Phytoremediation of Nutrients and Organic Carbon from Sago Mill Effluent using Water Hyacinth (*Eichhornia crassipes*)

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Abstract. The aim of this study was to investigate the ability of floating water hyacinth (*Eichhornia crassipes*) to survive under selected concentrations of sago mill effluent (SME) and determine the nutrient uptake by the plant. Phytoremediation at 10, 15, and 20% (V_{SME}/V_{water}) SME concentrations by water hyacinth was conducted under greenhouse conditions for 30 d in a batch type experiment. After 30 d of phytoremediation, the removal efficiency of chemical oxygen demand, ammonia and phosphorus from SME wastewater were (86.4% to 97.2%), (91.4% to 97.3%) and (80.4 to 97.2%), respectively. The results proved the efficiency of water hyacinth to polish SME wastewater.

Keywords: *ammonia; COD; nutrient uptake; phosphorus; phytoremediation; sago mill effluent; water hyacinth.*

1 Introduction

Metroxylon spp. is a species of palm that produces sago starch, locally also known as *rumbia*. Sago plants can grow well in South East Asia and can be found especially in Sarawak, Malaysia. As a functional component, sago starch is widely used as stabilizer, thickener, and gelling agent in the food industry [1]. Sago mill effluent (SME) produced by the manufacturing process of sago starch is a carbohydrate-rich liquid waste consisting mostly of macromolecules in the form of polysaccharides (starch and hemicelluloses). However, best practices have not been implemented in the manufacturing process and this waste has been improperly managed, resulting in severe pollution. Vigorous research is necessary to solve the present predicament faced by the Malaysian sago industry due to this waste problem [2]. Fast industrialization has caused the

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production and release of considerable quantities of wastes in water sources. Conventional water sources are easily contaminated by industrial wastewater [3]. Social activities can expedite the rate at which nutrients enter the environment. Water flow from agricultural regions and industrial developments, contamination from wastewater treatment plants and sewers, and other human-related activities increase both inorganic nutrients and organic substances in water and soil ecosystems. Moreover, the nitrogen availability can be increased by elevated nitrogen levels in atmospheric compounds. Meanwhile, phosphorus is often regarded as the main culprit for the occurrence of eutrophication in lakes due to contamination by wastewater [4].

Phytoremediation is an environmentally friendly, low-tech, low-cost and promising treatment approach for polluted soils, surface water, groundwater, and wastewater. It is known as an engineered plant-assisted remediation employing different species of plants to remove, contain, or render harmless such environmental contaminants as trace elements, organic compounds, hydrocarbon, heavy metals and radioactive compounds in water or soil. It is a method that can decrease remedial costs, remediation and restoration of hazardous waste in sites and it has a long-term applicability as well as aesthetic advantages [5,6].

The most important factor in investigation of phytoremediation is the selection of the appropriate plants. Several tropical native plant species in Malaysia have the ability to uptake contaminants from their growing environment, for example *Scirpus grossus* [7,8], *Lepironia articulate* [9,10] and *Ludwigia octovalvis* [11,12]. Plant selection is impacted by the condition of the site, which affects the plants' growth. In order to select the most suitable plants, a list of potentially useful plants for remediation should be prepared first [4].

Several studies have shown that water hyacinth has the potential to clean up various wastewaters due to its rapid growth, including phytoremediation of ethanol [13], sewage effluent [4], nutrients and heavy metals [14], and removal of nitrogen [15]. To this date, no research has been done on phytoremediation to treat SME using water hyacinth. The experiment in the present study emphasized the effectiveness of water hyacinth (*Eichhornia crassipes)* in removing chemical oxygen demand (COD), ammonia and phosphorus from SME.

2 Materials and Methods

2.1 Setup of Phytoremediation Test

This study was conducted in a greenhouse at Universiti Kebangsaan Malaysia using a floating water hyacinth (*E. crassipes*) plant collected from a local lake in Bukit Mahkota, Selangor, Malaysia. Real SME obtained from a sago mill in Batu Pahat, Johor, Malaysia was used. Nine glass aquariums, each with dimensions of 30 cm (length) × 30 cm (width) × 30 cm (depth), were used for the entire experiment with three controls containing water hyacinth in tap water without SME (PC), as illustrated in Figure 1(a). A phytoremediation test was executed to determine the maximum contaminant concentration of SME the floating water hyacinth (*E. crassipes*) plant can uptake while continuing to survive in contaminated medium for 30 d.

