Analysis of the pathogen mobility from wastewater to suspended duckweed

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Abstract

Global efforts are being made to ensure safe water sources, sanitation, food security, and sustainable agriculture through the United Nations Sustainable Development Goals. These efforts have attracted interest in the use of duckweed, a small angiosperm that thrives in nutrient rich waters across the globe, for polishing treatment of effluent from anaerobic baffled reactor (ABR) systems, and as a green-fertilizer or soil amendment. However, it is hypothesized that elevated concentrations of pathogenic bacteria may be present in the biomass of duckweed grown in ABR effluent. In order to address this, lab-scale batch and continuous-flow duckweed ponds were constructed with a 3 and 2-day hydraulic residence time (HRT), respectively to determine pathogen concentrations in *Lemna minor* and a mixed duckweed with both *L. minor* and *Wolffia arrhiza*. Analysis for Total Coliforms (TC) and *E. coli* indicator bacteria in ABR effluent and duckweed biomass was conducted using a spread plate method with 3M Petrifilms and Brilliance *E. coli*/coliform selective agar. Counts of *E. coli* and TC were lower in the effluent of ponds with duckweed than in controls without. After growth in ABR effluent, *L. minor* and mixed biomass contained *E. coli* and TC at concentrations of 10 and 8 log CFU/g, respectively. When *L. minor* and mixed duckweed was dried at 32°C, *E. coli* and TC present in duckweed biomass were reduced to a magnitude of 3 log CFU/g within the first 24 hours. During the next 48 hours *E. coli* and TC increased to 4.19(±0.07) and 4.78(±0.06) log CFU/g, respectively in *L. minor* biomass. In mixed biomass *E. coli* and TC increased to 4.04(±0.18) and 4.80(±0.11) log CFU/g respectively. These results indicate that pathogen counts in the ABR effluent are reduced and taken up by duckweed biomass, posing a potential health risk when used as a green-fertilizer or soil amendment.

1. Introduction

While anaerobic digestion is an efficient means of the removal of organic material and suspended solids, it has little effect on the nutrient concentrations and only partially removes pathogenic organisms (Collivignarelli et al., 1990). An ABR is an example of a low-maintenance anaerobic treatment system, efficient in the removal of organic material and suspended solids, but with little bacterial removal. This system is usually followed by one or more anaerobic filters (AF), creating the appropriate term, ABR-AF system. As a polishing treatment step, this system is usually followed by a constructed wetland, membrane filtration, or another natural system such as ponds or lagoons as a final treatment step to remove pathogenic bacteria. Natural systems have notably low cost and maintenance, rendering them suitable for developing countries where money and skilled workers are scarce (Conely et al., 1991). An example of a natural treatment system is described in a study done by Nasr et al. (2008), where duckweed was used in a pond as a polishing treatment for wastewater. Duckweed is a small, non-N-fixing, angiosperm with high reproduction rates that is naturally present in nutrient rich and brackish bodies of water. While duckweed has been the focus of many wastewater treatment studies due to its known ability to take up nutrients and contaminants, little information has been presented about its tendency to take up pathogenic bacteria into its cell tissue and on its surface (Goopy et al., 2003).

In ABR-AF systems, helminth eggs are effectively removed from through sedimentation. In addition, bacteria and viruses are inactivated to a great extent, although they still exist in

infectious concentrations in treated effluent. High inactivation rates of pathogenic bacteria have been reported in natural treatment processes such as constructed wetlands, shallow aerobic stabilizations ponds, algal ponds, or duckweed ponds. This is attributed to effects of starvation, exposure to UV rays, sedimentation, and various biochemical interactions.

In duckweed ponds, UV radiation is expected to have less effect in pathogen inactivation due to decreased sunlight penetration past matts of floating biomass. Additionally, duckweed biomass may serve as a surface for pathogen attachment. In effect, pathogens adsorbed to duckweed biomass may be shielded from UV light (MacIntyre et al., 2006). Additionally, pathogens may remain on the surface of duckweed biomass when removed during harvesting, or after sedimentation due to plant decay (El-Shafai et al. 2007). In addition to adsorption to the surface of duckweed biomass, it is also possible that pathogens may be internalized into the biomass. Pathogen internalization has not been studied in duckweed specifically, but a study by Hirneisen et al. (2012) discusses pathogen internalization of various root-based crops. Hirneisen reports that there is controversy whether internalization of pathogens is an active or passive process. However, authors suggest that motile bacteria position themselves close to the root-systems, increasing the potential for internalization into the plant biomass.

The goal of this study was to test the hypothesis that pathogenic bacteria may be present in duckweed biomass when grown in ABR-AF effluent. In order to address this hypothesis, this study attempted determine concentrations of TC and *E. coli* indicator bacteria in harvested duckweed biomass after several days of growth in ABR-AF system effluent. Additionally, in order to address the potential use of duckweed as a green-fertilizer or stock feed, inactivation of TC and *E. coli* were also determined in harvested duckweed biomass due to drying at ambient temperatures. Lastly, using batch and continuous flow bench-scale ponds containing duckweed and ABR-AF effluent, this study also attempted to determine the removal of TC and *E. coli* indicator bacteria present in ABR-AF system effluent treated with duckweed and compared with controls.

2. Methods and Materials

Preparation of Duckweed

Duckweed consisting of a mixture of *L. minor* and *W. arrhiza* was retrieved from a swine lagoon located at an estate 20 km outside of Pietermaritzburg, South Africa for use in this study. *L. minor* duckweed was retrieved from the mixture using a sieve to prepare containers with only *L. minor* without fronds of *W. arrhiza*. Clean tap water was poured over the sieve to separate any remaining *W. arrhiza* fronds from the *L. minor*. Both the *L. minor* and mixed duckweed were grown in effluent from the second anaerobic filter (AF2) in the ABR-AF system for three days prior to the start of the experiments to allow time to adjust to new growing conditions.

Growth Rate Kinetics

On the first day of each trial duckweed was dewatered using a manual centrifuge. An initial damp weight was recorded before the duckweed was added into its respective container. On the final day of each trial, pre-cut mousseline cloth was wetted with water and then dewatered using a manual centrifuge. The damp weight of each mousseline cloth was recorded. Duckweed was sieved out of each container using a hand sieve and mousseline cloth. The Duckweed was

contained in the mousseline cloth and weighed using a gravimetric scale. The damp weight of the mousseline cloth was subtracted from the total weight and recorded

$$Y = (DWC_f - C) - DW_i \quad \text{Eqn. 2.1}$$

Y = growth rate of duckweed $\left({}^{g}/_{d}\right)$

DWC_f = combined mass of dewatered mousseline cloth and final duckweed biomass

C = mass of dewatered mousseline cloth

DW_i = initial mass of dewatered duckweed biomass

2.1 Experimental Setups

Batch experimental setup

This study built upon the results of a report by Scolavino (2016) on nutrient removal by duckweed grown in diluted effluent sampled from different stages of an ABR-constructed wetland system. The first part of this study utilized a batch setup to analyze changes in water quality and indicator bacteria concentrations due to the presence of duckweed. Duckweed was grown in the growing tunnel at Newlands-Mashu DEWATS site for three days in three triplicate sets of 0.06 m^2 containers filled with 3 L of AF2 effluent. One set contained *L. minor*, another contained a mixture of *L. minor* and *W. arrhiza*, and finally a control was established consisting of ABR-AF effluent from AF2 without the presence of duckweed. An aerial load of 500 g/m^2 of duckweed was added to each container to achieve ideal growing conditions.

Figure 1: Control (C), *L. minor* (L), and Mixed (M) duckweed batch containers

Sampling and analytical methods – Batch setup

Each morning at 11:00 AM, 25 mL samples were retrieved after each container was stirred thoroughly. The water level of each container was recorded after sampling and replenished the next day with DI water before conducting water quality analysis and sampling to account for evapotranspiration. pH, dissolved oxygen (DO), electrical conductivity (EC), temperature, and turbidity were measured daily after sampling to assess the water quality in each container. A Fisher pH meter was used to measure pH, a WTW Oxi 3401 DO meter to analyze dissolved oxygen, a YSI EC meter used to test electro-conductivity and temperature, and a Hach DR900 spectrophotometer was used to test for turbidity.

Microbial analysis was conducted at the Durban University of Technology (DUT) Water and Wastewater Technology (WWT) laboratory using spread plate method with 3M petrifilms to determine changes in TC and *E. coli* concentrations over the 3-day HRT (Figure 2).

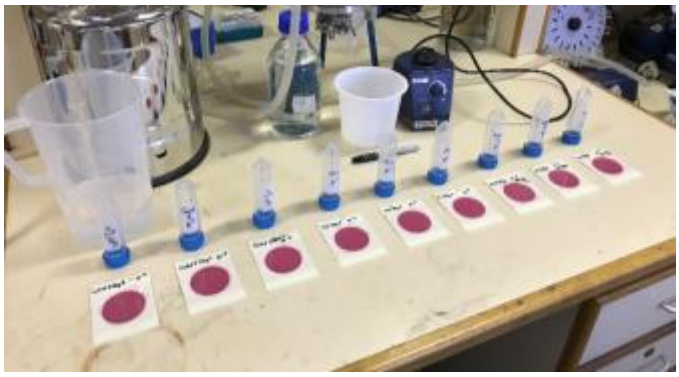


Figure 2: Spread plate method with 3M Petrifilms

IDEXX Colilert method was used to determine initial and final TC and *E. coli* concentrations in units of MPN/100 mL over the course of a 3-day HRT. A 10^{-5} serial dilution was prepared from a thoroughly vortexed 25 mL of sample. Microbial analysis was conducted using IDEXX Colilert-18 method (Figure 3).

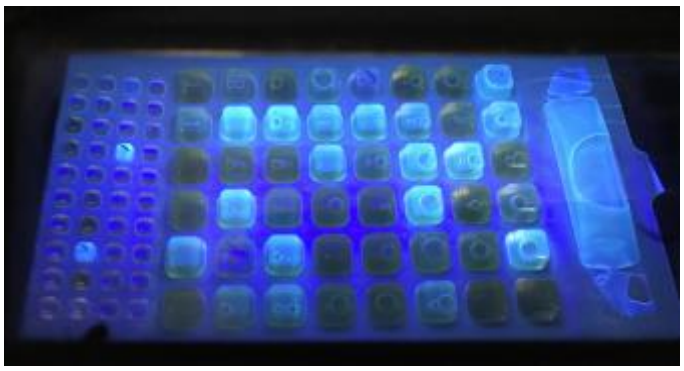


Figure 3: IDEXX Colilert-18 method. Fluoresced cells signifying the presence of E. coli.

