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Water Hyacinths (*Eichhornia crassipes*) – Application for Secondary Wastewater Treatment and Biomass Production

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Authors' contributions

This work was carried out in collaboration among all authors. Author KD supervised the study, wrote the final draft and approved the final manuscript.

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ABSTRACT

Clean water is one of the most significant challenges for our society. Efficient reuse of effluent water after treatment can becomes an effective solution to the shortage of water resources. The focus of this study is to investigate the use of *Eichhornia crassipes* plants for post treatment of clarified municipal residential sewage under natural conditions using a small pilot Laboratory Water Hyacinth Clarifier system. Twelve *Eichhornia crassipes* plants are used to investigate total phosphorus and ammonia nitrogen removal during a 20-day study period under various retention rates. The biomass gain of the *Eichhornia crassipes* plants was 2.4-fold from the initial weight of 1556.5 g to 3676.7 g. Total phosphorous reduction of 10.64%, 11.83%, 20.93%, 41.66%, 67.12%, and 40.13% for the 1.5, 9.0, 12.0, 24.0, 48.0 h, and 120.0-hour retention times respectively. Ammonia nitrogen removal was between 35.71%, 33.33% for the 1.50 and 9.0-hour retention time and 42.85% for the 12.0 and 24.0-hour retention time. A reduction of 71.43% resulted for the 48.0-hour retention time and an 85.71% reduction for the 120.0-hour retention time. Overall retention time of 24.0 h, 48.0 h and 120 h tend to give best removal rates for both total phosphorus and ammonia nitrogen removal. Factors

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such as climate, contaminant concentration, retention rate, and weather conditions play an important role for the application of *Eichhornia crassipes* in a tertiary treatment sequence of MRS.

Keywords: Ammonia; biomass production; Eichhornia crassipes; phosphor; secondary clarification; temperature; wastewater treatment; water hyacinth.

1. INTRODUCTION

Providing clean water is one of the most significant challenge our society faces in the near future. The increasing needs for water resources for residential, commercial, industrial and recreational use accelerate the depletion of the water resources [1] and increases waste water streams that are a combination of domestic, industrial, commercial or agricultural activities, surface runoff/storm water, and any sewer inflow/infiltration [2]. Wastewater consists of approximately 99.9% water and 0.1% waste [3]. The waste is comprised of organic and inorganic matter, dissolved and suspended solids, and microorganisms [3].

Reuse of the effluent water after treatment becomes an effective solution to the shortage of the water resources [4]. However, a modern wastewater infrastructure is needed for wastewater treatment to assure healthy river, streams, lakes and wetlands where discharged effluent and storm water often end up. According to the American Society of civil Engineers (ASCE) an estimated 56 million new users will be connected to the existing centralized treatment systems of 14,748 Public Operated Treatment Works (POTWs) in the next two decades [5]. This will require at least $271 billion in funding for modernization and additional plants to meet future demands [6].

Eichhornia crassipes (also known as water hyacinth), is an aquatic plant, and is native to South America, Amazon basin [7]. *Eichhornia crassipes* is one of the worst, highly invasive aquatic plant and can be found in 5 continents and more than 50 countries on earth [8]. Regions and area affected by *Eichhornia crassipes* including Central America, central and western Africa, south-east Asia, and south-eastern USA [9].

Optimal growth for *Eichhornia crassipes* happens at water temperature from 28°C to 30°C with rich nutrients like nitrogen, phosphorus, and potassium [10]. *Eichhornia crassipes* has an extremely high growth rate in freshwater system in an ideal condition. It can double in size every 5 to 7 days [9,11]. Other researcher show that *Eichhornia crassipes* can double its population in two 2 weeks, generating a very large amount of floating biomass. Once hectare of surface water can produce over 500 t of *Eichhornia crassipes* [12,13,14].

Eichhornia crassipes tolerates water level fluctuations, seasonal flow velocity variation, nutrient availability, pH, temperature and toxic substances [15]. *Eichhornia crassipes* was reported to tolerate salinities from 0 to 8.8 ppt [16]. It can survive at water temperatures of up to 30°C [10,13], and at water temperature of 5°C for a short period of time and weather conditions close to freezing and light snow as long as the water does not freeze and a warmer weather period follows. [17,13].

