

Nitrogen and BOD Removal from Municipal Wastewater Using a Constructed Vegetate Plug Flow Multiple Stages Series System

*Orvil Lugo Rosa
Civil Engineering
Roger Malaver, PhD.
Civil and Environmental Engineering Department
Polytechnic University of Puerto Rico*

Abstract — *Most wastewaters contain nitrogenous compounds that have given rise to various negative phenomena in water environments, such as growth of algae causing eutrophication with damage to aquatic life, being toxic to fish. Typical removal percentages of nitrogen in long term are only 25% on wastewater treatments. Several investigations have succeeded on Chemical and Biochemical Oxygen Demand (COD & BOD), turbidity, and suspended solids removal efficiency. This investigation shows that nitrates removal % from two stages plug flow system shows a maximum of 80.0% with a mean value of 30.4%. The nitrates removal % from three stages plug flow system varied between 21.4% and 76.2% with a mean value of 48.0%. The BOD removal % from three stages system varied between 74.7% and 19.4% with a mean value of 47.4%.*

Key Terms — *Constructed Wetlands, Natural System, Nitrates, Retention Time.*

INTRODUCTION

The following project continues the investigation named “Wastewater Treatment using Natural System” by Benny Albarrán / Roger Malaver, PhD as part of the Polytechnic University projects that works with the practice of natural systems to reduce water contamination [1]. This project is concentrated especially on the removal of biochemical oxygen demand (BOD) and nitrates and the role of retention time on wastewater treatment. BOD and nitrates are important element of waste water treatment removal. The presence of large amounts of phosphorous and nitrogen are sources of growth of algae’s on water bodies. These factors eventually lead to the eutrophication which is the lack of oxygen on water bodies. The eutrophication on water bodies causes bad odor,

death on the fishes and change water properties color. Phosphorous and nitrates are very common in agricultural activities which are main cause of rivers and tributaries contamination.

Biochemical oxygen demand (BOD) is the amount of oxygen consumed by the organism in the process of stabilizing waste. As such, it can be used to quantify the amount or concentration of oxygen-consuming substances that a wastewater may contain. Analytically, it is measured by incubating a sample in a refrigerator for five days at a temperature of 20°C and measuring the amount of oxygen consumed during that time [2]. The BOD is used to measure the point of organic of water pollution.

Since 1800’s, countries have been working on how to supply high quality water and remove waste from water to avoid public health problems and diseases outbreak. However, these restrictions were not available until the government has established some limitations to restrict tributaries, but these restrictions are not enough to improve river’s health to be modified in a near future. This government’s imposition helped to construct treatments plants to prevent discharges of inadequately treated sewage and other wastes into interstate waters or tributaries.

Several water technologies have been created, developed and implemented to treat water to comply with to the standard of the Water Pollution Act. The water treatment processes can be divided in three remove process, which are classified as physical, chemical and biological contaminants from wastewater. Generally, the wastewater treatment involves three stages called primary, secondary and tertiary treatment, but tertiary are not very common in Puerto Rico.

This project continued the behavior to treat wastewater as a physical/chemical secondary treatment using Cyperaceu Family plants with a Constructed Vegetate Plug Flow Multiple Stages Series System (CVPFMSSS) as part of the new challenge on having an environmentally-safe treated effluent. Constructed wetlands represent an important low impact alternative to conventional wastewater treatment especially to reduce energy, human labor, construction and maintenance costs and chemical cost. Wetlands by nature have a role in providing water quality protection in the catchment by filtering pollutants such as sediments, nutrients, organic/inorganic matter and bacteria.

The wastewater treated obtained from influent of the plant after grit chamber area of the San German Wastewater Treatment Plant (SGWWTP) at Puerto Rico as shown in Figure 1. Figure 2 shows the specific location of the sump pump used for this investigation. SGWWTP has a primary treatment (mechanical sewage screening, secondary treatment anoxic tanks, anaerobic tanks, aeration tanks, and clarifiers and a disinfection using UV Lights treatment). The SGWWTP expansion was completed at 2008 which serves as a secondary treatment facility to restore the water quality in the San German Rio Guanajibo's tributary. SGWWTP has a capacity of average of 2 MGD, but it is capable to handle 8 MGD peak. However, the current average influent of the plant was nearly 0.8 MGD. As part to reach the average 2.0 MGD on SGWWTP and as an economic strategy, later on 2012, Sabana Grande Waste Water Treatment Plant was closed and all residuals water was diverted to SGWWTP. Even though, the current influent to SGWWTP does not reach the average of 2.0 MGD.