Continuous-flow experimental setup

In addition to the batch setup, a continuous flow system was constructed to analyze changes in water quality and concentrations of indicator bacteria by duckweed in bench-scale duckweed ponds. Three 12 L, 7 cm deep, duckweed ponds were assembled to resemble full-scale ponds, and lab-scale ponds assembled in a previous experiment conducted by Nasr et al. 2008 (Figure 3.2); one with *L. minor*, another with a mixture of *L. minor* and *W. arrhiza*, and a control pond without the presence of duckweed. An aerial load of 600 g/m^2 of duckweed was added to the *L. minor* and Mixed ponds to achieve ideal growing conditions according to Nasr et al. 2008. This surface density provided a loose coverage that prevented algae growth while providing enough space for growth. Each pond was fed by an influent reservoir filled with effluent from the Newlands Mashu ABR-AF system at a flow rate of 0.25 L·hr^{-1} to achieve a 2-day HRT. The

influent reservoir was filled with AF2 effluent twice daily at 10:00 AM and 1:00 PM to provide two reference points for initial pathogen concentrations.

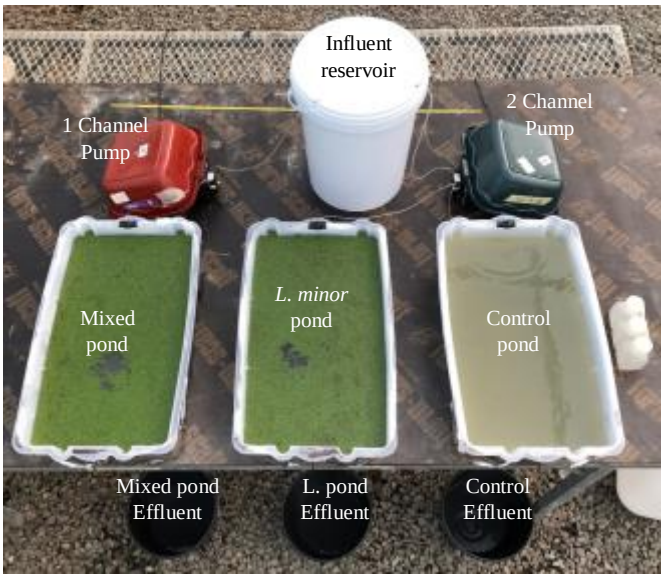


Figure 4: Continuous flow setup with Mixed, *L. minor*, and Control ponds fed by influent reservoir.

Sampling and analytical methods – Continuous-flow setup

The influent reservoir was re-filled with AF2 effluent at 10:00 AM and 1:00 PM on days 1 and 2. After each re-fill, 25 mL samples were collected in triplicate from the influent reservoir for initial water quality and microbial. At the end of each 2-day HRT, 25 mL samples were retrieved from the effluent of each pond 30 minutes before the 2-day HRT had been reached, at the HRT completion, and 30 minutes after the HRT had been reached to account for variance of effluent water quality (Figure 6).

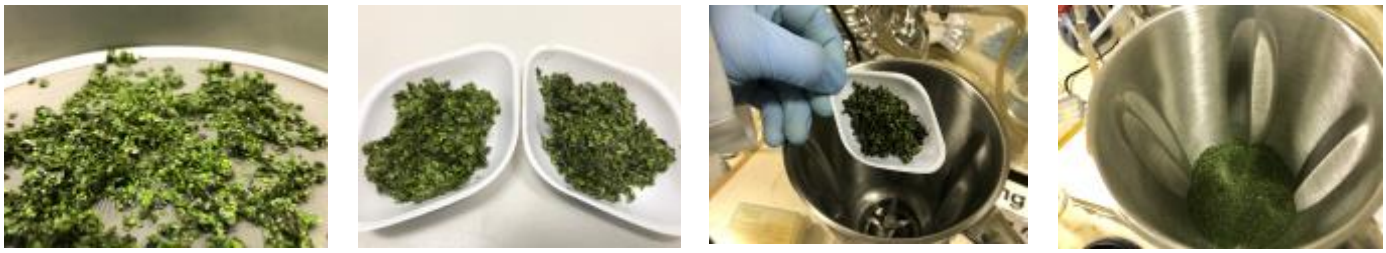
Figure 5: Sampling from continuous-flow effluent weir of control pond.

The pH, dissolved oxygen (DO), electrical conductivity (EC), temperature, and turbidity were measured in the influent reservoir at the time of each re-fill and at each sampling time from the pond effluent to assess the changes in water quality before and after treatment with a pond. A Fisher pH meter was used to measure pH, a WTW Oxi 3401 DO meter to analyze dissolved oxygen, a YSI Conductivity meter used to measure electro-conductivity and temperature, and a Hach DR900 spectrophotometer was used to determine turbidity.

Microbial analysis was conducted on influent samples, and on effluent samples after the HRT was reached using a spread plate method with *E. coli*/coliform selective agar. Triplicate samples were retrieved from the influent reservoir after being filled with AF2 effluent at 10:00 AM and 1:00 PM to determine influent TC and *E. coli* influent concentrations. Microbial analysis was conducted on samples retrieved from the pond effluent 30 minutes before the 2-day HRT, at the 2-day HRT, and 30 minutes after the 2-day HRT to account for the variance of effluent TC and *E. coli* concentrations.

2.2 Microbial study of duckweed biomass

L. minor and *W. arrhiza* were grown in AF2 effluent for 3 days prior to experimentation to allow the duckweed to acclimate to its new conditions. De-watered duckweed samples were retrieved from batch setups on the initial day of each trial and analyzed for TC and *E. coli* indicator bacteria present in the initial biomass. De-watered duckweed samples were also taken on the final day of each experiment to determine final TC and *E. coli* concentrations. Duckweed was dewatered using a manual centrifuge and a sieve. 5g of dewatered duckweed was weighed out and macerated in a blender for 3 minutes with 500 mL of DI water to obtain a 1:100 dilution (Figure 6). This liquid was further diluted to 10^{-5} in a 100-mL graduated cylinder. Each dilution was poured into a clean Nalgene bottle and analyzed for pathogens using IDEXX method *(Appendix section 6.1.3)*.



(A) (B) (C) (D)

Figure 6 Dewatering (A), weighing (B), and maceration (C) & (D) of duckweed biomass for microbial analysis.

2.3 Microbial study of duckweed biomass dried at ambient temperatures

Changes in *E. coli* and TC concentrations were determined in *L. minor* and mixed duckweed biomass harvested from continuous-flow experimental setups over the course of a three-day drying period at ambient temperatures. Dewatered duckweed was pre-weighed into 5 g samples, spread evenly onto metal weighing trays, and placed into an incubator at approximately 32°C to resemble drying conditions in direct sunlight in northern Africa, where average temperatures can reach up to 36°C in summer months. Each day, one of each duckweed sample was macerated,

prepared into 10^{-2} dilutions with DI water, and analyzed for pathogen concentration using a spread plate method with brilliance *E. coli*/coliform agar (Figure7) *(Appendix Section 6.1.2)*.

(A) (B)

Figure 7: 5 g dewatered duckweed in metal weighing trays before drying (A) and after one day of drying (B).

3. Results

In this study, changes in duckweed biomass in the batch and continuous-flow experiments are reported. In addition, bacterial enumeration was performed on treated effluent sampled from all ponds, harvested biomass, and biomass after drying at 32°C. Lastly, water quality analysis was conducted to assess the effects of the presence of duckweed in batch and continuous-flow ponds.

3.1 Microbial analysis of duckweed biomass

Microbial analysis with IDEXX Colilert method was conducted on initial duckweed biomass in batch containers without replication. Final microbial analysis of duckweed from batch containers was conducted in triplicate.

Batch setup

The results from the IDEXX Colilert method indicate that the initial TC concentrations in *L. minor* and mixed duckweed were 6.70×10^6 and 9.80×10^7 MPN/g, respectively. After growth in ABR-AF system effluent for 3 days, TC concentrations in *L. minor* remained unchanged at $6.15 \times 10^6 (\pm 2.71 \times 10^6)$ MPN/g, and decreased in the mixed biomass to $4.42 \times 10^6 (\pm 5.30 \times 10^5)$ MPN/g (Figure 11). Results indicate that initial *E. coli* counts in *L. minor* and mixed duckweed biomass were 1.55×10^6 and 5.73×10^7 MPN/g, respectively. After growth in ABR-AF system effluent for 3 days, *E. coli* counts in *L. minor* and mixed duckweed decreased to $9.43 \times 10^5 (\pm 3.62 \times 10^5)$ and $9.07 \times 10^5 (\pm 4.68 \times 10^5)$ MPN/g, respectively (Figure 8).

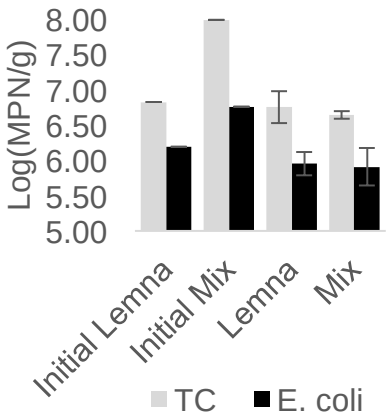


Figure 8: Initial and final IDEXX MPN of indicator bacteria in biomass of *L. minor* and mixed duckweed after growing in DEWATS effluent for four days.

Continuous-flow setup and subsequent drying at ambient temperatures

After growth in continuous-flow ponds, duckweed biomass was harvested and analyzed for final pathogenic bacteria concentrations and inactivation rates due to drying at ambient temperatures. Trends in *E. coli* and TC concentrations were similar in both harvested *L. minor* and mixed duckweed over a three-day drying time at 32°C. At the beginning of day-1, *E. coli* and TC concentrations were too numerous to count (TNTC) although estimated to be 10^8 and 10^{10} CFU/g, respectively. It is important to note the large inactivation observed within the first 24 hours, followed by an exponential regrowth in the following 48 hours. After the first 24-hour drying period a 5-log *E. coli* inactivation and a 6.5-log TC inactivation was observed followed by a 1-log *E. coli* regrowth and a 1.5-log TC regrowth over the next two days (Figure 9 and Table 1).

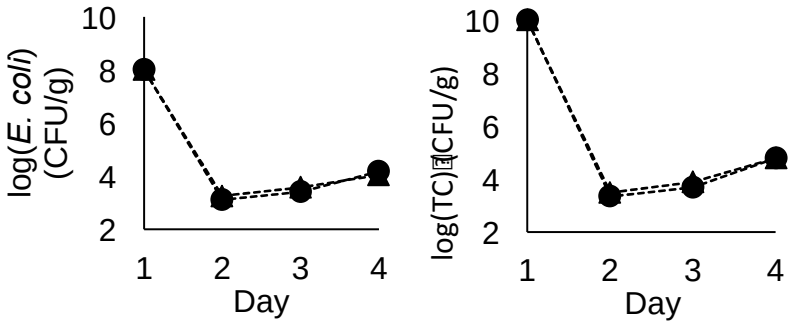


Figure 9: log inactivation and re-growth of *E. coli* and TC in duckweed biomass when dried at 32°C.

