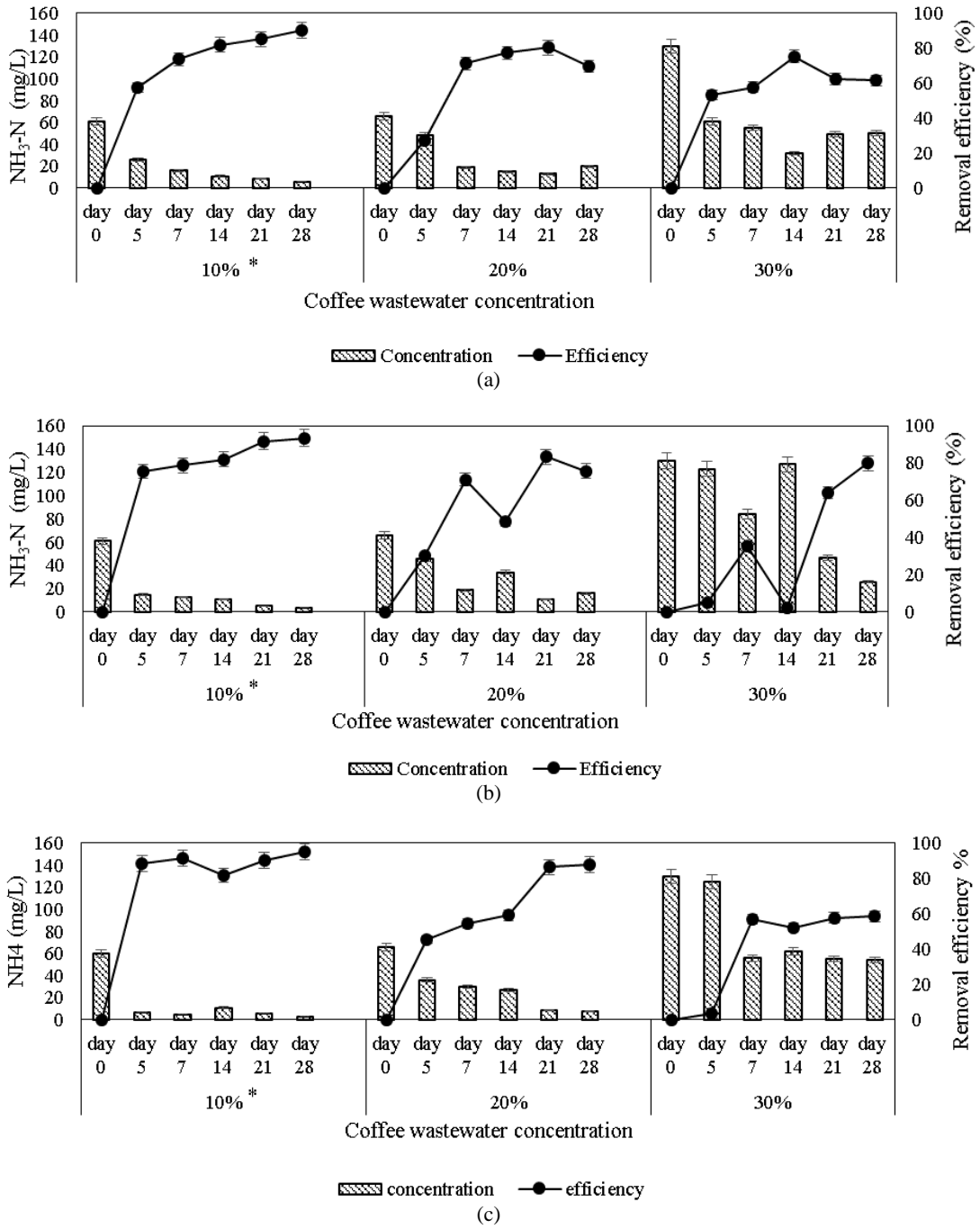
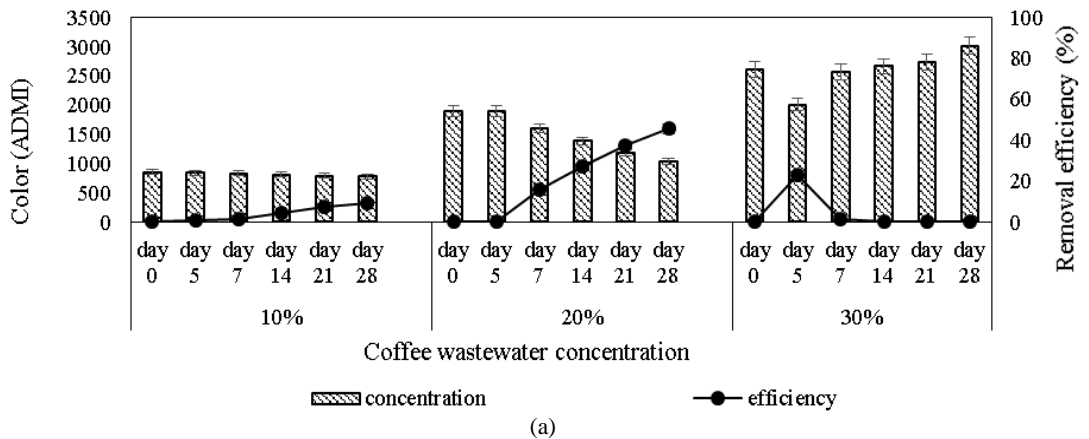


