VOL. 11, NO. 4, FEBRUARY 2016 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

www.arpnjournals.com

RHIZOFILTRATION FOR THE ENHANCEMENT OF BIOFUEL PRODUCTION

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ABSTRACT

The effectiveness of water hyacinth (*EichhorniaCrassipes*) in the remediation of municipal wastewater and production of bioethanol was compared with water lettuce (*Pistia Stratiotes*) and water spinach (*Ipomoea Aquatica*). Preliminary test using hydroponic system was conducted in order to measure the parameters required at the laboratory scale continuous experimental rig configuration (hydraulic retention time and flowrate). The experiment using the experimental rig was conducted for 18 d for each of the aquatic plant. Water lettuce showed a better removal performance compared with water hyacinth and water spinach. Water lettuce was able to remove 92% of nitrogen and 87% of phosphorus. Besides, water lettuce also showed higher BOD removal of 98.3% followed by water hyacinth 93.9% and then water spinach 89.6%. For COD removal performance, water lettuce treated COD up to 98% followed by water hyacinth 94.2% and water spinach 89.9%. The biomass harvested from water hyacinth, water lettuce and water spinach was 772 ± 37 g/kg, 794 ± 75 g/kg and 702 ± 69 g/kg, respectively. The DNS test was conducted to measure the reducing sugar from the plant biomass. The concentration of sugar produced by water hyacinth was 21.2 ± 2.7 g/L-m^2, while water lettuce was 23.5 ± 3.8 g/L which is higher than water hyacinth's sugar production. Water spinach produced the lowest sugar concentration with only 15.5 ± 3.3 g/L. The production of biofuel was estimated based on known theories. Water lettuce produced 0.13 ± 0.01 g/g bioethanol while water hyacinth produced 0.12 ± 0.01 g/g of bioethanol. Due to the low concentration of sugar, water spinach managed to produce only 0.10 ± 0.02 g/g bioethanol. Water lettuce showed better performance in comparison with water hyacinth in the remediation of wastewater and subsequent production of biofuel.

Keywords: aquatic weed, phytoremediation, rhizofiltration, wastewater treatment, biofuel production.

INTRODUCTION

A number of aquatic plants have been discovered efficient in removing contaminants from wastewater. These plants are called phytoremediators. Among them is water hyacinth, recognized due to its rapid growth and has been widely employed for treatment in a variety of wastewaters (Abbasi and Ramasami, 1999; Narayana and Parveen, 2000; Malik, 2007). It has also been identified as a good biogas producer (Singhal and Rai, 2002). Therefore, water hyacinth is a well-known aquatic remediator in phytoremediation and rhizofiltration field for its flexibility to adapt to various extreme conditions in order to decontaminate wastewater.

Intensive findings on the potentials and constrains of water hyacinth usage for a variety of applications have been reported (Chartchalerm *et al.* 2007). This research was conducted to compare water hyacinth with two other types of aquatic plants i.e. water lettuce and water spinach, in remediation of domestic wastewater.

The production of biofuel for replacement of fossil fuel production has caused severe conflict of interests with environmentalists and food industries. Environmentalists claims that although the production of biofuel sounds 'green', its consumption of plants and trees as its main resources is dangerous for the future in comparison with the demand for biofuel industries.

The real problem is regarding conversion of farmland for food to biofuel industries. In the US for example up until 2007, 25% of its corn farmland that is used for producing foods was converted to serve biofuel industries. Debates continued as biofuel industries had increased the price of food. As a solution, they had managed to propose a sustainable biofuel economy. The first nation that managed to achieve sustainability in this sector is Brazil (Nigam, 2002). Another approach is to introduce second generation of biofuels which may be sourced from non-food crops, crops residues and wastes. This method solves both food industries and environmentalist issues. Using crops residues and plant-based wastes, second generation biofuel production can combine with food industries as the wastes of food industries are recycled to biofuel. Moreover, electricity could also be generated by this source, which is beneficial for rural area or developing countries. This approach brings the idea of combining biofuel production while carrying out waste water treatment.