BACKGROUND

Constructed wetlands are considered as an alternative, but they are not fully understood. For example, Poland's Constructed Wetlands has been made, but the removal of nitrogen compounds are not as effective [3]. So further investigation, it has been made in Poland were include Hybrid systems.

Constructed wetlands are analyzed based on statistical method and mass balance, see Equation (1).

$$\text{accumulation} = \text{inflow} - \text{outflow} + \text{generation} \quad (1)$$



Figure 1

San German (SGWWTP) Grit Chamber Area



Figure 2

Grit Chamber Area after the Primary Treatment Plant

The US EPA prepared a manual to design constructed wetlands as treatment plant including in which the method to design is based on depends in different factors such as site selection, type of constructed wetland, performance expectations, hydraulic loading rate, vegetation selection, and retention time [4]. As base of the vegetation selection, plants should be available preferably locally; plants should be capable of reproducing and infilling rapidly; plants should exhibit vigorous roots that extend both laterally and vertically to have a better contact area for microbial bacteria and for introduction of oxygen into an otherwise

anaerobic root zone [1]. A constructed wetland provide an anaerobic zone surrounding the root zone that at the same time provides a mini-aerobic zone surrounding the root hairs that fix the oxygen pumped down by the stems and/or leaves of the aquatic vegetation.

As mentioned before, the removal of nitrates concentration efficiency has nearly a 25%. Nitrates produced through nitrification, is removed by denitrification and plant uptake. Nitrogen removal by plant uptake can only be accomplished if the plants are harvested [4]. The process of nitrification converts nitrogen compounds into the nitrate forms; the process of denitrification transforms the nitrate into a gaseous form so that it can be eliminated into the atmosphere. Denitrification is the microbial conversion of nitrate gaseous nitrogen, which results in the effective removal of nitrogen from wastewater [5]. Various types of constructed wetlands may be combined in order to achieve higher treatment effect, especially for nitrogen removal [6].

The other factor that we included in this project is BOD concentration removal efficiency. As mentioned in the U. S. Environmental Protection Agency (USEPA, 1988) [4] and the Water Pollution Control Federation (WPCF, 1990) [7]. Constructed Wetland is based on the assumptions of plug-flow hydrodynamics in which the analysis using mass and first-order biochemical oxygen demand (BOD) removal kinetics [8].

The removal efficiency of the wetland is a function of the surface area (length multiplied by width), while the cross-sectional area (width multiplied by depth) determines the maximum possible flow, as described on equation (2) [1]. A first order plug flow model based on BOD removal was used and it is described by the following equation:

$$\frac{dC}{dt} = -kC^n \quad (2)$$

Integrating and rearranging Equation (2) we obtained Equation (3):

$$A_s = \frac{Q * (\ln C_o - \ln C_e)}{k_t * d * \varepsilon} \quad (3)$$

The value of k_t is given by Equation (4):

$$k_t = k^{(T-20)} \quad (4)$$

where:

- A = area (m^2)
- Q = Average Flow (m^3/day)
- C_o = Influent BOD (mg/L)
- C_e = Effluent BOD (mg/L)
- k_t = Temperature-dependant rate constant
- d = Depth of gravel bed
- ε = porosity

Constructed wetlands for water treatment are complex-integrated systems of water, plants, animals, microorganisms, and the environment. The classification of constructed wetlands is based on: the vegetation type (emergent, submerged, floating leaved, free-floating); hydrology (free water surface and subsurface flow); and subsurface flow wetlands can be further classified according to the flow direction (vertical or horizontal) [9][1].

