Biodegradability via Fluorescence

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Abstract

In-depth analysis of the biodegradability of a Decentralized Wastewater Treatment System (DEWATS) plant in South Africa was performed using in-situ fluorescence spectroscopy. Tryptophan-like fluorescence was monitored throughout the system's three treatment streets with spatial and temporal variations to discover how biodegradability rates changed within individual chambers, treatment streets, and the system as a whole. A uniform biodegradation was found within individual chambers, Tryptophan concentrations were found to decrease throughout the system, especially within the anaerobic filters, and the efficiency of the third treatment system, with only four anaerobic baffled reactor chambers, was questioned. Results from this analysis will help to improve DEWATS plants around the world and were made in an effort to improve sanitation for underdeveloped areas.

Introduction

Throughout the world, wastewater treatment has been performed in a variety of methods. Conventionally, in the USA and other developed areas, it has been done using clarifiers and aerobic processes. The German NGO BORDA, however, has developed a treatment system using what is known as an anaerobic baffled reactor (ABR) to deliver sustainable sanitation solutions to underdeveloped areas. One such system was built through the partnership of the University of Kwazulu-Natal, the Ethekwini Municipality, and BORDA in the Newlands-Mashu area just outside of Durban, South Africa.

Serving approximately 83 houses, the Newlands-Mashu plant consists of three treatment streets. Each street contains an initial settler where scum is separated, an ABR, and anaerobic filters (AF). The ABR is a series of compartments (baffles) that force wastewater to flow down into each chamber, then up and over each baffle allowing organic matter to be digested anaerobically (microbial treatment) while particles settle to the bottom creating a sludge layer (physical treatment) (Tilley *et al.*, 2014). A schematic of the primary settler and ABR can be seen below.