A preliminary test specially based on observations of the physical growth of *E. crassipes* was performed for 14 d before proceeding to the phytoremediation test carried out for 30 d. The purpose of the preliminary test was to estimate the range of SME concentrations that can be tolerated by *E. crassipes*. This preliminary test using six SME concentrations (90, 70, 50, 30 20 and 10% (V_{SME}/V_{water})) found that the plant could survive on up to 20% of SME from the total volume of contaminated water. Therefore, diluted SME concentrations (20, 15 and 10%) of SME were used in this phytoremediation test with each aquarium having a total working volume of 17 L.

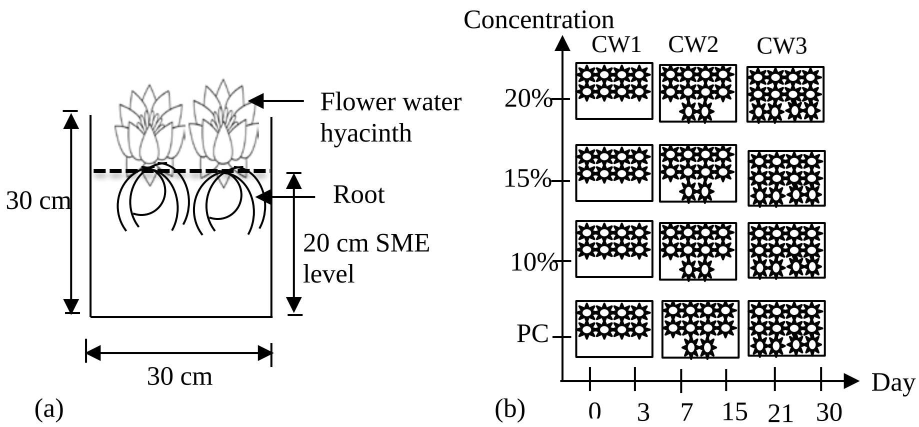

Figure 1 Aquarium setup for water hyacinth: (a) aquarium setup for water hyacinth; (b) experimental design for the whole phytoremediation test.

There were different numbers of water hyacinth plants in each aquarium, three groups coded as CW1 = 8 plants, CW2 = 10 plants and CW3 = 12 plants, as shown in Figure 1(b). The physicochemical characterization of raw SME was conducted of which the results are listed in Table 1. The pH, temperature and ORP were analyzed directly at the site after the raw SME had been collected from the main drain of the mill. The analysis of COD, ammonia and phosphorus of the raw SME was carried out under laboratory conditions.

Table 1 Physicochemical characterization of SME.

Parameter	Concentration
pH	4.2 ± 0.6
T	28 ± 5 °C
DO	7.36 ± 0.25 mg/L
ORP	-46.4 ± 5 mV
COD	8720 ± 256 mg/L
NH_4-N	52.5 ± 9 mg/L
P	2.1 ± 0.02 mg/L

2.2 Plant Biomass

The growth of a plant in an engineered phytoremediation experiment is essentially the same as its growth in the natural environment. There is a wide variety of plants that can be categorized as submerged, emergent or floating [16]. In this study, a floating plant (*E. crassipes*) was used to test the ability of *E. crassipes* to remediate contaminants in SME. *E. crassipes*, an aquatic floating species, spreads in tropical regions, visibly dominating wetlands, shallow lakes, and streams, as shown in Figure 2. At the beginning of the experiment, 120 young shoots of *E. crassipes* of the same size and with a mass of about 35-39 g were placed in an aquarium.

Figure 2 Photos of water hyacinth (*E. crassipes*) in Bukit Mahkota Lake, Selangor state, Malaysia.

2.3 Sample Collection and Physicochemical Analysis

During 30 d of exposure to SME, samples were collected on days 0, 3, 7, 15, 21, and 30. The physicochemical parameters of temperature, T (°C); and pH were monitored. The temperature and pH were measured using a (HACH, LPV2500.97.0002, China). About 100 mL of effluent sample from the growth medium in each aquarium was collected periodically in clean plastic bottles on the sampling days for an analysis of all parameters. COD analysis was conducted using digestive reagents of high range COD plus (3-150,000 ppm) (HACH, USA) incubated in the COD reactor series 8000 (HACH, USA) for 2 h at a temperature of 150 °C. After 2 h, the COD value was read using a portable data logging spectrophotometer (HACH, DR/2010, USA)[10]. Ammonia and phosphorus were analyzed using the Nessler method [4] and phosphorus reagent respectively, and read using a portable data logging spectrophotometer (HACH, DR/2010, USA) [17]. The effectiveness of the treatment system was calculated based on removal efficiency as expressed in Eq. (1):

$$\text{Removal Efficiency (\%)} = \left[\frac{(C_{inf} - C_{eff})}{C_{inf}}\right] \times 100 \quad (1)$$

with, C_{inf} is SME influent concentration and, C_{eff} is SME effluent concentration.