The hydraulic retention time (HRT) can be calculated as Equation (5). And hydraulic loading rate (HLR) can be calculated on Equation (6).

$$HRT = \frac{A * d * \varepsilon}{Q} \quad (5)$$

$$HLR = \frac{A}{Q} \quad (6)$$

VEGETATION CYPERACEAE FAMILY PLANT DESCRIPTION

The plants used are known from the species sedge (Cyperaceae Family) known by the common names “fragrant flatsedge” and “rusty flatsedge” as shown in Figure 3. The plants are seen from different areas in Puerto Rico especially at the roadside, near gutter, runoff areas [10][11]. This plant grows up in wet and muddy areas, including disturbed and altered sites. They are also found

around the Caribbean Islands, Mexico. Figure 3, right picture, shows the *Cyperus Odoratus* (“Fragrant flatsedge”) plants [10]. This species is variable and may in fact be more than one species included under one name. This plant is capable to reach half a meter in height on average. This plant adapts to coarse and medium texture soils and has a medium anaerobic and CaCO₃ tolerance in which help with nitrates removal.

CONSTRUCTED VEGETATE PLUG FLOW MULTIPLE STAGES SERIES SYSTEM

The Constructed Vegetate Plug Flow Multiple Stage Series System is one stage of horizontal subsurface flow constructed wetland (HSSFCWs) is not very common in North America. This HWSSFCW are often studied and used in Europe. A HSSFCW is a large gravel and sand-filled channel that is planted with aquatic vegetation. As wastewater flows horizontally through the channel below the gravel, the material filters out particles and microorganisms degrade organics. Organic compounds are effectively degraded mainly by microbial degradation under anoxic/anaerobic conditions as the concentration of dissolved oxygen in the filtration beds is very limited [12]. Suspended solids are retained predominantly by filtration and sedimentation and the removal efficiency is usually very high [13]. The systems cannot provide nitrification because of their limited oxygen transfer capacity [14]. In addition, HSSFCWs focused not only on common pollutants, but it also works on special parameters such as pharmaceuticals, industrial applications, oil refineries, chemical factories, pulp and paper production, tannery and textile industries, abattoir, distillery and winery and food-processing wastewaters (e.g., production and processing of milk, cheese, potatoes, sugar). It also works used to treat wastewaters from agriculture (e.g., pig and dairy farms, fish farm effluents) and various runoff waters (agriculture, airports, highway, greenhouses, plant nurseries). HF CWs have also effectively been used to treat landfill leachate [15].



Figure 3

Cyperaceu Family Plants

The bed is wide and shallow so that the flow path of the water is maximized. A wide inlet /outlet zone is used to evenly distribute the flow to avoid clogging or booting. Pre-treatment is crucial to prevent clogging and ensure efficient treatment. The bed should be lined with an impermeable liner (clay or geotextile) to prevent leaching. Small, round, evenly sized gravel (3–32 mm in diameter) is most commonly used to fill the bed to a depth of 0.5 to 1m. To limit clogging, the gravel should be clean and free of fines. Sand is also acceptable, but is more prone to clogging. A bad outlet zone designed could cause booting stage water which eventually leads that the water goes over the plants roots, therefor the water cannot be treated or may cause damage to the plant. The plant roots play an important role in maintaining the permeability of the filter. Any plant with deep, wide roots that can grow in the wet, nutrient-rich environment is appropriate [1].

The large gravel and sand-filled filter media works to remove the solids. The fixed bacteria will attach to the fixed surface upon and a base for the vegetation. Meanwhile, the facultative and anaerobic bacteria degrade most organics, the vegetation transfers a small amount of oxygen to the root zone so that aerobic bacteria can colonize the area and degrade organics as well. In the filtration beds, pollution is removed by microbial degradation and chemical and physical processes in a network of aerobic, anoxic, anaerobic zones with aerobic zones being restricted to the areas adjacent

to roots where oxygen leaks to the substrate [16][17].

METHOD AND MATERIAL

The project contains two in series HSSFCWs systems made of polyethylene, each with a large, tall, width of 0.689 m x 0.23 m x 0.45 cm used to treat the wastewater. Then a third one was added to the system. A slope of 1.0 % on each HSSFCWs were established to maintain the hydraulic gradient in all modules. All HSSFCW's inlet, outlet, middle zone were constructed of perforated PVC pipes. The perforated PVC pipes diameter is 0.019 m.