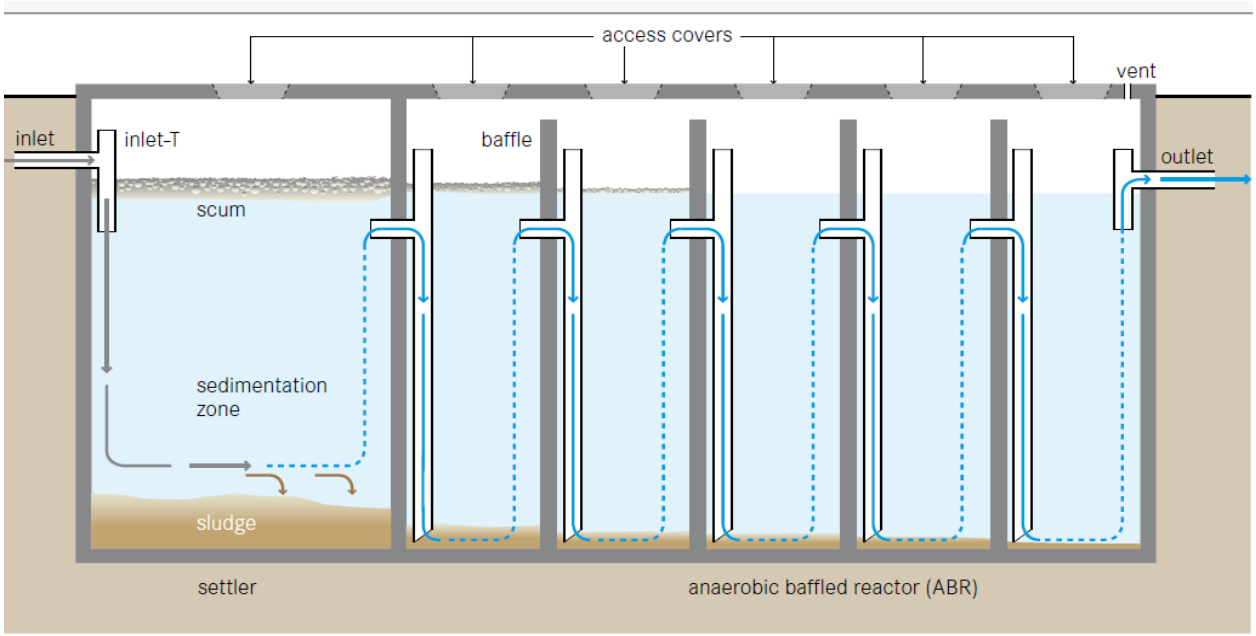

Figure 1. Schematic of Anaerobic Baffled Reactor. *Source: Tilley et al. 2014.*

Two of the streets have seven ABR chambers, two AF, and are then discharged into a wetlands area consisting of a vertical planted gravity filter (VGF) and a horizontal planted gravity filter (HGF). The third street consists of only four ABR chambers, two AF chambers, and effluent at this point is fed back into the municipal system (see picture below).

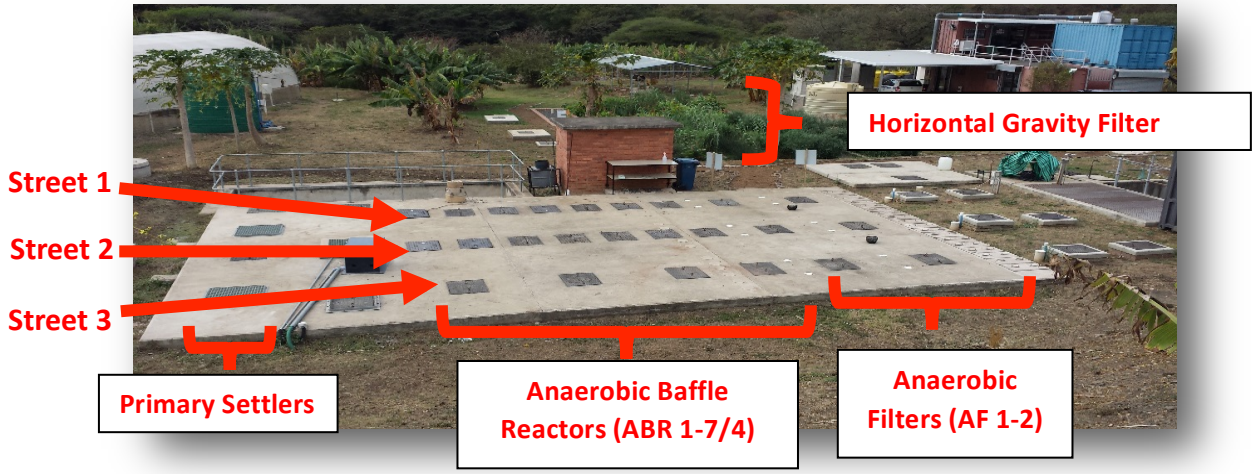

Figure 2. Newlands-Mashu DEWATS plant and on-site laboratory facilities. *Source: Monica Palomo.*

Research was conducted on this site to determine the amount of biodegradability occurring within each treatment system using in-situ fluorescence spectroscopy. This is a sensitive technique that attempts to monitor the concentration of tryptophan-like (TRP-like) substances and chromophoric dissolved organic matter (CDOM) in the water. TRP-like substances, in particular, were monitored as these have been more correlated to biodegradability experiments. Research done by Hudson, Naomi, *et al.* found that in aerobic treatment systems, fluorescence of TRP-like substances had a strong, positive relationship to the biochemical oxygen demand (BOD) of the wastewater, a direct measurement of the biodegradation occurring (Hudson *et al.* 2008). This meant that a higher concentration of TRP-like substances resulted in higher biodegradation rates occurring.

Additional research in the nearby waters of the Umgeni River and Msundunzi River in Kwazulu-Natal was conducted by A. Baker et al. that positively correlated the presence of E. coli to TRP-like fluorescence peaks. This research found that at lower TRP-like concentrations or low peak intensities, E. coli was also found in lower concentrations and vice versa (Baker *et al*, 2015).

Similar to these two research analyses, the research done at the Newlands-Mashu site was conducted based on the premise that higher TRP-like concentrations meant more biodegradation occurring and more microbial presence. The goal of the research was to answer three main questions: how was biodegradation changing within each chamber, how did it change throughout the whole system, and how did it change between streets.