2.4 Statistical Analysis

Statistical analysis was conducted with Statistical Product and Service Solutions (SPSS) 21.0 for Windows. Chemical oxygen demand (COD), ammonia (NH_3) and phosphorus (P) were analyzed according to one-way analysis of variance (ANOVA) and Pearson linear correlation to determine any significant effect of number of plants in the aquarium (CW) on the parameters. To compare COD, ammonia and phosphorus removals with different numbers of plants, retention time and SME concentration, the data were analyzed using two-way ANOVA. Duncan's multiple range tests were used to evaluate statistical differences of all parameters at the 0.05 probability level unless otherwise stated. The samplings were performed in duplicate and the results are presented as means with standard deviation.

3 Results and Discussion

3.1 Observation of Physicochemical Parameters

The selected physicochemical parameters were recorded throughout the phytoremediation test for all concentrations. In general, the temperature did not significantly change during the exposure period. Throughout the 30 d of the experiment, the temperature depended on the weather at the sampling time and varied from 28 °C to 30 °C during the treatments. According to Al-Sbani *et al.*

[10], these temperatures are significantly correlated with the climate of Malaysia. The pH ranged between pH 4.5 and 4.9 for the aquariums with plants at 0 days and between pH 6.9 and 7.6 for the aquariums with plants on 30 d. The increase of pH in the effluent may be due to the production of alkaline amylase by bacteria and rhizobacteria of *E. crassipes* [18,19]. The result indicates that temperature and pH did not differ significantly among the treatments in the aquariums with and without plants.

3.2 Plant Response to SME

All 120 *E. crassipes* plants survived under SME exposure and no death was recorded. Flowers of the plants bloomed after two weeks of exposure. The selected plant growth parameters were determined on day 0 and day 30 of the test. Generally, all of the plants showed increased growth after 14 d of exposure to SME and the plants survived under all three concentrations. Statistical analysis showed no significant difference between control and the three different concentrations (10%, 15% and 20%) at 0 d.

The wet weight was significantly different between control and the three different concentrations (10%, 15% and 20% (VSME/Vwater)) at 30 d, as shown in Figure 3. The loadings of the plant for the concentrations 10%, 15%, 20% (V_{SME}/V_{water}) and control were 90 g, 125 g, 85 g and 42 g respectively.

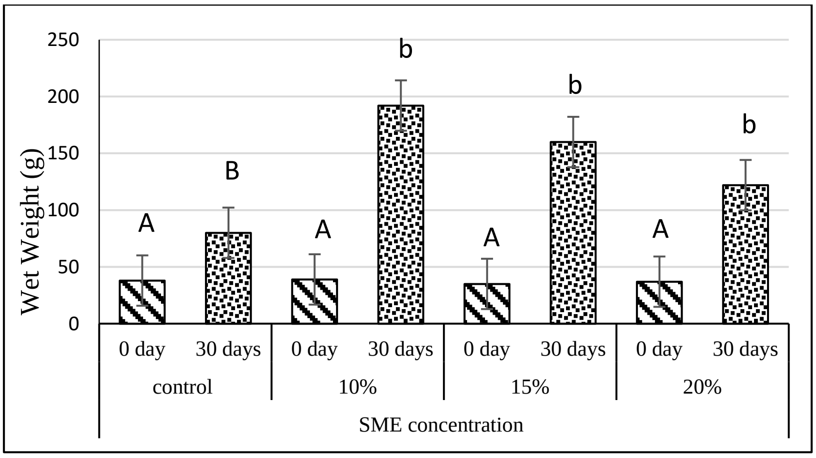

Figure 3 Wet weights of *E. crassipes* exposed to SME at different concentrations (10%, 15% and 20%) and control. A, A: no significant difference at $p > 0.05$ between the wet weight of the plants at zero days for control and different concentrations; B, b: significant difference at $p < 0.05$ between wet weight of the plants at 30 days of exposure.

3.3 Removal of Chemical Oxygen Demand (COD)

The organic strength of the wastewater was measured as COD, defined as the oxygen required to decompose organic and inorganic materials through chemical pathways. A high COD level is due to the toxic condition and the presence of biologically resistant organic substances [20].

Figure 4 illustrates the values of COD removal under the different treatments (CW1 = 8 plants, CW2 = 10 plants and CW3 = 12 plants) with SME concentrations of (20, 15 and 10%) during the 30-day treatment period. Statistical analysis showed a significant decrease at COD concentration 15% and 20% SME with respect to 10% for each group. The COD concentration from the SME significantly decreased at 15 d with (p < 0.05).