Table 1: Daily *E. coli* and TC concentrations in harvested *L. minor* and mixed duckweed after drying at 32°C

		Day 1	Day 2	Day 3	Day 4
L. minor (CFU/g)	*E. coli*	1.00E+08	1.30E+03	2.53E+03	1.55E+04
	TC	1.00E+10	2.23E+03	5.00E+03	6.07E+04
Mixed (CFU/g)	*E. coli*	1.00E+08	1.83E+03	3.87E+03	1.16E+04
	TC	1.00E+10	3.20E+03	7.80E+03	6.48E+04

3.2 Water quality and microbial analysis of treated effluent

Batch setup

A batch setup was used as shown in figure 1 to determine the changes in TC and *E. coli* pathogenic indicator bacteria in treated water, and any changes in water quality due to the presence of duckweed. Water added daily to each container to counter evapotranspiration did maintain steady water levels throughout the experiment. On the coolest day with a temperature of 12.7°C, 64(±12) mL, 87(±23) mL, and 44(±6) mL was lost due to evapotranspiration in *L. minor*, mixed, and control ponds, respectively. The largest losses due to evapotranspiration occurred between day 2 and 3 with a temperature of 14.2°C; 110(±17) mL, 110(±17) mL, and 107(±21) mL were lost in *L. minor*, mixed, and control ponds respectively.

Physical and chemical constituents of treated effluent in batch ponds (Figure 8)

The pH of each duckweed container increased from 7.18(±0.02) on day 1 to a maximum between day 3 and 4 of 7.88(±0.01), 7.85(±0.02), and 8.21(±0.01) for *L. minor*, mixed, and control ponds respectively. It is important to note that after day 1, pH readings of the control were consistently higher than both *L. minor* and mixed containers. Measurements of water temperature mirrored the trends of daily ambient temperatures. Ambient temperatures as well as temperatures of bins ranged from 12.6(±0.2) to 21.8(±0.1)°C with standard deviations of ±0.1, 0.2, 0.2, and 0.4 for days 1-4, respectively.

Turbidity rose in the first 24 hours of the experiment, but fell drastically in the following days. It is important to note that day 2 readings were higher in both duckweed ponds than in the control. Readings dropped from 215(±13) to 38(±2) NTU in *L. minor* containers, 228(±14) to 107(±11) NTU in mixed containers, and 211(±14) to 125(±4) NTU in control ponds.

Dissolved oxygen concentrations were high due to improper calibration of the DO meter. However, for comparison only within this study, we report the changes in DO for the different experiments. We observed sporadic DO concentrations throughout the experiment, ranging between 17.9(±0.2) – 23.2(±0.3) mg/L for *L. minor* containers, 18.2(±0.1) – 23.4(±0.2) mg/L for mixed containers, and 17.2(±0.3) – 22.5(±0.1) mg/L. Although they varied throughout the experiment, readings were higher at the end of the experiment than at the beginning. DO concentrations were consistently higher in *L. minor* ponds after the second day of the experiment.

Electrical conductivity readings were also sporadic, but followed the same trend for each container, throughout the experiment, ranging between 845 and 1143 µS with standard deviations of ±16.9, 8.3, 26.9, and 13.6 µS for days 1-4 respectively. Conductivity appears to be negatively correlated to DO concentrations. There was no significant difference in EC among the different *L. minor*, mixed, and control containers. Although trends varied throughout the week, readings were lower at the end of the experiment than at the beginning.

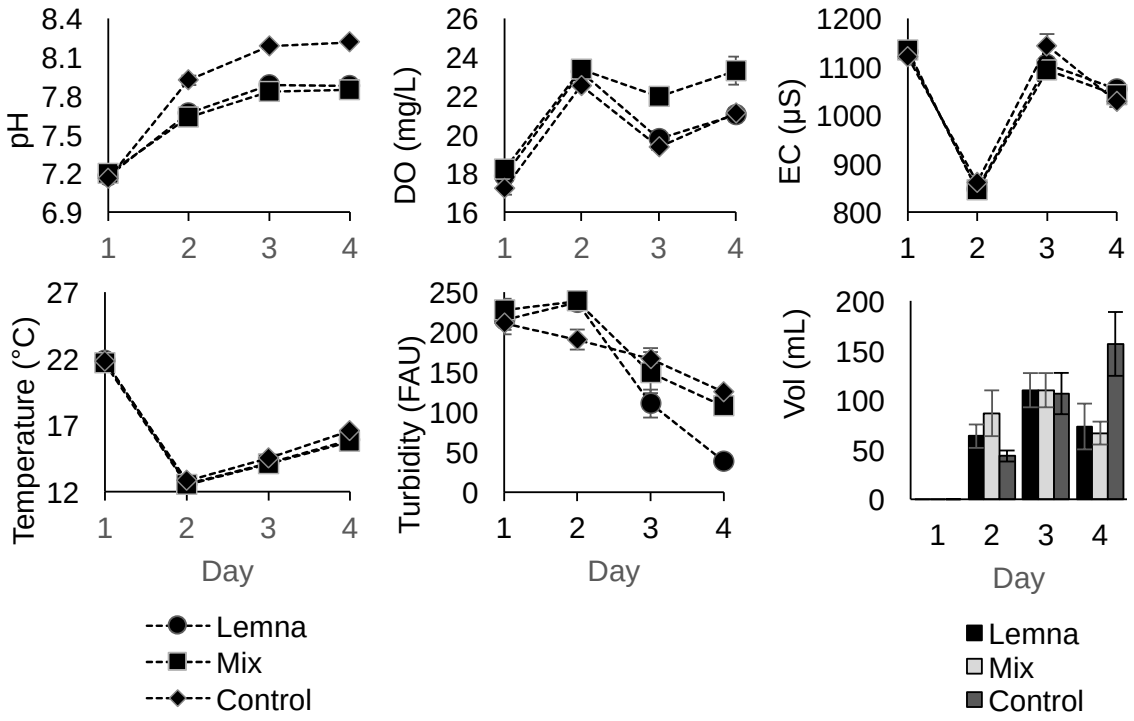


Figure 10: Daily pH, DO, EC, Temperature, Turbidity, and Evapotranspiration measurements of batch experiment.

Microbial analysis of treated effluent in batch ponds (Figure 9)

Results of replicate experiments show that TC and *E. coli* were reduced in all duckweed ponds as well as in the control. The log reduction allows us to clearly see the consistent drop in *E. coli* concentrations over the course of the first two days followed by a large reduction in the final 24 hours of the experiment. *E. coli* concentrations were reduced from $6.8x10^5(\pm1.01x10^5)$ to $1.61x10^4(\pm1.85x10^4)$ CFU/100 mL in *L. minor* containers, $3.63x10^4(\pm2.42x10^4)$ CFU/100mL in the mixed containers, and $1.57x10^5(\pm6.40x10^4)$ CFU/100 mL in control containers. TC concentrations were reduced from $1.80x10^6(\pm1.82x10^5)$ CFU/100 mL to $5.24x10^4(1.84x10^4)$ CFU/100 mL in *L. minor* containers, $1.01x10^5(\pm3.49x10^4)$ CFU/100 mL in mixed containers, and $2.78x10^5(\pm8.47x10^4)$ CFU/100 mL in control containers. It is important to note that the final reduction in TC and *E. coli* was lower in control containers than in containers with duckweed.

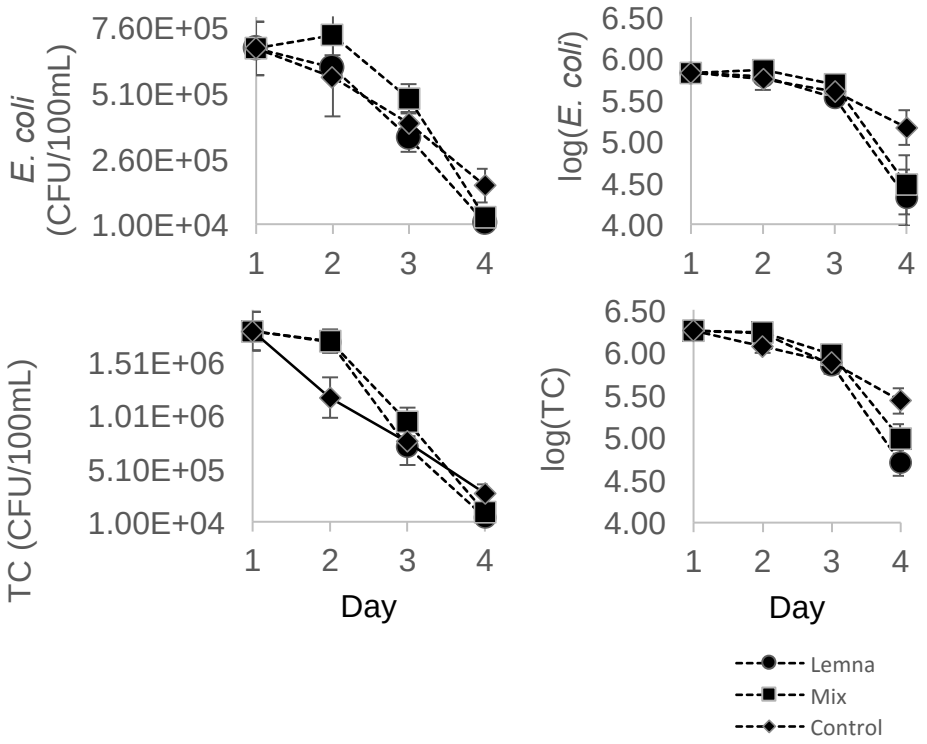


Figure 11: Inactivation of E. coli and TC over a four-day batch experiment determined with spread plate method

For the same experiments described above, results for initial and final IDEXX Colilert enumeration technique were compared with those of the spread plate method. Colilert results had an order of magnitude higher for both initial TC and *E. coli* (Figure 10) than the spread plate method (Figure 9). Results indicated higher initial pathogen counts, suggesting that initial TC and *E. coli* counts were $1.38x10^7(\pm1.17x10^7)$ and $3.16x10^6(\pm4.63x10^5)$, respectively in AF2 effluent. The Colilert method also suggested that TC and *E. coli* counts increased in the *L. minor* containers over the 3-day residence time to $1.31x10^8(\pm3.62x10^7)$ and $5.86x10^7(\pm4.69x10^6)$ MPN/100 mL, respectively. Meanwhile, TC and *E. coli* counts decreased in mixed and control containers. TC and *E. coli* counts decreased to $6.06x10^5(\pm4.91x10^5)$ and $2.42x10^5(\pm2.16x10^5)$ MPN/100 mL, respectively in mixed containers, and $1.92x10^6(\pm1.35x10^6)$ and $4.28x10^5(\pm3.88x10^5)$ MPN/100mL in controls. It is important to note the high standard deviations in the results of this method (Figure 10).

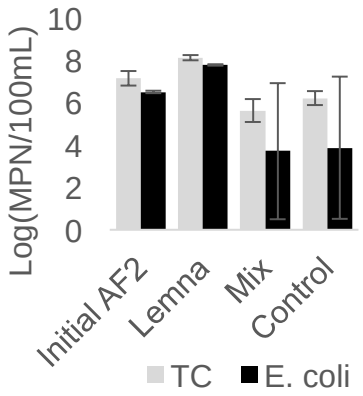


Figure 12: *E. coli* and TC concentrations in DEWATS effluent determined at the beginning and end of the four-day batch experiment determined with IDEXX Colilert method for comparison with spread plate method

Continuous-flow setup

A continuous-flow setup was used as shown in figure 5 to determine the changes in TC and *E. coli* pathogenic indicator bacteria in ABR-AF system effluent, and any changes in water quality due to the presence of duckweed. Each trial is indicated by influent reservoir (IR) 1-4, signifying that the influent reservoir was refilled to attain benchmark for initial water quality and microbial analyses.

