Tracking the Diurnal Variability of Organic Constituents in Anaerobic DEWATS with an Emphasis on Scum/FOG Accumulation

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ABSTRACT

The Anaerobic Baffled Reactor is a highly promising Decentralised Wastewater Treatment System (DEWATS) for nonpotable water purposes. However, two major issues that inhibit its performance are highly variable organic matter (OM) concentrations in wastewater influent, and excessive scum accumulation due to high FOG concentrations. The two experiments conducted for this study, Post Scum Removal and Diurnal Variability, aimed to find trends in daily variability with respect to FOG and scum accumulation, and to better understand the impact that FOG and scum accumulation has on OM degradation in the DEWATS. ABR performance was determined with temporal in-situ fluorescence measurements of tryptophan-like (TRP) and fulvic acid-like (CDOM) compounds using a C3 Submersible Fluorometer, and chemical oxygen demand (COD) concentrations. The C3 Submersible Fluorometer has been used in other studies at the Newlands Mashu Research Site, and has correlated well with the gold standard benchtop fluorometer. This study found that the variability of OM-laden wastewater was exacerbated by high FOG concentrations as well as scum accumulation and removal. COD measurements and fluorescence measurements often correlated, but greatly varied by chamber and with respect to scum accumulation and removal. If scum accumulation and removal are not properly managed, then the ABR will not biodegrade OM as efficiently. Scum accumulation can become so excessive that it infiltrates the ABR chambers, further affecting OM biodegradation. This phenomenon was observed in this study. Appropriate and individualized operation and maintenance strategies should be implemented for DEWATS to monitor OM and FOG concentrations as well as scum accumulation.

1. INTRODUCTION

Unimproved sanitation systems are prevalent in many rural and developing areas around the world. DEWATS can provide practical solutions for many sanitation issues. The Anaerobic Baffled Reactor (ABR) is a specific type of DEWATS that is low cost, low energy-usage, and low maintenance (Mladenov et al. 2017, in revision). The ABR is often paired with post-treatment processes such as Anaerobic Filters (AF) and Constructed Wetlands (CW) which further improve the wastewater effluent (Bigelow et al. 2017, in press). ABRs are also beneficial due to their biogas generation capability, infrequent sludge disposal, and their ability to produce water suitable for irrigation and other nonpotable purposes (Mladenov et al., in revision). Additionally, ABRs with a shorter average Hydraulic Retention Time (HRT), much like the ABR at the Newlands Mashu Research Site, have been shown to greatly reduce COD at values as high as 90% to 98% removal (Nachaiyasit and Stuckey 1997). Many variables are involved with the ABR and its post-treatment processes because it is a natural, gravity-fed anaerobic system. The ABR degrades OM efficiently under a wide, and often fluctuating, range of daily organic loadings (Foxon et al., 2004), but problems can arise from highly variable wastewater influent. The wastewater entering the Newlands Mashu DEWATS is in fact highly variable in terms of OM. The source of the site's wastewater is a peri-urban community that is speculated to have a largely meat-based diet (Pietruschka et al., 2015). High meat consumption contributes considerable concentrations of FOG to wastewater influent and is largely responsible for rapid and/or excessive scum accumulation (Pietruschka et al., 2015). The physical properties of FOG present problems for the ABR treatment process (Gutterer et al., 2009).

The motivation for this study stems from the consequences that can result from highly variable OM-laden wastewater influent. The ABR has a resilience to spikes in OM concentrations, but there is a level at which the degradation of OM begins to be less effective. The extent to which FOG, scum accumulation, and scum removal attribute to this decrease was another motivation for this study. According to Cammarota and Freire, high FOG concentrations in influent have caused lower OM removal efficiencies in anaerobic wastewater treatment systems (2006). Ziels et al. (2016) states that variable wastewater influent directly affects microbial community structure and biogas formation for anaerobic wastewater treatment systems. The anaerobic bacteria present in DEWATS are greatly impacted by the variable organic matter loadings present in the influent. Sudden spikes of OM concentrations, also known as organic shock loads, can impair the biodegradation rates of anaerobic systems for anywhere from a few days to a few weeks (Nachaiyasit and Stuckey 1997). The types of organics in the influent are a key determinant of the structural changes made to the microbial community.

High concentrations of FOG shifts microbial community structure, and can cause complete system failure in high enough concentrations (Ziels et al., 2016). FOG particles can infiltrate the ABR chambers which can disrupt microbial communities throughout the DEWATS (Pietruschka et al., 2015). Long-chain fatty acids (LCFA) are found in oils and are particularly dangerous for anaerobic systems. LCFA absorb to cell surfaces and "lead to direct toxicity and/or substrate transport limitations" for the bacteria in anaerobic systems (Ziels et al., 2016). High LCFA concentrations have been shown to cause excessive sludge floatation (scum) along with system clogging and unpleasant odors (Cammarota and Freire 2006). All three of these issues were observed in Street 1 and Street 2 of the Newlands Mashu Research Site. Jeganathan et al. (2006) found that scum formation and sludge washout can result from relatively low influent FOG concentrations, so scum accumulation occurs naturally. Scum-related issues arise only when the FOG and/or scum accumulation becomes excessive in the influent and/or settling chambers.

Some key theoretical principles of this study include the biodegradation of OM by anaerobic bacteria, and the problems caused by LCFA and FOG for the bacteria. The microorganisms present in the sludge blanket of the ABR chemically break down OM in wastewater. Each type of bacteria is suited to breakdown a different type of OM; Many types of bacteria are inhibited by highly variable loadings of OM and high concentrations of LCFA and FOG. The microbial community structure present in sludge tends to shift depending on the OM present in the wastewater influent (Ziels et al., 2016). High and variable OM concentrations can cause dramatic shifts and die-offs of the microbial community, and very low OM loading rates can result in the microorganisms eating each other (Gutterer et al., 2009). Longer carbon chains are difficult for many microorganisms to break down, specifically Carbon-8 to Carbon-13 (Pietruschka et al., 2015). LCFA in particular can cause major problems for the microbial community, and high concentrations of LCFA have been shown to cause complete system failure (Pietruschka et al., 2015). Another theoretical principle involved with this study involves TRP and CDOM. TRP and CDOM are not directly measured by the in-situ fluorometer. These two compounds were chosen based on the types of OM that are commonly found in domestic wastewater influent. TRP is an aromatic amino acid that has been positively correlated to E. coli concentrations, BOD, and much more (Bigelow et al., in press). "Higher TRP-like concentrations signaled a larger

microbial biomass presence and therefore higher biodegradation rates occurring" (Bigelow et al., in press). CDOM represents the larger, more recalcitrant OM such as humic and fulvic acids.

Two experiments were performed for this study: Post Scum Removal and Diurnal Variability. The main purpose of the Post Scum Removal experiment was to better understand the impact that FOG and scum accumulation in the settling chambers have on OM degradation in the ABR and AF chambers. This was achieved through temporal monitoring of TRP-like (TRP) and humic-like (CDOM) fluorescence paired with temporal total COD concentrations. The main purpose of the Diurnal Variability experiment was to discover daily trends of OM in the highly variable wastewater influent and how this impacts OM degradation. This was achieved using in-situ fluorescence and COD concentrations as well, but in a different manner. Both studies revolved around scum removal events.

2. METHODS

Street 3 was analyzed exclusively due to blockage issues with streets 1 and 2. Street 3 consists of four Anaerobic Baffled Reactor (ABR) chambers, two Anaerobic Filter (AF) chambers, and an initial settling chamber (Settler 1A). These streets and chambers are shown in Figure 1 below. Streets 1 and 2 differ from Street 3 because they have seven ABR chambers instead of four.

Figure 1: The Anaerobic DEWATS at the Newlands-Mashu Research Site

The Post Scum Removal experiment involved daily fluorescence measurements for the following chambers: Settler 1A, ABR 1, ABR 2, ABR 3, ABR 4 and AF 2. Grab samples of wastewater were taken from Settler 1A, ABR 1, and AF 2 daily for COD analysis. The collection times ranged from 11:00am to 2:30pm depending on the circumstances presented each day. The floating scum layer was pushed aside in order to collect the wastewater samples. Photographs of the scum layers of Settler 1A and ABR 1 can be found in Appendix 8.2, Figures A1 and A2. The experimental period began on July 12th and ended on July 20th with scum removal occurring on July 12th. The scum was removed from the settling chambers in the morning from approximately

9am to 11am, and the first samples were collected about one hour after scum removal was complete. Day 0 represents the day of scum removal and the initial day of the experiment. The data from Day 0 is considered the baseline for this experiment, since no fluorescence data was able to be collected on the day prior to scum removal. Days 3 and 4 were not included in the experiment because they fell on a weekend. Table 1 shows a visual timeline of the experimental period. Data was collected on the dates that are bolded.