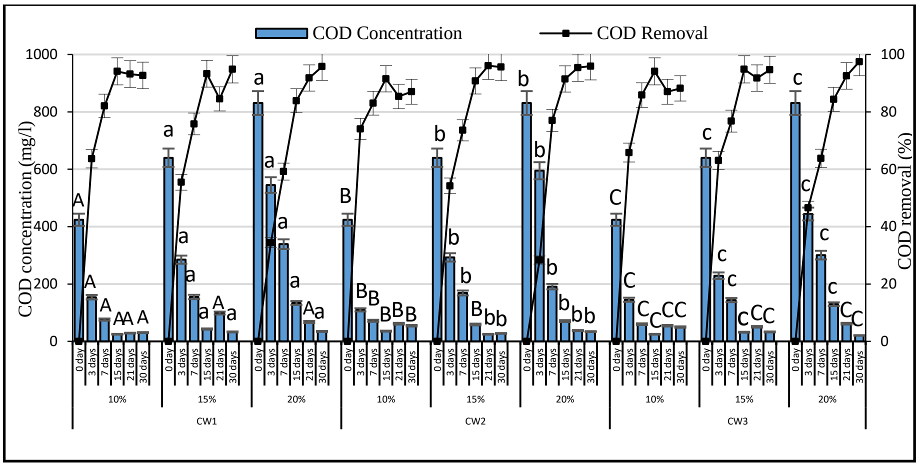

Figure 4 COD concentration and removal during 30 days. A, a: significant difference at p < 0.05 between concentrations at different days in CW1; B, b: significant difference at p < 0.05 between concentrations in CW2; C, c: significant difference at p < 0.05 between concentrations in CW3; significant difference of COD concentration when comparing 20% and 15% SME with respect to 10% SME on the same day for each CW group.

Table 2 shows that the removal efficiency was high at 30 d with different numbers of plants in the aquariums and three different concentrations. However, there was no significant removal effect through one-way ANOVA between the CW groups with different concentrations (p > 0.05).

Table 2 COD removal with CW1, CW2 and CW3 at the end of experiment period.

Concentration (%)	COD removal (%) at 30 days		
	CW1	CW2	CW3
20	95.62	95.86	97.18
15	94.47	95.87	94.36
10	92.32	86.35	89.44

3.4 Removal of Ammonia

Ammonia is an undesirable concentration of nutrients that can stimulate growth of microorganisms. When released to the aquatic ecosystem, these nutrients can lead to the growth of undesirable aquatic life and pollution in water [4,21]. Figure 5 depicts the concentrations of ammonia on sampling days (0, 3, 7, 15, 21 and 30) and efficiency of ammonia removal in SME by *E. crassipes.* The concentrations of ammonia decreased significantly between the three different concentrations with the sampling days ($p < 0.05$). The three different numbers of plants in the aquariums (CW1 = 8 plants, CW2 = 10 plants and CW3 = 12 plants) had high ammonia removal efficiency at all concentrations, as shown in Table 3. Through one-way ANOVA, there is no significant difference between the CW groups and the three different concentrations ($p > 0.05$).

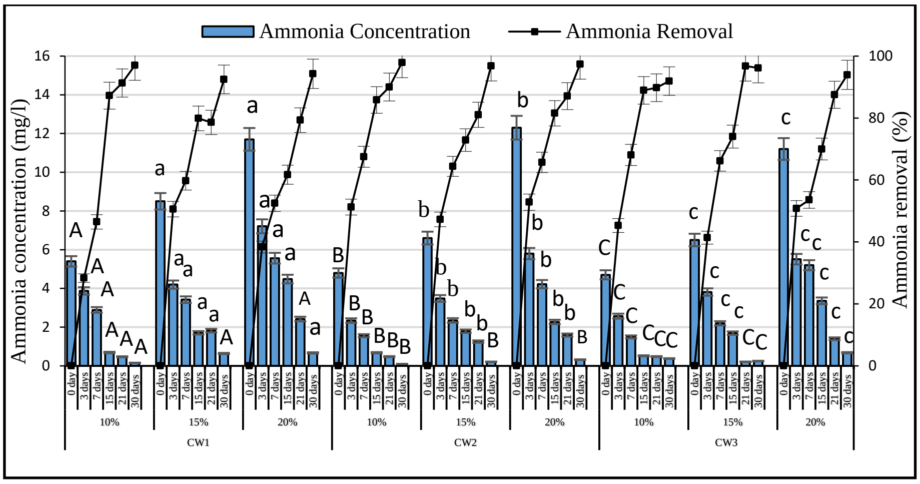

Figure 5 Ammonia concentration and removal during 30 days. A, a: significant difference at $p < 0.05$ between concentrations in CW1; B, b: significant difference at $p < 0.05$ between concentrations in CW2; C, c: significant difference at $p < 0.05$ between concentrations in CW3; significant difference in ammonia concentration when comparing 20% and 15% SME with respect to 10% SME on the same day for each CW group.