MATERIALS AND METHODS

Experimental Setup

Figure-1 shows the laboratory scale experimental rig used in this study. It is known as Lab-Scale Continuous Rhizofiltration System (Lab-Scale CRRS). The basis of this rig set-up was developed based on the rig configuration developed by the Department of Environmental Geosciences, Pukyong National University, Busan, South Korea (Minhee and Minjune, 2009). Appropriate modifications were made to suit the circumstances in Malaysia. The Lab-Scale CRRS rig was set up at the experiment area with 0.24 m^3 municipal wastewater poured into the rig's influent compartment. Different types of aquatic plant were used for the entire experimental run. 15 pieces of each water hyacinth, water spinach and water lettuce were selected and weighed about 25 to 35 grams each for every type of plant. The plants were then placed into the three compartments of the rig. Every batch contained 5 pieces of plants. Next, the flow control pump was set up to the flow rate of 0.013 m^3/day. The hydraulic retention time used in this experiment was 18 d. The organic loading rate applied was 1.083 kg/m^2-day while the hydraulic loading rate was 0.011 m^3/m^2-day. Figure-1 shows the influent tank with the influent wastewater poured into it and the effluent condition of the wastewater after 18 d.

Figure-1. The condition of influent poured inside the influent compartment of the rig (left) and the condition of effluent after 18 d (right).

The wastewater sampling for the experimentation was taken on daily basis starting from the 1^{st} day until the 18^{th} day of the rig experiment. The weight and length of the plant were also measured. Finally, the batches were cleaned and the procedure was repeated for 3 times to get the average results. The plants were harvested from the rig as phytoremediation biomass and were used to estimate the bioethanol production for each plant.

The monitoring of plant biomass was conducted by weighing the plant during the initial and final day of the treatment for every experimental stage. These steps were conducted in order to observe the plant's doubling time. The lengths of the plants were also measured for the same purpose. Extensive monitoring was also conducted in weighing and measuring the plant length and diameter during the experimental rig stage. This was because the plant's growth was directly influencing the production of bioethanol. After the 18^{th} day of treating municipal wastewater in the experimental rig, the plants were harvested from the rig. Figure-2 shows the size of water hyacinth before and after the experiment.

Figure-2. The initial size of water hyacinth (left) and the size of water hyacinth after 18 d (right).

The weights of the plants were measured in order to have the initial weight of the plants. The plants were then chopped into smaller pieces and were dried in the oven for 6 hr at 105 ^oC oven. Then, the dried phytoremediation plants were then weighed to obtain the dry weight of the biomass. After the biomass weight was calculated, the plant's biomass was then used for estimating the sugar production.

The sugar production was determined quantitatively by using 3,5-dinitrosalisyclic (DNS) acid method. Firstly, 0.1 g of sample was inserted into a dry 10 ml test tube. 1 ml of HCl was then added using 1 ml pipette into the sample tube. Besides, the blank tube was prepared by added 1 ml distilled water and 1 ml of HCl into a dry 10 ml test tube. The both test tubes were then added with 5 ml of distilled water and stirred using centrifugal laboratory machine for 2 minutes. After the sample was dissolved, the tubes were closed and boiled in 90 ^oC water using water bather for 30 minutes. 1 ml of iodine was then dropped into the tubes and waits until the light yellowish color appeared. After cooling it naturally, 1 ml of phenolphthalein and 1 ml of NaOH were added into the tubes. Next, 1 ml of distilled water and 1.5 ml of DNS acid were added and stirred again using the centrifugal laboratory machine for another 2 minutes. The tubes were boiled inside the water bather for 5 minutes. After the tubes are cooled naturally, the sample was ready to be tested using DR5000 with 540 nm color differentiation setting. The reading was then used to find the corresponding amounts of reducing sugar on the standard curve. Repeat the testing three times to have an average reading.

The estimation of the bioethanol produced by the plants began by conducting the physical pretreatment. The whole plants from the root to its leaves were dried for 6 hr in an oven with the temperature at 105 ºC. If the drying process has been done previously during the estimation of biomass weight, the biomass sample drying process was avoided. After the plants were dried, it was chopped until the size became 10 to 30 mm. Then, the chopped plants were grinded using a blender until the size of the biomass became 0.2 to 2 mm. The grinded biomass was then stored in a dry place with a normal room temperature before it was used for DNS test.