Physical and chemical constituents

The initial pH of the influent reservoir in each trial was 7.07(±0.02). In each trial pH increased more in the control than did in duckweed ponds. pH rose to 7.78(±0.06), 7.77(±0.05), and 7.94(±0.08) in *L. minor*, Mixed, and Control duckweed ponds (Figure 12). There was no significant difference in temperature between duckweed ponds at each sampling time (Figure 12).

In each trial, DO rose after treatment in each pond and the control. In trial 1 DO rose from 27.2(±0.1) mg/L in the influent reservoir to 31.0(±2.6), 31.0(±3.0), and 30.2(±0.9) mg/L in *L. minor*, mixed, and control duckweed ponds respectively. In trial 2, DO rose from 26.7(±0.3) mg/L in the influent reservoir to 29.6(±0.3), 29.5(±0.3), and 30.2(±0.5) mg/L in *L. minor*, mixed, and control duckweed ponds respectively. In trial 3 DO rose from 27.8(±0.2) mg/L in the influent reservoir to 32.8(±0.6), 31.5(±0.1), and 34.0(±0.6) mg/L in *L. minor*, mixed, and control duckweed ponds respectively. Lastly, in trial 4 Do rose from 27.9(±0.1) mg/L in the influent reservoir to 31.5(±0.5), 31.0(±0.6), and 31.6(±0.8) mg/L in *L. minor*, mixed, and control duckweed ponds respectively (Figure 12).

In trial 1, EC dropped from 1143(±2) μS/cm in the influent reservoir to 1062(±25), 1043(±68), and 1025(±19) μS in *L. minor*, mixed, and control duckweed ponds respectively. In trial 2, EC rose from 1142(±1) μS in the influent reservoir to 1151(±7), 1184(±10), and 1115(±49) μS in *L. minor*, mixed, and control duckweed ponds respectively. In trial 3, EC dropped from 1047(±1) μS in the influent reservoir to 981(±22), 994(±30), and 971(±23) μS in *L. minor*, mixed, and control duckweed ponds respectively. Finally, in trial 4, EC rose from 1058(±1) μS in the

influent reservoir to 1086(±14), 1083(±4), and 1084(±1) µS in *L. minor*, mixed, and control duckweed ponds respectively (Figure 3.3).

Turbidity results suggest that there was an increase in treatment efficiency throughout this experiment. In all four trials, ponds containing duckweed showed a decrease in turbidity over time compared to the controls. The first two trials resulted in an effluent turbidity of 76(±4) and 73(±4) NTU for *L. minor* duckweed ponds, 88(±3) and 82(±3) NTU for mixed duckweed ponds, and 144(±3) and 145(±3) NTU for control ponds respectively. The second two trials resulted in an even lower effluent turbidity of 54(±5) and 54(±4) NTU for *L. minor* duckweed ponds, 57(±2) and 59(±1) NTU for mixed ponds, compared to 120(±3) and 125(±4) NTU for control ponds, respectively (Figure 3.3).

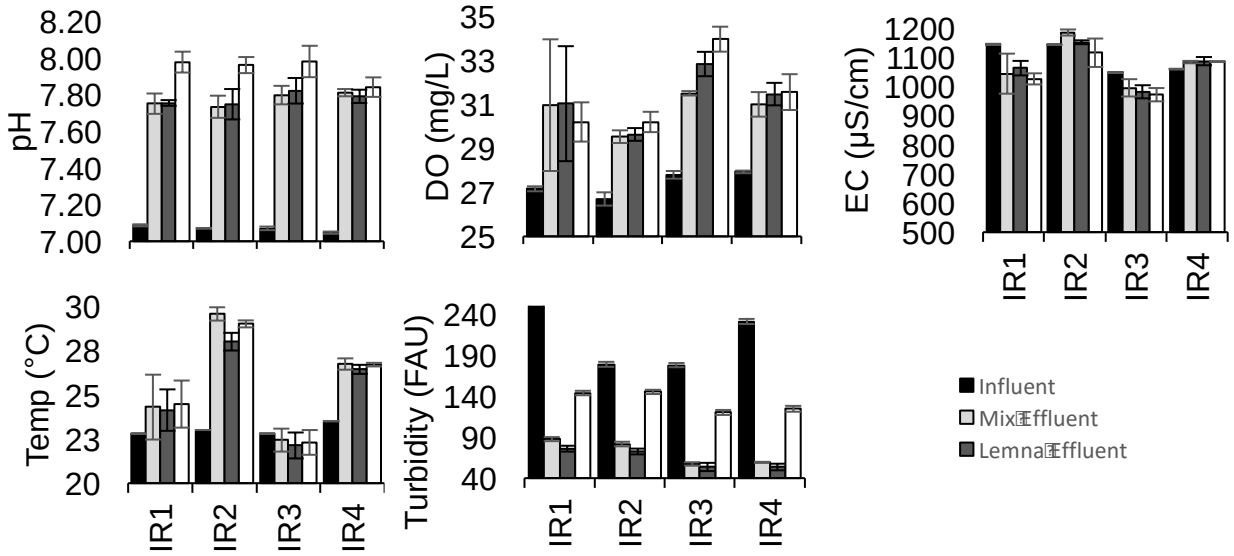


Figure 13: Water quality parameters taken from each influent reservoir, and from each effluent reservoir after the 2-day HRT was reached under continuous flow conditions.

Microbial analysis of treated effluent

An increase in pond treatment efficiency was observed over the course of three trials conducted over two days. Reductions of pathogenic bacteria ranged from 45-62 *E. coli* reduction and 24-66% TC reduction in *L. minor* ponds, 0-47% *E. coli* reduction and -71-52% TC reduction in mixed ponds, and -22-38% *E. coli* reduction and -61-51% TC reduction in control ponds (Figure 13).

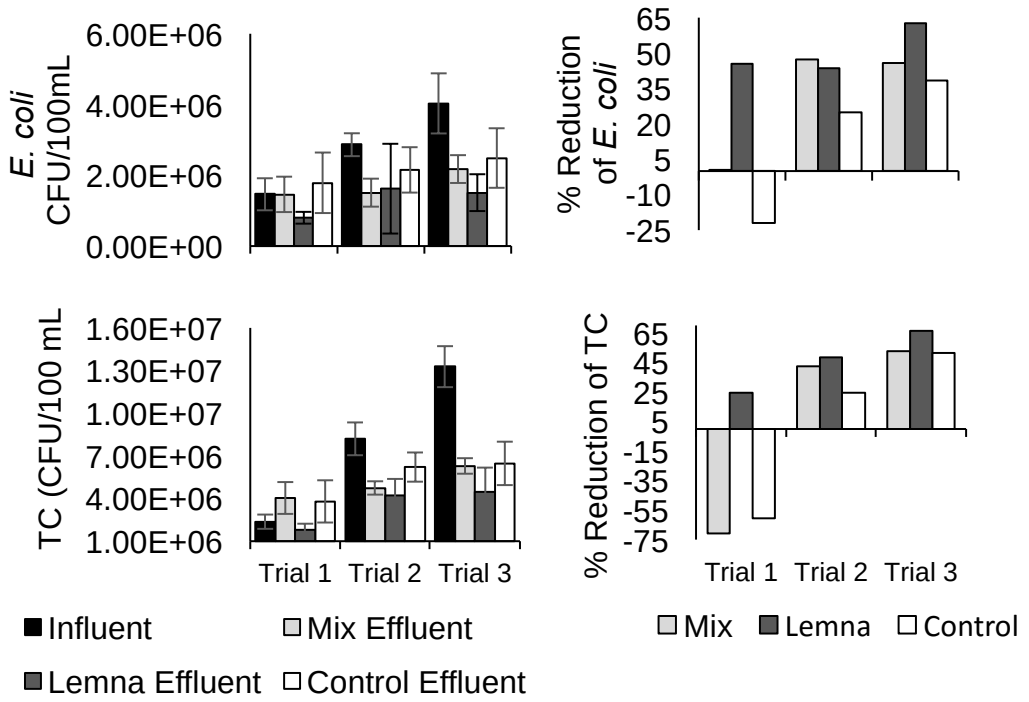


Figure 14: Changes in concentrations of indicator bacteria, *E. coli*, and TC in influent and in effluent after 2-day residence time in a continuous flow-setup.

3.3 Duckweed growth

In both batch and continuous-flow setups, a negative growth rate was recorded.

Growth rate kinetics – Batch setup

In batch experiments, 30.028(±0.007) g *L. minor* and 30.032(±0.023) g mixed duckweed were added into each container to achieve a 500 g/m^2 surface area density. The final biomass was recorded to be 29.817(±1.949) g for *L. minor* and 29.396(±3.614) g for mixed duckweed. This resulted in a -0.211(±1.942) g decrease in *L. minor* biomass, and a -0.636(±3.592) g decrease in mixed duckweed.

Growth rate kinetics – Continuous-flow setup

In continuous-flow experiments, 111 g *L. minor* and mixed duckweed was added to each pond to achieve a 600 g/m^2 surface area density. The final biomass was recorded to be 81.326 g *L. minor* and 94.583 g mixed duckweed. This resulted in a -29.674 g decrease in *L. minor* biomass, and a -16.417 g decrease in mixed duckweed biomass.

4. Discussion

4.1 Microbial analysis of duckweed biomass

Results from initial *L. minor* and mixed duckweed should be interpreted with caution because bacterial enumeration requires destructive sampling of the biomass. Additionally, there was not enough biomass to conduct replicate analyses of initial samples, although final biomass was destructively sampled and analyzed in triplicate.

Microbial analysis of duckweed biomass grown in

After treatment of ABR-AF system effluent in both batch containers and continuous-flow ponds, duckweed contained high concentrations of *E. coli* and TC fecal indicator bacteria adsorbed to the biomass surface and within the cell tissue. It was expected that the concentration of fecal indicator bacteria would reach a consistent concentration in the duckweed biomass when grown in treated wastewater. This was observed by the similar initial and final *E. coli* and TC concentrations in the *L. minor* biomass (Figure 8) after treatment in batch containers filled with ABR-AF system effluent. However, a decrease in *E. coli* and TC fecal indicator bacteria in mixed duckweed biomass was observed in batch containers. Because the mixed duckweed was not as thoroughly rinsed in the sieving process as *L. minor*, it is expected that excess fecal indicator bacteria located on the damp mixed biomass may have washed off when added into the ABR-AF system effluent, resulting in lower *E. coli* and TC counts in the final mixed biomass.