Methods

In-situ fluorescence was conducted using a submersible C3 fluorimeter (Cyclops) manufactured by Turner Designs that was equipped with three sensors to detect the following: chlorophyll *in vivo* for blue excitation (465 nm ex/696 nm em), CDOM (325 nm ex/470 nm em), and tryptophan (285 nm ex/350 nm

em). Before use, the Cyclops was calibrated for both TRP and CDOM concentrations using TRP powder and Pony Lake CDOM powder respectively in ultrapure water.

To take measurements, the Cyclops was submerged into the wastewater, slightly swung to remove any accumulated air bubbles, and then held as motionless as possible for a specific amount of time. Duplicate measurements, when performed, simply consisted of repeating this process a second or third time.

Four measuring or sampling plans were created for use with the Cyclops including initial, secondary, 24-hour, and comparison measurement methods. The initial sampling plan was constructed to detect differences in depth and location within an ABR chamber. Three different locations were measured within each ABR chamber, inlet, middle, and outlet, at three different depths: 0.5m, 1.0m, and 1.5m (see ABR diagram below). Inlet, middle, and outlet of each anaerobic filter (AF) were measured as well by dropping the Cyclops to the rocks then lifting slightly (<0.5m). In addition, the influent into the horizontal gravity filter (HGF) was measured by filling a 5L pitcher with influent, covering this in a black plastic bag (to diminish adverse effect of outside light with fluorescence), then submerging the Cyclops at an angle to account for air bubbles and slightly lifting it from the bottom. The Cyclops was held at each location for one minute with readings at one second interval.

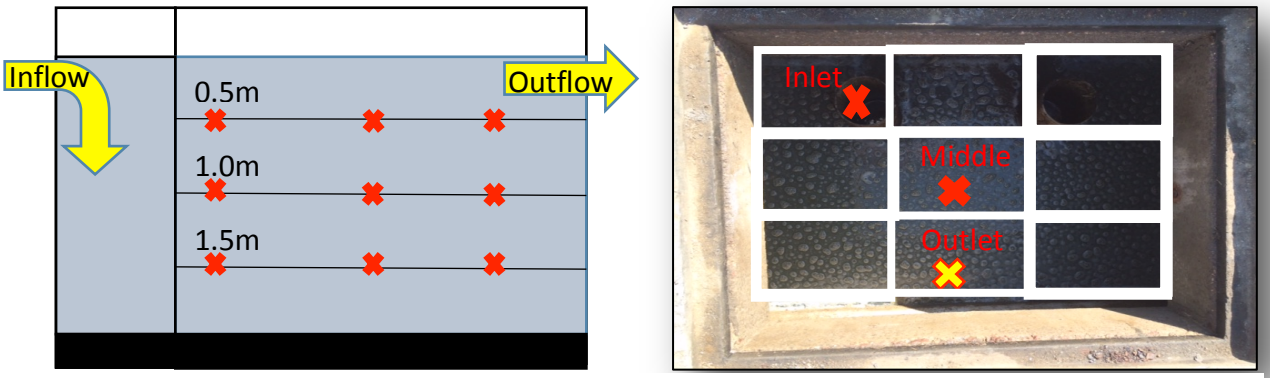

Figure 3. Profile and top view of an ABR chamber. *Source: Amy Bigelow*

The secondary sampling plan consisted of submerging the Cyclops at the outlet only of each chamber with the same minute, one second interval procedure as for the initial sampling plan. HGF measurements were performed in exactly the same manner as in the initial plan. The 24-hour plan consisted of leaving the Cyclops submerged in one chamber (either ABR 1 or ABR 7/4) for an entire 24 hour period with readings taken every five minutes. Lastly, the comparison sampling method was used to compare ABR 1, ABR 7/4, and AF 2 in Streets 1-3. This was done by submerging into the outlets of these chambers and taking the same minute, one second interval readings.

Results

The first week of Cyclops measurements were done to assess the biodegradability at different depths and different locations within each chamber of street one. Measurements were taken on July 1, 2015 over the course of the entire day with duplicate measurements taken at all locations in AF2 and ABR 1.

