ABSTRACT

High ammoniacal nitrogen (AN) in industrial effluent must be treated before final discharge to prevent eutrophication phenomenon. Phytoremediation is recommended to be a better solution to treat wastewater with high AN content due to its cost-effective, environmental friendly and sustainable characteristics. Water hyacinth (*Eichhornia crassipes*) has been widely applied in phytoremediation technology to remove various types of pollutants. In this study, AN synthetic wastewater with varied AN concentrations of 10-200 mg/L was prepared to conduct tolerance limit test of water hyacinth for 10 days. The effect of pH on the physico-chemical parameters of AN synthetic wastewater and water hyacinth biomass growth was also investigated. Under sunlight exposure, it was found that water hyacinth was able to survive up to 150 mg/L of AN concentration for a duration of 10 days. The results showed that pH factor posed a significant impact on biochemical oxygen demand (BOD₅) and biomass growth of water hyacinth whereas less significant impact was exhibited on chemical oxygen demand (COD) and total suspended solids (TSS). Overall, water hyacinth has been shown to be a feasible macrophyte for phytoremediation of AN in wastewater.

Key words: Phytoremediation, Water hyacinth, Ammoniacal nitrogen, Macrophyte

INTRODUCTION

In Malaysia, industrial effluent containing high level of ammoniacal nitrogen (AN) must be treated to achieve the concentration below 20 mg/L before discharged into waterbodies, as imposed by the Environmental Quality (Industrial Effluent) Regulations 2009. Instead of using conventional physico-chemical and biological treatment methods, phytoremediation technology is recognized as a better solution due to its cost effective, sustainable and environmental friendly characteristics as well as the efficiency in removing various pollutants in wastewater (Lu *et al*., 2013; Paz-Alberto & Sigua, 2013; Rezania *et al*., 2015). Phytoremediation technology is a treatment process by making use of plant (phyto) to clean up (remediate) polluted soil, sediment, surface water and groundwater environments (Fox *et al*., 2008). Water hyacinth (*Eichhornia crassipes*) has been considered as the suitable candidate for phytoremediation to remove pollutants present in wastewater due to its characteristics of rapid proliferation, adaptation to a wide range of environmental condition and a large nutrient uptake capacity (Fox *et al*., 2008; Lu *et al*., 2008; Wang *et al*., 2011; Hu *et al*., 2012; Rezania *et al*., 2015). From the literature, several studies had reported the utilization of water hyacinth to treat wastewater which contained AN concentration ranging from 4.7 mg/L to 130 mg/L (Sooknah & Wilkie, 2004; Wang *et al*., 2011; Hu *et al*., 2012; Wang *et al*., 2013; Anandha & Kalpana, 2015; Fazal *et al*., 2015; Valipour *et al*., 2015; Mayo & Hanai, 2014; Rezania *et al*., 2016). However, the AN tolerance limit of water hyacinth remains unknown. Thus, this study aims to investigate the feasibility of using water hyacinth for phytoremediation of AN in wastewater through determination of the tolerance capability of this macrophyte at varied AN concentrations, and to evaluate the effect of pH on the physico-chemical parameters of AN synthetic
wastewater throughout the phytoremediation process.

MATERIALS AND METHODS

Preliminary works

Phytoremediation tanks were constructed using 2 mm thickness acrylic glass in lab scale condition with dimension of 30 cm (length) × 15 cm (width) × 15 cm (height). Native water hyacinth plants were collected and washed gently prior to the experimental use.

Tolerance limit study

AN synthetic wastewater was prepared using ammonium chloride according to AN concentration range of 10 mg/L to 200 mg/L. The working volume for each phytoremediation tank was 10 L. Six young water hyacinth plants were placed in each tank and tolerance limit test lasted for 10 days duration. The tanks were placed in outdoor or indoor condition to study the effect of sunlight exposure towards the tolerance limit of water hyacinth plants at different AN concentrations. The pH reading of AN synthetic wastewater for each tank was recorded daily and water was added into the tank regularly to maintain 10 L of water volume. The initial and final conditions of the plants which included the physical appearance, fresh biomass weight and number of leaves were observed and measured.

pH study

The effect of pH (4 to 9) on the physico-chemical parameters of the wastewater, namely total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD₅) and biomass growth were investigated. 3M hydrochloric acid (HCl) solution and 3M sodium hydroxide (NaOH) solution were used for pH adjustment of AN synthetic wastewater. For each experiment (pH = 4, 5, 6, 7, 8, 9), three replicates and one control experiment were prepared. The initial AN concentration (35 mg/L) and macrophyte density (400 g plants/ tank) were kept as constant for all experiments. The tanks were placed in outdoor condition for duration of 10 days. The fresh biomass weight was measured before and after each phytoremediation process.

