



San Diego State University

## **Duckweed and Wastewater Analysis**

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## **Abstract**

*This research was done to see how the inclusion of duckweed to treated wastewater from the DEWATS would affect the quality of the water. Wastewater collected from different points in the DEWATS in Newlands-Mashu was made into dilutions and inoculated with duckweed. The water was then tested for phosphate, turbidity, chemical oxygen demand (COD), total solids and volatile solids over the retention time of two weeks. Daily measurements of pH, electroconductivity, and temperature were made. Finally, after two weeks of growth, duckweed was harvested and measured for dry biomass. Plant uptake resulted in an average of 93% reduction in turbidity with average final values of 7.02 NTU for 100% wastewater dilutions. COD reduction was present in most cases of 100% wastewater dilutions, and total solids showed an overall trend of increasing after 14 days while volatile solids showed a trend of decreasing.*

## **1. Introduction**

The decentralized wastewater anaerobic treatment system (DEWATS) at Newlands-Mashu in Durban, South Africa, receives wastewater (WW) from approximately 80 households. It is composed of a settling chamber, seven anaerobic baffled reactors (ABRs), two anaerobic filters (AFs), a siphon tank, and a vertical planted gravity filter (VPGF) as shown in figure 2. Wastewater accumulates in the siphon tank until the tank fills and triggers an automatic discharge of the contents onto the VPGF. In a study by Singh et al. (2008) a DEWAT system has been shown to remove up to 96% total suspended solids, 90% chemical oxygen demand, and 26% total phosphorous. This makes initial treatment of the water very successful, however, further treatment of the water can be done.

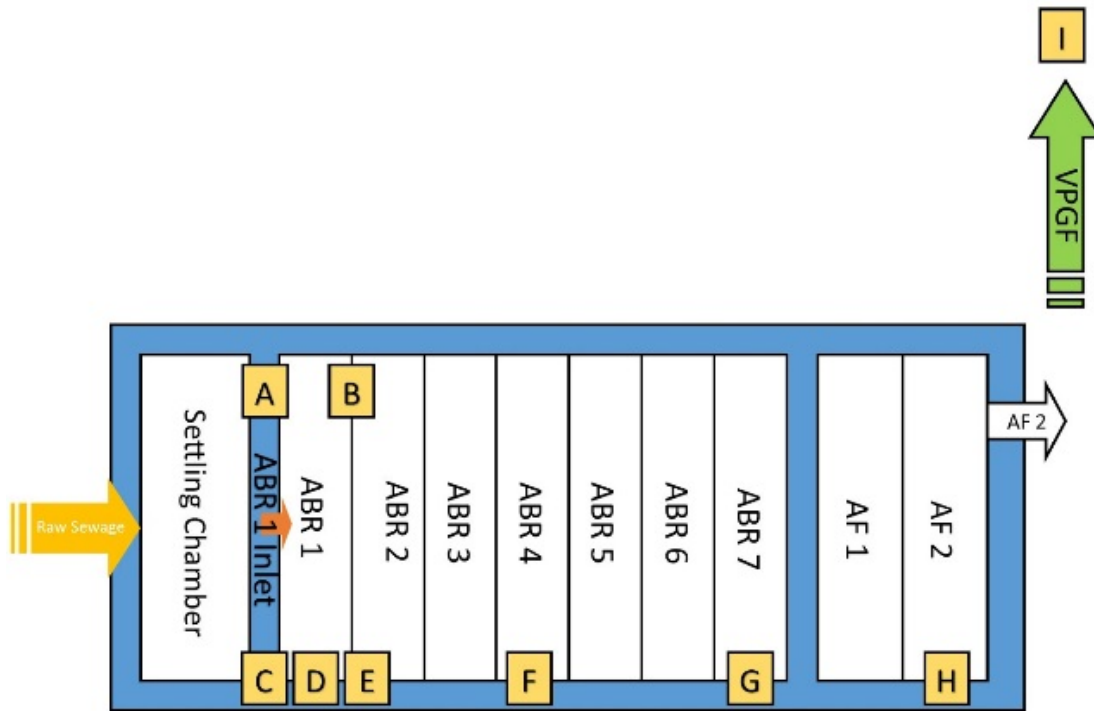


Figure 2. Sampling points and diagram of the DEWATS, Street 1.

After initial treatment of wastewater by the DEWAT system, aquatic plants, such as duckweed, can be used to further polish water quality. Duckweed is a small, green, freshwater plant with a leaf like frond only a few millimeters wide and a short root that is usually less than 1 cm long and is also among the smallest and simplest angiosperms and has high reproduction rates (Zirschky et al, 1988). Duckweed has been the focus of many wastewater treatment studies over the past 40 years. However, little study has been done on the effects of duckweed to pretreated wastewater from a DEWATS.

*Lemna minor* is a common duckweed genera used in the treatment of wastewater, and is the focus species of this study. Duckweeds are unique and can break down organic molecules such as various amino acids, directly (Hillman et al. 1978). Nitrogen and phosphorous are essential in the metabolic process for duckweed and some percentage of these nutrients are incorporated into new cell mass (Harvey et al.1973). The goal of this project was evaluate the influence of duckweed treatment on removal of nutrients and overall water quality of the partially treated wastewater from the DEWATS. Some major advantages of duckweed over other aquatic plants is their low sensitivity to cold climates, however, their shallow root system and sensitivity to wind are considered drawbacks (Zimmels et al. 2004).

## 2. Methods

The stability of the systems was monitored with daily measurements of pH, electroconductivity, and temperature. Water quality changes were determined by measuring total solids, volatile solids, turbidity, COD, phosphate, nitrate, and ammonium-N. Duckweed mass in each system was determined using a dry mass gravimetric approach.