Table 1: Timeline for the Post Scum Removal Experiment

July 12	**July 13**	**July 14**	July 15	July 16	**July 17**	**July 18**	**July 19**	**July 20**
Day 0 (W)	Day 1 (Th)	Day 2 (F)	Day 3 (Sat)	Day 4 (Sun)	Day 5 (M)	Day 6 (T)	Day 7 (W)	Day 8 (Th)
Scum Removal	Day 1 After Removal	Day 2 After Removal	No Data	No Data	Day 5 After Removal	Day 6 After Removal	Day 7 After Removal	Day 8 After Removal

The Diurnal Variability experiment involved eight in-situ fluorescence measurements per day for ABR 1 and AF 2. Measurements occurred at 45-minute intervals starting at 9:30am and ending at 2:45pm. This experiment lasted for four total days (July 24th, 25th, 27th, and 31st) with scum removal occurring on July 26th. The experimental period falls on two days before (July 24th and 25th) and two days after (July 27th and July 31st) scum removal, but not on the day of removal.
Grab samples of wastewater were taken from ABR 1 and AF 2 three times per day at 9:30am, 11:45am, and 2:00pm for COD analysis. Table 2 shows a visual timeline of the experimental period. Data was collected on the dates that are bolded in the table.

Table 2: A Visual Timeline for the Diurnal Variability Experiment

July 24	**July 25**	July 26	**July 27**	July 28	July 29	July 30	**July 31**
1st day of data collection	2nd day of data collection	Scum removal (Wed)	3rd day of data collection	No data collection (Fri)	No data collection (Sat)	No data collection (Sun)	4th day of data collection

2.1 IN-SITU FLUORESCENCE

2.1.1 Sample Collection

Fluorescence measurements were collected once per day, at approximately midday for the Post Scum Removal experiment. The samples were measured as soon as possible with respect to their collection time. Due to complications with the 30-foot long Extender Cable©, a shorter Continuous Data Cable© was used to lower the fluorometer into the wastewater. Due to the short length of the cable and the location of the power source, the *Sample Displacement Method* was created (see below).

Approximately eight liters of sample was collected from the DEWATS chambers for fluorescence measurements. The samples were collected from the top 0.5 meters, and nearest to the middle, of the chambers. Any floating scum that infiltrated the chambers (mainly in ABR 1 and Settler 1A) was moved aside before sample collection to prevent large solids from interfering with the optical sensors on the fluorometer. The samples were placed into plastic buckets and then carried to the designated measuring area. This location was shaded to avoid the interference of sunlight with the optical sensors and the temperature readings.

The same procedure was implemented for the Diurnal Variability Experiment but the frequency of sampling times was increased. Eight samples each were collected from ABR 1 and AF 2 for the Diurnal Variability experiment.

2.1.2 Experimental Procedure and Analysis

After sample collection, the buckets were lined up starting with AF 2 and ending with Settler 1A. After all cables were joined successfully, the fluorometer was connected to the laptop and the C-Soft software was opened. The fluorometer was then lowered into the bucket containing the sample. The fluorometer was held with zip ties for stability purposes (See Appendix 8.2, Figure A3). The fluorometer was agitated upon insertion to eliminate any air bubbles trapped in the Shade Cap©. Air bubbles can interfere with the optical sensors and introduce error to the fluorescence data. After agitation, the fluorometer was held as motionless as possible for two minutes while the C-Soft program collected fluorescence data for TRP-like and humic-like OM in Relative Fluorescence Units (RFU). After two minutes and a 15-second buffer, data collection was stopped and the fluorometer was removed from the bucket. The fluorometer was rinsed with tap water and the data file was saved. These steps were repeated until Settler 1A was measured, making sure to rinse the fluorometer before measuring each wastewater sample.

Averages for TRP and CDOM were found for each chamber in RFU for a two-minute measuring interval. The first five data points were omitted due to a calibration procedure performed by the fluorometer upon insertion in the samples. Fluorescence values were also converted from RFU to mg/L. The equations necessary for this conversion were developed from Bigelow et al. (in press). Bigelow et al. (in press) used "commercially available tryptophan (Sigma Aldrich, L-Tryptophan, reagent grade) and Suwanee River (International Humic Substances Society) CDOM" to make the calibration, and the following equations resulted for TRP (1) and Suwanee River CDOM (2):

$$\mathbf{Y} = 0.0025\mathbf{X} - 0.4732 \qquad \textbf{(1)}$$

$$\mathbf{Y} = 0.0093\mathbf{X} - 1.052 \qquad \textbf{(2)}$$

where **X** represents the data in RFU and **Y** represents the data in mg/L. These equations were used to convert all fluorescence data from this project into mg/L.

2.2 CHEMICAL OXYGEN DEMAND (COD)

2.2.1 Sample Collection

For both experiments, COD grab samples were collected from the same buckets used for fluorescence measurements. Collection time of the grab samples occurred immediately after the conclusion of fluorescence measurements. Samples were collected in 50 mL plastic tubes and at least 25 mL of sample was collected. Due to a malfunction of the COD digester, the samples could not be analyzed immediately after collection; all samples were kept in a refrigerator with a preservative solution until the digestion process could proceed.

2.2.2 Experimental Procedure and Analysis

The Spectroquant® Solution A and B method was used according to the standard operating procedure for the range of 100-1500 mg/L COD. After the samples were prepared, they were digested and then analyzed in a spectrophotometer.

The MERCK digester was heated to 148 ^{o}C before preparing the samples for COD analysis. Empty glass vials were labeled accordingly. 0.3 mL of solution A, 2.3 mL of solution B, and 3 mL of sample were added to each vial. A blank was prepared once per day for calibration purposes; 3 mL of deionized water instead of 3 mL of sample was used to create a blank. Standardized potassium hydrogen phthalate (KHP) solution was used to make the COD standard curve. Standards were with the following concentrations of KHP: 100, 300, 600, 900, 1200 and 1500 mg/L (See Appendix 8.2, Figure A4). These standards were prepared in the same way as the samples (3 mL of standard in each vial).

After all vials were prepared and lids were fastened, the vials were mixed vigorously for 10 seconds and then placed into the digester for 2 hours. After digestion was complete, the vials were set aside to cool before being placed in the LASEC spectrophotometer. The spectrophotometer was set to mode 51 and a wavelength of 605 nm. Before any analysis could proceed, the machine was "zeroed" and the blank would read an emission of 0.000 and a transmission of 100.0%. All samples, including the blanks, were wiped with a Kim wipe before analysis began. Every sample was analyzed individually in the spectrophotometer and then the blank was re-analyzed to make sure the readings had not diverged too far from an emission of 0.000 and a transmission of 100.0%. All vials were measured in triplicate. After the emissions of the standards were found, a calibration curve was created with the following equation:

$$\mathbf{Y} = 0.0006\mathbf{X} + 0.0133, \qquad \mathbf{(3)}$$

where the emission (**Y**) of each sample was used to find the concentration of COD (**X**) for the samples. Please refer to the standard curve in Appendix 8.2, Figure A5.

3.1 RESULTS FOR POST SCUM REMOVAL EXPERIMENT

Results for fluorescence are displayed in units of RFU in the following figures. Any future reference to concentrations of CDOM in mg/L actually represents humic fluorescence as mg/L of

Suwanee River CDOM. Any future reference to concentrations of TRP in mg/L actually represents TRP-like fluorescence as mg/L of TRP.

Scum was removed from Settler 1A on the first day of the experimental period (Day 0). Shortly after scum removal occurred, in-situ fluorescence was measured throughout the DEWATS chambers. Figure 2 shows data for TRP and CDOM concentrations and fluorescence for the following chambers: Settler 1A, ABR 1, ABR 2, ABR 3, and AF 2. ABR 4 and AF 1 were not measured. The average concentrations and fluorescence units of TRP and CDOM on Day 0 are similar in terms of their general trend throughout the measured chambers (See Figure 2). The large decrease from Settler 1A to ABR 1, and the larger decrease from ABR 3 to AF 2, are both observed for TRP and CDOM. Additionally, TRP and CDOM both gradual increase from ABR 1 to ABR 3. All points of intersection observed in the figures are coincidental and are attributed to using a secondary axis for TRP.