Table 3 Ammonia removal with CW1, CW2 and CW3 at the end of the experimental period.

Concentration (%)	Ammonia removal (%) at 30 days		
	CW1	**CW2**	**CW3**
20	91.97	96.68	93.69
15	91.61	94.91	95.71
10	95.51	97.28	91.43

3.5 Removal of Phosphorus

Figure 6 shows the concentration of phosphorus and its removal efficiency from SME by water hyacinth. Phosphorus is a nutrient that can stimulate growth of undesirable aquatic life and pollution in water bodies [22]. The concentrations of phosphorus in most treatments were significantly different between the three concentrations and sampling days (0, 3, 7, 15, 21 and 30) ($p < 0.05$), as shown in Figure 6.

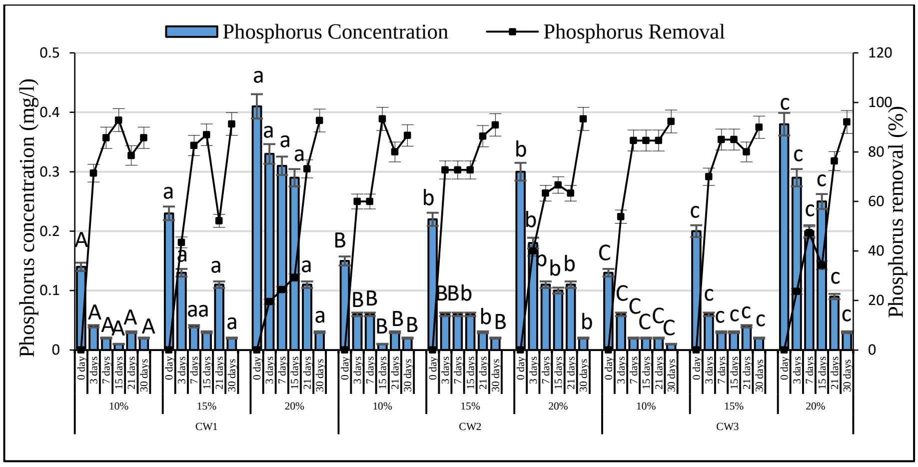

Figure 6 Phosphorus concentration and removal during 30 days. A, a: significant difference at $p < 0.05$ between concentrations in CW1; B, b: significant difference at $p < 0.05$ between concentrations in CW2; C, c: significant difference at $p < 0.05$ between concentrations in CW3; significant difference in phosphorus concentration when comparing 20% and 15% SME with respect to 10% SME on the same day for each CW group.

There was a significant difference between the treatments with different numbers of plants (CW1 = 8 plants, CW2 = 10 plants and CW3 = 12 plants) in the aquariums with high removal efficiency, as shown in Table 4, through one-way ANOVA between CW groups ($p < 0.05$).

Table 4 Phosphorus removal with CW1, CW2 and CW3 at the end of the experimental period.

Concentration (%)	Phosphorus removal (%) at 30 days		
	CW1	**CW2**	**CW3**
20	90.52	90.41	97.18
15	87.65	95.87	94.36
10	80.35	86.35	89.44

4 Conclusions

Water hyacinth (*E. crassipes*) showed good growth and development during the experimental period of 30 days. Moreover, the *E. crassipes* showed the ability to tolerate and survive under three concentrations of nutrients (20%, 15% and 10% (V_{SME}/V_{water})). In this experiment, there was significant effect of *E. crassipes* in decreasing the concentrations of COD, ammonia and phosphorus within the 30 days of exposure. The different CW groups showed no significant effect on the removal of COD and ammonia, and a significant effect of the different CW groups on phosphorus removal. Removals of 86.4-97.2%, 91.4-97.3% and 80.4-97.2% for respective COD, ammonia and phosphorus were obtained using *E. crassipes* plants, proving that *E. crassipes* is capable of removing COD, ammonia and phosphorus from sago mill effluent. Utilization of *E. crassipes* can help to reduce eutrophication effects in receiving streams and also improve its water quality. It can be used in the future in the post-treatment of effluent that can be introduced to polish these nutrients after biogas processing or bioremediation.

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