Microbial analysis of duckweed dried at ambient temperatures

Reductions of *E. coli* and TC fecal indicator bacteria were similar both in harvested *L. minor* and mixed duckweed when dried at 32°C for a four-day period. The initial decrease in *E. coli* and TC in both *L. minor* and mixed duckweed biomass is consistent with the results of Mondini et al. (2002), who found a similar response of microbial biomass to air-drying. In this study, Mondini et al. explained that after an initial period of microbial die-off, regrowth of both *E. coli* and TC would occur, suggesting that surviving pathogenic bacteria were re-growing. This phenomenon was also described in other studies (Bottner, 1985; Shen et al., 1987) and attributed to the phenomenon that after initial die-off nutrients and organic matter become more readily available to surviving microorganisms.

Microbial analysis of wastewater in batch and continuous flow experimental setups

Results reported in Figures 9 and 13 suggest greater inactivation of TC and *E. coli* fecal indicator bacteria occurred within *L. minor* and mixed duckweed ponds than in the control containers in both batch and continuous flow setups. The mechanisms by which these fecal indicator bacteria are removed in both batch containers and continuous-flow ponds are explained in reviews by Maynard et al., 1999 and Davies-Colley et al., 2000. These reviews suggest that temperature, starvation, and interactions of sunlight with pH and oxygen radicals, predation, and sedimentation have a significant role in the removal of fecal coliforms. The oxygen around the root zone of the duckweed may react with UV-light, producing oxygen radicals (Maynard et al., 1999), thereby inactivating pathogens nearby. This indicates that adsorption and oxidation due to the presence of duckweed may have a greater effect on the removal of pathogens present in ABR-AF system effluent. Additionally, studies by Reed and Crites (1984) and Reed et al. (1988) suggest that pathogenic organisms and viruses retained in root zone beds through sorption and filtration mechanisms may be destroyed by die-off and predation.

An initial increase in TC and *E. coli* was observed in the first trial of the continuous flow experimental setup (Figure 13). This may be a result of the initial transfer of pathogens that had already been adsorbed to the surface of the *Leman minor* and mixed duckweed.

These lab-scale experimental setups were only operable for three days. Although the duckweed species were each grown in ABR-AF system effluent for a three-day period prior to

experimentation to allow the duckweed to acclimate to new growing conditions, variance in water quality between the acclimation pond and batch and continuous-flow setups likely affected the growth rate of duckweed present in each pond. Different results would likely be observed in a system in which the duckweed is already acclimated to its growing conditions when the experiment begins. A high growth rate would signify that the duckweed has acclimated to its growing conditions.

4.2 Water quality analysis

No identifiable trends in water quality parameters measured were influenced by volumes lost due to evapotranspiration. It was expected that evapotranspiration rates would be up to four times higher in containers with duckweed as reported by Sasse (1998).

The changes in water quality of this study were consistent with previous studies that found an increase in pH in duckweed ponds. Nasr et al. (2008) reported a pH rise from 7.2 in the ABR to 8.5 in the pond effluent due to photosynthetic activity. Similarly, the pH in our study rose from 7.18(±0.02) to 7.88(±0.01). However, the pH in control ponds (without photosynthetic activity), experienced a higher increase in our study, 8.21(±0.01), which indicates the increase may be due to other factors.

Although DO measurements were high due to calibration errors, the trend in DO showed that DO rose in all experimental setups. A potential explanation is given in a study done by Korner et al. (2003), that duckweed may provide additional surface are for microbial growth, oxygenating the water.

EC readings depended largely on the ambient temperature in both batch and continuous-flow experiments. A large decrease in EC was observed on the second day of the batch experiment, which can be attributed to the lower ambient temperature that day.

It is not expected that duckweed ponds with duckweed present on them had any effect on the overall temperature of the pond. The temperature of each pond was greatly affected by changing ambient temperatures.

Turbidity decreased in all duckweed ponds in both the batch and continuous flow setups. Because the batch setup was thoroughly mixed each time before samples were taken sedimentation is not expected to have any effect on turbidity reduction in the batch setup. However, in the continuous flow setup the reduction in turbidity is expected to be due partially to sedimentation because mixing was not considered to be a significant factor. In the continuous flow setup, the greater reduction in *L. minor* and mixed duckweed ponds is due to the adsorption of pathogenic bacteria to the fronds and root structures of suspended. In a study by Scolavino et al. (2016) turbidity reductions were reported as high as 96% when duckweed flourished in Scolavino's experiment. Although, duckweed may have an important influence on turbidity reductions, that role may have been less apparent in this experiment due to the lower growth of duckweed.

Indeed, in both continuous flow and batch experimental setups, negative biomass growth was observed. This may be due to the lack of ideal growing conditions. Although the duckweed

acclimated to AF2 effluent for at least three days prior to experimentation, little growth was observed. The negative biomass accumulation may be due to the decay of dead fronds into the wastewater or the lack of time necessary to establish growth.

5. Conclusion

Duckweed was found to produce greater log reductions of pathogenic bacteria from ABR-AF system effluent wastewater in both batch and continuous-flow systems. After treatment in ABR-AF system effluent, duckweed biomass contained pathogenic bacteria in significant quantities. After duckweed was harvested from a pond and laid out to dry in ambient temperatures, pathogenic bacteria that may have been internalized on the biomass or adsorbed to the plant surface itself, experienced an initial die-off as low as $2.23x10^3$ CFU/g, but regrew to concentrations as high as $6.48x10^4$ CFU/g 48 hours later.

With this knowledge, duckweed may be used in the future as a polishing treatment for ABR-AF system effluent to reduce pathogenic bacteria concentrations. Additionally, it is now known that there is a health risk associated with the reuse of duckweed that is grown in ABR-AF system effluent as a green fertilizer or feed stock. Using duckweed grown in ABR-AF system effluent as a green fertilizer presents a health risk to the consumer if there is any possibility that pathogens may be transmitted into the crop. Duckweed grown in DEWATS effluent may also present a risk to animals when used as a stock feed. With drying times of only 4 days, non-treated duckweed grown in ABR-AF system effluent should only be used as a green fertilizer for crops not intended for human consumption. Finally, it has been shown that pathogen concentrations are significantly reduced after 24 hours of drying at ambient temperatures. However, surviving bacteria grow back with widely available substrate. Further studies must be conducted to assess the reduction of fecal indicator bacteria due to extended drying times at ambient temperatures and the potential of transmission of pathogens from duckweed into crops for human consumption.to infect agricultural animals.

6. Recommendations

It would be beneficial to conduct this experiment with a system that has allowed proper time for the duckweed to acclimate to its growing conditions. With ideal growth rates, the effects that duckweed has on ABR-AF system effluent will be clearly. Additionally, conducting experiments on larger systems will mitigate the error that comes with minor biomass losses, and prevent large daily temperature fluctuations. Lastly, it is recommended that thorough rinsing of both mixed and *L. minor* duckweed be conducted before experimentation to prevent the transmission of excess fecal indicator bacteria into the system.

Future studies will be necessary to assess the reduction of fecal indicator bacteria due to the drying of duckweed over a longer span of time at ambient temperatures. The duckweed-drying experiment in this study took place over a four-day period. It will be beneficial to observe the trends that occur after two weeks to a month of drying time. Perhaps fecal indicator bacteria will die off as the moisture content of the biomass continues to deplete.

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Appendix

6.1 Methods

6.1.1 Spread plate method with 3M Petrifilms

- 25 mL of sample was vortexed until thoroughly mixed.
- A 10^{-2} dilution was prepared by serial 1:10 dilutions in sterile falcon tubes.
- 1mL of each 10^{-2} dilution was pipetted onto labeled 3M petrifilms
- Petrifilms were incubated at 35°C for 24 hours.
- Colonies were counted and recorded in units of CFU/100mL

6.1.2 Spread plate method with Brilliance E. coli/Coliform selective agar

- 25mL of sample was vortexed until thoroughly mixed
- A 10^{-2} dilution was achieved by serial dilution
- 100µL was pipetted onto each petri dish and spread plate method was conducted
- Incubate at 35°C for 24 hours
- Colonies counted and recorded in units of CFU/100mL

6.1.3 IDEXX Colilert-18

- Add IDEXX Colilert-18 pillow packet to 100mL sample in sterile container
- Cap and stir thoroughly until dissolved
- Pour sample/reagent mixture into a Quanti-Tray* or Quanti-Tray*/2000 and seal in an IDEXX Quanti-Tray* Sealer.
- Incubate sealed tray at 35°C for 18 hours.
- Read results according to IDEXX interpretation table:

Table 2: IDEXX Colilert-18 Interpretation Guide

Result Interpretation

Appearance	Result
Less yellow than the comparator[1] when incubated at 35±0.5°C or 44.5±0.2°C	Negative for total coliforms and *E. coli*; Negative for fecal coliforms
Yellow equal to or greater than the comparator when incubated at 35±0.5°C	Positive for total coliforms
Yellow equal to or greater than the comparator when incubated at 44.5±0.2°C	Positive for fecal coliforms
Yellow and fluorescence equal to or greater than the comparator when incubated at 35±0.5°C	Positive for *E. coli*

6.2 Water Quality Analysis

Table 7.2.1: Daily water quality parameters – Batch Setup

Parameters: 7/17/17 11:00 AM						
L.	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)
1	7.17	17.7	1105	21.7	0	201
2	7.18	17.8	1145	22.1	0	224
3	7.18	18.1	1140	21.8	0	221

Avg	7.18	17.9	1130	21.9	0	215
Std Dev.	0.01	0.2	22	0.2	0	13
Mix	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)
1	7.20	18.1	1146	21.8	0	243
2	7.20	18.2	1142	21.7	0	224
3	7.19	18.3	1116	21.4	0	216
Avg	7.20	18.2	1135	21.6	0	228
Std Dev.	0.01	0.1	16	0.2	0	14
Control	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)
1	7.17	17.5	1135	21.8	0	199
2	7.16	17.2	1119	21.8	0	208
3	7.14	16.9	1105	21.7	0	226
Avg	7.16	17.2	1120	21.8	0	211
Std Dev.	0.02	0.3	15	0.1	0	14
Parameters: 7/18/17 10:00 AM						
L.	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)
1	7.68	22.9	848	12.5	50	233
2	7.67	23.4	846	12.6	70	234
3	7.65	23.4	848	12.6	70	244
Avg	7.67	23.2	847	12.6	63	237
Std Dev.	0.02	0.3	1	0.1	12	6
Mix	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)
1	7.61	23.5	847	12.6	60	246
2	7.64	23.4	844	12.4	100	237
3	7.65	23.2	844	12.5	100	232
Avg	7.63	23.4	845	12.5	87	238
Std Dev.	0.02	0.2	2	0.1	23	7
Control	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)
1	7.89	22.5	856	12.9	41	205
2	7.91	22.5	858	12.8	50	186
3	7.96	22.6	869	12.7	40	181
Avg	7.92	22.5	861	12.8	44	191
Std Dev.	0.04	0.1	7	0.1	6	13
Parameters: 7/19/17 11:00 AM						
L.	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)
1	7.89	19.3	1109	14.2	130	111
2	7.90	19.9	1105	14.1	100	128
3	7.86	20.1	1102	14.1	100	93
Avg	7.88	19.8	1105	14.1	110	111
Std Dev.	0.02	0.4	4	0.1	17	18
Mix	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)