### 2.1 Duckweed Set Up

Dark, square containers with a surface area of  $0.06 \text{ m}^2$  is filled with dilutions of wastewater and tap water in triplicate. Dilutions of 0%, 25%, 50%, 75%, and 100% wastewater (WW) were used to make 3 liter dilutions. For example, 100% contained 3 L WW, 75% contained 2.25 L WW and 0.75 L tap water, and so on (Figure 1). A control was created in triplicate with 0% WW and contained 3 L of tap water. Approximately 36 grams of wet, clean duckweed was added to each container.



Figure 1. Dilution Row of ABR 7 (first 3 containers contain 100% WW and so on).

### 2.2 Sampling

Samples were taken from locations in ABR1 (D), ABR 7(G), AF 2(H), and the VPGF(I), as shown in figure 2. All samples of the wastewater were taken from Street 1. Samples from the Duckweed dilution systems were taken at Day 0, Day 7, and Day 14 from the dilution row. Turbidity of the starting dilutions was determined immediately. Samples of the initial dilutions were frozen for later COD analysis, and total solids testing. Duplicate samples were frozen separately for nutrient analysis test kits. Measurements for pH, electroconductivity (EC), and temperature were taken every weekday, between the hours of 11:00-13:00. On day 7, 90 mL of sample was taken from each container and 45 mL of tap water was returned to the container to account for evaporation.

### *2.3 Total and Volatile Solids*

Dilutions samples were tested for total and volatile solids. Solids are classified as suspended and dissolved matter in water. Total Solids is the term used for the material that remains in a crucible after evaporation from the sample that is dried in an oven. Volatile Solids is the weight loss after ignition of the total solids. Crucibles were labeled and then dried by placing in the oven at 105°C for one hour. Crucibles were cooled to room temperature and weighed. Only the masses of the crucibles were recorded ( $W_1$  = weight of crucible). Sample was mixed so the particles are suspended evenly throughout sample. Thirty mL of sample was measured and added to the crucible. Mass of the sample was recorded. The crucibles with the sample were placed in the oven at 105°C for 24 hours. Crucibles and contents were cooled and then weighed. Results were recorded ( $W_2$ = weight of crucible and sample after oven). Equation 1 is then used to calculate total solids.

$$\text{(EQ. 1) Total Solids (mg/g)} = \frac{(W_2 - W_1)g}{W_{\text{Sample}}(g)} \times 1000$$

Crucibles were then placed in the furnace at 550°C for 2 hours. After cooling, the crucibles were weighed again ( $W_3$  = weight of crucible and sample after ignition). Equation 2 is then used to calculate volatile solids.

$$\text{(EQ. 2) Volatile Solids (mg/g)} = \frac{(W_2 - W_3)g}{W_{\text{Sample}}(g)} \times 1000$$

### *2.4 Chemical Oxygen Demand*

The Chemical Oxygen Demand (COD) expresses the amount of oxygen originating from potassium dichromate that reacts with the oxidizable substances contained in 1 L of water under the working conditions of the specified procedure. Organic and inorganic compounds oxidizable by dichromate are measured. Samples of ABR 1, ABR 7, AF 2, and VPGF were tested for COD at Day 0, Day 7 and Day 14. The digester was heated to 150°C before preparing the samples. Measurements were obtained by referencing the standard operating procedures for a measuring range of 100 - 1500 mg/L COD. Empty cells were labeled according to sample, 0.3 mL of solution A, 2.3 mL of solution B, and 3 mL of the sample are added to the cells. A blank is prepared by adding 0.3 mL of solution A, 2.3 mL of solution B, and 3 mL of distilled water into an empty cell. Standardized potassium hydrogen phthalate (KHP) solution was used to make the standard curve (Figure 3), the cells were prepared using 0.3 mL of solution A, 2.3 mL of solution B, and 3 mL of the KHP. Lids were securely fastened to the cells and the contents of the cell were mixed vigorously and placed into the digester for 2 hours. After digestion is complete, the samples were cooled for 10 minutes and then swirled. Samples were cooled for another 20 minutes. The spectrophotometer wavelength was set to 605 nm, in the mode 51 for the range of 100-1500 mg/l COD. The blank was inserted and the machine was zeroed. Once the blank has been tested the remaining samples can be tested using the spectrophotometer. After the last sample was measured the blank was reinserted and tested again.

## *2.5 Nutrient Analysis*

### Phosphate

Samples collected at day 0, 7 and 14 were tested for nutrients. A Spectroquant® Phosphate Test kit was used to determine orthophosphate. 5.0 mL of each sample was added to a test tube along with 1.2 mL of Reagent PO4-1 and then mixed. The mixture was then measured promptly using a Spectroquant®.

## *2.6 Dry Mass of Duckweed*

Duckweed growing in the dilution rows was harvested after 14 days of water treatment. Duckweed was harvested using a strainer then placed into already weighed paper bags. The weight of the wet duckweed was recorded and the weight of the paper bag was subtracted out. Duckweed was dried in the oven at 80 degrees Celsius for 4 days or until duckweed is completely dried. The dry weight of the duckweed was then recorded and the weight of the paper bag was subtracted out.

### 3. Results

Figure 4 shows the average trend in COD reduction for the 100% WW dilutions throughout the system during the 14 day study. Trends are most clear when looking at 100% WW dilutions. It was not possible to test all samples at day 7, however it is seen that for low dilutions (100 and 75%), there is an overall trend of decrease in COD, while for high 25% WW dilutions there is a trend of increase from day 7 to day 14 which can be seen in table 1.