Wastewater analysis

For each wastewater sample, pH was measured on site using pH meter (Model pH 5+, Eutech Instruments). For pH study, 300 mL of water sample was collected after each phytoremediation process and preserved at 4°C before analysis. The analysis of physico-chemical parameters of the wastewater samples were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 2005). For COD analysis, water sample solution was placed for digestion under 150°C for 2 hours in COD reactor block (Model DRB 200, Hach USA) prior to titration using 0.1M ferrous ammonium sulphate (FAS) solution. For BOD₅ analysis, water sample was incubated at 20°C for 5 days with measurement of both initial and final dissolved oxygen (DO) level. For TSS analysis, filtration method was applied by using glass-microfibre discs 47 mm and then followed by drying process in oven at 103-105°C for at least 1 hour.

RESULTS AND DISCUSSION

Tolerance limit study

Figure 1 tabulates the biomass growth and change in number of leaves for initial AN concentration of 10-200 mg/L after 10 days. A positive correlation between biomass growth and change in number of leaves was obtained. The gradual increase in biomass growth was observed for initial AN concentration of 10-30 mg/L. The highest biomass growth achieved was 207.42 g attributed by 30 mg/L initial AN concentration after 10 days. However, for AN concentration of 40 mg/L and above, the biomass growth and the increase in number of water hyacinth leaves demonstrated declining trend. Moderate biomass growth was observed for 80-110 mg/L initial AN concentration with achievement between 62.72 g to 94.55 g biomass growth. For initial AN concentration of 120-170 mg/L, stunted biomass growth between 13.14 g to 45.61 g was obtained. As extremely high amount of AN was stored in the plants, ammonium toxicity might occur with symptoms of curling of lower leaves and yellowing between the veins of older leaves (Mattson et al., 2009). These symptoms of ammonium toxicity were observed on water hyacinth plants with initial AN concentration of 160 mg/L and above after 10 days of exposure to sunlight. Besides, negative biomass growth was attained for AN concentration ranging from 180 mg/L to 200 mg/L, which corresponded with intolerant physical appearance such as yellowish and withered water hyacinth plants. Apart from the tolerance limit study of water hyacinth at varied AN concentrations under sunlight exposure, another set of tolerance limit experiment had been conducted without sunlight exposure, in which the results showed that the leaves of the plants turned yellowish at 40 mg/L AN concentration after 10 days of exposure. As compared to the leaves of the plants that turned yellowish at 160 mg/L AN concentration under sunlight exposure condition, this showed the
significant impact of sunlight to facilitate the plants in carrying out photosynthesis process by utilizing the nutrients in the wastewater.

A study conducted by Sooknah and Wilkie (2004) which used three different macrophytes in removing nutrient from undiluted dairy manure wastewater containing 130 mg/L of AN for 31 days reported that water hyacinth was the sole survivor species by the end of first week while both water lettuce and pennywort were crisping, browning, became necrotic and finally wilting. Besides, it was also found that biomass yield of water hyacinth in the undiluted dairy manure wastewater was double the diluted medium (ratio = 1:1) which proved that high AN concentration could be stressful for biomass growth of water hyacinth. Similar phenomenon were presented by Reddy et al. (1983) that feedlot runoff and methane digester wastewater containing high AN concentration (>200 mg/L) acted as inhibitor for macrophytes growth. Casabianca-Chassany et al. (1992) also stated that high AN concentration which exceeded 200 mg/L contributed to inhibition of water hyacinth growth while optimum plant growth was obtained at AN concentration of 100-150 mg/L. The inhibition of biomass growth in medium containing high AN level was caused by anion transport inhibition due to saturation and depolarization of cell membranes (Caicedo et al., 2000). In addition, Karpiscak et al. (1999) reported that AN level of 188 mg/L and organic nitrogen level of 114 mg/L were painstaking for constructed wetlands system.

When ammonium chloride dissolves in water, hydrogen ions will be released which contribute to the formation of a slightly acidic solution (Flowers et al., 2015). Therefore, the higher the AN concentration, the more acidic is the AN solution. For solution with AN concentration of 10 mg/L, the initial pH was 6.80 whereas the initial pH for AN concentration of 200 mg/L was 6.38. Figure 2 demonstrates the pH change at varied AN concentration for continuous 10 days. Referring to Figures 2(a) and 2(b), it was clearly shown that the pH values gradually decreased with time. The gradual decrease of pH was also reported by Edaigbini et al. (2015) in treating produced water using water hyacinth. This was due to the releasing of hydrogen ions from plant roots into the water environment during AN absorption (Mattson et al., 2009; Gupta et al., 2012). However, further uptake of AN from growing medium was inhibited when plants reached their storage capability limit. This explained the reason for obtaining the steep slope of pH trendline in Figures 2(a) and 2(b) from Day 1 to Day 7, and followed by shallow slope until Day 10. In the comparison, control set contained only AN synthetic wastewater without water hyacinth plant, thus there was no AN absorption from the water medium to the plant. In other words, there was no releasing of hydrogen ions from the plant roots into the water medium, which resulted in consistent pH values of the water medium for control set from Day 1 to Day 10, as displayed in Figures 2(c) and 2(d). From this tolerance limit study, the results overall showed that water hyacinth was feasible in treating wastewater with AN concentration of 10-150 mg/L. Other than AN concentration, pH was another significant parameter that required careful monitoring since continuous decrease of pH of the growth medium due to AN absorption would become the limiting factor for growth of water hyacinth (Caicedo et al., 2000; Gupta et al., 2012).
Fig. 2. pH change at initial AN concentration for experiment set (a) 10-100mg/L, (b) 110-200mg/L and control set (c) 10-100mg/L, (d) 110-200mg/L (retention time = 10 days, initial macrophyte density = 400g/tank).