The following two graphs show the TRP-like concentration results of ABR chambers 3 and 4 and can be taken as representative data for all chambers measured.

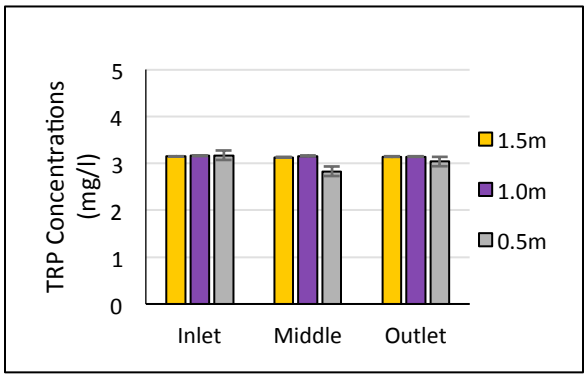

Figure 4. ABR 3 TRP concentrations plotted by location and depth within the chamber

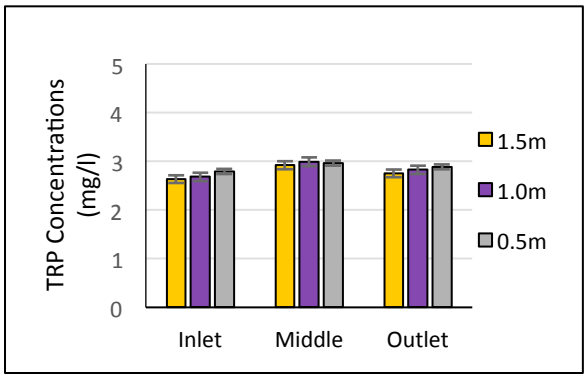

Figure 5. ABR 4 TRP concentrations plotted by location and depth within the chamber

Standard deviations for ABR 3 data were fairly low ranging from 0.0108 mg/l from the outlet at 1m to 0.701 mg/l in the middle at 1m depth. Standard deviations for ABR 4 were similarly low ranging from 0.009 mg/l at the inlet at 0.5m to 0.055 mg/l at the outlet at 1m. Lower standard deviations in ABR 4 suggest greater precision in these measurements than in ABR 3.

During the second week, only the outlet at 0.5m of each chamber was measured but was measured at 9:00, 11:00, and 13:00 on July 7, 2015. The following graphs are the TRP-like concentrations and CDOM concentrations for all three times for the entire treatment street from ABR 1 to the HGF.

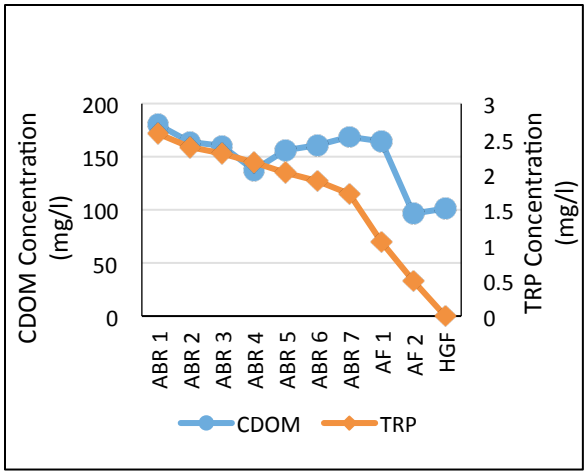

Figure 6. 9:00 sampling at the outlet of each ABR chamber, AF chamber, and HGF at 0.5m.

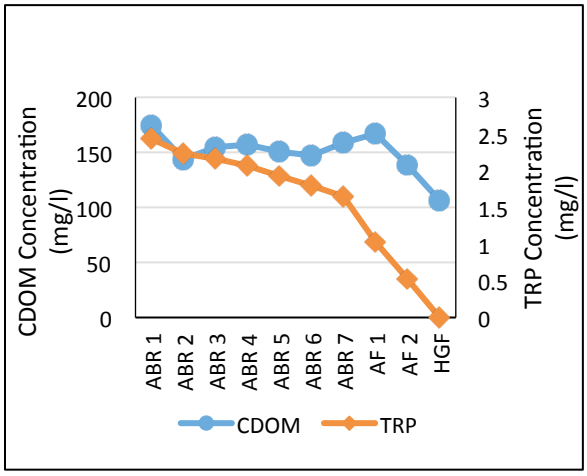

Figure 7. 11:00 sampling at the outlet of each ABR chamber, AF chamber, and HGF at 0.5m.