1	7.81	21.7	1109	14.0	100	133
2	7.83	22.1	1080	14.0	130	136
3	7.85	22.1	1089	14.2	100	179
Avg	7.83	22.0	1093	14.1	110	149
Std Dev.	0.02	0.2	15	0.1	17	26
Control	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)
1	8.18	19.2	1132	14.8	100	182
2	8.20	19.4	1127	14.5	130	156
3	8.18	19.5	1171	14.2	90	160
Avg	8.19	19.4	1143	14.5	107	166
Std Dev.	0.01	0.2	24	0.3	21	14
Parameters: 7/20/17 10:00 AM						
L.	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)
1	7.87	20.7	1057	15.8	60	38
2	7.89	21.0	1048	16.0	100	37
3	7.87	21.4	1056	15.9	60	41
Avg	7.88	21.0	1054	15.9	73	39
Std Dev.	0.01	0.4	5	0.1	23	2
Mix	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)
1	7.83	22.5	1050	15.8	60	107
2	7.85	23.5	1044	15.6	60	97
3	7.86	23.9	1031	15.9	80	118
Avg	7.85	23.3	1042	15.8	67	107
Std Dev.	0.02	0.7	10	0.2	12	11
Control	pH	DO (mg/L)	EC(uS)	Temp (°C)	Evap. Vol. (mL)	Turbidity (FAU)
1	8.22	20.8	1030	16.7	120	128
2	8.22	21.3	1016	16.5	170	121
3	8.20	21.2	1037	16.4	180	126
Avg	8.21	21.1	1028	16.5	157	125
Std Dev.	0.01	0.3	11	0.2	32	4

Table 7.2.2: Water quality analysis from Influent reservoir (influent reservoir) – continuous flow setup

IR1_7.31.17					
10:45 AM					
Sample	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
A	7.08	27.3	1141	22.8	254
B	7.09	27.1	1144	22.8	254
C	7.09	27.1	1145	22.8	260
Avg	7.09	27.2	1143	22.8	256

Std Dev.	0.01	0.12	2	0.0	3
IR2_7.31.17					
13:00:00 PM					
Sample	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
A	7.07	27.0	1142	23.0	179
B	7.07	26.4	1142	23.0	176
C	7.07	26.7	1143	23.0	182
Avg	7.07	26.7	1142	23.0	179
Std Dev.	0.00	0.3	1	0.0	3
IR3_8.01.17					
10:00 AM					
Sample	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
A	7.08	27.6	1046	22.8	176
B	7.06	27.9	1047	22.8	181
C	7.07	27.9	1047	22.8	177
Avg	7.07	27.8	1047	22.8	178
Std Dev.	0.01	0.2	1	0.0	3
IR4_8.01.17					
13:00:00 PM					
Sample	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
A	7.04	28.0	1057	23.5	232
B	7.05	27.9	1059	23.5	228
C	7.05	27.9	1059	23.5	234
Avg	7.05	27.9	1058	23.5	231
Std Dev.	0.01	0.1	1	0.0	3

Table 7.2.3: Trial 1&2 water quality analysis – continuous flow setup

pond_Eff_IR1_8.02.17					
10:30 AM					
	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
Mix	7.81	28.3	971	22.7	85
L.	7.77	28.8	1034	22.8	75
Control	8.04	29.2	1005	23.0	145
11:00 AM					
	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
Mix	7.70	34.2	1051	23.9	88

L.	7.74	33.9	1082	24.6	73
Control	7.93	30.9	1027	24.8	141
11:30 AM					
	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
Mix	7.74	30.4	1107	26.3	90
L.	7.75	30.4	1070	25.0	80
Control	7.96	30.5	1043	25.6	146
Mix					
avg	7.75	31.0	1043	24.3	88
std dev	0.06	3.0	68	1.8	3
L.					
avg	7.75	31.0	1062	24.1	76
std dev	0.02	2.6	25	1.2	4
Control					
avg	7.98	30.2	1025	24.5	144
std dev	0.06	0.9	19	1.3	3
pond_Eff_IR2_8.02.17					
12:40 PM	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
Mix	7.67	29.2	1195	30.0	80
L.	7.72	29.3	1154	28.5	73
Control	7.94	29.7	1063	28.8	143
1:10 PM					
Mix	7.74	29.7	1175	29.4	85
L.	7.68	29.8	1155	28.0	76
Control	7.93	30.6	1160	29.2	145
1:40 PM					
Mix	7.79	29.7	1182	29.3	80
L.	7.84	29.8	1143	27.5	69
Control	8.01	30.3	1121	29.0	148
Mix					
avg	7.73	29.5	1184	29.6	82
std dev	0.06	0.3	10	0.4	3
L.					
avg	7.75	29.6	1151	28.0	73
std dev	0.08	0.3	7	0.5	4
Control					
avg	7.96	30.2	1115	29.0	145
std dev	0.04	0.5	49	0.2	3

Table 7.2.4: Trial 3&4 Water quality analysis - continuous flow setup

pond_Eff_IR3_8.03.17					
9:35 AM					
	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
Mix	7.85	31.6	972	21.8	59
L.	7.89	32.3	977	21.3	59
Control	8.07	33.5	946	21.5	123
10:05 AM					
	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
Mix	7.79	31.4	1028	23.1	58
L.	7.82	33.4	1005	22.7	53
Control	7.97	34.6	991	22.6	121
10:35 AM					
	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
Mix	7.75	31.5	983	22.4	55
L.	7.75	32.8	961	22.4	49
Control	7.9	33.8	975	22.8	117
Mix					
avg	7.8	31.5	994	22.4	57
std dev	0.1	0.1	30	0.7	2
L.					
avg	7.8	32.8	981	22.1	54
std dev	0.1	0.6	22	0.7	5
Control					
avg	7.98	34.0	971	22.3	120
std dev	0.1	0.6	23	0.7	3
pond_Eff_IR4_8.03.17					
12:35 PM					
	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
Mix	7.83	30.4	1082	27.1	59
L.	7.83	30.9	1071	26.7	50
Control	7.9	31.1	1083	26.6	125
1:05 PM					
	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
Mix	7.79	31.5	1087	26.5	59
L.	7.76	31.6	1099	26.2	58
Control	7.8	31.1	1084	26.7	128

1:35 PM					
	pH	DO (mg/L)	EC (µS)	Temp (°C)	Turbidity (FAU)
Mix	7.81	31.1	1079	26.6	60
L.	7.78	31.9	1088	26.4	53
Control	7.82	32.5	1085	26.8	121
Mix					
avg	7.81	31.0	1083	26.7	59
std dev	0.02	0.6	4	0.3	1
L.					
avg	7.79	31.5	1086	26.4	54
std dev	0.0	0.5	14	0.3	4
Control					
avg	7.84	31.6	1084	26.7	125
std dev	0.1	0.8	1	0.1	4

6.3 Microbial analysis

Table 7.3.1: Spread plate counts of WW with 3M petrifilms – Batch setup

3M Counts: 7/17/17 11:00 AM (10^-2)							
AF2	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	121	70	191	7.00E+05	1.91E+06	5.85	6.28
B	102	57	159	5.70E+05	1.59E+06	5.76	6.20
C	113	77	190	7.70E+05	1.90E+06	5.89	6.28
Avg	112	68	180	6.80E+05	1.80E+06	5.83	6.25
Std Dev.	10	10	18	1.01E+05	1.82E+05	0.07	0.05
3M Counts: 7/18/17 10:00 AM (10^-2)							
L.	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
1	102	57	159	5.70E+05	1.59E+06	5.76	6.20
2	109	66	175	6.60E+05	1.75E+06	5.82	6.24
3	117	59	176	5.90E+05	1.76E+06	5.77	6.25
Avg	109	61	170	6.07E+05	1.70E+06	5.78	6.23
Std Dev.	8	5	10	4.73E+04	9.54E+04	0.03	0.02
Mix	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
1	120	63	183	6.30E+05	1.83E+06	5.80	6.26
2	91	76	167	7.60E+05	1.67E+06	5.88	6.22
3	82	80	162	8.00E+05	1.62E+06	5.90	6.21

Avg	98	73	171	7.30E+05	1.71E+06	5.86	6.23
Std Dev.	20	9	11	8.89E+04	1.10E+05	0.05	0.03
Control	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
1	58	40	98	4.00E+05	9.80E+05	5.60	5.99
2	68	68	136	6.80E+05	1.36E+06	5.83	6.13
3	56	63	119	6.30E+05	1.19E+06	5.80	6.08
Avg	61	57	118	5.70E+05	1.18E+06	5.74	6.07
Std Dev.	6	15	19	1.49E+05	1.90E+05	0.12	0.07
3M Counts: 7/19/17 11:00 AM (1:121)							
L.	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
1	27	23	50	2.78E+05	6.05E+05	5.44	5.78
2	22	30	52	3.63E+05	6.29E+05	5.56	5.80
3	45	31	76	3.75E+05	9.20E+05	5.57	5.96
Avg	31	28	59	3.39E+05	7.18E+05	5.53	5.85
Std Dev.	12	4	14	5.27E+04	1.75E+05	0.07	0.10
Mix	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
1	46	43	89	5.20E+05	1.08E+06	5.72	6.03
2	32	35	67	4.24E+05	8.11E+05	5.63	5.91
3	36	43	79	5.20E+05	9.56E+05	5.72	5.98
Avg	38	40	78	4.88E+05	9.48E+05	5.69	5.97
Std Dev.	7	5	11	5.59E+04	1.33E+05	0.05	0.06
Control	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
1	33	33	66	3.99E+05	7.99E+05	5.60	5.90
2	32	29	61	3.51E+05	7.38E+05	5.55	5.87
3	26	36	62	4.36E+05	7.50E+05	5.64	5.88
Avg	30	33	63	3.95E+05	7.62E+05	5.60	5.88
Std Dev.	4	4	3	4.25E+04	3.20E+04	0.05	0.02
3M Counts: 7/20/17 10:00 AM (1:121)							
L.	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
1	3	3	6	3.63E+04	7.26E+04	4.56	4.86
2	2	1	3	1.21E+04	3.63E+04	4.08	4.56
3	4	0	4	0.00E+00	4.84E+04	0.00	4.68
Avg	3	1	4	1.61E+04	5.24E+04	4.32	4.70

Std Dev.	1	2	2	1.85E+04	1.85E+04	0.34	0.15
Mix	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
1	7	3	10	3.63E+04	1.21E+05	4.56	5.08
2	4	1	5	1.21E+04	6.05E+04	4.08	4.78
3	5	5	10	6.05E+04	1.21E+05	4.78	5.08
Avg	5	3	8	3.63E+04	1.01E+05	4.47	4.98
Std Dev.	2	2	3	2.42E+04	3.49E+04	0.36	0.17
Control	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
1	11	15	26	1.82E+05	3.15E+05	5.26	5.50
2	11	17	28	2.06E+05	3.39E+05	5.31	5.53
3	8	7	15	8.47E+04	1.82E+05	4.93	5.26
Avg	10	13	23	1.57E+05	2.78E+05	5.17	5.43
Std Dev.	2	5	7	6.40E+04	8.47E+04	0.21	0.15