Wastewater

Wastewater for this study was taken from Universiti Teknologi Malaysia (UTM) municipal wastewater treatment pond. This pond covers large number of municipal wastewater from several colleges and faculties in UTM. Sampling was carried out after the peak hour, between 3.00 PM to 5.00 PM in order to obtain the maximum concentration of pollutants and nutrient contaminants in the wastewater (James, 1985).

Plants

Since it was hot season during April, the source for the plant materials (water hyacinth, water spinach and water lettuce) was easily found. Water lettuce and water hyacinth were collected at UTM, while water spinach was collected at Nenas Food Tech Industry Sdn Bhd (NFT) wastewater treatment pond. The plants of average stalk width 5 – 10 cm were harvested from growth area in facultative pond where the wastewater was taken.

Water Analysis

The removal efficiency for domestic wastewater was assessed by monitoring several parameters such as five day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), nitrogen (N) and total phosphorus (TP). Water analysis checker was used to measure other parameters such as temperature (T), alkalinity-acidity (pH), NH_4-N, and Dissolved Oxygen (DO), for the observation of the additional data. Nitrogen and phosphorus concentration in the wastewater develop the production of phytoplankton and higher the concentration of organics limit heterotrophic growth (Thomas, 1984). Thus, both parameters needs to be reduced to remediate the wastewater. In the experiment, nitrogen and phosphorus removal were monitored by comparing the removal effect of a treatment against the control.

RESULTS AND DISCUSSION

Nitrogen Removal Performance

Figure-3 shows the concentration and removal rate for total nitrogen for the water hyacinth. The concentration of nitrogen in the influent was compared with the removal performance. The concentration of nitrogen in the influent sample was between 50-60 mg/L throughout the experiment. Meanwhile, the concentration of nitrogen steadily decreased in the effluent sample. At the end of 18 d, the content of nitrogen left in the effluent was only 5 mg/L equivalent to 92% removal of nitrogen. The nitrogen removal from the wastewater was a result of water hyacinth taking up nitrogen and storing them or absorbing them as the source of growth for the plant.

In the case of water lettuce, the removal performance is illustrated in Figure-3. The nitrogen concentration treated by water lettuce showed a rapid decrease at the beginning of the treatment. The removal performance by water lettuce can be considered faster as it stabilized on the 12th day of the treatment. On the 12th day, the removal percentage of the treatment reached 98.3%. This result may have been due to its capability to grow faster and have a longer root than water hyacinth. As water lettuce grew larger in size, it requires a larger amount of nitrogen to support its growth (Chongrak, 1996). This finding therefore proved that water lettuce has a greater potential than water hyacinth in removal of nitrogen from municipal wastewater. The removal performance of nitrogen by water spinach can be found also in the Figure-4. As shown in the figure, the nitrogen concentration decreased slowly in the first 5 d of the treatment. However, the removal performance of nitrogen rapidly decreased between the 5th till the 13th day. The removal of nitrogen reached 93.3% on the 17th day and stabilized on the 20th day.

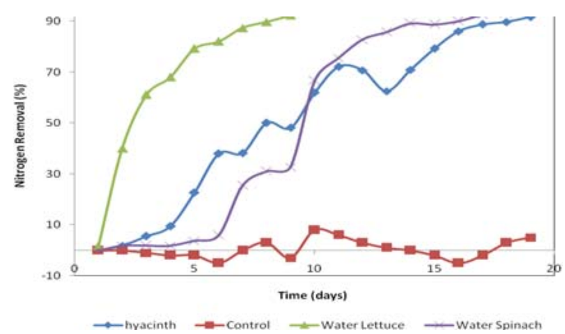

Figure-3. Nitrogen removal performance in lab-scale CRRS.

The observation done during the experiment is that the slow start of nitrogen removal performance by water spinachis was due to the small root that the plant had. The small physical condition of the root influenced the contact surface area between the root and the wastewater. As the treatment progressed beyond the 5th day, the water spinach grew twice the initial size and the new root which had grown together helped the plant to absorb more nitrogen. As a result of this, the nitrogen removal performance increased rapidly until the concentration of nitrogen nearly depleted on the 17th day of the treatment.