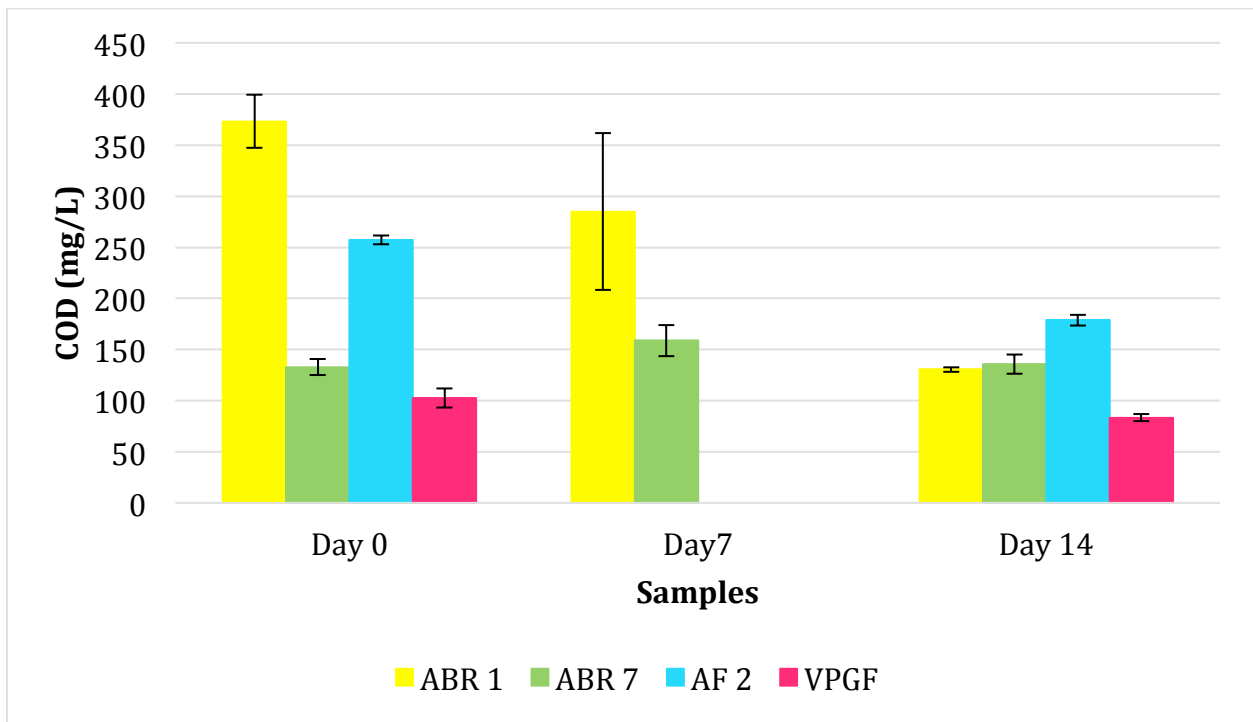


Figure 4. COD averages over time for 100% WW dilutions.

Figure 5 shows the average total and volatile solids measured at day 0 and day 14 for the 100% WW dilutions. The trends show an overall increase in the amount of total solids and a decrease in the amount of volatile solids.

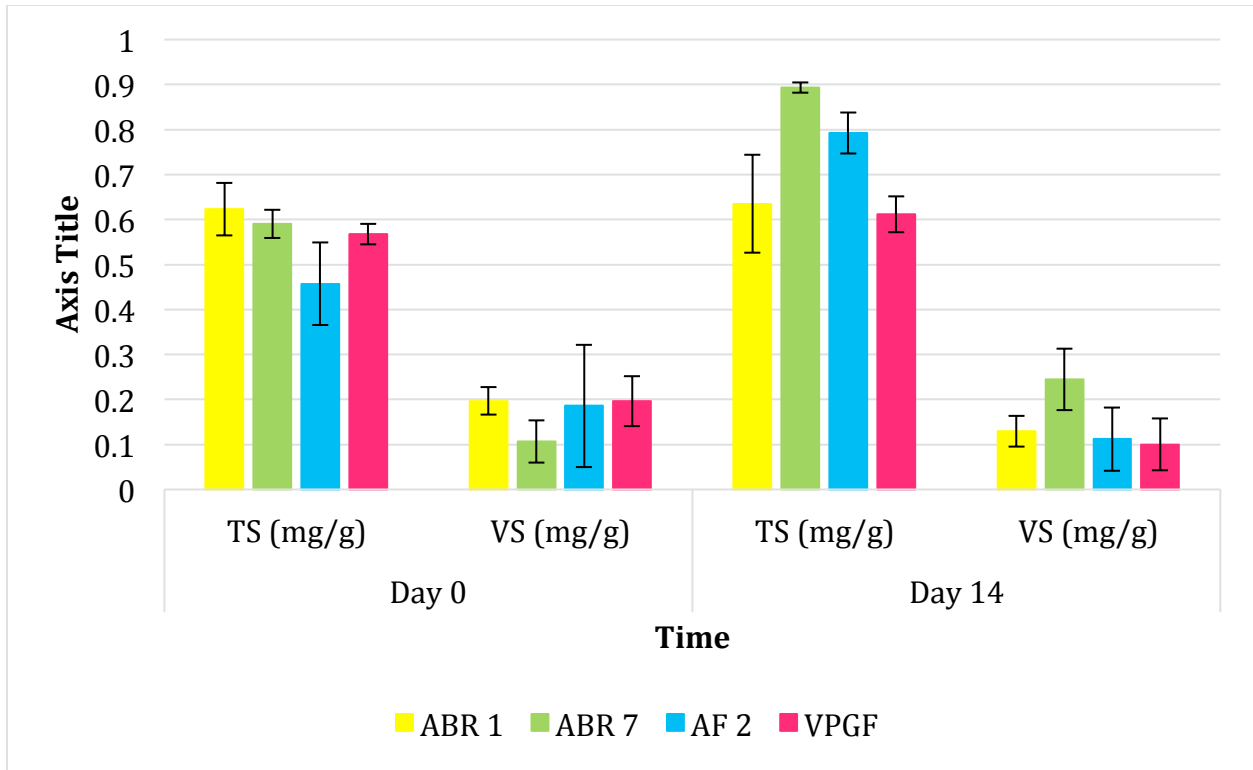


Figure 5. TS and VS averages over time for 100% WW dilutions.