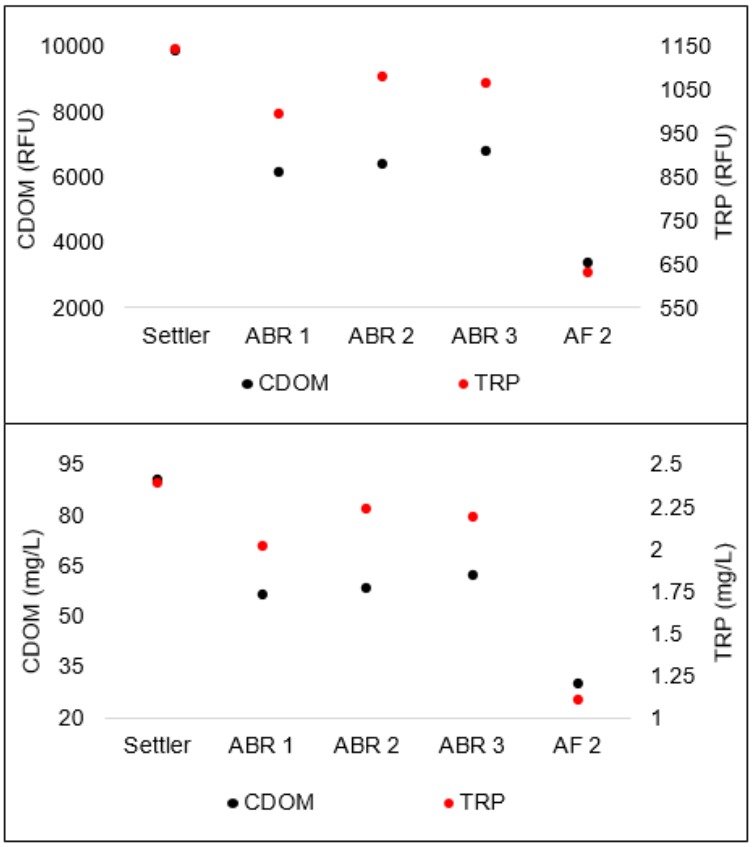

Figure 2: TRP and CDOM fluorescence data in RFU (top) and mg/L (bottom) on Day 0

These trends observed on Day 0 are considered to be the baseline for this experiment; Fluorescence values of TRP and CDOM are assumed to represent typical fluorescence values for these chambers. On Day 1 (the first day after scum removal), CDOM and TRP fluorescence values show an overall increased in RFU, and data from Settler 1A differs the most from the values observed on Day 0. Despite these differences, the general trend is quite similar to the

baseline. Day 1 measurements included ABR 4, and ABR 4 is included in all measurements from Day 1 to Day 8. Figure 3 shows the fluorescence data collected on Day 1.

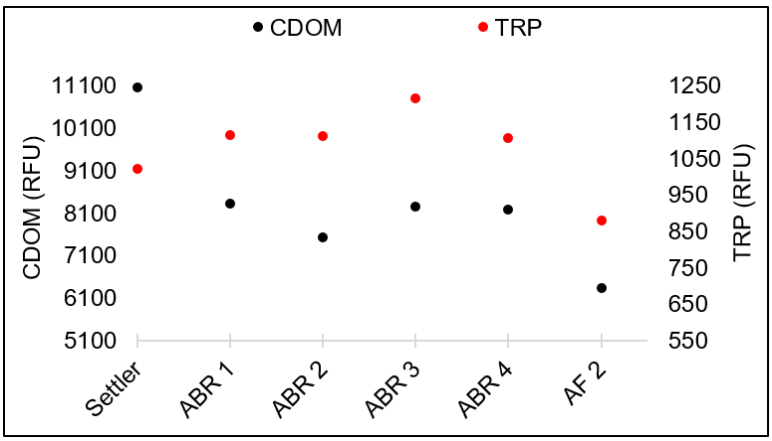

Figure 3: Average Fluorescence Values on Day 1 of TRP and CDOM

On Day 2 (the second day after scum removal), the TRP fluorescence values did not vary much throughout the chambers. CDOM fluorescence reached the lowest value observed in the entire experimental period in AF 2; the values in the other chambers aligned with the general trend. No data was collected on days 3 and 4. On Day 5 (the fifth day after scum removal), CDOM and TRP fluorescence both increase from ABR 4 to AF 2; This phenomenon is only observed on Day 5. On Day 6, the values for TRP and CDOM in Settler 1A are the lowest observed in the Settler for all days. On Day 7, TRP remains relatively constant for all chambers except AF 2 where a sharp decrease from ABR 4 to AF 2 is observed. Additionally on Day 7, CDOM fluorescence is lower in Settler 1A than in ABR 1; This phenomenon also occurs for TRP each day except for Day 0 and Day 8. On Day 8, both CDOM and TRP begin to resemble the baseline trend observed on Day 0; The values are much more variable, but a similar general trend is still present. Figure 4 shows the average TRP and CDOM fluorescence values in RFU from Day 2 to Day 8.

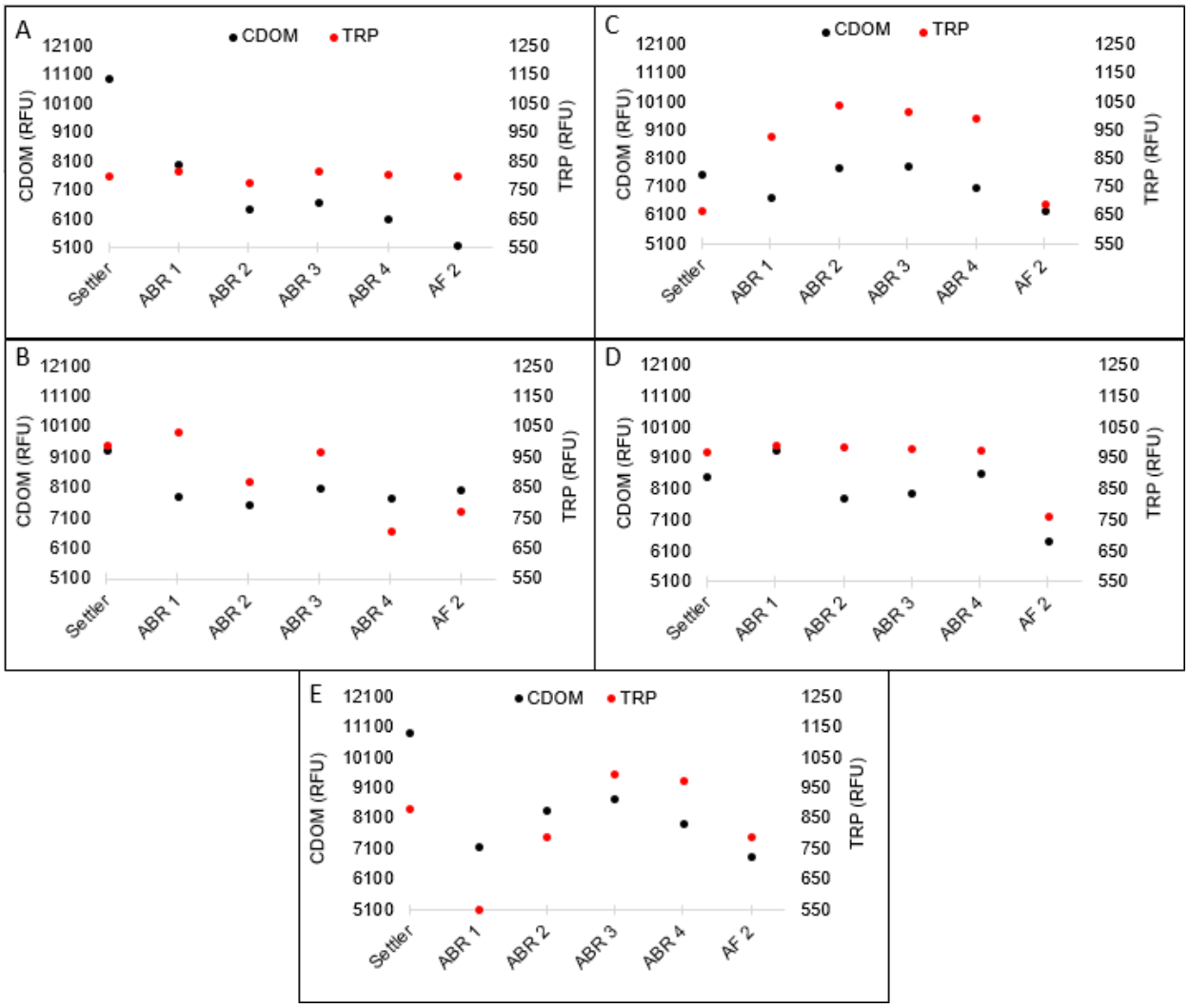

Figure 4: CDOM and TRP Values on Day 2 (A), Day 5 (B), Day 6 (C), Day 7 (D) and Day 8 (E)