These characteristics make Eichhornia crassipes Fig. 1 an invasive weed that can adjust to a diversity of environmental conditions in many parts of the world.

Eichhornia crassipes adjustability can cause several serious problems. It can disrupt native species' diversity through hybridization, ecosystem modifications and functioning [19,20] It may change the aquatic habitat structure, and negatively affecting biological communities [21]. *Eichhornia crassipes* can also have a risk for human health. Its mat-like nature leads to the concentration of micro-organisms around the plant roots and shoots, it can increase the pest's population (like mosquitos) and may bring many diseases, such as ancephalitis, filariasis, malaria, and schistosomiasis [15,22,23,24,25,26].

Despite those disadvantages *Eichhornia crassipes* may have or cause for fresh water body/ environmental, we could, on the contrary, use its special characteristics like tolerating water level fluctuations, seasonal flow velocity variation, nutrient availability, pH, temperature and toxic substances [13] and apply *Eichhornia crassipes* adjustability and characteristics to the treatment of waste water (WW) of domestic and agricultural origin [9,11,13].

Floating Aquatic Macrophyte-based Treatment System (FAMTS) have been explored in the past for the purpose of removing and recovering nutrients in wastewater from animal-based agriculture operations. These systems have high potential, as they are able to treat the water through multiple methods including solid settling, plant uptake, biotransformation, and physio-chemical reactions. Also, these systems contain relatively simple technology and have low costs [27,28]. *Eichhornia crassipes* in particular show promise as a model plant for FAMTS. According to a study, Water Hyacinths can be grown in undiluted wastewater whereas water lettuce and vetiver grass Pennywort could only survive in diluted wastewater. In addition, the report claims that water Hyacinths were more effective than both of these plants in removing nutrients, COD, solids, and salinity than the other two plants [29].

In this study the potential of *Eichhornia crassipes* for the remediation of Phosphorous (P) and Ammonia (NH_3-N) from pretreated Municipal Residential Sewage (MRS) including biomass growth potential was carried out under natural conditions using a small pilot clarifier system.

2. MATERIALS AND METHODS

The *Eichhornia crassipes* plants used for this study came from fishpond Fig. 1 were they were used as ornamental pants and to control water quality in Syracuse, NY, USA. The plants were originally obtained as ornamental plants from a nursery in Florid, USA. During the wintertime when temperatures drop below 10°C some of the plants were nurtured in a 100 l fish tank at 20°C using UV growth lamps. As a nutrient source 250 ml, 100-fold diluted cow manure was added every 28 days. The plants were reintroduced into the fishpond-starting end of April when temperatures are above 10°C.

2.1 Laboratory Water Hyacinth Clarifier System

A small Laboratory Water Hyacinth Clarifier (LWHC) system was designed, build and installed according to Fig. 2.at a municipal wastewater treatment plant at the secondary clarifier (SC). The LWHC system consisted of a 160l Polyvinyl Chloride (PVC) tank (1) with mm in length, 465 mm in width and mm in height. Two wooden boards (2) with 25.4 mm x 152.4 mm (1in x 6 in) with a width of 465 mm were used to create the influent and effluent area with a 465 mm width and 76.2 mm (18.3 in x 3 in) in length. The growth area for the *Eichhornia crassipes (9)* between the two boards is 70cm x 46.5 cm = 0.325 m² (581.25 in²) in size. The wastewater (8) is pumped from the secondary clarifier with small 25-Watt pond pump (4). The pond pump has a maximal flow rate of 4.40 gal/min (16.66 l/min) at a head of 5.5 ft. (1.67 m) at a rate of 0.5 l/min trough a 3/8 in Inside Diameter (ID) Polyvinyl Chloride (PVC) hose (5) is connected to the PVC influent port (3) and effluent port (4). The influent port is located above the LWHC water level, the effluent port is located at the water leaves so the treated WW can exit by gravity back into the clarifier (8). Board 2 at the exit prevents that floating material can exit the LWHC system.