In comparison, water hyacinth, water spinach and water lettuce application for the treatment of municipal wastewater showed that water lettuce had the best removal capability with 98.3% of nitrogen removed. This was followed by water spinach which removed 93.3% and water hyacinth which removed 92% of nitrogen. Water

VOL. 11, NO. 4, FEBRUARY 2016 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

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lettuce also showed a rapid decrease in terms of nitrogen removal compared to water hyacinth and water lettuce.

Phosphorus Removal Performance in Lab-Scale CRRS

Figure-4 shows the removal performance of phosphorus done by water hyacinth, water lettuce and water spinach in 18 d of treatment in Lab-Scale CRRS. With respect to the control performance, the removal of phosphorus by these 3 plants were; water hyacinth 17.9±3.1 mg/L, water lettuce 19.8±0.1 mg/L and water spinach 15.6±1.4 mg/L. Water lettuce shows the highest removal performance with 86.99%. The replication also proved that water lettuce removal performance hold the lowest standard deviation of 0.1 differences between the replications. Therefore, by observing these data trend, it indicated that water lettuce is the best phytoremediation plant to remove phosphorus.

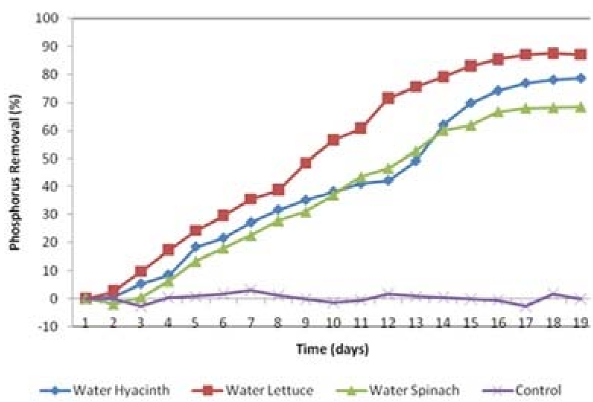

Figure-4. Phosphorus removal performance in lab-scale CRRS.

Table-1 shows the determination of significant level for water lettuce in removing phosphorus. The ANOVA analysis found that there was a significant difference between water lettuce and other plants with a value of p lesser than 0.05. In comparison, there was no significant difference between water spinach and water hyacinth where the p value was larger than 0.05. At 95% confidence level, the ANOVA analysis subsequently proved that water lettuce hold a slight difference in the removal of phosphorus when compared to water hyacinth and water lettuce.

COD and BOD Removal Performance in Lab-Scale CRRS

Figure-5 shows the removal performance of COD and while BOD_5 removal performance is shown in Figure-6. The BOD removal performance illustrates that water lettuce removed 98.3% followed by water hyacinth 93.9% and then water spinach 89.6%. For COD removal performance, water lettuce treated COD up to 98% followed by water hyacinth 94.2% and water spinach 89.9%. All plants succeeded in the remediation of BOD and COD until it reached the level required by the Malaysian Environmental Quality Act (1974).

Table-1. ANOVA analysis for phosphorus removal performance.

Phosphorus	Sum of Squares	df	Mean Square	F	Significant
Between Groups	34.667	25	1.387	4.992	0.003
Within Groups	3.333	12	0.278		
Total	38.000	37			

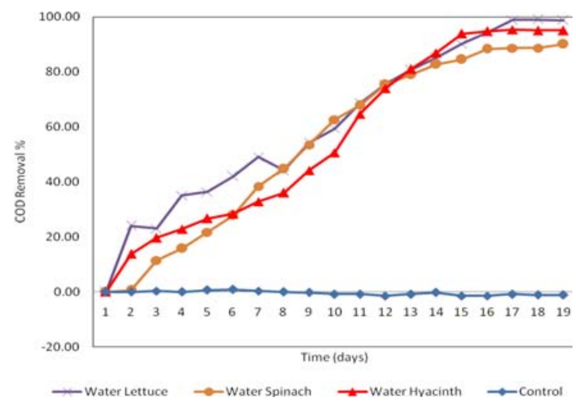

Figure-5. COD removal performance in lab-scale CRRS.