Table 7.3.2: IDEXX Colilert MPN of WW – Batch setup

Intial WW IDEXX counts: 7/17/17 11:00 AM								
AF2	Lg Wells	Sm Wells	Lg Wells (F)	Sm Wells (F)	MPN (TC)	MPN (*E. coli*)	Log(MPN TC)	Log(MPN *E. coli*)
A	49	16	25	2	2.73E+07	3.64E+06	7.44	6.56
B	39	2	21	1	7.44E+06	2.79E+06	6.87	6.45
C	34	7	21	3	6.70E+06	3.05E+06	6.83	6.48
Avg	41	8	22	2	1.38E+07	3.16E+06	7.14	6.50
Std Dev.	8	7	2	1	1.17E+07	4.36E+05	0.34	0.06
Final WW IDEXX counts: 7/20/17 11:00 AM								
L.	Lg Wells	Sm Wells	Lg Wells (F)	Sm Wells (F)	MPN (TC)	MPN (*E. coli*)	Log(MPN TC)	Log(MPN *E. coli*)
A	49	44	49	30	1.57E+08	6.19E+07	8.20	7.79
B	49	39	49	28	1.06E+08	5.53E+07	8.02	7.74
C	0	0	0	0	0.00E+00	0.00E+00	0.00	0.00
Avg	49	42	49	29	1.31E+08	5.86E+07	8.11	7.77
Std Dev.	0	4	0	1	3.62E+07	4.69E+06	0.12	0.03
Mix	Lg Wells	Sm Wells	Lg Wells (F)	Sm Wells (F)	MPN (TC)	MPN (*E. coli*)	Log(MPN TC)	Log(MPN *E. coli*)
A	4	1	3	0	6.36E+05	3.13E+05	5.80	5.50
B	1	0	0	0	1.01E+05	0.00E+00	5.00	0.00

C	7	3	3	1	1.08E+06	4.14E+05	6.03	5.62
Avg	4	1	2	0	6.06E+05	2.42E+05	5.61	3.70
Std Dev.	3	2	2	1	4.91E+05	2.16E+05	0.54	3.21
Control	Lg Wells	Sm Wells	Lg Wells (F)	Sm Wells (F)	MPN (TC)	MPN (*E. coli*)	Log(MPN TC)	Log(MPN *E. coli*)
A	6	1	0	0	7.47E+05	0.00E+00	5.87	0.00
B	22	4	7	0	3.39E+06	7.58E+05	6.53	5.88
C	14	0	5	0	1.63E+06	5.25E+05	6.21	5.72
Avg	14	2	4	0	1.92E+06	4.28E+05	6.21	3.87
Std Dev.	8	2	4	0	1.35E+06	3.88E+05	0.33	3.35

Table 7.3.3: Initial and final biomass microbial analysis with IDEXX Colilert method – Batch setup

Initial Biomass IDEXX counts: 7/17/17 11:00 AM								
AF2	Lg Wells	Sm Wells	Lg Wells (F)	Sm Wells (F)	MPN (TC)	MPN (*E. coli*)	Log(MPN TC)	Log(MPN *E. coli*)
L.	34	7	10	4	6.70E+06	1.55E+06	6.83	6.19
Mix	49	38	32	5	9.80E+07	5.73E+06	7.99	6.76
Final Biomass IDEXX counts: 7/20/17 11:00 AM								
L.	Lg Wells	Sm Wells	Lg Wells (F)	Sm Wells (F)	MPN (TC)	MPN (*E. coli*)	Log(MPN TC)	Log(MPN *E. coli*)
A	39	6	8	0	8.36E+06	8.60E+05	6.92	5.93
B	36	5	11	1	6.97E+06	1.34E+06	6.84	6.13
C	23	1	6	0	3.13E+06	6.30E+05	6.50	5.80
Avg	33	4	8	0	6.15E+06	9.43E+05	6.75	5.95
Std Dev.	9	3	3	1	2.71E+06	3.62E+05	0.23	0.16
Mix	Lg Wells	Sm Wells	Lg Wells (F)	Sm Wells (F)	MPN (TC)	MPN (*E. coli*)	Log(MPN TC)	Log(MPN *E. coli*)
A	31	1	11	1	4.79E+06	1.34E+06	6.68	6.13
B	49	48	3	1	TNTC	4.10E+05	TNTC	5.61
C	27	2	8	1	4.04E+06	9.70E+05	6.61	5.99
Avg	36	17	7	1	4.42E+06	9.07E+05	6.64	5.91
Std Dev.	12	27	4	0	5.30E+05	4.68E+05	0.05	0.27

Table 7.3.4: Spread plate counts of influent reservoir – Continuous flow setup

IR1_7.31.17							
10:45 AM							
Sample	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)

A	10	19	29	1.90E+06	2.90E+06	6.28	6.46
B	8	15	23	1.50E+06	2.30E+06	6.18	6.36
C	9	10	19	1.00E+06	1.90E+06	6.00	6.28
Avg	9	15	24	1.47E+06	2.37E+06	6.15	6.37
Std Dev.	1	5	5	4.51E+05	5.03E+05	0.14	0.09
IR2_7.31.17							
13:00:00 PM							
Sample	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	TNTC	TNTC	TNTC	N/A	N/A	N/A	N/A
B	TNTC	TNTC	TNTC	N/A	N/A	N/A	N/A
C	TNTC	TNTC	TNTC	N/A	N/A	N/A	N/A
Avg	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Std Dev.	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IR3_8.01.17							
10:00 AM							
Sample	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	61	30	91	3.00E+06	9.10E+06	6.48	6.96
B	44	25	69	2.50E+06	6.90E+06	6.40	6.84
C	55	31	86	3.10E+06	8.60E+06	6.49	6.93
Avg	53	29	82	2.87E+06	8.20E+06	6.46	6.91
Std Dev.	9	3	12	3.21E+05	1.15E+06	0.05	0.06
IR4_8.01.17							
13:00:00 PM							
Sample	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	79	37	116	3.70E+06	1.16E+07	6.57	7.06
B	106	34	140	3.40E+06	1.40E+07	6.53	7.15
C	92	50	142	5.00E+06	1.42E+07	6.70	7.15
Avg	92	40	133	4.03E+06	1.33E+07	6.60	7.12
Std Dev.	14	9	14	8.50E+05	1.45E+06	0.09	0.05

Table 7.3.5: Spread plate counts of pond effluent Trail 1&2 – continuous flow

pond_Eff_IR1_8.02.17							
10:30 AM							
Mix	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	24	16	40	1.60E+06	4.00E+06	6.20	6.60
B	26	16	42	1.60E+06	4.20E+06	6.20	6.62
C	22	13	35	1.30E+06	3.50E+06	6.11	6.54

11:00 AM							
Mix	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	21	7	28	7.00E+05	2.80E+06	5.85	6.45
B	14	12	26	1.20E+06	2.60E+06	6.08	6.41
C	30	11	41	1.10E+06	4.10E+06	6.04	6.61
11:30 AM							
Mix	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	40	19	59	1.90E+06	5.90E+06	6.28	6.77
B	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
C	29	23	52	2.30E+06	5.20E+06	6.36	6.72
Average	26	15	40	1.46E+06	4.04E+06	6.14	6.59
Std. Dev.	8	5	11	4.98E+05	1.12E+06	0.16	0.12
L.	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	7	5	12	5.00E+05	1.20E+06	5.70	6.08
B	7	8	15	8.00E+05	1.50E+06	5.90	6.18
C	8	9	17	9.00E+05	1.70E+06	5.95	6.23
L.	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	7	6	13	6.00E+05	1.30E+06	5.78	6.11
B	12	10	22	1.00E+06	2.20E+06	6.00	6.34
C	11	9	20	9.00E+05	2.00E+06	5.95	6.30
L.	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	9	9	18	9.00E+05	1.80E+06	5.95	6.26
B	17	9	26	9.00E+05	2.60E+06	5.95	6.41
C	11	7	18	7.00E+05	1.80E+06	5.85	6.26
Average	10	8	18	8.00E+05	1.79E+06	5.89	6.24
Std. Dev.	3	2	4	1.66E+05	4.40E+05	0.10	0.11
Control	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	21	12	33	1.20E+06	3.30E+06	6.08	6.52
B	20	17	37	1.70E+06	3.70E+06	6.23	6.57
C	13	9	22	9.00E+05	2.20E+06	5.95	6.34
Control	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	35	27	62	2.70E+06	6.20E+06	6.43	6.79
B	17	22	39	2.20E+06	3.90E+06	6.34	6.59

C	28	35	63	3.50E+06	6.30E+06	6.54	6.80
Control	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	17	12	29	1.20E+06	2.90E+06	6.08	6.46
B	15	15	30	1.50E+06	3.00E+06	6.18	6.48
C	15	12	27	1.20E+06	2.70E+06	6.08	6.43
Average	20	18	38	1.79E+06	3.80E+06	6.21	6.55
Std. Dev.	7	9	15	8.55E+05	1.48E+06	0.19	0.16
pond_Eff_IR2_8.02.17							
12:40 PM							
Mix	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	14	7	21	7.00E+05	2.10E+06	5.85	6.32
B	16	11	27	1.10E+06	2.70E+06	6.04	6.43
C	11	13	24	1.30E+06	2.40E+06	6.11	6.38
1:10 PM							
Mix	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	10	12	22	1.20E+06	2.20E+06	6.08	6.34
B	20	11	31	1.10E+06	3.10E+06	6.04	6.49
C	25	13	38	1.30E+06	3.80E+06	6.11	6.58
1:40 PM							
Mix	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	9	11	20	1.10E+06	2.00E+06	6.04	6.30
B	16	9	25	9.00E+05	2.50E+06	5.95	6.40
C	10	16	26	1.60E+06	2.60E+06	6.20	6.41
Avg	15	11	26	1.14E+06	2.60E+06	6.05	6.41
Std Dev.	5	3	6	2.55E+05	5.61E+05	0.10	0.09
L.	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	13	6	19	6.00E+05	1.90E+06	5.78	6.28
B	13	7	20	7.00E+05	2.00E+06	5.85	6.30
C	11	15	26	1.50E+06	2.60E+06	6.18	6.41
L.	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	10	4	14	4.00E+05	1.40E+06	5.60	6.15
B	4	5	9	5.00E+05	9.00E+05	5.70	5.95
C	7	16	23	1.60E+06	2.30E+06	6.20	6.36