Figure 6 shows the trends in turbidity for 100% WW dilutions for day 0, 7, and 14. There was a dramatic decrease in turbidity for all samples from day 0 to day 7, however there was an insignificant amount of change in turbidity from day 7 to day 14 for most cases (table 3). From day 0 to day 7, a 90-96% reduction of turbidity can be seen from all 100% WW samples, however, from day 7 to day 14, a minor increase in turbidity can be noted for AF 2 and the VPGF dilutions.



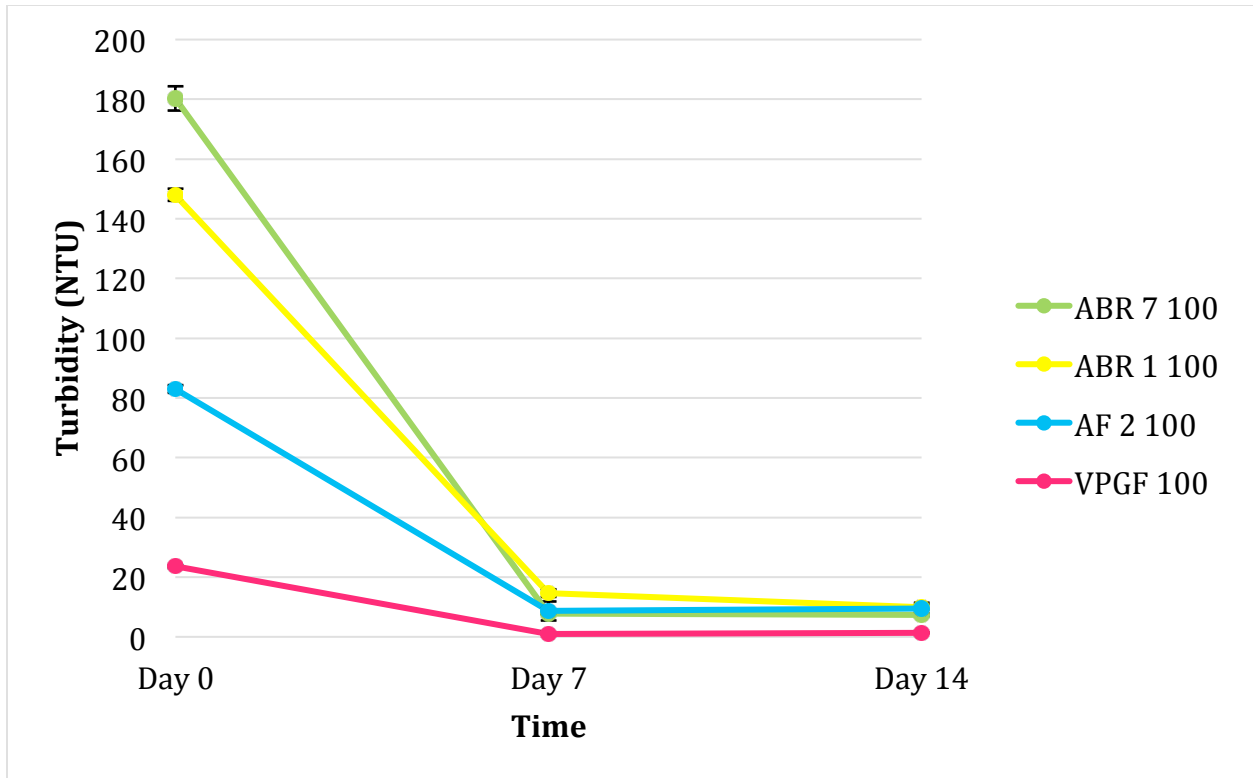


Figure 6. Turbidity averages over time for 100% WW.

Figures 7 and 8 show the average daily measurements of pH and electroconductivity for 100% WW dilutions. A decrease in the electroconductivity and increase in pH can be seen over the first weekend from day 0 to day 3. This trend is then followed by a steady increase in the electroconductivity and pH. This suggests there is an evaporative concentration effect since no new water is added, so water evaporates and ions become more concentrated. That means that everything becomes slightly more concentrated. In some cases, there are higher concentrations on day 14 than on day 0 (COD for example), and this is likely due to the evapoconcentration effect.