Two kinds of stone were located inside of all basins. The inlet and outlet zones of each HSSFCW have the large stones with the size of 3-5 cm. The large stone will produce a uniform distributed flow and to avoid clogging at the start and/or end zones. The remainder area is filled with gravel with the size of 1 cm and a 31.2% of porosity. Figures 4 and 5 show the schematics view of the module system design.

The idea of this project is based on simulate continuous flow as the practice to simulate real life situation. In the practice, it is normal to has peak flow during the days, and during the night, the waste water that arrived to the plant is less than during the day. Therefore two cylinders basin that it is called Wastewater Storage Tanks (WWSTs) were installed with a volume of 0.53 m³ and 0.03 m³ as show in Figure 6. These WWSTs were filled in twice in a day with a constant flow.

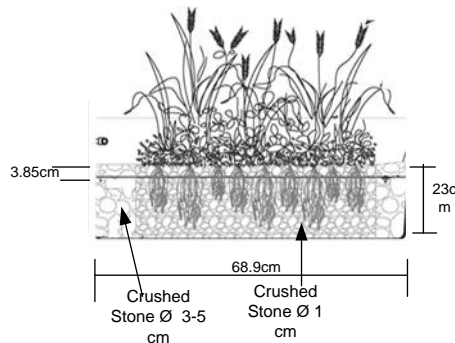


Figure 4

HSSFCW Schematic Lateral View

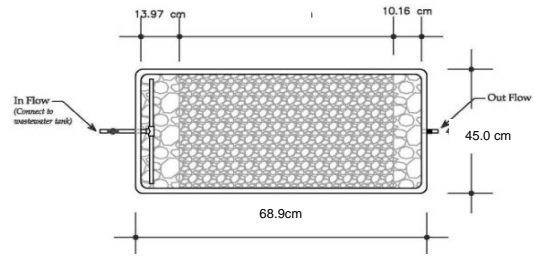


Figure 5

HSSFCW Schematic Top View



Figure 6

Wastewater Storage Tanks (WWSTs)

The sampling points of the projects were divided in which will be called zones as shown on Figure 8.

- Zone 1 – corresponds to the influent wastewater (untreated)
- Zone 2 – corresponds to the vegetate zone basin #1
- Zone 3 – corresponds to the exit of the first system (gravel and aquatic plants)
- Zone 4 – corresponds to the vegetate zone basin #2
- Zone 5 – corresponds to effluent collection zone (outflow)system
- Zone 6 – corresponds to the vegetate zone basin #3 system (implemented after day 78)
- Zone 7 – corresponds to effluent collection zone (outflow)system (implemented after day 78).

The collected wastewater was poured into WWSTs which have a total volume of 0.56 m³. The

two plastic containers simulate a continuous flow. Wastewater at the container was filled in manually using a sump pump. The containers were filled twice a day. The average flow is about 1.12 m³/day on the multiple stages. The container which has a control valve was adjusted and set the influent flow. However, set an exact influent control flow had not been made, solid waste affect the constant inflow. A slow flow rate was partially controlled by the influent valve, during the experiment period, giving an average net hydraulic loading rate and hydraulic retention time of 0.47m³/m²/day and 1.61 hours, respectively, on the planted constructed wetland. Clogging at the influent system at basin #1 was frequently. The removal efficiency % was calculated as Equation (7). As part of the nitrates efficient experiment, the planted HSSFCW systems were operated during these conditions continuously for 105 days, respectively, with the purpose of reducing the nitrates. It is important to mention that the third planted HSSFCW was added to the system after 65 days. The HSSFCW systems BOD removal efficiency experiment was performed not simultaneously with the nitrates experiments. It was experimented three months later at the day 218 and culminated on day 267. No sludge was removed from both systems at any time during the long-term experiment. Some of the parameters measured during that time included influent and effluent pH, temperature, turbidity, BOD and nitrates, using HACH technologies that are part of the Standard Methods from the U.S.E.P.A [18].

$$\% \text{Eff} = \frac{C_i - C_e}{C_i} \times 100\% \quad (7)$$

Where

%Eff : Removal efficiency of the concentration between influent and effluent

C_i: Initial concentration

C_e: final concentration

Two different plants from the Cyperaceae family plants were planted acclimate to the basin. The roots of the plant were planted to make contact with the waste water. Originally, it was planted 15 plants to each basins. First sampling analysis was made after 5 days. Figure 7 shows the third stage added on day 78. Figure 8 shows a the whole basin in lateral schematic view.