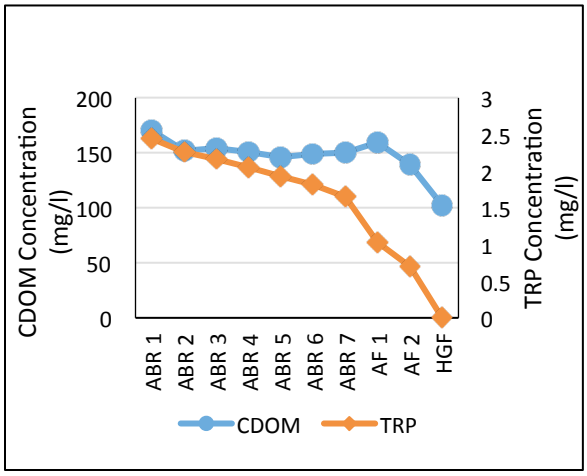

Figure 8. 13:00 sampling at the outlet of each ABR chamber, AF chamber, and HGF at 0.5m.

In addition to these results, measurement results for a 24-hour time lapse in ABR 1 and ABR 7 (the inlet and outlet of the ABR system) can be seen in the following graphs. It is important to note what days of the week these measurements were taken as a weekly pattern may be able to be established. ABR 1 measurements were taken from 16:30 July 8, 2015 to 15:35 July 9, 2015 (a Wednesday to a Thursday). ABR 7 measurements were taken from 16:00 July 9, 2015 to 15:10 July 10, 2015 (a Thursday to a Friday).

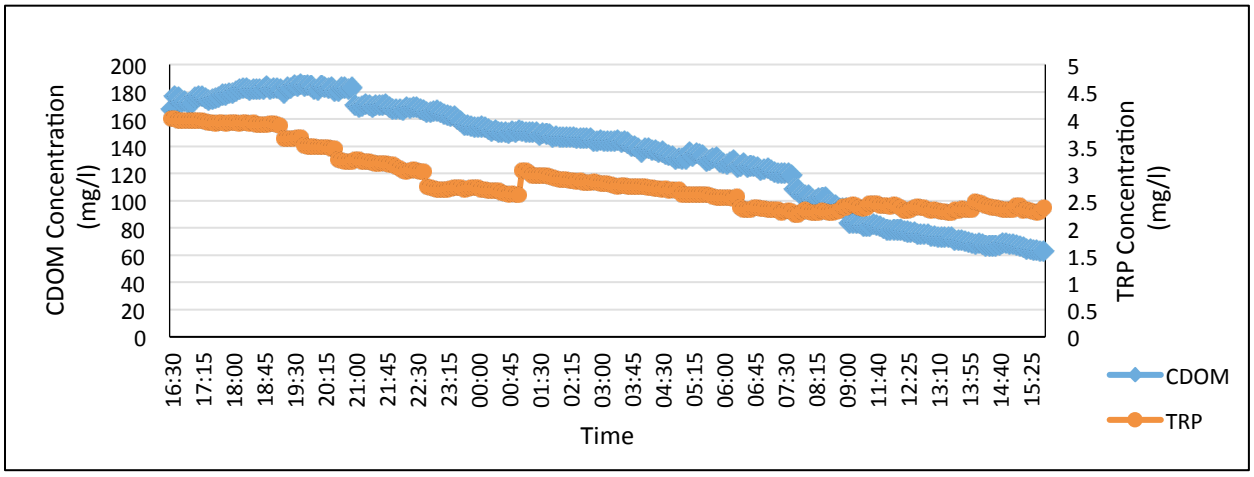

Figure 9. TRP-like and CDOM concentrations from 24-hour time lapse of ABR 1, Street 1. Measured at the outlet at 0.5m.