The Pearson R Test was used to correlate TRP and CDOM (in mg/L) to COD concentrations (in mg/L). The correlation coefficient determines the strength of the relationship between two variables and it ranges from negative one to positive one; Negative coefficients represent negative correlations, positive coefficients represent positive correlations, and a coefficient of zero implies that no relationship exists between the two variables. The formula used is listed in Appendix 8.1, equation 4. COD concentrations were calculated only for Settler 1A, ABR 1, and AF 2 due to time and equipment constraints. COD emission values can be found in Appendix 8.3.1, Tables A1 and A2. Average concentrations of TRP, CDOM, and COD for the entire experimental period are listed in Appendix 8.3.2, Table A3. Table 3 below shows the correlation coefficients of TRP to COD and CDOM to COD for each chamber from Day 0 to Day 8. The strongest correlation for the entire experimental period is between COD and TRP in ABR 1 at 0.7369; There are no other strong positive correlations. The strongest negative correlation for the experimental period is between COD and CDOM in AF 2 at -0.6091; There are no other strong negative correlations. Settler 1A has the lowest correlations due to higher amounts of solids in

the wastewater which influence COD more so than fluorescence. All correlation values for the Post Scum Removal experiment are listed below in Table 3.

Table 3: Correlation Coefficients of TRP to COD and CDOM to COD from Day 0 to Day 8

Chamber	TRP	CDOM
Settler 1A	0.2369	-0.0899
ABR 1	0.7369	0.0292
AF 2	-0.0279	-0.6091

The strongest positive correlation for this experiment is shown in Figure 5. The table used to create this scatterplot is listed in Appendix 8.3.2, Table A4. The R^2 value is the square of the correlation coefficient. The data correlates most strongly in the beginning of the experimental period and the data spread farther apart as the days after scum removal continue.

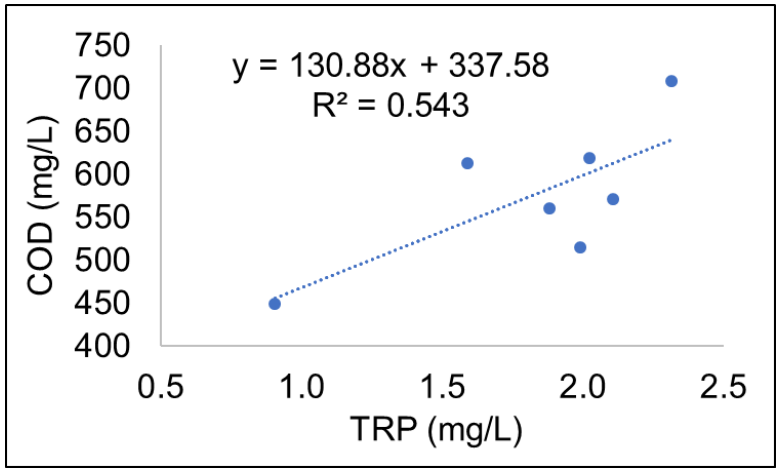

Figure 5: Correlation between TRP and COD in ABR 1 for entire Experimental Period

Correlation coefficients were also calculated for an overall correlation between TRP and COD (0.4913) and between CDOM and COD (0.6193) across all chambers and days. The corresponding scatterplots can be found in Appendix 8.2, Figure A6, and the data tables are found in Appendix 8.3.2, Table A5.

3.2 RESULTS FOR DIURNAL VARIABILITY EXPERIMENT

Over the course of the experimental period (four days of data collection), the fluorescence and COD values drastically varied by time of day and by chamber. On the first day of experimentation (two days before scum removal), the highest values of TRP in ABR 1 and AF 2 were both observed at 11:45pm, while the highest values of CDOM in ABR 1 and AF 2 were observed at 2:45pm and 9:30am, respectively. For the remainder of the experimental period, the times of the highest concentrations continued to vary. Due to the large volume of data collected for this experiment, COD concentrations were only measured at the beginning, middle, and end

of each day (9:30am, 11:45am, and 2:00pm). Table A6 in Appendix 8.3.3 lists the average concentrations for TRP, CDOM, and COD at all timepoints over the entire experimental period. Average diurnal values for TRP, CDOM, and COD for ABR 1 and AF 2 are listed below in Table 4. For each variable, the highest and lowest values observed in both ABR 1 and AF 2 are shaded in gray.

Table 4: Average Diurnal Values for CDOM, TRP, and COD in ABR 1 and AF 2

Date	Chamber	CDOM (RFU)	TRP (RFU)	COD (mg/L)
July 24th	ABR 1	9472.640	1035.459	433.944
July 24th	AF 2	7303.550	731.393	288.019
July 25th	ABR 1	8909.702	958.224	465.056
July 25th	AF 2	6607.152	706.447	292.833
July 27th	ABR 1	8719.711	979.769	544.685
July 27th	AF 2	6425.217	662.184	340.426
July 31st	ABR 1	8451.678	855.115	442.093
July 31st	AF 2	6745.806	677.269	288.389

Due to the high variation observed for fluorescence and COD values throughout the experimental period, there are no distinct trends; this agrees with the idea that the OM-laden wastewater influent is indeed highly variable on a daily basis. Scum removal occurred on July 26th, but the impact that this removal had on the diurnal variability cannot be discerned from the data. Occasionally, CDOM and TRP values would not vary too much throughout the day, but the opposite case was most often observed. Examples of this diurnal variability can be seen below in Figure 6 (ABR 1) and Figure 7 (AF 2). These figures display the fluorescence results for the entire experimental period. In ABR 1, TRP fluorescence was observed to be more variable after scum removal occurred, whereas CDOM fluorescence was observed to be more stable after scum removal. In AF 2, both TRP and CDOM fluorescence varied greatly on all days measured. Additionally, TRP fluorescence often increased sharply from 9:30am to 10:15am and initial CDOM fluorescence greatly varied from day to day.

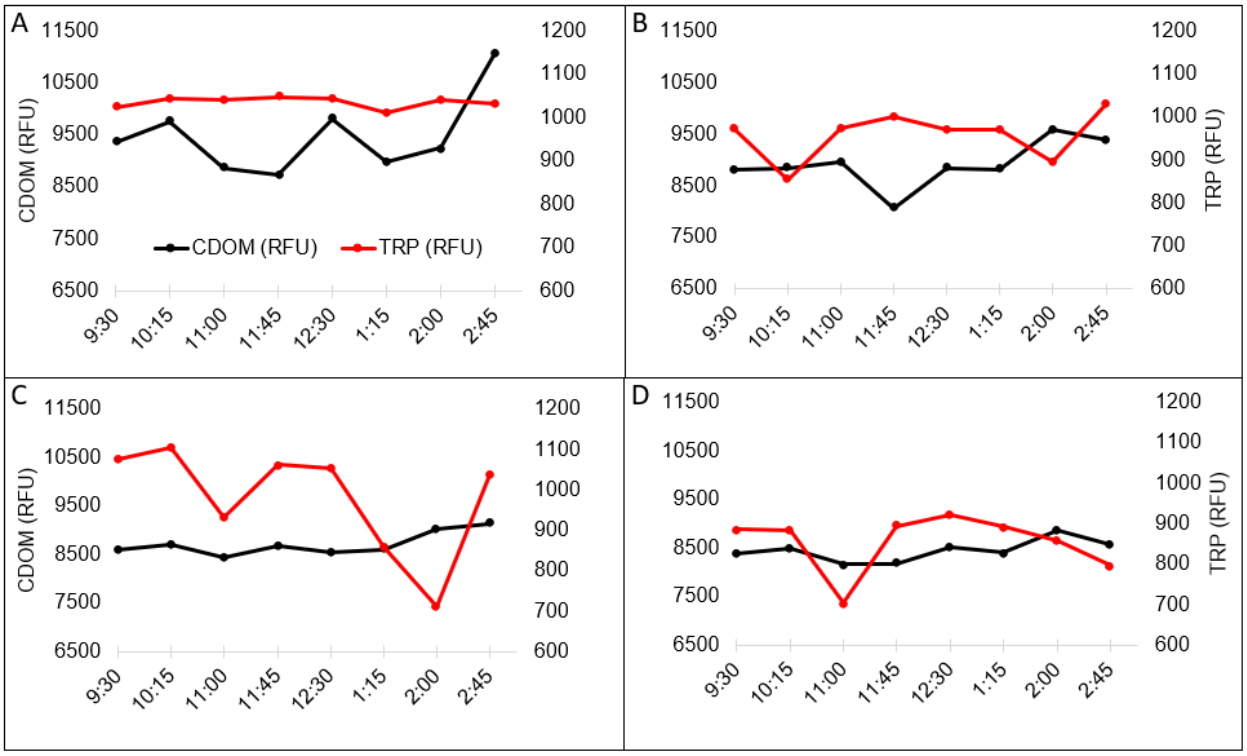

Figure 6: Average TRP and CDOM values for ABR 1 on July 24th (A), July 25th (B), July 27th (C), and July 31st (D)

