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The Phytoremediation using Water Hyacinth and Water Lettuce: Correlation between Sugar Content, Biomass Growth Rate, and Nutrients

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ABSTRACT

Degradation of water quality due to the presence of pollutants in water is an emerging issue in many countries, including Malaysia. Phytoremediation is one of the environmentally friendly, cost-effective conventional technologies that are still used in modern times. However, the selection of plant species is the most important aspect for the application of phytoremediation in wastewater treatment. Nevertheless, there are species of floating aquatic macrophytes that are capable of coping with various pollutants present in wastewater. Among the various floating aquatic macrophyte species, water hyacinth (WH) and water lettuce (WL) have been described as effective phytoremediators in reducing water pollution through bioaccumulation in their body tissues. Hence, WH and WL were chosen in this study as it is easily found, propagated, and cultivated. This paper aims to determine the biosorption capacity of these species in eliminating various pollutants present in wastewater as well as to define the optimum harvesting time for each species. Although these floating aquatic macrophytes are considered the most problematic plants due to their uncontrollable growth in water bodies worldwide, their ability to remove pollutants from wastewater has created a sustainable approach for their use in phytoremediation. In this sense, the use of phytoremediation by implementing the invasive floating aquatic macrophytes can certainly support the sustainable management of wastewater treatment in the future. Based on the results, it was found that WH efficiently removed higher PO_4^{3-}, NO_3^- and NO_2^- concentrations compared to WL from the wastewater. Both WH and WL showed the same trend of correlation between the growth rate and sugar content, where the sugar content increased when the plants reached the highest growth rate. The maximum nutrient uptake occurred in 14-17 days, proving that nutrient availability is critical for plant growth. This study concludes that the sugar content of WH and WL are increased with the biomass growth rate, and both plants species are competent in eradicating the nutrient pollution in wastewater. On top of that, this study infers that the maximum harvesting period for WH biomass is on day 18, while WL biomass is on day 21; based on the highest sugar content and biomass weight of each species.

Keywords: Biomass growth rate; nutrients; phytoremediation; sugar content; water hyacinth; water lettuce

INTRODUCTION

The high amount of nutrients released from domestic wastewater is of concern because it may trigger eutrophication in water bodies. Eutrophication causes excessive growth of algae in surface water and subsequently leads to the deterioration of water quality (Sabeena et al. 2018). Therefore, the excessive amount of nutrients in wastewater must be removed to protect the environment (Mulling et al. 2014). Nowadays, several treatment processes, such as physical, chemical, and biological processes have been used for the removal of nutrients from wastewater. (Nizam et al. 2020). However, these treatment methods require high operational and maintenance costs (Ali et al. 2020). Among all these treatment methods, phytoremediation is a promising technique for removing nutrients from wastewater owing to the ecofriendly, cost-effective, and efficient process.

Phytoremediation refers to a new plant-based technology that can be used as an alternative option to purify contaminated water. This technology focuses on the use of floating aquatic macrophytes as a tool to eliminate pollutants from wastewater (Rezania et al. 2015). The selection of aquatic plants is important as it is the main tool for removing contaminants (De Stefani et al. 2011; Ansari et al. 2020). The selection of aquatic plants is based on the high contaminant uptake, and also fast and easy growth (Roongtanakiat et al. 2007; Mustafa and Hayder, 2021). Microorganisms attached to plant roots can oxidize biodegradable materials present in contaminated wastewater (Nayanthara and Bindu, 2017).

Eichhornia crassipes, also known as water hyacinth (WH), is a fast-growing invasive plant that can double its reproduction in less than 13 days (Rezania et al. 2015; Arefin et al. 2021). Due to that, this plant can disturb aquatic ecosystems and activities, thus causing problems for navigation. However, due to their high hemicellulose content and low lignin content, the use of WH for bioethanol production is quite feasible (Arefin et al. 2021). Therefore, future work will pay special attention to researching WH as a substrate for bioethanol production.