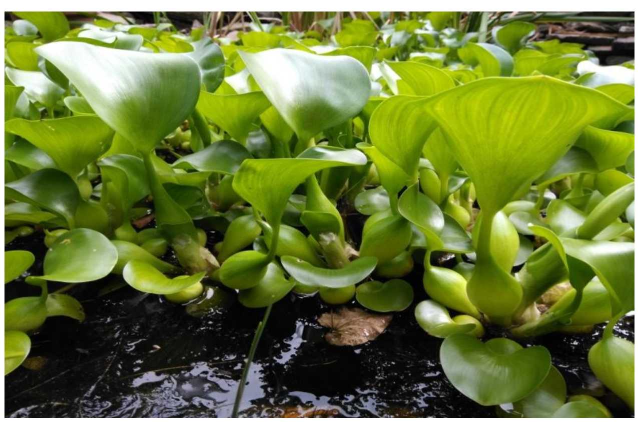


Fig. 1. Water hyacinth *(Eichhornia crassipes)* [18]

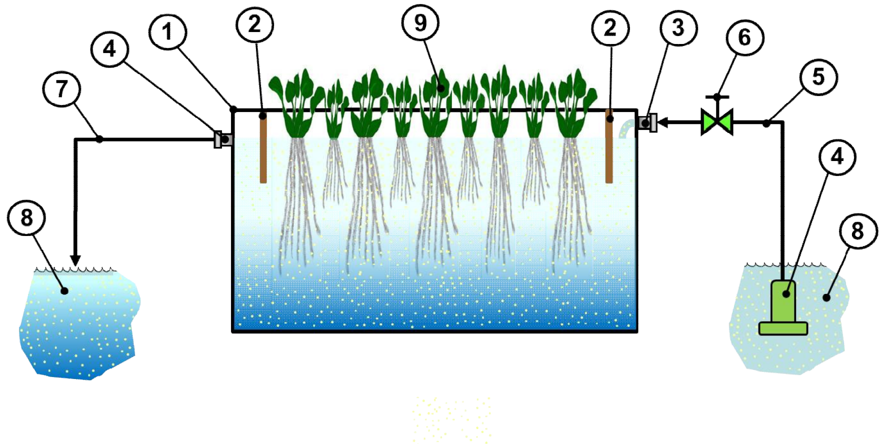


Fig. 2. Laboratory water hyacinth (*Eichhornia crassipes*) Clarifier [30]

2.2 Water Hyacinth Growth in the Laboratory Water Hyacinth Clarifier System

The growth study with *Eichhornia crassipes* was carried under natural conditions for 20-days using influent water from the SC which approximately receives approximately 475,000l/d pretreated Municipal Residential Sewage (MRS) from a trickling filter (TF). After the SC the treated MRS passes through a secondary clarifier and a disinfection unit before it is discharged into a stream [14,30,31].

The LWHC installed at the SC received the pretreated MRS by pumping it from the SC into the LHWC and discharged the LWHC effluent by gravity flow back into the SC. The retention time of the MRS in the LHWC was adjusted to 1.5 h, 9.0 h, 12.0 h, 24.0 h, 48.0 h, and 120.0 h during the trial period.

Twelve water hyacinths plants were placed inside this tank and their biomass yield was recorded.

Prior to weighing the plants where carefully removed and dried with a paper towel. After drying each of the *Eichhornia crassipes* plant placed on an analytical scale with an empty container large enough to hold the *Eichhornia crassipes* plant. Prior to weighting, the scale was tared to account for the weight of the empty container.

The outside air Temperature (t) in [ºC], Relative Humidity (RH) in [%] and Precipitation (PCPN) in [mm] was recorded during the 20-day study.

The WWTP influent MRW stream fluctuates based on the time and day of the year an can be in the range between 1.0 to 12.0 mg/l for Total Phosphorous (TP), 10 to 30 mg/l of ammonium (NH_3), 15 to 45 mg/l for Total Kjeldal Nitrogen (TKN), Chemical Oxygen Demand (COD) from 50 to 200 mg/l, Total Suspended Solids (TSS) are in the range of 150 to 250 mg/l, and the 5-day Biological Oxygen Demand (BOD_5) ranges from 50 to 350 mg/l at an average temperature of 15°C [32].

Phosphorous levels expected at the TF can be as high as 5.0 mg/l and as low as 1.2 mg/l.

Effluent parameters permitted to reach a maximum for TP of 0.8 mg/l, NH_3 of 0.5 mg/l, TKN of 1.3 mg/l, CBOD <4 mg/l, TSS of <4 mg/l and BOD_5 of <4 mg/l [32].