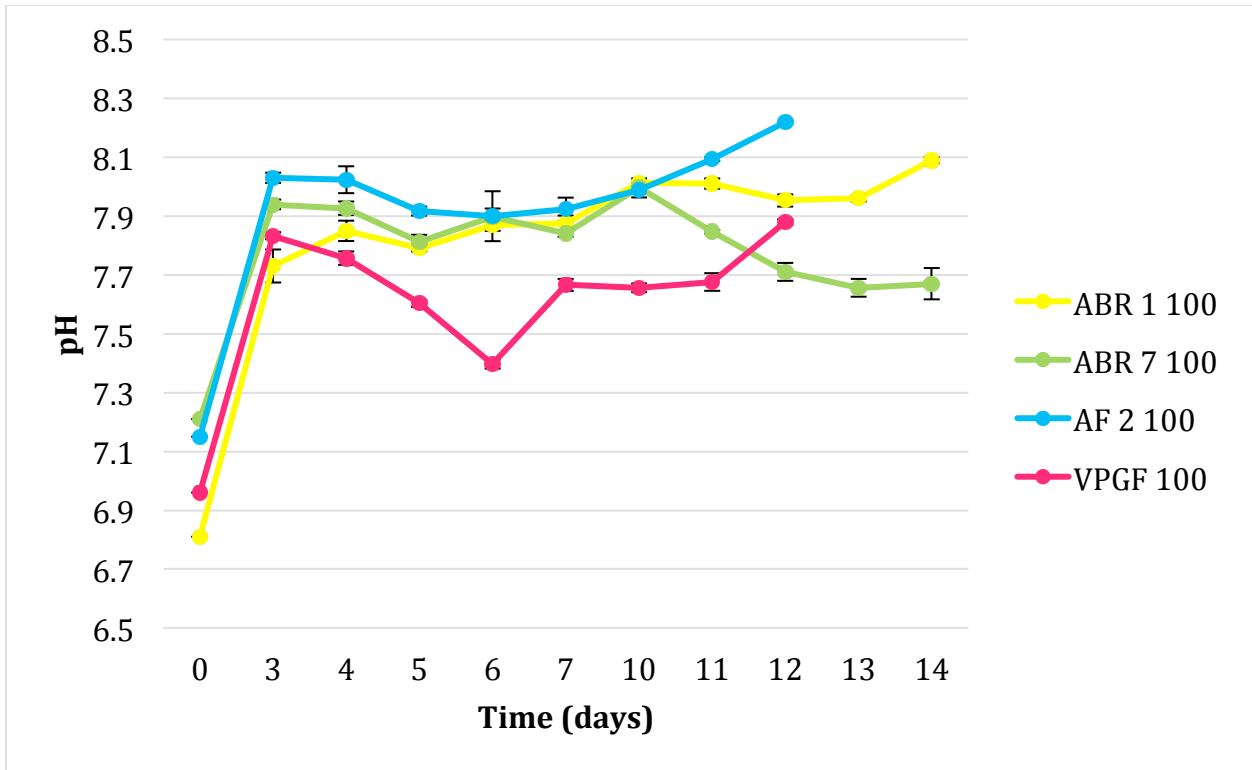


Figure 7. pH averages over time of 100% WW.

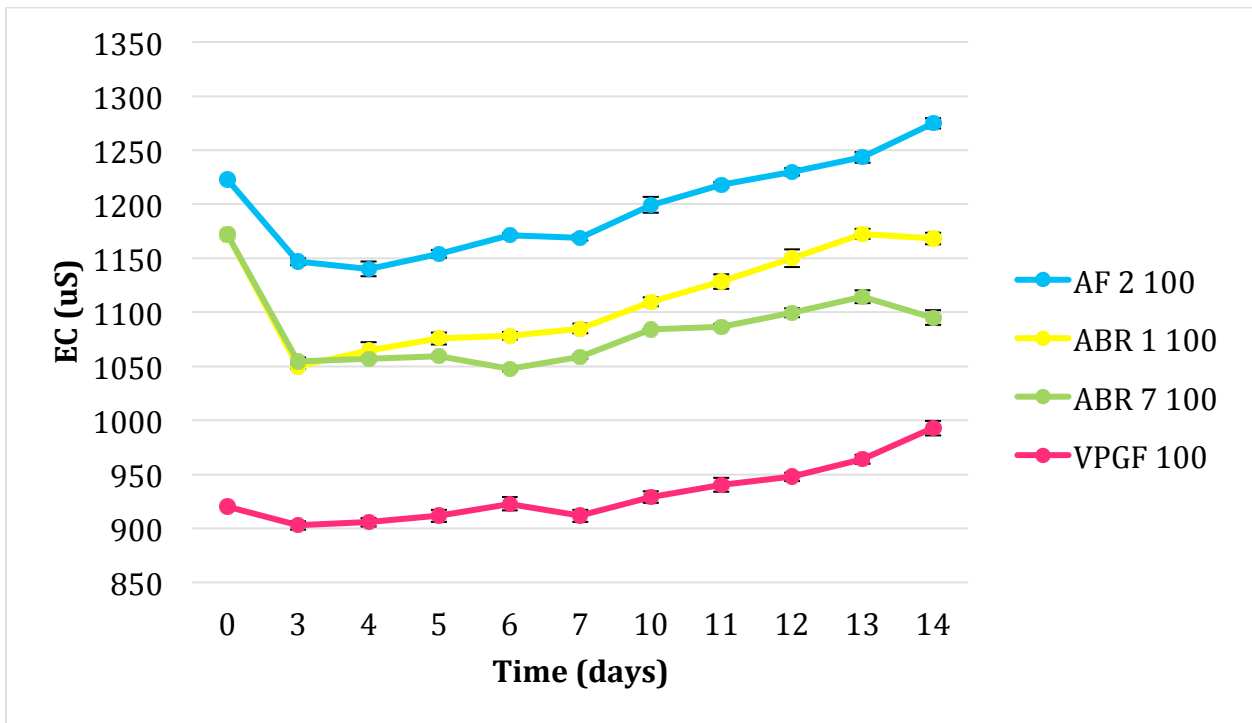


Figure 8. Electrical conductivity averages over time of 100% WW.

Figure 9 compares the 100% WW dilution duckweed dry mass accumulation and figure 10 shows the average duckweed dry mass accumulation for all samples. It is noted that for most sampling points, the duckweed performed better in higher dilutions except for in the VPGF, where the duckweed performed best at a 100% WW dilution. This trend can be seen in the duckweed dry mass accumulation where it is seen that duckweed prefers WW in higher dilutions in all cases except the VPGF. Figure 11 shows the bright green color of the VPGF 100% WW dilution duckweed on day 14 and figure 12 shows the brown color of the AF 2 100% WW dilution on the same day.