Pistia stratiotes L. is known as water lettuce (WL) and the plant has been widely used to mitigate pollutants from contaminated water (Gupta et al. 2012). This plant can be categorized in the Araceae family, which can reproduce rapidly and vegetatively on the water surface (Hussnera et al. 2014; Walsh and Maestro, 2014). WL floats on the water surface and its roots submersed beneath water bodies. The size of WL leaves can reach up to 13 cm long (Dipu et al. 2011). WL also has a soft body that can increase biochemical responsiveness and low labor harvesting due to its small body size (Mishima et al. 2008).

Several studies have described the effectiveness of WH and WL to remove various pollutants from wastewater (Qin et al. 2016). For example, microorganisms attached to plant roots can oxidize the biodegradable substances in contaminated wastewater (Nayanthara and Bindu, 2017). Nevertheless, very few studies have been performed to determine the effectiveness of WH and WL for removing nutrients from wastewater (Qin et al. 2016). The purpose of this study is to evaluate the efficiency of two different floating aquatic macrophyte species (i.e., WH and WL) in eliminating nutrients from wastewater. Moreover, this study intends to examine the critical harvesting time of plants based on the correlation between sugar content, biomass growth rate, and nutrients recovered from WH and WL.

METHODOLOGY

EXPERIMENTAL SET-UP

A pilot fabricated tank treatment system was assembled at a domestic sewage treatment plant located in Skudai, Johor (see Figure 1). The fabricated tank treatment system consists of (1) five tanks, (2) PVC pipes, (3) valves, and (4) pumps. Each storage tank with a dimension of 90 cm in length, 90 cm in width, and 48 cm in height has a volume of 380 L. The tank treatment system was connected with PVC pipes. Each tank was filled with water using a pump and the valve installed between each container was closed when the water overflows. Two types of floating aquatic plants were used in this study, namely WH and WL. The plants were cultivated in a tank, as shown in Figure 1. WH and WL are floating-leaved aquatic plants; hence, their leaves are watertight and filled with air so that they can float on the water surface. All the fabricated tanks were filled with WH (first substrate) and WL (second substrate) to observe the removal of nutrients from water. A tank without WH and WL was set up as a control.

FIGURE 1. Pilot fabrication tank system

WATER ANALYSIS

The water was sampled from the fabricated tank and analyzed to determine the nutrient removal efficiency of the aquatic plants in comparison to the control. The fresh biomass of WH and WL was weighed on the selected day to observe the biomass growth rate of each plant. Both treatment efficiency and plant growth rate were determined for 21 days at three-day intervals and were conducted in triplicates.

BIOMASS PREPARATION

The fresh aquatic plant was harvested and thoroughly rinsed with tap water to ensure that the remaining dirt was removed. The plant was then segregated and categorized into leaves and rhizomes. The plant was cut into the desired size in the range of 1–2 cm and then dried at 105 °C until the weight was constant. Later, the dried plant was ground, sieved through an 850 µm sieve, and kept in a closed container. The steps in preparing biomass include A) harvesting plants, B) separating leaves and rhizomes, and C) drying the biomass (see Figure 2).

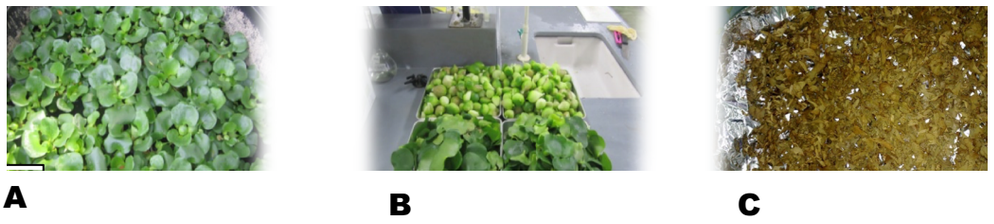

FIGURE 2. The steps in preparing water hyacinth

ANALYSIS METHOD

In this study, the nutrient parameters of phosphate (PO_4^{3-}), nitrate (NO_3^-), and nitrite (NO_2^-), were determined following the Standard Methods for the Examination of Water and Wastewater (APHA, 2005) (Table 1).