Fig. 5. $\text{NH}_3\text{-N}$ in wastewater for (a) 8 plants (b) 10 plants (c) 12 plants systems. Asterisk symbol (*) indicates the significantly higher $\text{NH}_3\text{-N}$ removal between wastewater concentrations.



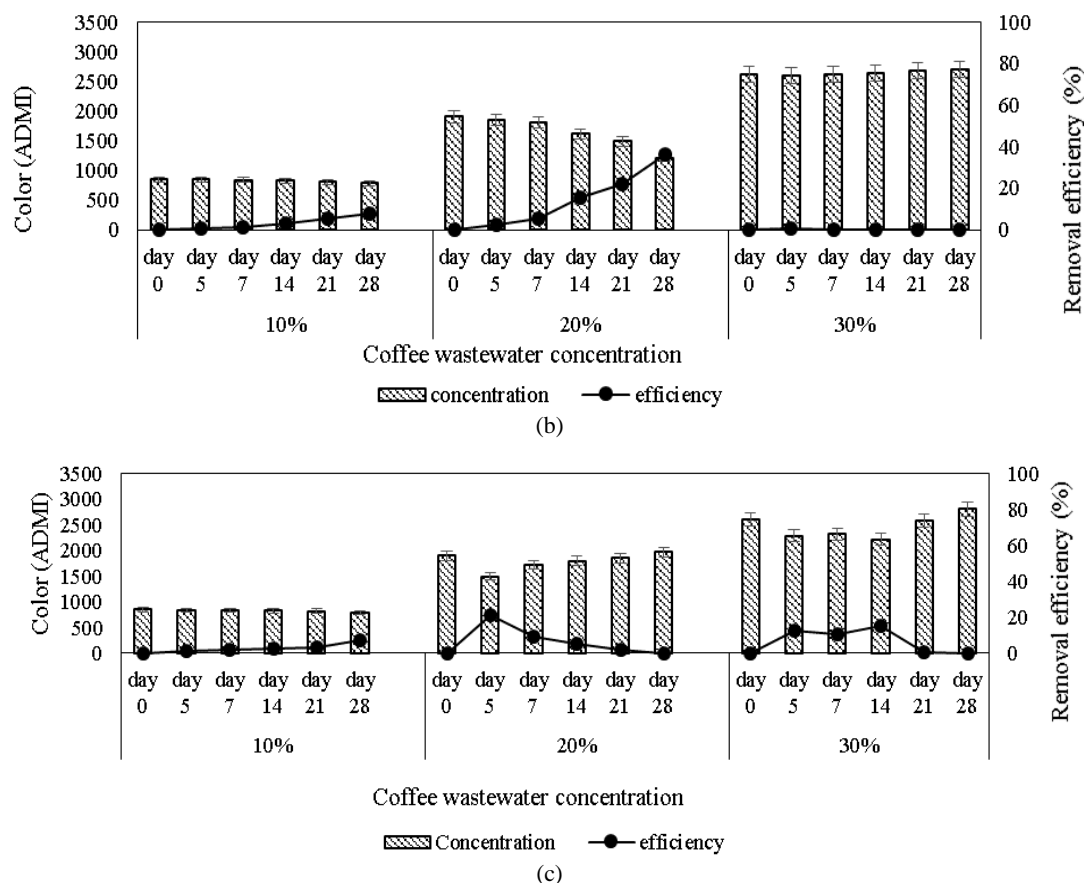


Fig. 6. Colour of wastewater for (a) 8 plants (b) 10 plants (c) 12 plants systems

4. Conclusions

To summarize the obtained results, the utilization of water hyacinth to polish coffee wastewater effluent before discharge into water bodies is highly reliable. Great performance of pH neutralization, COD (highest removal of 93.8%), TSS (highest removal of 93.0%) and NH₃-N removals (highest removal of 93.4%) were obtained in this study.

The 10% was concluded to be the most suitable concentration of coffee wastewater to be treated with this method using water hyacinth and at this concentration, in which COD to wet weight of plants within a range of 2 - 2.667 mg COD/g showed to be the best set up on this research. It can also be concluded that the additional plants inside the system did not increase the efficiency of treatment.

Acknowledgments

The authors would like to acknowledge Universiti Kebangsaan Malaysia (UKM) for supporting this research work through MI-2020-003 grant.

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