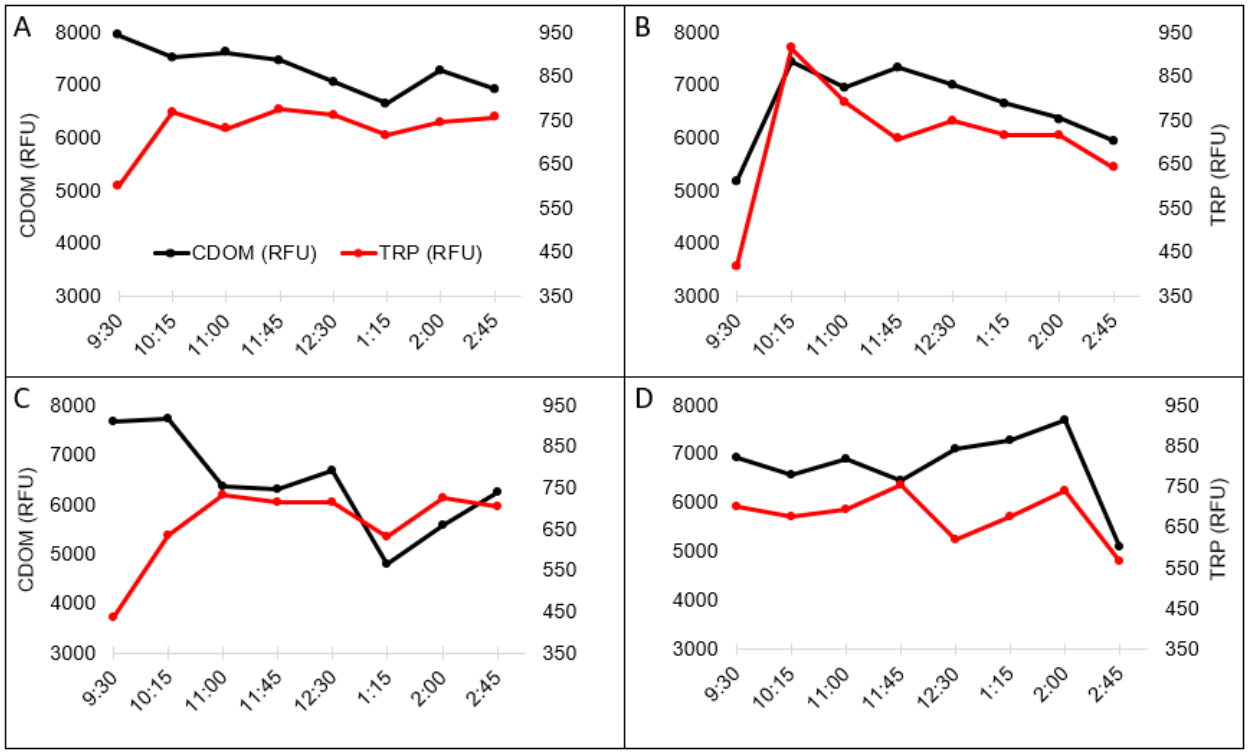

Figure 7: Average TRP and CDOM values for AF 2 on July 24th (A), July 25th (B), July 27th (C), and July 31st (D)

The Pearson R Test was again used to correlate TRP and CDOM (in mg/L) to COD concentrations (in mg/L). The formula used is listed in Appendix 8.1, equation 4. COD concentrations were calculated only at the following times due to time and equipment constraints: 9:30am, 11:45am, and 2:00pm. Average COD concentrations for each chamber at the three times can be found in Appendix 8.3.3 Table A6, along with average TRP and CDOM fluorescence values for all eight times. Due to the small amount of COD data collected per day, correlation coefficients were initially calculated for the entire experimental period. There were no strong positive or negative correlations, as seen in Table 5 below.

Table 5: Correlation Coefficients of TRP and CDOM to COD for all Days and Times

	ABR 1	AF 2
TRP	-0.1215	0.0347
CDOM	0.0928	-0.1748

Since no correlations were observed for the entire experimental period, the data was split between pre-scum removal (July 24th and July 25th) and post-scum removal (July 27th and July 31st). Higher correlations were found when comparing the data in this manner. The highest positive correlation is between TRP and COD in ABR 1 before scum removal occurred; after scum removal, the correlation decreased and became negative. The highest negative correlation is between CDOM and COD in AF 2 after scum removal; before scum removal, the correlation was lower and positive. These changes before and after scum removal indicate that scum removal had an impact on diurnal variability. Overall, the correlations were still only moderately high; No strong relationships between fluorescence measurements and COD concentrations were observed. All correlation coefficients are shown below in Table 6.

Table 6: Correlation Coefficients Before and After Scum Removal

Before or After Scum Removal	Chamber	TRP	CDOM
Before	ABR 1	0.4198	0.2658
Before	AF 2	-0.3630	0.1704
After	ABR 1	-0.3704	0.3176
After	AF 2	0.0281	-0.5256

The highest positive and negative correlations for this experiment are shown below in Figures 8 and 9, respectively. The R^2 value is the square of the correlation coefficient.