TABLE 1. Analytical methods of wastewater

Parameter	Measurement Method
Phosphate, PO_4^{3-}	Phosver3 (Ascorbic acid) Method: 8048
Nitrate, NO_3^-	Cadmium reduction Method: 8192
Nitrite, NO_2^-	Ferrous Sulfate Method: 8153

Meanwhile, the sugar content in the biomass was determined using the hydrolysis method (Mishima et al. 2008). About 200 mg of dried biomass was hydrolyzed with 2 mL of 72% sulfuric acid for 1 h at 30 °C, added to 56 mL of water, and autoclaved for 1 h. Upon completion, the supernatant was collected and analyzed by implementing the 3,5-dinitrosalicyclic acid (DNS) method. The sugar content was determined based on colorimetric changes, where the sample was measured at a wavelength of 540 nm (Miller, 1959).

RESULTS AND DISCUSSION

BIOMASS GROWTH RATE

The biomass growth rate of aquatic plants is an important factor affecting the effectiveness of phytoremediation. In general, phytoremediation is a plant-based approach that utilizes plants to remove pollutants from wastewater. Thus, monitoring the growth rate of plants is vital to determine the efficiency of WH and WL as bioindicators in phytoremediation.

In this study, the growth rate of WH and WL was calculated based on the plants' growth increment (in percentage) within 21 days of the experimental period with three-day intervals (Figure 3). From Figure 3, the growth rate of WH increased approximately 7.40% per day, whereas WL grew three-fold more than WH, which is about 21.37% per day. The finding signifies that the growth rate of both plants increased progressively with an excellent linear regression value approaching one (WH: 0.9984 and WL: 0.9954). Thus, the formed equations are relevant and fit for use in the growth rate assessment in this study.

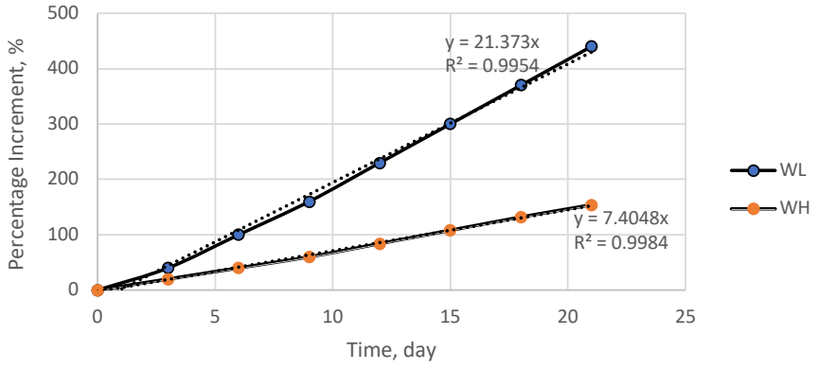

FIGURE 3. Growth rate profile of WH and WL (%)

The batch experiment was conducted for 21 days. During the batch experiment, about 340g of each plant was placed in the tank, and the performance of WH and WL based on the biomass growth rate was assessed every 72 h. Figure 4 illustrates the comparison of the percentage of biomass growth rate between WH and WL. It was found that the biomass of WH increased up to day 18, with a 20% increment from day 1 to 9, improving up to a 24% increment on day 12, and maintained until day 18, before experiencing a slight reduction to 22% on day 21. Based on this observation, it is deduced that the WH growth rate started to decline after 18 days. This finding is supported by Su et al. (2018), where the growth rate of WH biomass increased progressively and started to decline after day 20 due to the aging of the plants.

Meanwhile, the growth rate of WL exhibited a notable increment compared to WH. The biomass growth rate of WL increased from 40% (day 3) to 60% (day 6), became stagnant until day 9, before continuing to increase up to 70% on day 12; afterward, the growth rate remained unchanged until the end of the experimental period. This outcome is supported by Farnese et al. (2014), where the growth rate of WL would increase at the earlier stage and became stagnant after 24 days.