Figure 7

Third Basin Added Without plants

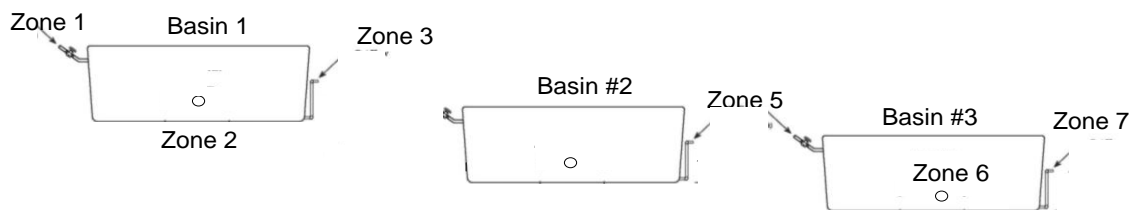


Figure 8

Lateral Schematic of the System with their Zones and Basin

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental results for the influent (zone 1) nitrates varied between 7.4 mg/L and 1.9 mg/L with a mean value of 4.77 mg/L, and, respectively, for raw wastewater in the planted module system. The zone 5's nitrates varied as follows 7 mg/L and 3.18 mg/L with a mean value of 3.24 mg/L, and . The zone 7's nitrates varied as follows 5.5 mg/L and 1.6 mg/L with a mean value of 2.7 mg/L.

The nitrates removal % from zone 1 to zone 7 varied between 21.4% and 76.2% with a mean value of 48.0%. The nitrates removal % from zone 1 to zone 5 shows a maximum of 80.0% with a mean value of 30.4%. The nitrates between vegetation zones has higher concentration numbers than the influent's concentration, it was seen 82.6% of the time between zone 1 and zone 2, and meanwhile it was seen 39.1 % of the time between zone 4 and zone 3. This might be associated with the microenvironments produced in the vegetation zone and the roots absorption process that provides additional attachment sites for the organic matters and suspended solids particles [1]. The suspended solid accumulates in the gravel, therefore, the

nitrification increases along with an aged HSSFCW, and then the nitrates concentration increases. Figure 9 shows how the nitrates concentration is removed along with the days and how it is affected. Figure 10 shows how retention time is a factor when the flow was set lower.

. The influent's BOD₅ varied between 453 mg/L and 237 mg/L with a mean value of 343 mg/L. The zone 5's BOD₅ varied as follows 282 mg/L and 158 mg/L with a mean value of 226 mg/L, and . The zone 7's BOD₅ varied as follows 256 mg/L and 69 mg/L with a mean value of 226 mg/L.

As shown figure 11, the BOD₅ removal % from zone 1 to zone 7 varied between 74.7% and 19.4% with a mean value of 47.4%. The BOD₅ removal % from zone 1 to zone 5 varied between 44.3% and 15.6% with a mean value of 33.4%. The BOD₅ removal % from zone 1 to zone 3 varied between 29.9% and 12.7% with a mean value of 21.6%. This shows how clearly the concentration removal percentage increase along the distance showing that the retention time is affected with the area.