2.3 Testing and Recording

100 ml Nalgene bottles were used to collect the influent of the small LWHC system influent and effluent samples. The samples were stored in a cold room at 4.0°C (39.2°F) before, during and between testing.

A HACH DR900 Spectrophotometer and a HACH DRB200 Reactor was used for analyzing the exact concentration of Total Phosphorous (TP), and Ammonia Nitrogen (NH_3-N).

The analyzation of the collected 30 ml samples followed HACH Method 10127 [33] for TP using HACH-TNT Reagent Set (1-100.0 mg/L), and HACH Method 10031 [34] for NH_3-N using HACH-TNT Reagent Set (0.4-50.0 mg/L). Both

HACH Testing procedures have according to HACH a 95% confidence interval [33,34].

3. RESULTS AND DISCUSSION

Fig. 3 shows the outside Temperature (t), Relative Humidity (RH) and Precipitation (PCPN) for the 20-day trial period measured at 12:00 pm. During the test period, a total of 44.2 mm precipitation was received with over 27 mm of precipitation received during a one-time rain event.at the 12th day of testing. The outside temperature was between 11.3ºC and 26.7ºC, and the relative humidity was between 77.1% and 98.8%. The temperature of the MRS fluctuated between 16.0ºC and 18.0ºC at the time of sampling.

The individual biomass gain for each of the twelve *Eichhornia crassipes* plants during the 20-day test phase is shown in Fig. 4. Each of the plants was weighted and labeled separately prior to testing. During testing each of the plants was carefully removed after influent and effluent water sampling were taken and dried with a paper towel prior to weighting.

As shown in Fig. 4, Plant one grew from its initial weight of 37.4 g to 87.6 g representing a 2.3-fold increase in biomass. Plant two grew from its initial weight of 71.5 g to 190.1 g representing a 2.6-fold increase in biomass. Plant three grew from its initial weight of 106.4 g to 420.0 g representing a 3.9-fold increase in biomass. Plant four grew from its initial weight of 207.7 g to 364.3 g representing a 1.7-fold increase in biomass. Plant five grew from its initial weight of 184.5 g to 312.5 g representing a 1.7-fold increase in biomass. Plant six grew from its initial weight of 68.6 g to 208.3 g representing a 3.0-fold increase in biomass. Plant seven grew from its initial weight of 127.5 g to 278.2 g representing a 2.2-fold increase in biomass. Plant eight grew from its initial weight of 247.2 g to 643.0 g representing a 2.6-fold increase in biomass. Plant nine grew from its initial weight of 249.6 g to 525.3 g representing a 2.1-fold increase in biomass. Plant ten grew from its initial weight of 98.9 g to 193.2 g representing a 2.0-fold increase in biomass. Plant eleven grew from its initial weight of 39.2 g to 131.5 g representing a 3.4-fold increase in biomass. Plant twelve, grew from its initial weight of 118.0 g to 322.7 g representing a 2.7-fold increase in biomass.

The overall gain in biomass of all twelve plants is 2.4-fold from the initial weight of 1556.5 g to 3676.7 g during the 20-day test period, similar to a growth study in a primary clarifier that used seven *Eichhornia crassipes* plants [13].

The *Eichhornia crassipes* plants TP and NH_3-N uptake in the LWHC under a 1.5, 9.0, 12.0, 24.0, 48.0 and 120.0 hour retention times is shown Fig. 5 and 6. Water samples were tested using laboratory bench testing.

Fig. 5 shows that the influent Total Phosphorous (TP) equivalent (P_2O_5) decreased from a 9.40 to 8.40 mg/L for the 1.50 h retention time. A 9.0 h, 12.0 h, 24h and 120 h retention time decreased the TP in the effluent from 9.30 to 8.20 mg/L, 8.60 to 6.80 mg/l, 7.20 to 4.20 mg/l, 7.30 to 2.40 mg/l and 7.35 to 4.4 mg/l respectively. Tis correlates to a phosphorous reduction of 10.64%, 11.83%, 20.93%, 41.66%, 67.12%, and 40.13% for the 1.5, 9.0, 12.0, 24.0, 48.0 h, and 120.0-hour retention times respectively.