Figure 4 shows different growth rates of WH and WL. This phenomenon is called acclimatization, where the plants have to adapt for a while to a new environment under the influence of nutrient conditions, temperature, pollutants, and others. (Krishnan et al. 2020). The growth rate of plants significantly increased due to the crop root mats were fully developed and thereby increasing he capacity of roots to uptake the pollutants in the wastewater (Rezania et al. 2015). Therefore, in terms of rapid multiplication, WL is the preferred species compared to WH, where the former can provide sufficient biomass substrate for bioethanol production.

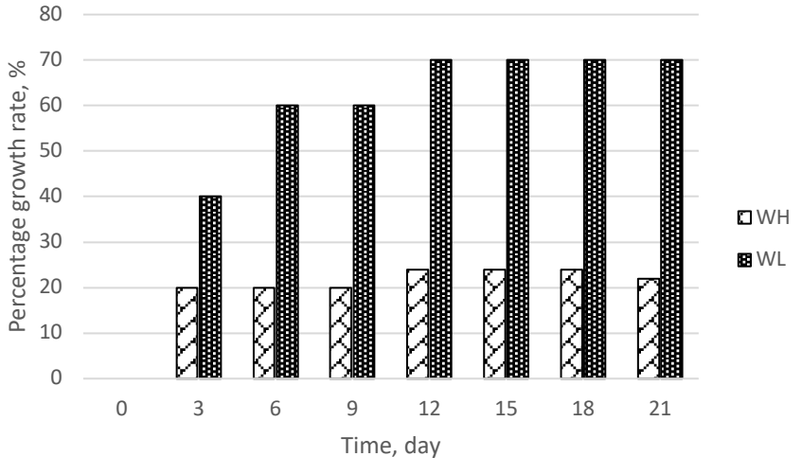

FIGURE 4. Biomass growth rate

WATER CONSUMPTION MEASUREMENT

The volume of water in the tank system was measured every 3 days to observe the total volume of water consumption by the aquatic plants. This study was conducted to assess the effects of biomass plant growth rate on water consumption. Table 2 shows the recorded volume of water remaining in the tanks systems for control, WH, and WL. The data shown in Table 2 expressed a similar water reduction pattern for WH and WL. The water level in the tank filled with WH decreased from 380 L to 153 L throughout 21 days of the experiment, meaning that a 59.7% reduction in the water volume level has been achieved. A similar trend was recorded for the tank filled with WL, as the water volume dropped from 380 L to 145 L, indicating approximately 61.8% water loss within the same experimental period. This reflects that the sufficient volume of water stimulates the biomass growth rate of WH and WL (Rezania et al. 2013). Meanwhile, the control tank reported water loss of 51.8% from the initial water volume, probably due to evaporation. This is because the surface of the control tank was not covered with any aquatic plant; hence, the water evaporated into the atmosphere.

TABLE 2. Wastewater analysis measurement methods

Day		Volume of water, L (Control)	Volume of water, L
0		380	380
3		372	360
6		300	280
9	Water hyacinth (WH)	270	265
12		260	248
15		210	200
18		211	186
21		183	153
0		380	380
3		372	375
6		300	320
9	Water lettuce (WL)	270	300
12		260	237
15		210	214
18		211	175
21		183	145

NUTRIENT UPTAKE IN THE BATCH SYSTEM

The utilization of WL and WH as bioindicators in phytoremediation technology is not new. Dixit et al (2011) introduced four different species of plants (i.e., Cattail, J. Americana, A. Philoxeroides and WH) that were considered feasible in removing nutrients from contaminated wastewater. Among the four listed species, WH showed excellent growth response, in which the plant was able to fill about 71% of the water surface with their growth, as well as removed 2.9 g phosphorus and 6.9 g nitrogen from the contaminated wastewater. This highlights that WH is feasible in removing nutrients in a water treatment system. Furthermore, Qin et al. (2016) also demonstrated the ability of WH and WL in removing nutrients from contaminated wastewater. Based on the study, the authors concluded that WH was preferred over WL based on the finding where WH exceeded the WL performance in removing nitrogen with 58.64% removal efficiency. In this section, the performance of WH and WL in removing nutrients will be explained and discussed.