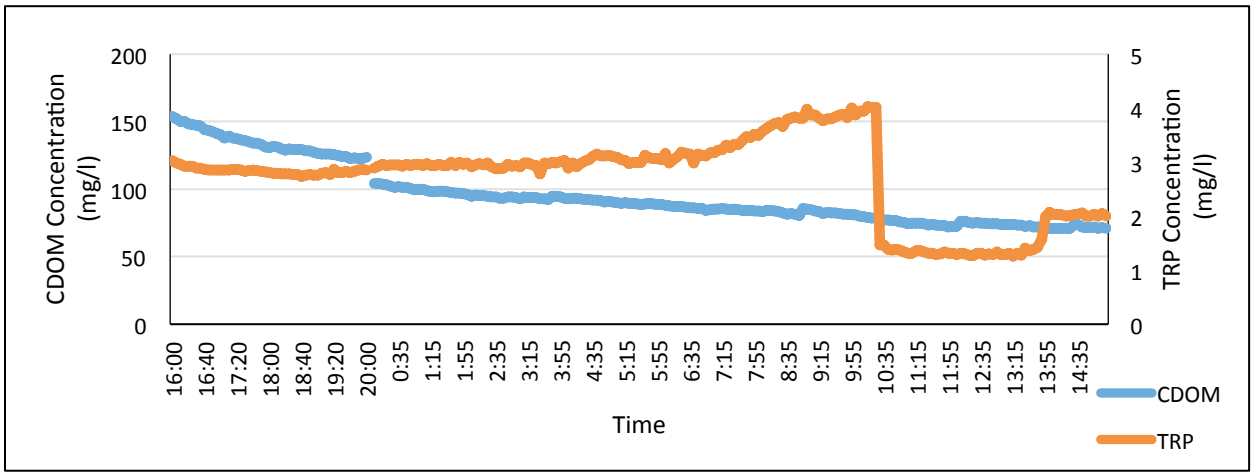

Figure 10. TRP-like and CDOM concentrations from 24-hour time lapse of ABR 7, Street 1. Measured at the outlet at 0.5m. There is a noticeable, abrupt decrease in TRP at 9:55am.

The third week of measurements resulted in data for Street 3. The following graphs indicate the results for full Street 3 measurements at 9:00, 11:00, and 13:00 without readings from the HGF as Street 3 does not empty into the gravity filters but rather goes back into the municipal system. All measurements were taken on July 13, 2015 at 0.5m at the outlet of each chamber

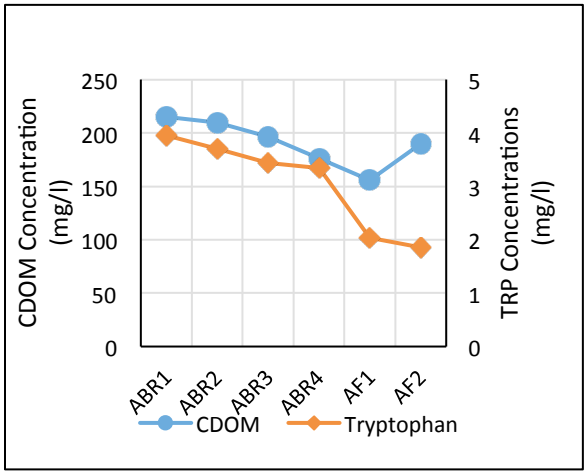

Figure 11. TRP-like and CDOM concentrations for 9:00 sampling of Street 3. Measured at outlet, at 0.5m.