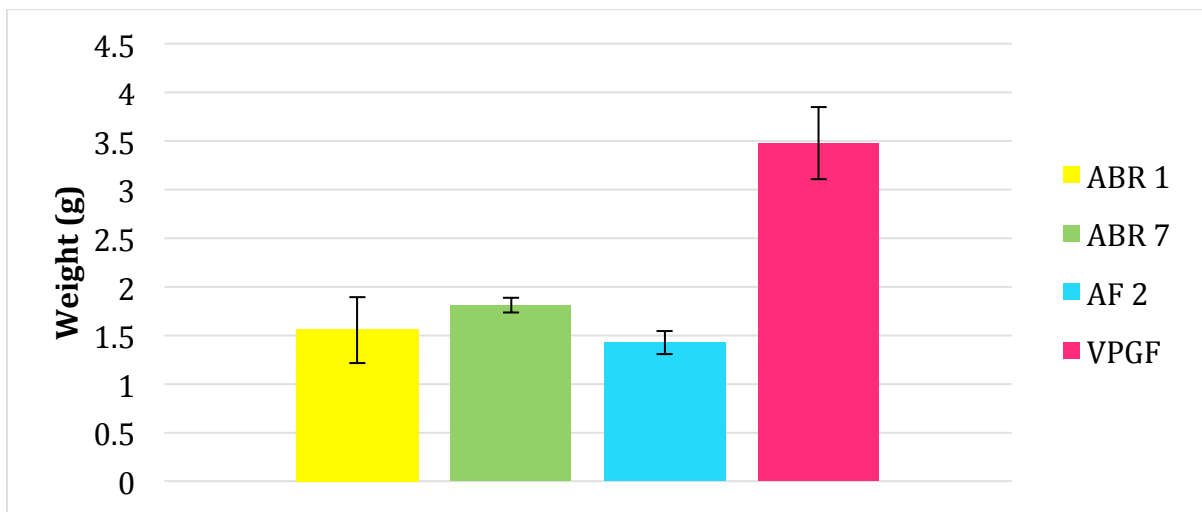


Figure 9. Dry mass averages for 100% WW after 14 days of study.

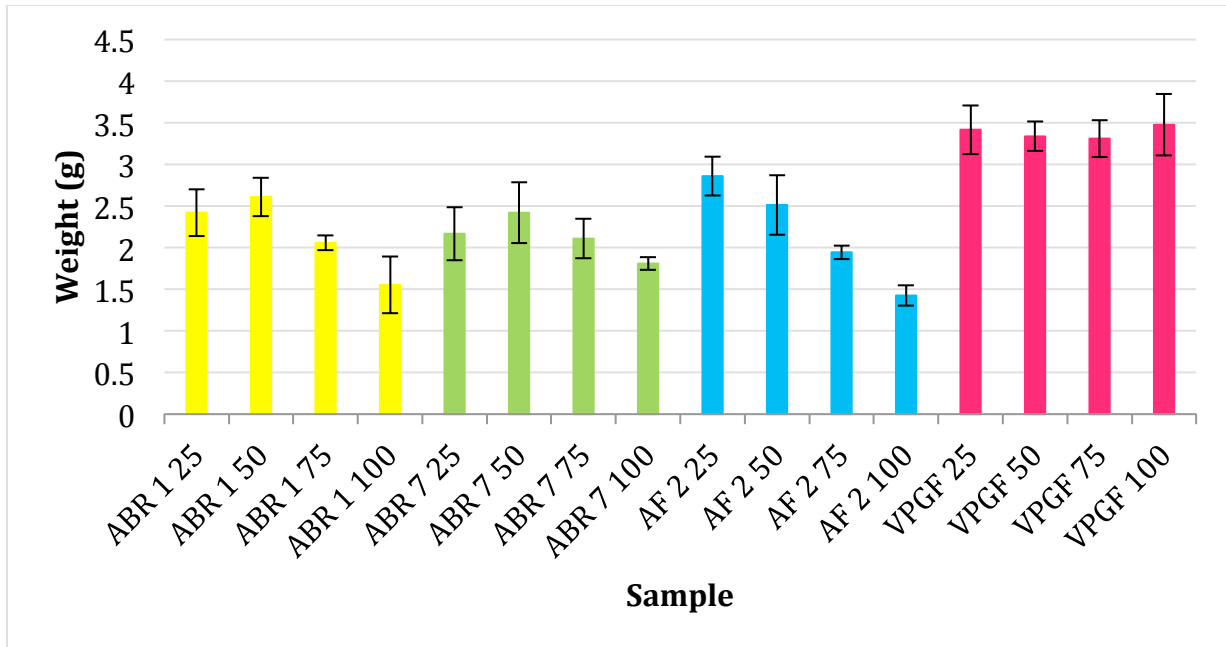


Figure 10. Dry Mass averages of all samples.