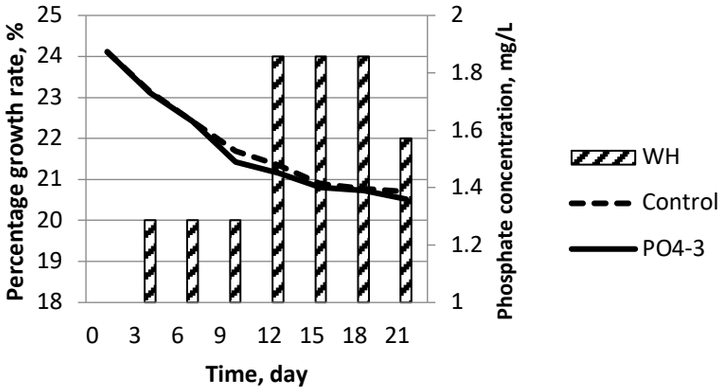

FIGURE 5. Phosphate profile of WH

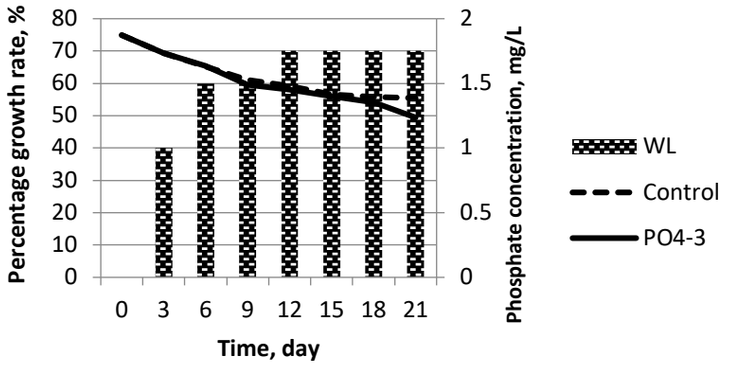

FIGURE 6. Phosphate profile of WL

The phosphate concentration profiles of WH and WL are shown in Figure 5 and 6, respectively. From Figure 5, it was found that the phosphate concentration decreased from 1.873 mg/L to 1.36 mg/L. This marks that WH reduced the PO_4^{3-} concentration by 27.4% after 21-day operation. Meanwhile, WL recorded a slightly higher PO_4^{3-} reduction with 34% removal, where PO_4^{3-} decreased from 1.873 mg/L to 1.236 mg/L. This finding agrees with the previous study by Qin et al. (2016), where WL worked better in bioremediation for treating PO_4^{3-} from polluted wastewater.

The results showed that WL degraded. PO_4^{3-} is also a critical factor for biomass energy and carbohydrate metabolism (Nayanthara and Bindhu 2017). Therefore, as the biomass growth rate increases, PO_4^{3-} removal will also increase. Younger macrophytes can effectively take up PO_4^{3-} from domestic wastewater, which is not the case for adult macrophytes.

The nitrate profiles of WH and WL are depicted in Figure 7 and 8, respectively. Based on Figure 7, the nitrate concentration inside the water tanks filled with WH reduced from 1.6 mg/L to 0.6 mg/L, representing about 62.5% nitrate removal within 21 days of operation. Meanwhile, Figure 8 shows that the nitrate concentration decreased from 1.275 mg/L to 0.79 mg/L and reached 38% nitrate reduction. Based on these profiles, it can be clarified that WH performs efficiently in removing nitrate compared to WL. The same finding was also reported by Nivetha et al. (2016). This is because the roots of WH are longer than WL, allowing better microorganism activity that can boost the absorption of nutrients and pollutants from wastewater (Akinbile and Yusoff 2012). The profile of nitrate concentration for both plants show similar trends, where the concentration decreases with the increment of biomass growth. This indicates the balance of nitrification and nutrient uptake from the wastewater.