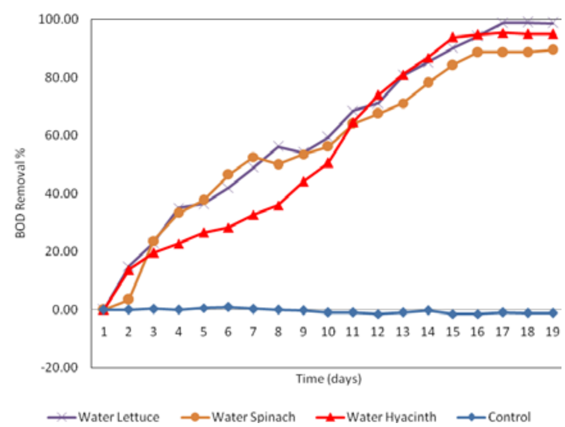

Figure-6. BOD_5 removal performance in lab-scale CRRS.

The result shows that water lettuce removed COD and BOD higher than water hyacinth while water spinach scores the lowest removal performance. However, there is no significant difference between water lettuce, water spinach and water hyacinth in removing COD and BOD. This was proved in the ANOVA analysis where the value of p exceeded 0.05. Therefore, even though water lettuce shows that it has the highest removal performance of COD and BOD, the statistical analysis proved that the removal performance between water hyacinth, water lettuce and water spinach were even.

Other Removal Performance in Lab-Scale CRRS

In addition to nitrogen, phosphorus, COD and BOD, there were also other parameters that were monitored during this study. Parameters such as colour, dissolved oxygen, pH, total suspended solid, total dissolved solid, volatile suspended solid and salinity were also observed in order to display the overall removal performance by water lettuce in comparison with water hyacinth and water spinach. Table-2 shows the data acquired from the observation of all parameters mentioned. From Table-2, it is proven that when compared with water hyacinth and water spinach, water lettuce showed a better overall treatment. Other than removing COD, BOD, phosphorus and nitrogen, it also dissolved more oxygen into the wastewater. The mean difference from water lettuce is higher compared to water spinach and water hyacinth. As a result, water lettuce showed a significant differences in ANOVA analysis where it was 95.7% more than the required 95% of confident level. Also, the results of DO by water lettuce were higher than in other plants. Other than that, pH, total dissolved solid, volatile suspended solid and salinity also showed a significant difference with $p < 0.05$ in the ANOVA Analysis. Parameters like pH, VSS and salinity indicates that water hyacinth contributes to the significant differences in which water hyacinth showed the highest removal percentage compared to water lettuce and water spinach.

Phytoremediation Byproduct: Biomass

Table-3 shows the results of biomass collected from the phytoremediation plants that underwent the treatment of the municipal wastewater in the Lab-Scale CRRS. The Lab-Scale CRRS contained 3 batches of plant compartment in which every batch contained 5 numbers of phytoremediation plants during the initial treatment process. All plants from the rig were collected for the biomass production. As shown in Table-3, water hyacinth holds the highest value of total weight after 18 d of municipal wastewater treatment. The weight of water hyacinth increased nearly 6 times higher than its initial weight compared to water lettuce and water spinach which only managed to grow about 5 times of the initial weight.
However, the water lettuce showed a higher value of dry weight than water hyacinth. This is due to its physical condition that water lettuce does not have sponge type cellulose compared to water hyacinth. Although water spinach produces less biomass, the ANOVA analysis shows no significant difference between water lettuce, water hyacinth and water spinach in terms of biomass production.

Table-2. Color, DO, pH, TSS, TDS, VSS and salinity removal performance.

Parameters	Influent	Effluent			Significant
		Water Hyacinth	Water Lettuce	Water Spinach	
Colour	193±3	21±1	9±2	19±1	0.767
Dissolved oxygen (DO)	3.70±0.10	6.00±0.02	6.81±0.25	5.11±0.29	0.043
pH	8.72±0.11	7.14±0.02	7.77±0.22	7.74±0.10	0.013
Total suspended solid (TSS)	1.41±0.02	1.41±0.09	1.43±0.07	1.41±0.02	0.417
Total dissolved solid (TDS)	111±5	140±3	104±5	120±3	0.000
Volatile suspended solid (VSS)	1.41±0.01	1.38±0.01	1.40±0.01	1.39±0.01	0.038
Salinity	0.08±0.02	0.05±0.03	0.08±0.00	0.06±0.02	0.000

Table-3. Biomass from the phytoremediation plants in the lab-scale CRRS.