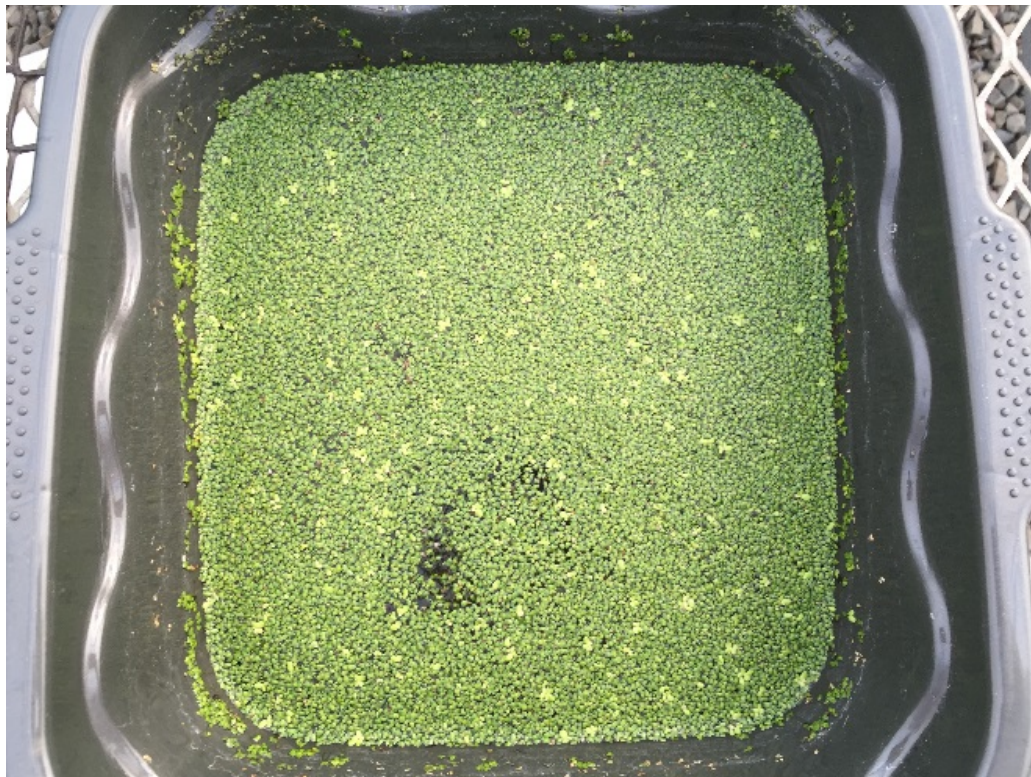


Figure 11. Duckweed growing in VPGF 100% WW on day 14.

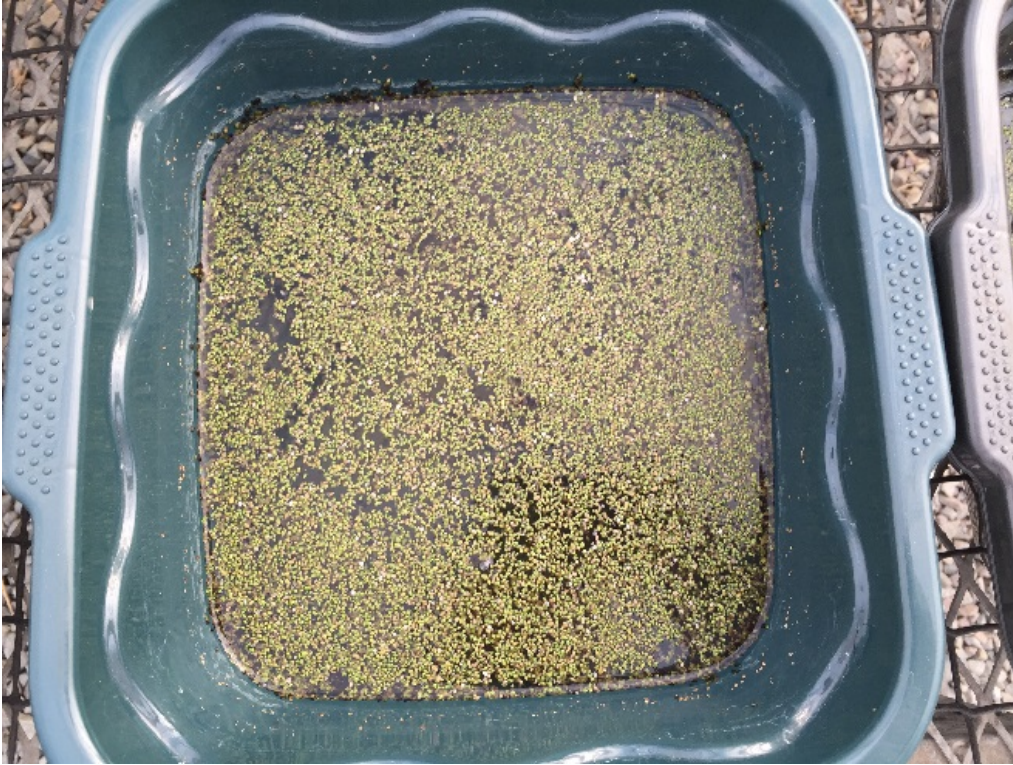


Figure 12. Duckweed growing in AF 2 100% WW on day 14.

#### 4. Discussion

A study done by Oron (1990) shows that the optimal retention time for duckweed tends to be 4-8 days. This may be due to the fact that after the first week, the dead duckweed debris may have an adverse effect on the wastewater samples, which is in agreement with the results of this study. The largest reduction in turbidity was from ABR 7, in which the 100% WW dilution decreased from 180.3 NTU to 7.35 NTU for a 96% reduction. However, the second largest reduction in turbidity was from the VPGF, in which the 100% WW dilution decreased from its already low NTU value of 23.6 to 1.38 NTU for a 94% reduction. The increase in total solids can also be attributed to similar causes as the increase in turbidity.

The increase in pH was likely due to the photosynthetic activity of the duckweed, which in most cases raised the pH by one factor. A similar study done by Nasr et al. (2008) showed this same effect where photosynthetic activity of duckweed caused a raise in the pH from 7.2 in the ABR effluent to 8.5 in the duckweed effluent. This study also showed that as long as pH levels did not exceed 8.7, the duckweed could be used to treat water.

As observed in figure 11, the VPGF 100% WW dilution shows the adult (or larger size) fronds being the dominant subjects, however, it should also be noted that the white leaves mean that



the duckweed is running low on nutrients, suggesting that a retention time of 14 days is too long for the VPGF sample. In figure 12, it can be noted that the fronds, which tend to be younger, take on a brown color and are even beginning to let sunlight through to the water. When the containers were cleaned out at the end of the study, it should be noted that a small amount of algae was beginning to line the inside of the container for the AF 2 100% WW dilutions. The inhibited growth in AF 2 may have been caused by nitrogen deficient wastewater or an imbalance in preferential nutrients.

The duckweed from the VPGF dilution row showed the largest amount of dry mass accumulation, suggesting that it had the most preferential qualities for duckweed growth. However, more analysis should be done on the nutrient removal from those samples to see where the duckweed performed best as a source of water treatment. Further study should be done on the nutrient quality of the wastewater after treatment with duckweed as well as analysis of the duckweed itself. Future studies should include total and fecal coliforms analysis to better understand the fertilizer potential of duckweed.