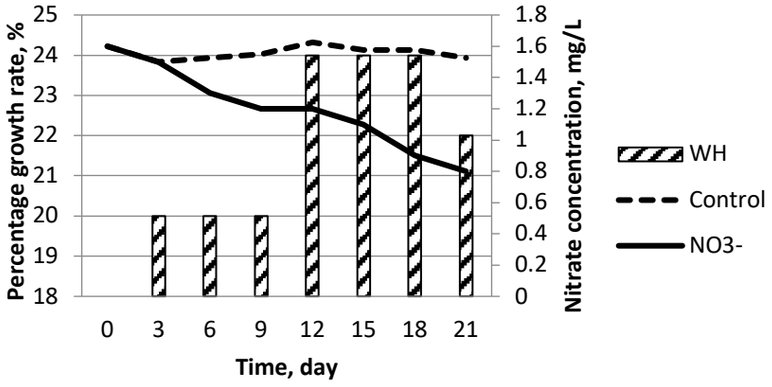

FIGURE 7. Nitrate profile of WH

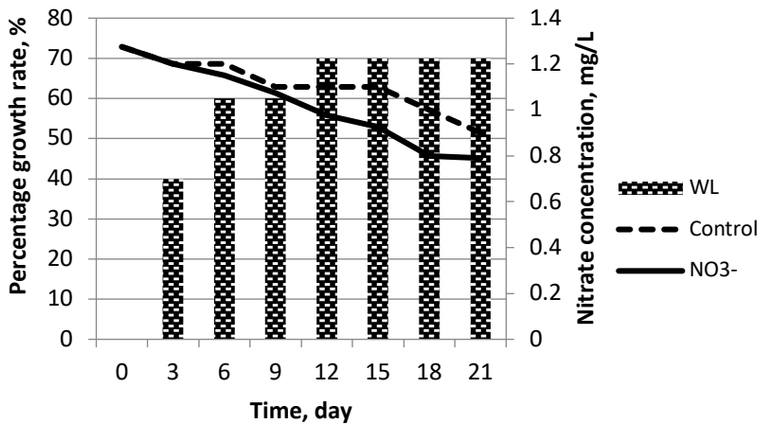

FIGURE 8. Nitrate profile of WL

Figures 9 and 10 depict the profiles of nitrite concentration of WH and WL, respectively. Both profiles show similar declining trends, indicating that both aquatic plants are capable of removing nitrite from wastewater. The nitrite concentration of WH and WL decreased by 60% (from 0.0245 mg NO_2^-/L to 0.009 mg NO_2^-/L) and 25% (0.0127 0245 mg NO_2^-/L reduces to 0.0095 mg NO_2^-/L), as shown in Figure 9 and 10, respectively.

The finding shows that a higher concentration of nitrite has been achieved using WH compared to WL. This is because the roots of these two aquatic macrophytes are very effective in absorbing aquatic nutrients (Akinbile and Yusoff, 2012). In terms of plant growth rate, the performance of WH is more efficient than that of WL in releasing oxygen to harness solar energy during the day. However, to achieve optimal bioremediation of water and plant density, adult plants should be harvested from water bodies before decomposition begins.