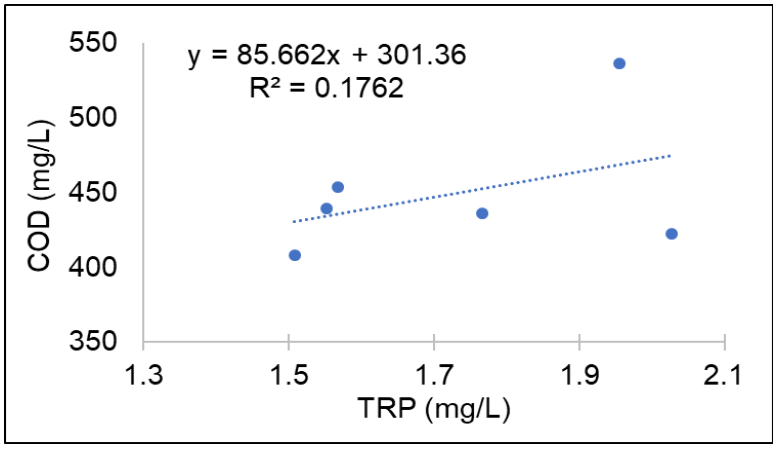

Figure 8: Correlation between TRP and COD in ABR 1 Before Scum Removal

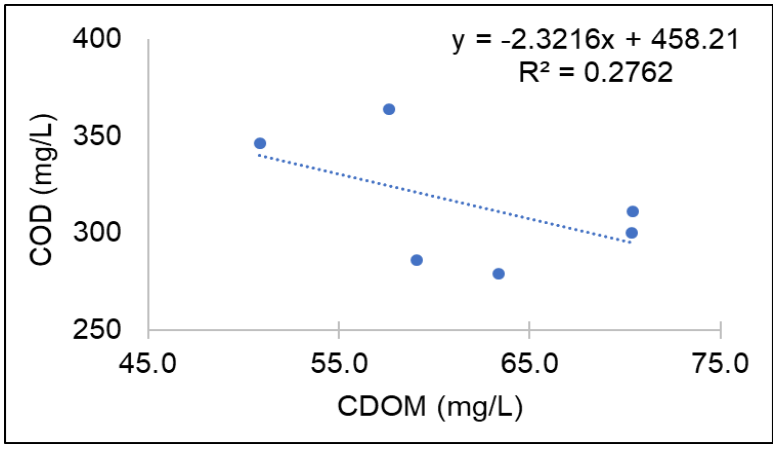

Figure 9: Correlation between CDOM and COD in AF 2 After Scum Removal

4.1 DISCUSSION OF POST SCUM REMOVAL RESULTS

The baseline trend observed on Day 0 behaved as predicted; the amount of TRP-like and humic-like organic material was highest in Settler 1A and was lowest in AF 2. The similarities of the trends for CDOM and TRP on Day 0 signify that the DEWATS chambers are effectively degrading OM. The ABR chambers do not degrade OM in a steadily decreasing fashion due largely in part to the flowrate. Theoretically, the DEWATS should exhibit continuous flow, but in actuality, treating domestic wastewater simulates more of a batch system behavior. There is little to no flowrate overnight while many residents are asleep, and there are peak flowrates during the morning and late afternoon hours. This variable flow of wastewater affects the contact time between the wastewater and sludge, thereby affecting the contact time between OM and bacteria. Decreased contact times may account for increased COD concentrations. According to Tegley, “Flow rate affects total COD based on contact time. The higher the flow rate, the higher the concentrations of COD will be because the wastewater has less time in contact with the sludge layers” (2015). Therefore, the increasing TRP and CDOM fluorescence values observed

from ABR 1 to ABR 3 on Day 0 may be largely attributed to the variable flowrate of the wastewater influent.

For the remainder of the experimental period, TRP and CDOM values and trends greatly fluctuate; This is attributed to in part by the nature of anaerobic biodegradation. According to Barker et al., "For both aerobic and anaerobic treatment only a small fraction of the effluent COD is originally from the influent substrate… the majority of the organic material is of microbial origin" (1999). The microorganisms present in sludge produce byproducts when they break down OM. In fact, humic-like compounds are often byproducts of the degradation of labile, TRP-like compounds. This creates difficulty in discerning the removal efficiency of OM in the DEWATS chambers. This phenomenon helps to explain why on certain days TRP and CDOM fluorescence values in AF 2 were higher than in ABR 4; other contributors include the variable flowrate and scum accumulation/removal. Only on the eighth day after scum removal did the baseline trend (from Day 0) begin to reappear. The ABR and AF chambers may have started to stabilize, or this could be a mere coincidence. According to Barker et al., certain types of OM "may take as long as 11 days for their degradation" (1999); Therefore, in order to confidently state whether a stabilization of the chambers was occurring on the eighth day after scum removal, a longer experimental period is necessary.

The strongest correlation with COD for the entire experimental period is seen for TRP in ABR 1. This was expected since many studies have shown a correlation between TRP-like fluorescence with BOD and soluble COD. What was not expected, however, was the higher overall correlation between CDOM and COD compared to the correlation between TRP and COD (0.6193 compared to 0.4914). This is partially due to the fact that total COD was measured as opposed to soluble COD, which would include more recalcitrant (humic-like) types of OM. LCFA are also difficult for microorganisms to break down, so high FOG concentrations may also contribute to the higher correlation between CDOM and COD. In a study performed by Mladenov et al. (in revision), a significant relationship was noted between TRP fluorescence and COD. It was suggested that "TRP fluorescence could be used as a surrogate for chemical oxygen demand (COD) and soluble COD concentrations" (Mladenov et al., in revision). If soluble COD was able to be measured for this study, then perhaps a similar argument could be made about the correlation between TRP fluorescence and COD. For the Post Scum Removal experiment, the trend between TRP fluorescence and COD was high in ABR 1 but not for any other chambers. Additionally, TRP did not have a high overall correlation to COD, but this might have changed if soluble COD had been used instead of total COD. The extent to which scum removal affected OM degradation is uncertain, but scum removal certainly instigated a sort of stress on the system as evidenced by the drastic changes made to general fluorescence trends.

4.2 DISCUSSION OF DIURNAL VARIABILITY RESULTS

In a study performed previously at the Newlands Mashu Research Site, Mladenov et al. found that "TRP fluorescence intensities ranged from 900 to 1,900 RFU… with a mean of ~1,500 RFU" and that CDOM fluorescence intensities "ranged from ~6,500 to 9,000 RFU, with a mean of ~8,000 RFU" (in revision). TRP fluorescence values are often lower than 900 RFU for AF 2 and the highest value seen in ABR 1 (around 1105 RFU) was less than the mean for the comparison study. These differences in TRP fluorescence values are likely a result of many

variables such as the flowrate, blockage issues with the DEWATS, FOG concentrations, scum accumulation, and scum removal. CDOM fluorescence values more closely resemble the data from Mladenov et al. (in revision). Despite the large fluctuations in TRP and CDOM fluorescence, this comparison study shows that the majority of values fall within a reasonable range.

The results indicate that the wastewater influent exhibits a high diurnal variability with respect to OM. Throughout the four days of experimentation, no consistent trends for TRP-like and humic-like fluorescence could be discerned in either chamber at any time; This was observed visually and is supported by the extremely low correlations of CDOM and TRP to COD. Although fluorescence values and COD both varied daily throughout the experimental period, they did not vary in the same manner. The variable flowrate as well as scum accumulation and removal largely contribute to the lack of discernable trends. The flowrate remains stagnant overnight, peaks during the early morning hours, and peaks again in the late afternoon. Large jumps of fluorescence values were observed more often in the morning and afternoon hours than during midday which can be attributed largely to the flowrate. The large influx of wastewater influent during the morning is demonstrated by the sharply increasing initial TRP values in AF 2 (See Figure 7, panels A-C). While longer cell retention times give the bacteria more time to degrade OM, the sudden increases in flowrate can disrupt biodegradation; this may also contribute to the scum infiltration from the settling chambers into ABR 1 and ABR 2. Wastewater present in one chamber likely has a different OM composition than the next chamber due to the discontinuous flowrate. Scum removal also had a noticeable effect on diurnal variability; When the data was categorized into pre and post scum removal, stronger correlations of the variables were observed. In ABR 1, visual changes in TRP and CDOM trends can be seen upon close observation before and after scum removal (See Figure 6). The extent of the impact that FOG concentrations scum accumulation, and scum removal have on the DEWATS is still uncertain, but this study shows that an impact definitely exists.

4.3 QUALITY CONTROL AND ERROR ANALYSIS

The ABR and AF systems are both living anaerobic systems that involve many unknowns. Additionally, in-situ instruments have a great deal of variability. Even the benchtop instruments (the COD digester and spectrophotometer) had many steps and many chances for error. To reduce instrumentation and measuring errors, COD was measured in triplicate, and all fluorescence data are averages of a 2 minute and 15 second measuring period. The sampling process introduced another level of error; Each time that the scum was moved aside to take a water sample, the system was disturbed. This disturbance likely altered the data to some extent for the Post Scum Removal experiment, and to a large extent for the Diurnal Variability experiment since measurements were taken 8 times per day. However, this procedure was consistently performed when sampling, so the data obtained in the two experiments should not be largely affected. The scum itself is quite inhomogeneous and presents many uncertainties as well. Due to all the variables involved, the discussion of the two experiments focuses on trends and correlations more than the actual quantitative results.

5. CONCLUSIONS AND RECOMMENDATIONS

Based on the in-situ fluorescence and total COD data for both experiments, it can be concluded that the wastewater influent is indeed highly variable in terms of its diurnal flowrate as well as the types and concentrations of OM. It can also be concluded that this variability is exacerbated by scum accumulation and removal. The Post Scum Removal experiment revealed a glimpse of the stress that scum removal causes for the ABR and AF chambers. General trends observed on Day 0 for TRP and CDOM fluorescence began to dissipate not long after the scum was removed. Although this experiment did not investigate the effects on microorganisms, it can be assumed that the microbial community structure was disturbed by the highly variable, LCFA-containing, and OM-laden wastewater. The Diurnal Variability experiment revealed how much the OM concentrations can vary in the ABR and AF throughout the day. This experiment also affirmed the idea that scum accumulation and removal can have a large impact on OM degradation.