pH study

Figure 3 shows the effect of pH on TSS, COD and BOD₅ of synthetic wastewater samples and the biomass growth of water hyacinth throughout the phytoremediation process. Overall, pH posed insignificant impact on TSS of the AN synthetic wastewater, in which only 3-6 mg/L of TSS was obtained. Comparing to the experimental set with water hyacinth plants, the TSS values obtained for the set of control experiment without water hyacinth plants was lower, which was only 0-1 mg/L TSS. Higher TSS values in final AN synthetic wastewater with water hyacinth plants were possibly due to the biomass decomposition and algae formation in the wastewater (Saeed & Al-Nagaawy, 2013).

However, it was found that pH posed significant impact on BOD₅ of the wastewater. Extremely low or high pH (pH = 4 and 9) gave low BOD₅ values as compared to other pH values, which was probably due to the inhibition of microbes, biomass and algae growth at extreme pH condition. Higher BOD₅ values were obtained in experimental set compared to that in control experiments as depicted in Figure 3(b) was due to the algae and plants decomposition during phytoremediation process which contributed as the sources of biodegradable organic matter for microbial community which subsequently increased the oxygen demand (Saeed & Al-Nagaawy, 2013). Chukwuka et al. (2008) also found that the presence of water hyacinth on water surface could cause rapid depletion in dissolved oxygen level in the infested area. For control set, the BOD₅ values were possibly contributed by the oxidation of AN mediated by microorganisms (APHA, 2005).

For COD analysis, fluctuating trendline is shown in Figure 3(c). Similar to TSS results, it was found that pH posed less significant impact on COD in the wastewater. For experimental set, COD values were measured as 6.67-26.67 mg/L. As for control experiments, the COD values varied from 0-13.33 mg/L. Similar findings were reported by Chukwuka et al. (2008) that higher COD and BOD values were obtained for freshwater area with water hyacinth infestation (BOD range: 0.38-19.20 mg/L; COD
PHYTOREMEDIATION OF AMMONIACAL NITROGEN IN WASTEWATER USING *Eichhornia crassipes*

**Fig. 3.** Effect of pH on (a) TSS, (b) COD, (c) BOD\textsubscript{5} of synthetic wastewater samples and (d) biomass growth of water hyacinth (retention time = 10 days, initial macrophyte density = 400g/tank, initial AN concentration = 40 mg/L).

The results of water hyacinth biomass growth as shown in Figure 3(d) indicated the significant impact of pH towards the water hyacinth biomass growth. The highest biomass growth of 217.89 g was obtained for wastewater at pH 7. The lower biomass growth attained at pH 4 (99.63 g) and pH 9 (144.39 g) as compared to biomass growth obtained at other pH values were consistent with the low BOD\textsubscript{5} values obtained at the relevant pH. After 10 days of phytoremediation process under pH 4 and pH 9, the physical appearance of the water hyacinth plants showed intolerant characteristics, which included yellowing and wilting of plants. This also proved that extremely low or high pH acted as the limiting factor for biomass growth of water hyacinth plants (Gupta *et al.*, 2012). These results were supported by the study conducted by Caicedo *et al.* (2000) which reported that extreme pH (<5 and >8) caused detrimental effect to growth of duckweed (*Spirodela polyrrhiza*). From this pH study, it could be concluded that water hyacinth was a suitable macrophyte to be employed in phytoremediation process since it could tolerate a wide range of pH condition (4 to 9) and posed only minor impacts on the physico-chemical properties of the treatment medium.

**CONCLUSION**

This study showed that it was feasible to use water hyacinth to treat wastewater with AN content which ranged from 10 mg/L to 150 mg/L under recom-
mended operating conditions which included appropriate pH range (5-9) and sunlight exposure. Besides, it was possible to employ water hyacinth to treat wastewater which contained AN concentration not more than 30 mg/L under the condition without sunlight exposure. For pH study, it was shown that pH factor gave significant impact towards water hyacinth biomass growth and BOD₅ of AN synthetic wastewater. Meanwhile, the TSS and COD values of AN synthetic wastewater were insignificantly influenced by pH. The overall results showed that the phytoremediation process using water hyacinth did not contribute extra pollutant loads to the treated wastewater. In conclusion, water hyacinth has been shown to be a feasible macrophyte for phytoremediation of AN in wastewater.

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REFERENCES