The plant biomass needed for *Eichhornia crassipes* to remove one milligram TP are 2,034 g, 1,849 g, 1,563 g, 938 g, 750g, and 953 g for the 1.5, 9.0, 12.0, 24.0, 48.0 h, and 120.0-hour retention times respectively. This shows that the 24, 48, and 120-hour retention times are most efficient for TP removal for the LWHC.

The larger decrease in TP for the 24-hour study at the same *Eichhornia crassipes* plant biomass can be explained due to the large rainfall of over 27 mm received on day 12 of the study prior to testing the TP removal.

Due to the large biomass growth of the *Eichhornia crassipes* plants overgrowing the LWHC system at the end of the study time, plant 6, 9, and 12 with a biomass weight of 208.3 g, 525.3 g, and 322.7 g respectively. This resulted in a total *Eichhornia crassipes* plant biomass of 2,620.4 g in the LWHC system. This explains the large decrease in TP of 67.12% for the 48.0-hour retention time and the nearly same TP removal of 41.66% and 40.13% for the 24.0 and 120.0-hour retention time. However, the 120.0- hour TP removal time is lower based on retention time compared to the 24.0-hour retention time. This can be explained by the up to 96-hour combined lag time of the treated MRS entering the SC after the large rain event on day 12 of the study as well as the additional rainfall of nearly 8 mm received on day 19 during the 120-0-hour study.

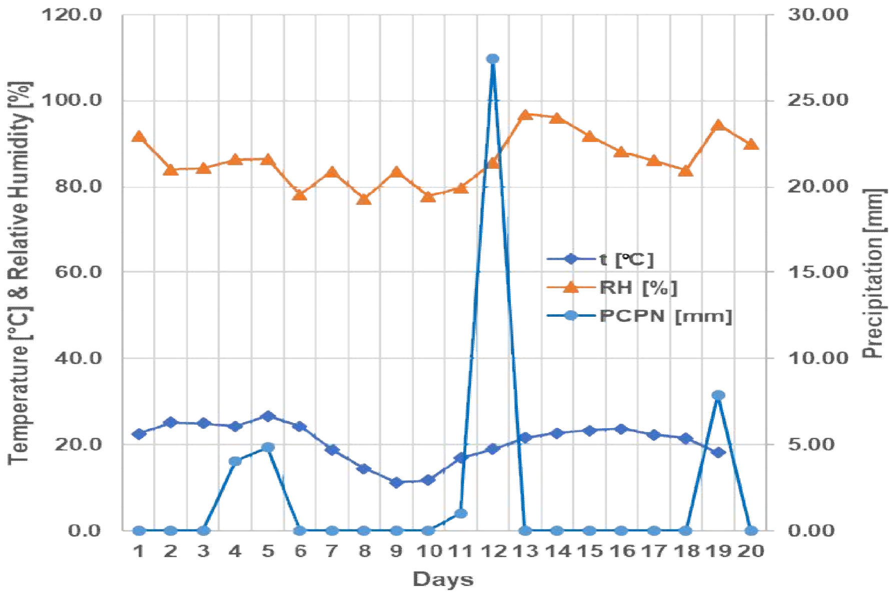
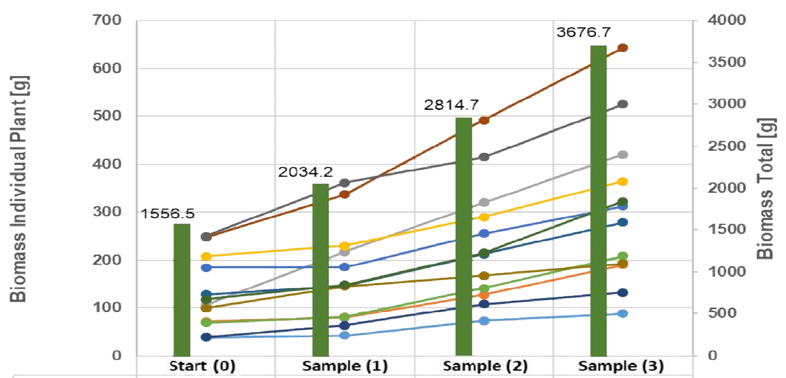