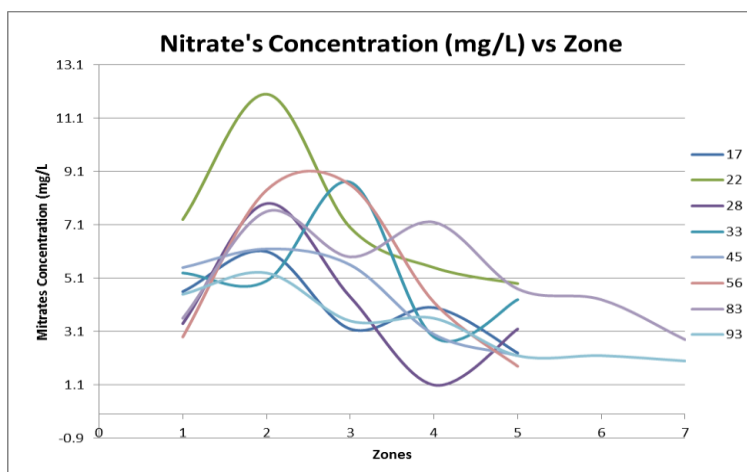


Figure 9
Nitrates Concentration Removal on by Zones perr Day

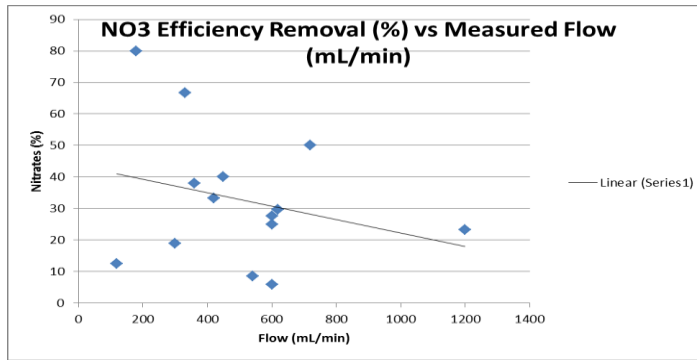


Figure 10

Nitrates Concentration Removal Efficiency % Between Zones 1-5 vs Flow

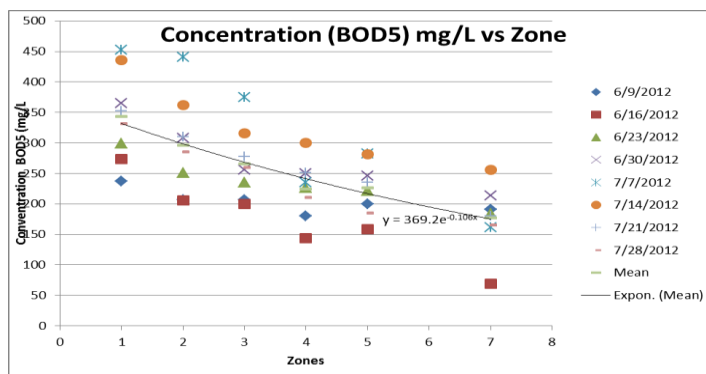


Figure 11

BOD Concentration Removal per Zone

The influent's turbidity varied 144 NTU and 46.8 NTU with a mean value of 96.7 NTU. The zone 3's turbidity varied as follows 93.4 NTU and 28.3 NTU with a mean value of 55.47 NTU. The zone 5's turbidity varied as follows 85.5 NTU and 21.9 NTU with a mean value of 42.7 NTU. The zone 7's turbidity varied as follows 48.6 NTU and 29.6 NTU with a mean value of 36.6 mg/L.

The turbidity removal % from zone 1 to zone 7 varied between 76.1% and 47.3% with a mean value of 60.2%. The turbidity removal % from zone 1 to zone 5 varied between 81.4% and 19.2% with a mean value of 53.7%. The turbidity removal % from zone 1 to zone 3 varied between 62.2% and 11.8% with a mean value of 40.4%. Same as the nitrates removal %, the turbidity has an increment between zone 2 and zone 1 which means that at

certain point zone #2 has more turbidity value due to the microenvironment where, get attached. In figure 12, the graph shows the physical behavior shows the behavior of Figure 13, where it shows that effluent physical characteristic is clearer than the effluent, but the vegetate zones are darker than their respective influent. In addition it shows that trans cure the days, water get darker with time. From left to right, the first picture is from day #28, day #210 and #237.

The influent pH varied between 7.64 and 6.52 with a mean value of 7.19 and the effluent zone #5 varies between 7.60 and 6.49 with a mean value of 7.26 during the experiment as shown Figure 14. The pH has an average increment between zone 1 and zone #5 of 1.1%. The observed results show that from zone 2 and 1 tends to decrease its value.