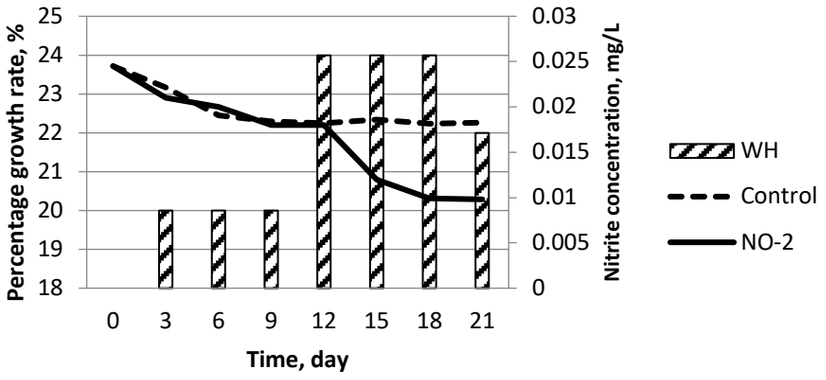

FIGURE 9. Nitrite profile of WH

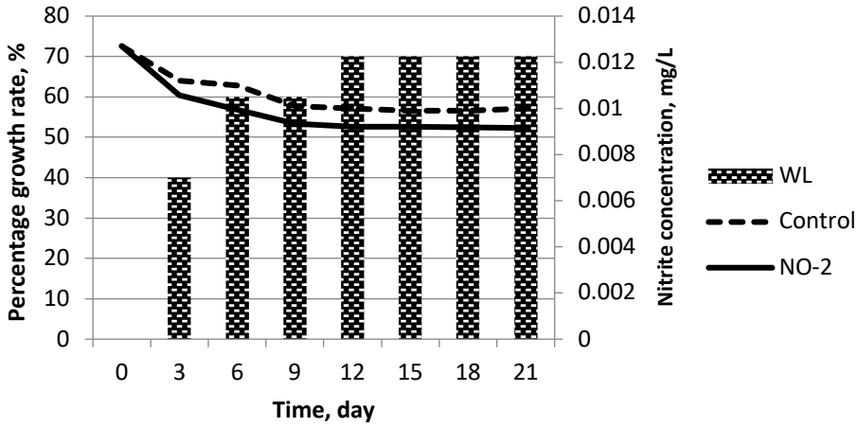

FIGURE 10. Nitrite profile of WL

CORRELATION OF SUGAR CONTENT AND BIOMASS GROWTH RATE

The results of plotting either the percentage growth rate or sugar content versus time for WH are depicted in Figure 11. The figure shows that the sugar content WH biomass increased as the percentage growth rate increased. WH absorbed the nutrients from the wastewater during photosynthesis, thus increasing its sugar content. As shown in Figure 11, the sugar content of WH biomass increased rapidly from 5.4 mg/g to 27 mg/g (i.e., 80 % increase) during the first 18 days of the experiment, and then decreased to 26 mg/g on day 21 of the experiment. The rapid increment of sugar content during 18 days of the experiment may be due to the increased fresh weight of WH biomass. The reduction of sugar content from 27 mg/g to 26 mg/g after 18 days of the experiment indicates the decomposition of WH, which directly affecting the reduction of the fresh weight of WH biomass.

Figure 12 depicts the plot of growth rate and sugar content in WL biomass against time. As shown in the figure, the curve shows the value of sugar content in WL biomass started to increase rapidly from day 3 to day 18, followed by a slow increment until equilibrium was achieved on day 21, where the sugar content increased from 4.2 mg/g to 23 mg/g (i.e., 81.7% increase). This finding shows a close correlation between the biomass growth rate and the sugar content of WL.