Fig. 3. Temperature, relative humidity and precipitation



	Start (0)	Sample (1)	Sample (2)	Sample (3)
WH 1	37.4	42.3	72.7	87.6
WH 2	71.5	79.8	127.3	190.1
WH 3	106.4	216.6	319.9	420
WH 4	207.7	229.6	290.0	364.3
WH 5	184.5	184.8	255.1	312.5
WH 6	68.6	80.7	141.3	208.3
WH 7	127.5	146.5	212.5	278.2
WH 8	247.2	337.2	491.8	643.00
WH 9	249.6	361.2	414.4	525.3
WH 10	98.9	144.5	167.6	193.2
WH 11	39.2	63.1	107.6	131.5
WH 12	118	147.9	214.5	322.7
WH total	1556.5	2034.2	2814.7	3676.7

Fig. 4. Total biomass yield of *Eichhornia crassipes* in the laboratory clarifier

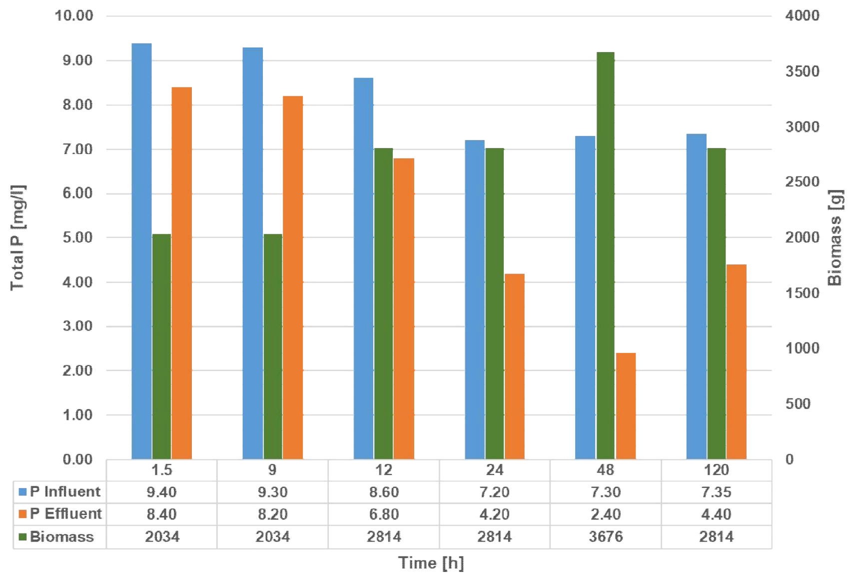


Fig. 5. Total phosphor reduction based on *Eichhornia crassipes biomass*

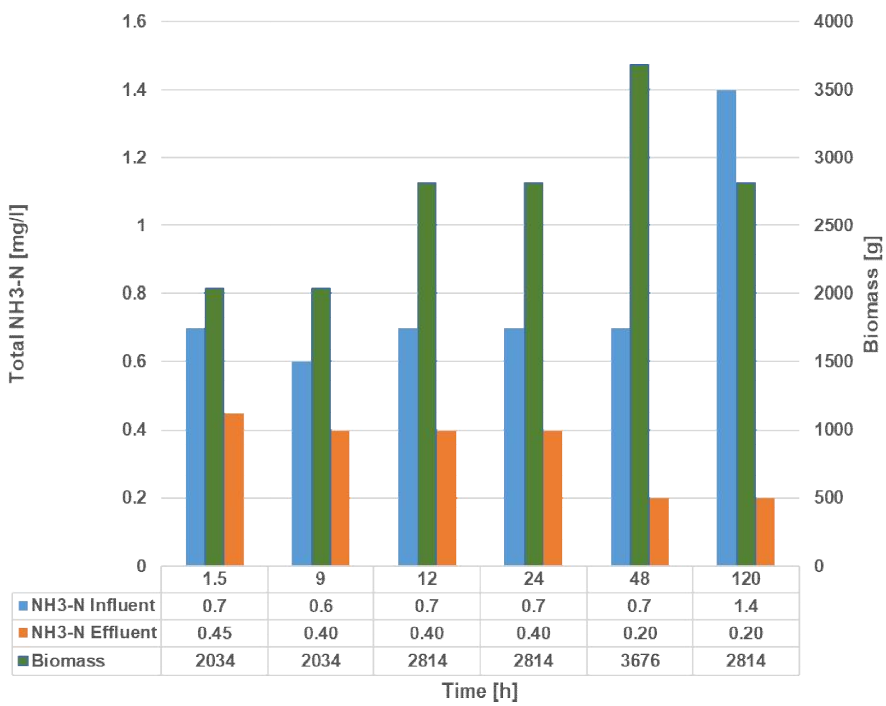


Fig. 6. Total NH_3-N reduction based on *Eichhornia crassipes biomass*

NH_3-N uptake decreased from a 0.70 to 0.45 mg/L for the 1.50 h retention time. The 9.0 h, 12.0 h, 24.0 h, 48.0 h and 120 h retention time decreased the NH_3-N in the effluent from 0.60 to 0.40 mg/L, 0.70 to 0.40 mg/l, 0.70 to 0.40 mg/l, 0.7 to 0.20 mg/l and 1.40 to 0.20 mg/l respectively. Tis correlates to a phosphorous reduction of 35.71%, 33.33% for the 1.50 and 9.0-hour retention time and 42.85% for the 12.0 and 24.0-hour retention time. A reduction of

71.43% resulted for the 48.0-hour retention time and an 85.71% reduction for the 120.0-hour retention time.

The plant biomass needed of *Eichhornia crassipes* to remove one milligram NH_3-N are 8,136, 10,170,780 g, 9,380 g, 9,380 g, 7,352 g, and 2,345 g for the 1.5, 9.0, 12.0, 24.0, 48.0 h, and 120.0-hour retention times respectively. This shows that the 24, 48, and 120-hour retention times are most efficient for TP removal for the LWHC.

Due to the large biomass growth of the *Eichhornia crassipes* plants overgrowing the LWHC system at the end of the study time, plant 6, 9, and 12 with a biomass weight of 208.3 g, 525.3 g, and 322.7 g respectively. This resulted in a total *Eichhornia crassipes* plant biomass of 2,620.4 g in the LWHC system.