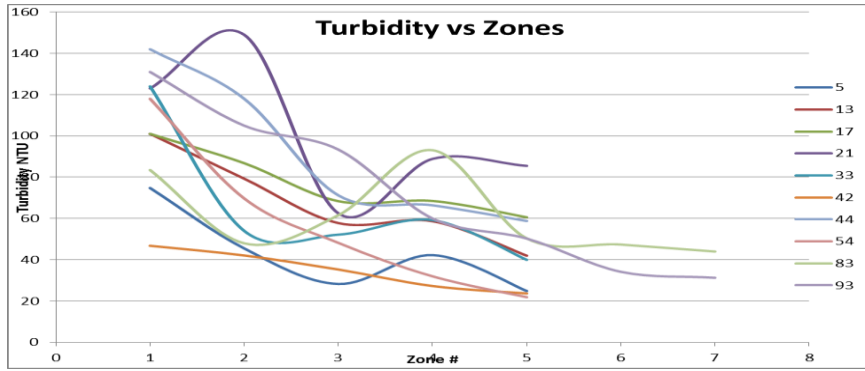


Figure 12

Turbidity Concentration per Zone

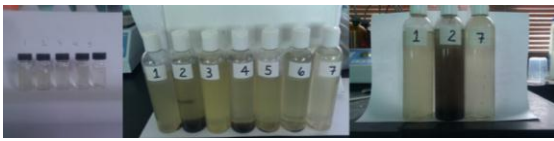


Figure 13

Sampling taken from day 28, 210 and 237 Respectively

The first Cyperaceu Family plants were planted on November 5, 2011. During those days, the plants were not stronger. The first sampling data was taken on day #5. On Day 14, basin #1 started to look greener and some of the first plant dies. Basin #2 started to look greener as shown in Figure 15. The percentage removal of nitrates on basin #2 was highly effective with the high flow provided. The experiment shows that nitrates and turbidity tend to increment on the vegetate zone which reflect the physical views on Figure 16. During the days of experiment other plants emerged.

CONCLUSION

This study demonstrated that the HSSFCW system has the capability to removes nitrates and BOD. It also shows HRT and HLT have an effect on the BOD and nitrates. The system shows the capability to remove over 25% of nitrates in which compare with other studies. It also shows within time vegetate zones tends to stabilize but it is also tends to saturate, to decrease the pH, increase nitrates concentration and turbidity in which explain the microenvironment where the solid

attached to the environment in this case to the gravel.

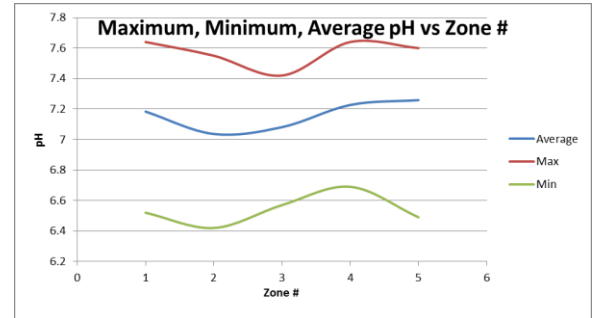


Figure 14

pH Maximum, Minimum and Average Values per Zones

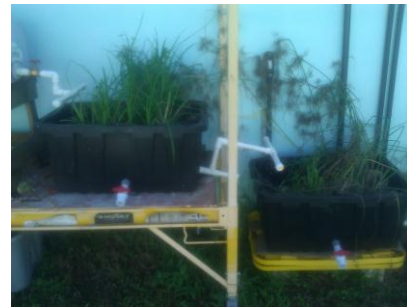


Figure 15

CVPFMSS Day 14



Figure 16

CVPFMSS Day 197 and Day 240 Respectively

Constructed wetlands are a viable alternative for wastewater treatment for sources of pollution. Moreover, this type of treatment system provide better range of economic benefits, than conventional wastewater treatment plants, in terms of electricity, human labor, construction and maintenance costs. Further studies shall include not only on common pollutants such phosphorous, ammonia, but special parameters such as pharmaceuticals, industrial applications, oil refineries, chemical factories, pulp and paper production, tannery and textile industries [14]. Different design methods shall be include recirculation, flushing on the vegetate zones.