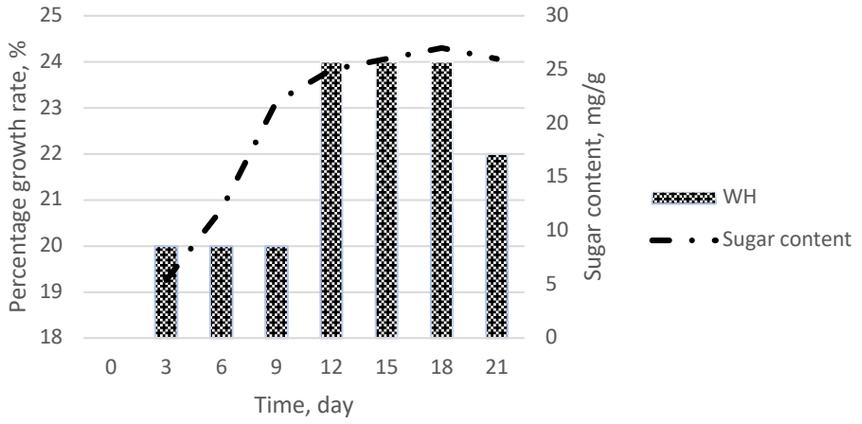

FIGURE 11. Growth rate and reducing sugar of WH

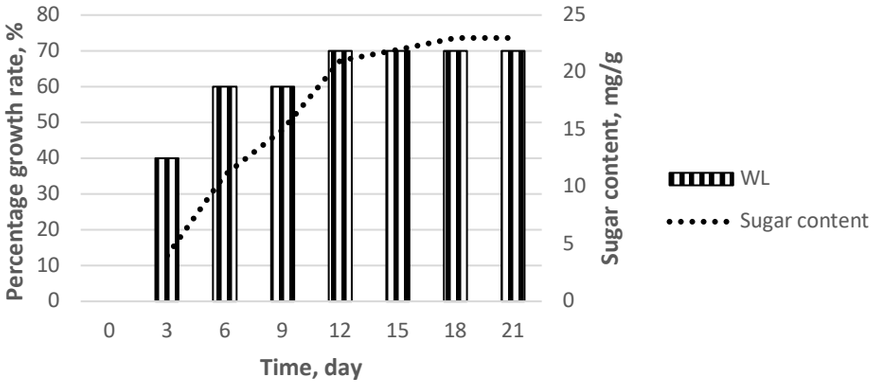

FIGURE 12. Growth rate and reducing sugar of WL

Sugar content is an important source for bioethanol production. Bioethanol can be used as a potential biofuel in the future. The optimal collecting/harvesting time of WH biomass is determined by the highest sugar content in the WH. Therefore, this finding shows that the optimum collecting/harvesting time of WH and WL is on day 18 (see Figure 11) and day 21 (see Figure 12), respectively.

RELATIONSHIP BETWEEN NUTRIENT UPTAKE AND SUGAR CONTENT IN WH AND WL

The increment of fresh weight and sugar content in the biomass of WH and WL is dependent on the nutrient uptake by these floating plants from wastewater. This is because wastewater composition varies depending on the source. Therefore, nutrient components significantly influence the WH and WL growth rate. For instance, Nizam et al. (2020) stated that the uptake of ammonium by lignocellulosic biomass is higher than that of nitrate. Thus, the presence of ammonium nitrogen in sewage could be favorable for biomass growth. Besides, nitrate matter in sewage could be used by plants for biomass growth. In addition, an excessive amount of ammonium can suppress wastewater parameters (Sharma et al. 2015). Nayanthara and Bindhu, (2017) found that phosphate is consumed by aquatic plants for energy and carbohydrate metabolism.

The highest nutrient uptake by plants took place within 14 to 17 days; hence the availability of nutrients can increase the plant growth rate. However, after the maximum growth rate, growth and sugar production declined even in the presence of nutrients. This proves that there is a strong correlation between sugar content and biomass growth rate.

CONCLUSION

In this study, the concentrations of PO_4^{3-}, NO_3^-, and NO_2^- in the batch treatment system have been evaluated at a certain time. The treatment system was regulated without water flow. The results showed that WH reduced PO_4^{3-}, NO_3^-, and NO_2^- concentrations by 27.4%, 62.5%, and 60%, respectively, as the growth rate of WH increased. Meanwhile, the reduction of PO_4^{3-}, NO_3^- and NO_2^-, concentration reached 34%, 38%, and 28%, respectively, using WL due to the increase in the growth rate of WL in the batch treatment system. Thus, it was found that WH efficiently removed higher PO_4^{3-}, NO_3^- and NO_2^- concentrations compared to WL.

Both WH and WL showed the same trend of correlation between the growth rate and sugar content, where the sugar content increased when the plants reached the highest growth rate. The maximum nutrient uptake occurred in 14-17 days, proving that nutrient availability is critical for plant growth. However, after the WH and WL reached the maximum growth rate, growth and sugar production declined even in the presence of nutrients. This proves that there is a strong correlation between sugar content and biomass growth rate. This finding proves that the maximum collecting/harvesting period for WH biomass is on day 18, based on the highest sugar content and biomass weight. In contrast, the optimum collecting/harvesting period for WL biomass is on day 21, based on the highest sugar content.

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DECLARATION OF COMPETING INTEREST

None

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