L.	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	11	5	16	5.00E+05	1.60E+06	5.70	6.20
B	6	10	16	1.00E+06	1.60E+06	6.00	6.20
C	9	15	24	1.50E+06	2.40E+06	6.18	6.38
Average	9	9	19	9.22E+05	1.86E+06	5.91	6.25
Std. Dev.	3	5	5	4.89E+05	5.39E+05	0.23	0.14
Control	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	9	7	16	7.00E+05	1.60E+06	5.85	6.20
B	11	15	26	1.50E+06	2.60E+06	6.18	6.41
C	11	16	27	1.60E+06	2.70E+06	6.20	6.43
Control	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	9	22	31	2.20E+06	3.10E+06	6.34	6.49
B	18	16	34	1.60E+06	3.40E+06	6.20	6.53
C	17	17	34	1.70E+06	3.40E+06	6.23	6.53
Control	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	20	14	34	1.40E+06	3.40E+06	6.15	6.53
B	22	13	35	1.30E+06	3.50E+06	6.11	6.54
C	15	15	30	1.50E+06	3.00E+06	6.18	6.48
Average	15	15	30	1.50E+06	2.97E+06	6.16	6.46
Std. Dev.	5	4	6	3.94E+05	6.06E+05	0.13	0.11

Table 7.3.6: Spread plate counts of pond effluent Trail 3&4 – continuous flow setup

pond_Eff_IR3_8.03.17							
9:35 AM							
Mix	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	23	19	42	1.90E+06	4.20E+06	6.28	6.62
B	38	10	48	1.00E+06	4.80E+06	6.00	6.68
C	35	13	48	1.30E+06	4.80E+06	6.11	6.68
10:05 AM							
Mix	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	32	10	42	1.00E+06	4.20E+06	6.00	6.62
B	39	13	52	1.30E+06	5.20E+06	6.11	6.72
C	36	20	56	2.00E+06	5.60E+06	6.30	6.75

10:35 AM							
Mix	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	29	19	48	1.90E+06	4.80E+06	6.28	6.68
B	25	18	43	1.80E+06	4.30E+06	6.26	6.63
C	34	14	48	1.40E+06	4.80E+06	6.15	6.68
Average	32	15	47	1.51E+06	4.74E+06	6.17	6.67
Std. Dev.	6	4	5	3.95E+05	4.67E+05	0.12	0.04
L.	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	45	10	55	1.00E+06	5.50E+06	6.00	6.74
	10	24	34	2.40E+06	3.40E+06	6.38	6.53
	28	8	36	8.00E+05	3.60E+06	5.90	6.56
L.	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	31	12	43	1.20E+06	4.30E+06	6.08	6.63
	41	8	49	8.00E+05	4.90E+06	5.90	6.69
	16	47	63	4.70E+06	6.30E+06	6.67	6.80
L.	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	21	15	36	1.50E+06	3.60E+06	6.18	6.56
	23	15	38	1.50E+06	3.80E+06	6.18	6.58
	19	7	26	7.00E+05	2.60E+06	5.85	6.41
	26	16	42	1.62E+06	4.22E+06	6.13	6.61
	11	13	12	1.27E+06	1.16E+06	0.27	0.12
Control	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	37	13	50	1.30E+06	5.00E+06	6.11	6.70
	34	19	53	1.90E+06	5.30E+06	6.28	6.72
	36	15	51	1.50E+06	5.10E+06	6.18	6.71
Control	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	62	18	80	1.80E+06	8.00E+06	6.26	6.90
	47	26	73	2.60E+06	7.30E+06	6.41	6.86
	29	28	57	2.80E+06	5.70E+06	6.45	6.76
Control	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	44	20	64	2.00E+06	6.40E+06	6.30	6.81
	33	33	66	3.30E+06	6.60E+06	6.52	6.82

	43	22	65	2.20E+06	6.50E+06	6.34	6.81
	41	22	62	2.16E+06	6.21E+06	6.32	6.79
	10	6	10	6.42E+05	1.03E+06	0.13	0.07
pond_Eff_IR4_8.03.17							
12:35 PM							
Mix	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	42	27	69	2.70E+06	6.90E+06	6.43	6.84
B	38	18	56	1.80E+06	5.60E+06	6.26	6.75
C	44	21	65	2.10E+06	6.50E+06	6.32	6.81
1:05 PM							
Mix	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	44	22	66	2.20E+06	6.60E+06	6.34	6.82
B	38	25	63	2.50E+06	6.30E+06	6.40	6.80
C	41	14	55	1.40E+06	5.50E+06	6.15	6.74
1:35 PM							
Mix	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
A	39	21	60	2.10E+06	6.00E+06	6.32	6.78
B	36	25	61	2.50E+06	6.10E+06	6.40	6.79
C	48	23	71	2.30E+06	7.10E+06	6.36	6.85
Avg	41	22	63	2.18E+06	6.29E+06	6.33	6.80
Std Dev.	4	4	5	3.96E+05	5.46E+05	0.09	0.04
L.	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	9	7	16	7.00E+05	1.60E+06	5.85	6.20
	18	12	30	1.20E+06	3.00E+06	6.08	6.48
	16	10	26	1.00E+06	2.60E+06	6.00	6.41
L.	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	37	14	51	1.40E+06	5.10E+06	6.15	6.71
	30	18	48	1.80E+06	4.80E+06	6.26	6.68
	43	24	67	2.40E+06	6.70E+06	6.38	6.83
L.	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	45	14	59	1.40E+06	5.90E+06	6.15	6.77
	32	18	50	1.80E+06	5.00E+06	6.26	6.70
	37	19	56	1.90E+06	5.60E+06	6.28	6.75
	30	15	45	1.51E+06	4.48E+06	6.15	6.61

	13	5	17	5.18E+05	1.69E+06	0.16	0.20
Control	Red	E. coli	TC	E. coli*P (CFU/100mL)	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	50	23	73	2.30E+06	7.30E+06	6.36	6.86
	28	31	59	3.10E+06	5.90E+06	6.49	6.77
	36	31	67	3.10E+06	6.70E+06	6.49	6.83
Control	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	45	22	67	2.20E+06	6.70E+06	6.34	6.83
	56	25	81	2.50E+06	8.10E+06	6.40	6.91
	46	33	79	3.30E+06	7.90E+06	6.52	6.90
Control	Red	*E. coli*	TC	*E. coli*P (CFU/100mL)*	TC*P (CFU/100mL)	log (E. coli*P)	log (TC*P)
	41	34	75	3.40E+06	7.50E+06	6.53	6.88
	33	9	42	9.00E+05	4.20E+06	5.95	6.62
	23	16	39	1.60E+06	3.90E+06	6.20	6.59
	40	25	65	2.49E+06	6.47E+06	6.37	6.80
	11	8	15	8.42E+05	1.53E+06	0.19	0.12

6.4 Duckweed drying microbial analysis

Table 7.4.1: Spread plate counts of DW biomass after drying at ambient temperatures

L. biomass analysis							
Day 1	Red	E. coli	TC	E. coli*P (CFU/g)	TC*P (CFU/g)	log (E. coli*P)	log (TC*P)
A	1.00E+08	1.00E+06	1.00E+08	1.00E+08	1.00E+10	8.00	10.00
B	1.00E+08	1.00E+06	1.00E+08	1.00E+08	1.00E+10	8.00	10.00
C	1.00E+08	1.00E+06	1.00E+08	1.00E+08	1.00E+10	8.00	10.00
Avg	1.00E+08	1.00E+06	1.00E+08	1.00E+08	1.00E+10	8.00	10.00
Std Dev.	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00	0.00
Day 2	Red	E. coli	TC	E. coli*P (CFU/g)	TC*P (CFU/g)	log (E. coli*P)	log (TC*P)
A	7	10	17	1.00E+03	1.70E+03	3.00	3.23
B	11	14	25	1.40E+03	2.50E+03	3.15	3.40
C	10	15	25	1.50E+03	2.50E+03	3.18	3.40
Avg	9	13	22	1.30E+03	2.23E+03	3.11	3.34

Std Dev.	2	3	5	2.65E+02	4.62E+02	0.09	0.10
Day 3	Red	E. coli	TC	E. coli*P (CFU/g)	TC*P (CFU/g)	log (E. coli*P)	log (TC*P)
A	26	27	53	2.70E+03	5.30E+03	3.43	3.72
B	34	25	59	2.50E+03	5.90E+03	3.40	3.77
C	14	24	38	2.40E+03	3.80E+03	3.38	3.58
Avg	25	25	50	2.53E+03	5.00E+03	3.40	3.69
Std Dev.	10	2	11	1.53E+02	1.08E+03	0.03	0.10
Day 4	Red	E. coli	TC	E. coli*P (CFU/g)	TC*P (CFU/g)	log (E. coli*P)	log (TC*P)
A	432	144	576	1.44E+04	5.76E+04	4.16	4.76
B	520	184	704	1.84E+04	7.04E+04	4.26	4.85
C	404	136	540	1.36E+04	5.40E+04	4.13	4.73
Avg	452	155	607	1.55E+04	6.07E+04	4.19	4.78
Std Dev.	61	26	86	2.57E+03	8.62E+03	0.07	0.06
Mix biomass analysis							
Day 1	Red	E. coli	TC	E. coli*P (CFU/g)	TC*P (CFU/g)	log (E. coli*P)	log (TC*P)
A	1.00E+08	1.00E+06	1.00E+08	1.00E+08	1.00E+10	8.00	10.00
B	1.00E+08	1.00E+06	1.00E+08	1.00E+08	1.00E+10	8.00	10.00
C	1.00E+08	1.00E+06	1.00E+08	1.00E+08	1.00E+10	8.00	10.00
Avg	1.00E+08	1.00E+06	1.00E+08	1.00E+08	1.00E+10	8.00	10.00
Std Dev.	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00	0.00
Day 2	Red	E. coli	TC	E. coli*P (CFU/g)	TC*P (CFU/g)	log (E. coli*P)	log (TC*P)
A	20	24	44	2.40E+03	4.40E+03	3.38	3.64
B	11	16	27	1.60E+03	2.70E+03	3.20	3.43
C	10	15	25	1.50E+03	2.50E+03	3.18	3.40
Avg	14	18	32	1.83E+03	3.20E+03	3.25	3.49
Std Dev.	6	5	10	4.93E+02	1.04E+03	0.11	0.13
Day 3	Red	E. coli	TC	E. coli*P (CFU/g)	TC*P (CFU/g)	log (E. coli*P)	log (TC*P)
A	34	35	69	3.50E+03	6.90E+03	3.54	3.84
B	45	40	85	4.00E+03	8.50E+03	3.60	3.93
C	39	41	80	4.10E+03	8.00E+03	3.61	3.90
Avg	39	39	78	3.87E+03	7.80E+03	3.59	3.89
Std Dev.	6	3	8	3.21E+02	8.19E+02	0.04	0.05

Day 4	Red	E. coli	TC	E. coli*P (CFU/g)	TC*P (CFU/g)	log (E. coli*P)	log (TC*P)
A	450	77	527	7.70E+03	5.27E+04	3.89	4.72
B	476	100	576	1.00E+04	5.76E+04	4.00	4.76
C	668	172	840	1.72E+04	8.40E+04	4.24	4.92
Avg	531	116	648	1.16E+04	6.48E+04	4.04	4.80
Std Dev.	119	50	168	4.96E+03	1.68E+04	0.18	0.11