Plant	Batch No.	Initial Weight (kg)	Total Weight (kg)	Dry Weight (g)	Biomass (g/kg)
Water Hyacinth	1	0.157	0.897	694.65	772±37
	2	0.160	0.921	726.65	
	3	0.155	0.879	661.42	
Water Lettuce	1	0.152	0.799	599.72	794±75
	2	0.149	0.823	679.51	
	3	0.159	0.845	681.23	
Water Spinach	1	0.101	0.459	298.95	702±69
	2	0.107	0.551	404.69	
	3	0.112	0.427	307.65	

Reducing Sugar

The result for sugar concentration obtained from the biomass of the phytoremediation plant is shown in Table-4. The linear equation produced by the standard curve graph is used to estimate the sugar concentration abstracted from the biomass of water hyacinth, water lettuce and water spinach. Dinitrosalicyclic method (Sumner and Graham, 1921) was used to quantify the level of sugar concentration produced by the phytoremediation's biomass.

Table-4. Sugar concentration obtained from the biomass of phytoremediation plant.

Plant	Batch No.	Absorbance (540nm)	Sugar (g/L)	Sugar Content (g/L-m^2)
Water Hyacinth	1	0.56	5.92	23.5
	2	0.43	4.59	18.2
	3	0.52	5.54	22.0
Water Lettuce	1	0.62	6.60	26.2
	2	0.59	6.32	25.1
	3	0.45	4.82	19.2
Water Spinach	1	0.29	3.15	12.5
	2	0.35	3.77	15.0
	3	0.45	4.80	19.1

The results shown indicate that water lettuce attained the highest sugar concentration, followed by water hyacinth and water spinach. The average sugar that water hyacinth produced was 5.35 g/L, water lettuce 5.92 g/L and water spinach 3.91g/L of sugar. From the concentration of sugar, the sugar content obtained with respect to the area covered by the plant treatment, for water hyacinth, water lettuce and water spinach are 21.2±2.7 g/L-m2, 23.5±3.8 g/L-m2 and 15.5±3.3 g/L-m2, respectively.

In comparison, Radhika and Murugesan (2012) reported that to produce 35 g/L-m2 of total sugar concentration from mixed cellulosic biomass, a level of sugar content that is higher than the sugar content acquired from the result of this study is required. Meanwhile, a study on the production of sugar and bioethanol from water hyacinth showed that water hyacinth managed to produce 29 g/L-m2 of sugar content (Nigam, 2002). In this study, the result shows that the highest sugar concentration obtained was 23.5±3.8 g/L-m2. The lower performance of producing total sugar concentration compared with previous study may have been due to the climate differences and the differences in municipal wastewater characteristics (Wyman *et al.* 2005). Other than that, the characteristics of the municipal wastewater treated by phytoremediation plants in other places may have been higher in nutrient than that in this study.

Production of Bioethanol by Phytoremediation Plant

In this study, water lettuce and water spinach are compared with water hyacinth in order to prove the hypothesis that both water lettuce and water spinach also hold a competitive performance in the bioethanol production. Table-5 shows the estimation of biofuel production from the sugar concentration using Holzberg equation (Holzberg *et al.* 1967). Earlier study confirmed that for semi-submersible aquatic plant, water hyacinth hold the most efficient plant for producing bioethanol. Mishima *et al.* (2008) reported that due to the high percentage of cellulose and hemicellulose, water hyacinth biomasses are able to yield a high concentration of sugar and thus higher bioethanol production. Mishima *et al.* (2008) also reported that water hyacinth does not have a large percentage of lignin. Lignin is an element contained in the plant that is not usable for bioethanol production. The low concentration of lignin made water hyacinth as one of the most popular plants selected for phytoremediation and bioethanol production study.

Table-5. Biofuel estimation from sugar concentration using Holzberg equation (Holzberg *et al.* 1967).