Scum accumulation and removal have a large potential to negatively impact DEWATS. Therefore, operation and maintenance issues involving scum should be of great concern to the communities and individual residences that implement DEWATS technologies. Communities and residences should be surveyed to gauge how much diet-based FOG will enter the DEWATS, *before* it is built. Perhaps redesigning settling chambers or installing FOG/scum trapping systems could ameliorate the issue. Engaging the community that the DEWATS serves is paramount for ensuring its smooth operation. Historically, operation and maintenance for the ABR was believed to be infrequent for scum/sludge floatation issues. According to Tilley et al., "process operation [for the ABR] in general is not required, and maintenance is limited to the removal of accumulated sludge and scum every 1 to 3 years" (2014). This is simply not feasible for the Newlands Mashu DEWATS as evidenced by the observations of this study. Additionally, Tilley et al. (2014) advocates the use of "Motorized Emptying and Transport technology" for scum removal, but this is not often feasible for communities in developing countries or in more impoverished communities. These facts presented in Tilley et al. (2014) are relatively recent yet they fail to address the issues caused by high FOG concentrations, scum accumulation, and scum removal. It is recommended that these issues should be further investigated to better understand system responses. Longer and more repetitive experimental periods should be implemented, and more variables should be addressed. More information is needed about the recovery time of the system after scum removal. Lastly, all components of FOG (not just oils) should be taken into account to explore which component, if any, has the most detrimental effect on OM degradation.

6. ACKNOWLEDGEMENTS

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8. APPENDIX

8.1 Equations

For TRP Concentrations: $\mathbf{Y} = 0.0025\mathbf{X} - 0.4732$ **(1)**

For CDOM Concentrations: $\mathbf{Y} = 0.0093\mathbf{X} - 1.052$ **(2)**

Equation of standard curve: $Y = 0.0006X + 0.0133$ **(3)**

Pearson Correlation Coefficient Formula **(4)**

$$R = (N\Sigma xy - (\Sigma x)(\Sigma y)) / [(N\Sigma x^2 - (\Sigma x)^2) * (N\Sigma y^2 - (\Sigma y)^2)]^{1/2}$$

Where N = Number of pairs of scores x and y

Where x and y = data for variable x and variable y

8.2 Photographs and Figures

Figure A1: Scum Layer in ABR 1 of Street 3

Figure A2: Scum Accumulation in Settler 1A

Figure A3: Fluorometer held by zip ties while connected and collecting real-time data

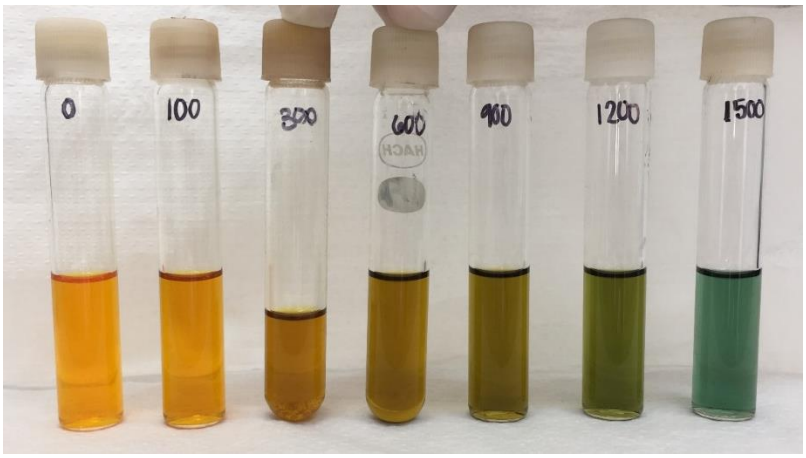

Figure A4: Standard COD solutions (mg/L) after spectrophotometer analysis

*The 300 mg/L standard leaked due to a faulty cap which is why the volume appears lower than the rest of the vials.

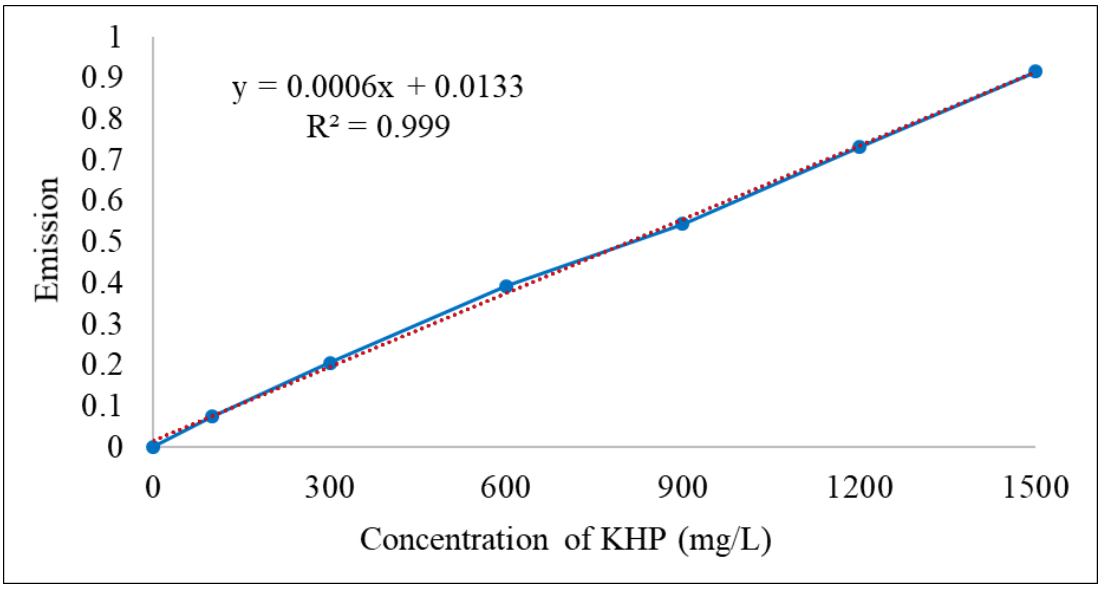

Figure A5: Standard Curve for COD Concentrations

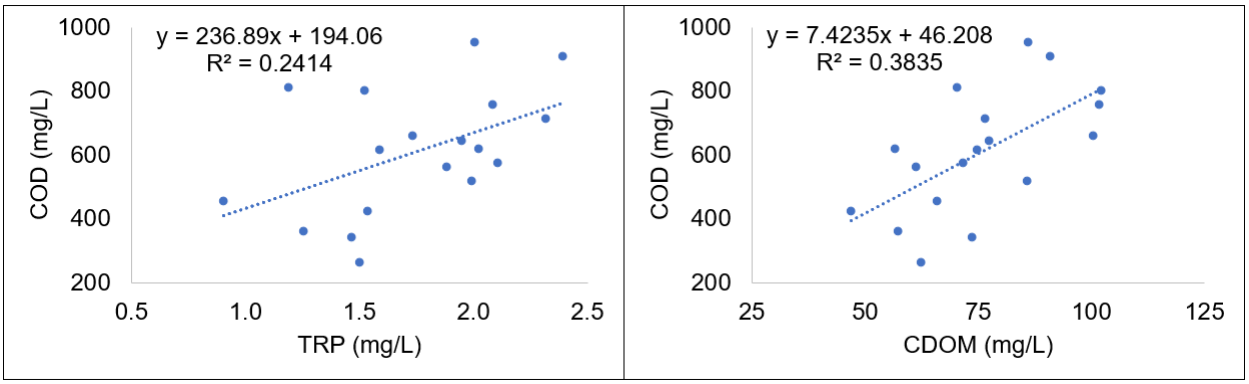

Figure A6: Correlations between TRP and COD (left) and between CDOM and COD (right) for entire Experimental Period

8.3 Raw Data

8.3.1 COD Emission Data

Table A1: Average Triplicate COD Emission Values for the Post Scum Removal Experiment

Date	Weekday	Chamber	Emission
July 11th	T	S 1A	0.603
		ABR 1	0.340
July 12th	W	S 1A	0.558
		ABR 1	0.385
July 13th	Th	S 1A	0.465
		ABR 1	0.439
July 14th	F	S 1A	0.493
		ABR 1	0.381
		AF 2	0.267
July 17th	M	S 1A	0.584
		ABR 1	0.356
		AF 2	0.216
July 18th	T	S 1A	0.498
		ABR 1	0.350
		AF 2	0.229
July 19th	W	S 1A	0.399
		ABR 1	0.323
July 20th	Th	S 1A	0.407
		ABR 1	0.283
		AF 2	0.170

Table A2: Average Triplicate COD Emission Values for the Diurnal Variability Experiment