This explains the increase in NH_3-N removal of 9.52% (increase from 33.33% to 42.85%) for the 48.0-hour retention time study at the same influent level of 0.70 mg/l of NH_3-N for the 12.0-h retention time study. However, the large rainfall event prior to the 24.0-hour study did have an effect on the NH_3-N removal for the 120-hour study, which showed a decrease of 1.20 mg/l (85.71%) in NH_3-N concentration to the influent level of 1.4 mg/l. This can be explained by combination of effects, such as: a) the up to 96-hour combined lag time of the treated MRS entering the SC after the large rain event on day 12 of the study, b) the additional rainfall of nearly 8 mm received on day 19 during the 120-0-hour study, and c) the larger NH_3-N load the MRS is carrying due to Inflow and Infiltration (I&I) of groundwater and storm water into the sanitary sewer system, and d) the larger amount of MRS the WWTP had to handle based on the storm event.

4. CONCLUSION

This study under natural conditions with a small pilot LWHC system showed the potential of *Eichhornia crassipes* for the remediation of Phosphorous (P) and Ammonia (NH_3-N) as well as potential biomass generation using pretreated MRS.

The overall gain in biomass of twelve *Eichhornia crassipes* plants during the 20 day study time is 2.4-fold from the initial weight of 1556.5 g to 3676.7 g during the 20-day test period, this is similar to a growth study conducted in a primary clarifier [13].

TP reduction of 10.64%, 11.83%, 20.93%, 41.66%, 67.12%, and 40.13% for the 1.5, 9.0, 12.0, 24.0, 48.0 h, and 120.0-hour retention times respectively.

NH_3-N removal was between 35.71%, 33.33% for the 1.50 and 9.0-hour retention time and 42.85% for the 12.0 and 24.0-hour retention time. A reduction of 71.43% resulted for the 48.0-hour retention time and an 85.71% reduction for the 120.0-hour retention time.

Overall retention time of 24.0 h, 48.0 h and 120 h tend to give best removal rates for both TP and NH_3-N removal using *Eichhornia crassipes* plants in a small pilot LWHC.
However, factors for a tertiary treatment sequence of MRS using *Eichhornia crassipes* plants are climate, contaminant concentration, retention rate, and weather conditions, which play an important role for the application of *Eichhornia crassipes* in a tertiary treatment sequence of MRS.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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