REFERENCES

- Odoratus L. (Fragrant Flatsedge) Plant Profile, <http://plants.usda.gov/java/profile?symbol=CYOD>
- [1] Albarrán, B., Malaver, R., Wastewater Treatment using Natural System, Polytechnic University, San Juan, 2011
- [2] Sincero, G.A., Sincero, A.P., Physical and Chemical Treatment of Water and Wastewater, IWA Publishing, 2003, Department of the Environment State of Maryland
- [3] Obarska-Pempkowiak, H., Gajewska M., The Removal of Nitrogen Compounds in Constructed Wetlands in Poland, Faculty of Hydro & Environmental Engineering, Technical University of Gdańsk,
- [4] U.S. Environmental Protection Agency Office of Research and Development, Design Manual Constructed Wetlands and Aquatic Plant Systems for Municipal Wastewater Treatment, <http://www.epa.gov/owow/wetlands/pdf/design.pdf>
- [5] Campbell, C.S., Ogden, M.H., *Constructed Wetlands in the Sustainable Landscape*, 1999, pp. 21-22.
- [6] Kantawanichkul, S., Newamkam, P., *Nitrogen removal in a combined system: Vertical vegetated bed over horizontal flow sand bed*, Water Sci. Technol. 44 (2001), pp. 137-142.
- [7] Water Pollution Control Federation (WPCF). (1990). *Natural systems for wastewater treatment, manual of practice FD-16*. Water Pollution Control Federation, Alexandria, VA
- [8] Chen, S., Wang, G., and Xue, S. (1999). "Modeling BOD Removal in Constructed Wetlands with Mixing Cell Method." *J. Environ. Eng.*, 125(1), 64–71.
- [9] Vymazal, J., *Constructed Wetlands for Wastewater Treatment*, Water 2010, 2, pp. 530-549.
- [10] United States Department of Agriculture (U.S.D.A): Natural Resources Conservation (NRCS) – Cyperus
- [11] Acevedo, P., Strong, M. T., Monocotyledons and Gymnosperms of Puerto Rico and the Virgin Islands, Volume 52: 1-415, Department of Botany
- [12] Vymazal, J., Kröpfelová, L., Is Concentration of Dissolved Oxygen a Good Indicator of Processes in Filtration Beds of Horizontal-flow Constructed Wetlands? In *Wastewater Treatment, Plant Dynamics and Management*, Vymazal, J., Ed., Springer: Dordrecht, The Netherlands, 2008, pp. 311-317.
- [13] Vymazal, J., Kröpfelová, L., *Wastewater Treatment in Constructed Wetlands with Horizontal Sub-Surface Flow*, The Netherlands, 2008.
- [14] Vymazal, J., Horizontal Sub-surface Flow and Hybrid Constructed Wetlands Systems for Wastewater Treatment, *Ecological Engineering*, Volume 25, Issue 5, December 2005, Pages 478–490
- [15] Vymazal, J., The use constructed wetlands with horizontal sub-surfaceflow for various types of wastewater, *Ecological Engineering* Volume 35, Issue 1, 8 January 2009, Pages 1–17
- [16] Vymazal, J., Types of Constructed Wetlands for Wastewater Treatment: Their Potential for Nutrient Removal. In *Transformations of Nutrients in Natural and Constructed Wetlands*, Vymazal, J., Ed.; Backhuys Publishers: Leiden, The Netherlands, 2001, pp. 1-93.
- [17] Cooper, P.F., Job, G.D., Green, M.B. and Shutes, R.B.E., *Reed Beds and Constructed Wetlands for Wastewater Treatment*, WRc Publications: Medmenham, UK, 1996.
- [18] United States Environmental Protection Agency (U.S.E.P.A): Clean Water Act Analytical Methods. <http://water.epa.gov/scitech/methods/cwa/index.cfm>
- [19] *Guide to Identify Common Wetland Plants in the Caribbean Area: Puerto Rico and the U.S. Virgin Islands*. University of Puerto Rico Press, October, 2010, pp.118-119.