## **5. Conclusion**

When added to pretreated wastewater from the DEWATS, duckweed provided a 90-96% reduction in turbidity. A reduction in COD can be seen for all dilutions except ABR 7. Increase in total solids and decrease in volatile solids for all cases except ABR 7 can be noted and possibly due to interference from duckweed fronds or other organic material. The greatest dry mass accumulation was seen in low dilutions of wastewater except for the VPGF where the greatest accumulation was seen in the 100% WW dilution. The findings of this study contribute to a better understanding of the optimal condition for duckweed growth and where nutrient removal was best performed. This study can serve a further importance in understanding nutrient recovery from wastewater. Duckweed collected from this study has been stored and can be tested for further analysis for its qualities as a fertilizer. Duckweed is shown to be a promising tool in nutrient recovery and water treatment.

## **6. References**

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## 7. Appendix

Table 1. COD averages

COD Averages				
	Day 0	Day 7	Day 14	
Sample	Concentration	(mg/L)		% reduction
ABR 1 25		25.56	28.33	
ABR 1 50		75.00	54.17	
ABR 1 75		240.00	88.89	
ABR 1 100	373.33	285.00	130.56	65.03
ABR 7 25		26.11	46.11	
ABR 7 50		100.56	37.22	
ABR 7 75		161.67	107.22	
ABR 7 100	132.78	158.89	135.56	-2.09

AF 2 25			65	
AF 2 50			132.78	
AF 2 75			166.11	
AF 2 100	257.22		178.89	30.45
VPGF 25			46.11	
VPGF 50			62.78	
VPGF 75			76.11	
VPGF 100	102.78		83.33	18.92
Control 1		3.33	25	
Control 2			51.67	

Table 2. TS and VS averages

Total and Volatile Solids					
Day 0			Day 14		
Sample	TS (g/g)	VS (g/g)	Sample	TS (g/g)	VS (g/g)
ABR 1 25	0.000196	1.1E-05	AF 2 25	0.000213	3.35E-05
ABR 1 50	0.000323	4.81E-05	AF 2 50	0.000383	7.9E-05
ABR 1 75	0.000532	0.000132	AF 2 75	0.000645	0.00018
ABR 1 100	0.000635	0.000129	AF 2 100	0.000793	0.000112
ABR 7 25	0.000252	2.21E-05	VPGF 25	0.000146	0
ABR 7 50	0.000445	9.79E-05	VPGF 50	0.000347	4.46E-05
ABR 7 75	0.000632	0.000109	VPGF 75	0.000454	0.000122
ABR 7 100	0.000893	0.000245	VPGF 100	0.000612	0.0001
Control 1	0.000167	4.44E-05	Control 2	4.39E-05	0

Table 3. Turbidity averages

Turbidity Averages							
Sample	Day 0		Day 7		Day 14		% reduction
	NTU	SD	NTU	SD	NTU	SD	
ABR 1 25			1.17	0.02	0.76	0.20	
ABR 1 50			2.48	0.06	3.13	0.63	
ABR 1 75			5.95	1.33	8.87	1.99	
ABR 1 100	148.0	2.00	14.63	1.24	9.89	0.72	93
ABR 7 25			1.14	0.02	0.48	0.09	
ABR 7 50			2.49	0.12	1.85	0.24	
ABR 7 75			5.41	0.25	5.92	0.92	
ABR 7 100	180.3	4.04	7.75	0.34	7.35	0.50	96
AF 2 25			1.95	1.08	1.33	0.59	
AF 2 50			2.30	0.25	2.50	0.46	



AF 2 75			5.02	0.98	7.94	0.86	
AF 2 100	83.0	1.32	8.71	3.22	9.46	1.90	89
VPGF 25			0.51	0.11	0.72	0.22	
VPGF 50			0.55	0.08	0.91	0.13	
VPGF 75			0.77	0.43	1.33	0.08	
VPGF 100	23.6	0.52	0.97	0.03	1.38	0.06	94
Control 1			0.74	0.28	0.52	0.08	
Control 2			0.70	0.20	0.64	0.15	

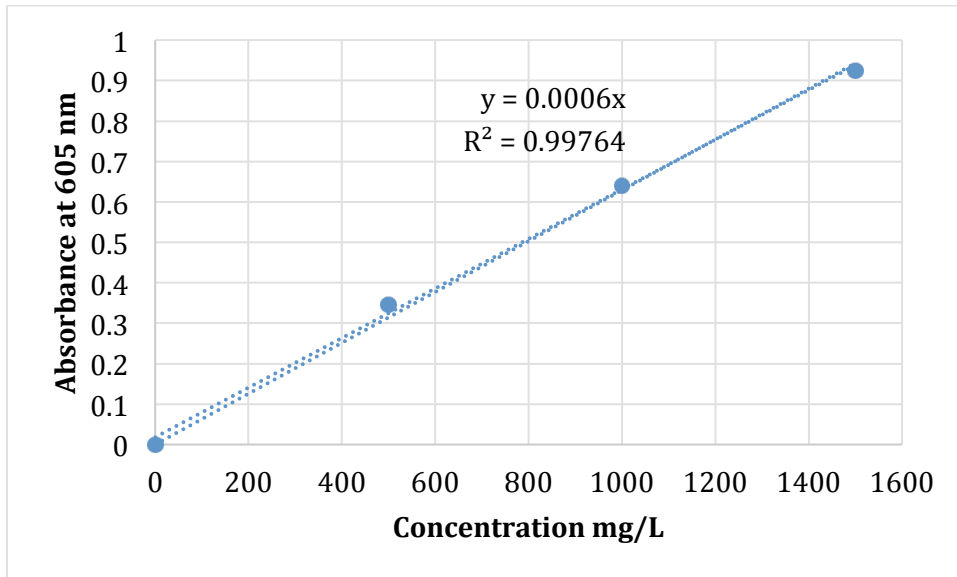


Figure A1. COD standard curve at 605 nm.