Plant	Batch No.	Sugar Content (g/L-m^2)	Biofuel Concentration (g/g)
Water Hyacinth	1	23.50	0.12
	2	18.22	0.09
	3	21.99	0.11
	4	23.25	0.12
	5	26.27	0.13
	6	26.86	0.14
	7	23.25	0.12
	8	23.84	0.12
	9	20.91	0.11
Water Lettuce	1	26.19	0.13
	2	25.10	0.13
	3	19.15	0.10
	4	23.17	0.12
	5	23.75	0.12
	6	26.19	0.13
	7	31.05	0.16
	8	23.42	0.12
	9	26.02	0.13
Water Spinach	1	12.52	0.06
	2	14.96	0.08
	3	19.06	0.10
	4	18.39	0.09
	5	24.76	0.13
	6	20.91	0.11
	7	24.05	0.12
	8	20.78	0.11
	9	23.25	0.12

As shown in Table-5, nine replications for each of the plant sample are conducted in order to obtain a more accurate result for biofuel production. Based on the Holzberg equation, the minimum level of bioethanol production for each plant sample was estimated. The result showed that water hyacinth produces 0.12±0.01 g/g bioethanol concentration. In comparison, water lettuce and water spinach produces 0.13±0.02 g/g and 0.10±0.02 g/g, respectively. The comparison of bioethanol production between each plant is shown in Figure-7 where the data of the biomass versus bioethanol is presented in scattered graph.

From Figure-7, a clear comparison can be observed between the bioethanol production from water hyacinth, water lettuce and water spinach. The trend of the data concentration from water lettuce bioethanol production can be seen as nearly the same with water hyacinth. However, water spinach is observed to have a low bioethanol production where the bioethanol produced by water spinach are lower than the bioethanol concentration produced by water hyacinth's bioethanol production. Therefore, ANOVA analysis proved that water spinach also hold at least the same performance with water hyacinth in terms of production of bioethanol.

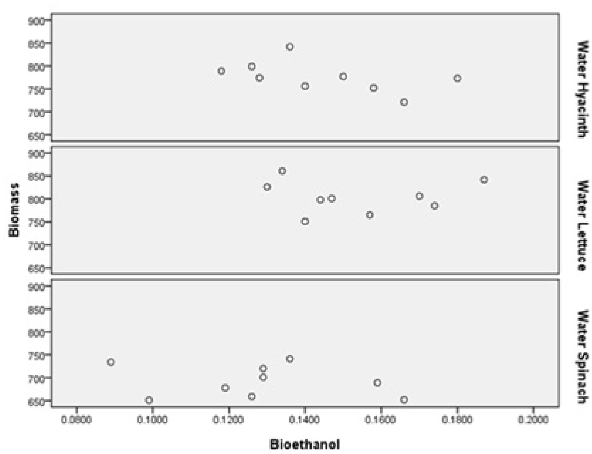

Figure-7. Scattered graph of biomass versus bioethanol with respect to phytoremediation plants.

CONCLUSIONS

This study indicates that water lettuce holds a better potential than water hyacinth in both performance for phytoremediation of municipal wastewater and biofuel production. ANOVA analysis showed that water lettuce is significantly better than water hyacinth in removing nitrogen and phosphorus ($p < 0.05$). The phytoremediation performance in remediating municipal wastewater using Lab-Scale CRRS solidify the earlier argument that water hyacinth does have a stable removal efficiency in all parameter tested in this study. Especially in removing nitrogen, water hyacinth showed a steady and unwavering data trend line throughout the 19 d of treatment.

Other than that, most of the parameter tested shows that water hyacinth remediation performances were nearly the same with water lettuce. Water hyacinth generates 6 times higher than its initial weight, while water lettuce and water spinach only managed to increase 5 times from its initial weight. However, water lettuce was the highest weight of biomass production. This study shows that water hyacinth and water spinach produces about the same amount of sugar concentration. The highest concentration of sugar was produced by water lettuce, followed by water. Water spinach produced the lowest sugar concentration yielded from its biomass. Finally, this study proved that water lettuce shares the same level of potential with water spinach in municipal wastewater treatment and bioethanol production. Water lettuce produced 0.13±0.01 g/g while water hyacinth produced 0.12±0.01 g/g of bioethanol production.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Science, Technology and Innovation (MOSTI) (Grant Project No: 4S032).and Universiti Teknologi Malaysia (UTM) for financially support this research.

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