Date	Weekday	Time	Chamber	Emission
July 24th	M	9:30	ABR 1	0.258
			AF 2	0.207
		11:45	ABR 1	0.286
			AF 2	0.183
		2:00	ABR 1	0.277
			AF 2	0.168
July 25th	T	9:30	ABR 1	0.335
			AF 2	0.197
		11:45	ABR 1	0.267
			AF 2	0.207
		2:00	ABR 1	0.275
			AF 2	0.163
July 27th	Th	9:30	ABR 1	0.283
			AF 2	0.200
		11:45	ABR 1	0.336
			AF 2	0.232
		2:00	ABR 1	0.401
			AF 2	0.221
July 31st	M	9:30	ABR 1	0.315
			AF 2	0.181
		11:45	ABR 1	0.288
			AF 2	0.185
		2:00	ABR 1	0.233
			AF 2	0.193

8.3.2 Post Scum Removal

Table A3: Average Concentrations of TRP, CDOM, and COD for the Entire Experimental Period

Days After Scum Removal	Chamber	TRP(mg/L)	CDOM(mg/L)	COD(mg/L)
0	Settler 1A	2.394	90.896	907.278
	ABR 1	2.025	56.472	619.500
	AF 2	1.110	30.341	-
1	Settler 1A	2.084	101.786	752.278
	ABR 1	2.315	76.409	708.944
	AF 2	1.730	57.822	-
2	Settler 1A	1.520	102.212	800.056

	ABR 1	1.591	74.595	613.389
	AF 2	1.533	47.003	423.389
5	Settler 1A	2.005	85.906	951.167
	ABR 1	2.108	71.739	571.722
	AF 2	1.464	73.753	338.389
6	Settler 1A	1.190	70.297	808.389
	ABR 1	1.881	61.528	561.167
	AF 2	1.253	57.410	360.056
7	Settler 1A	1.949	77.429	642.833
	ABR 1	1.989	85.667	515.611
	AF 2	1.421	58.210	-
8	Settler 1A	1.733	100.447	655.611
	ABR 1	0.905	65.732	450.056
	AF 2	1.410	62.515	261.167

Table A4: Average Concentrations of TRP in ABR 1 for Entire Experimental Period

	TRP (mg/L)	COD (mg/L)
Day 0	2.0	620
Day 1	2.3	709
Day 2	1.6	613
Day 5	2.1	572
Day 6	1.9	561
Day 7	2.0	516
Day 8	0.9	450

Table A5: Correlation Coefficients Calculated from Data from Settler 1A, ABR 1, and AF 2 for all days in Experimental Period

	TRP (mg/L)	COD (mg/L)	CDOM (mg/L)
	2.394	907.278	90.896
	2.084	752.278	101.786
	1.520	800.056	102.212
	2.005	951.167	85.906
	1.190	808.389	70.297
	1.949	642.833	77.429
	1.733	655.611	100.447
	2.025	619.500	56.472
	2.315	708.944	76.409
	1.591	613.389	74.595
	2.108	571.722	71.739

	1.881	561.167	61.528
	1.989	515.611	85.667
	0.905	450.056	65.732
	1.533	423.389	47.003
	1.464	338.389	73.753
	1.253	360.056	57.410
	1.500	261.167	62.515
Correlation Coefficient	**0.4913**		**0.6193**

8.3.3 Diurnal Variability

Table A6: Average Concentrations of CDOM, TRP, and COD for Entire Experimental Period

July 24	**ABR 1**	**CDOM (mg/L)**	**CDOM (RFU)**	**TRP (mg/L)**	**TRP (RFU)**	**COD (mg/L)**
	9:30	85.966	9356.760	1.508	1023.841	408.389
	10:15	89.539	9740.992	1.557	1043.435	
	11:00	81.311	8856.231	1.552	1041.478	
	11:45	80.177	8734.248	1.567	1047.775	453.944
	12:30	90.100	9801.322	1.556	1043.336	
	1:15	82.490	8983.008	1.475	1010.797	
	2:00	84.888	9240.860	1.552	1041.676	439.500
	2:45	101.878	11067.703	1.526	1031.332	
July 24	**AF 2**	**CDOM (mg/L)**	**CDOM (RFU)**	**TRP (mg/L)**	**TRP (RFU)**	**COD (mg/L)**
	9:30	72.677	7927.868	0.449	7927.868	322.833
	10:15	68.913	7523.140	0.869	7523.140	
	11:00	69.746	7612.661	0.774	7612.661	
	11:45	68.383	7466.149	0.885	7466.149	282.833
	12:30	64.568	7055.934	0.849	7055.934	
	1:15	60.881	6659.438	0.735	6659.438	
	2:00	66.515	7265.256	0.816	7265.256	258.389

	2:45	63.285	6917.950	0.835	6917.950	
July 25	**ABR 1**	**CDOM (mg/L)**	**CDOM (RFU)**	**TRP (mg/L)**	**TRP (RFU)**	**COD (mg/L)**
	9:30	80.825	8803.967	1.955	971.408	536.722
	10:15	81.107	8834.248	1.663	854.380	
	11:00	82.234	8955.471	1.959	972.979	
	11:45	73.921	8061.653	2.028	1000.417	422.278
	12:30	81.240	8848.628	1.953	970.539	
	1:15	80.893	8811.339	1.953	970.390	
	2:00	88.079	9584.000	1.766	895.597	436.167
	2:45	86.166	9378.314	2.102	1030.079	
July 25	**AF 2**	**CDOM (mg/L)**	**CDOM (RFU)**	**TRP (mg/L)**	**TRP (RFU)**	**COD (mg/L)**
	9:30	47.054	5172.724	0.572	418.128	305.611
	10:15	68.098	7435.438	1.810	913.382	
	11:00	63.540	6945.355	1.507	791.917	
	11:45	67.183	7337.058	1.292	705.917	323.389
	12:30	63.978	6992.430	1.402	750.069	
	1:15	60.881	6659.438	1.314	714.760	
	2:00	58.187	6369.818	1.314	714.945	249.500
	2:45	54.236	5944.959	1.133	642.456	
July 27	**ABR 1**	**CDOM (mg/L)**	**CDOM (RFU)**	**TRP (mg/L)**	**TRP (RFU)**	**COD (mg/L)**
	9:30	78.822	8588.595	2.219	1076.823	450.056
	10:15	80.010	8716.397	2.290	1105.415	
	11:00	77.531	8449.785	1.857	932.129	
	11:45	79.558	8667.702	2.184	1063.005	537.833
	12:30	78.478	8551.636	2.164	1054.714	

	1:15	79.030	8611.008	1.673	858.674	
	2:00	82.911	9028.298	1.303	710.460	646.167
	2:45	83.990	9144.264	2.119	1036.932	
July 27	**AF 2**	**CDOM (mg/L)**	**CDOM (RFU)**	**TRP (mg/L)**	**TRP (RFU)**	**COD (mg/L)**
	9:30	70.427	7685.950	0.617	435.997	311.167
	10:15	70.920	7738.876	1.113	634.496	
	11:00	58.075	6357.752	1.356	731.712	
	11:45	57.621	6308.893	1.318	716.545	363.944
	12:30	61.104	6683.438	1.315	715.230	
	1:15	43.494	4789.845	1.104	630.945	
	2:00	50.866	5582.565	1.345	727.441	346.167
	2:45	57.114	6254.413	1.290	705.104	
July 31	**ABR 1**	**CDOM (mg/L)**	**CDOM (RFU)**	**TRP (mg/L)**	**TRP (RFU)**	**COD (mg/L)**
	9:30	77.066	8399.736	1.742	886.165	503.389
	10:15	77.968	8496.760	1.740	885.233	
	11:00	74.862	8162.843	1.287	703.894	
	11:45	74.999	8177.488	1.761	893.769	457.278
	12:30	78.285	8530.843	1.835	923.464	
	1:15	77.090	8402.380	1.757	892.099	
	2:00	81.513	8877.950	1.675	859.441	365.611
	2:45	78.606	8565.421	1.519	796.856	
July 31	**AF 2**	**CDOM (mg/L)**	**CDOM (RFU)**	**TRP (mg/L)**	**TRP (RFU)**	**COD (mg/L)**
	9:30	63.355	6925.488	1.275	699.1702479	278.944
	10:15	59.918	6555.947	1.216	675.5471074	
	11:00	63.069	6894.678	1.263	694.4859504	

	11:45	59.060	6463.603	1.406	751.6429752	286.167
	12:30	64.855	7086.810	1.071	617.5206612	
	1:15	66.626	7277.223	1.216	675.6991736	
	2:00	70.323	7674.777	1.372	738.0561983	300.056
	2:45	46.266	5087.921	0.942	566.0297521	