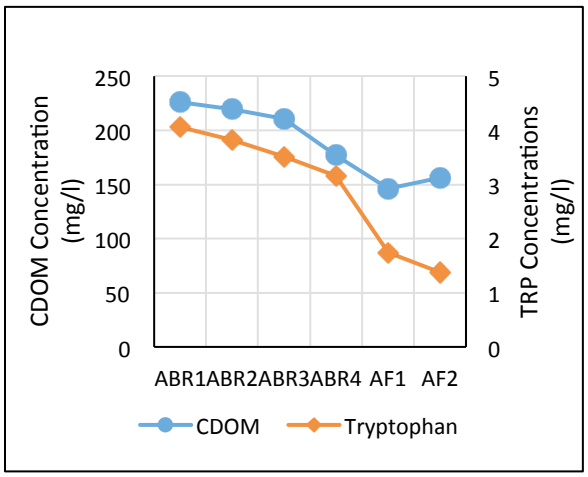

Figure 12. TRP-like and CDOM concentrations for 11:00 sampling of Street 3. Measured at the outlet, at 0.5m.

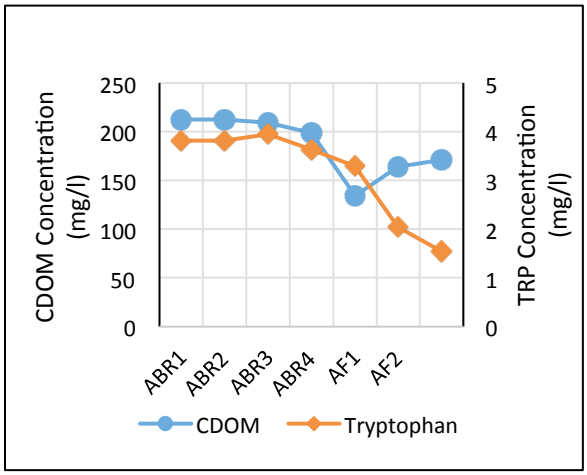

Figure 13. TRP-like and CDOM concentrations for 13:00 sampling of Street 3. Measured at outlet, at 0.5m.

A 24-hour time lapse was also taken of the inlet and outlet of the ABR (ABR chambers 1 and 4). ABR 1 was measured from 11:50 July 16, 2015 to 8:25 July 17, 2015 (Thursday to Friday) and ABR 4 was measured from 10:55 July 15, 2015 to 9:40 July 16, 2015 (Wednesday to Thursday). The results can be seen in the following graphs.

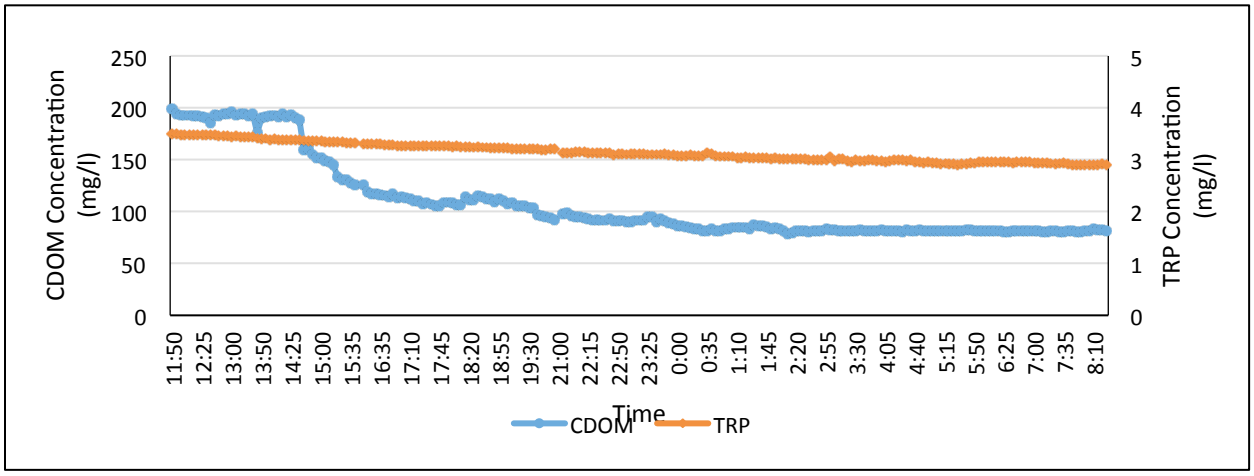

Figure 14. TRP-like and CDOM concentrations for 24-hour time lapse of ABR 1 in Street 3. Measured at outlet, at 0.5m.

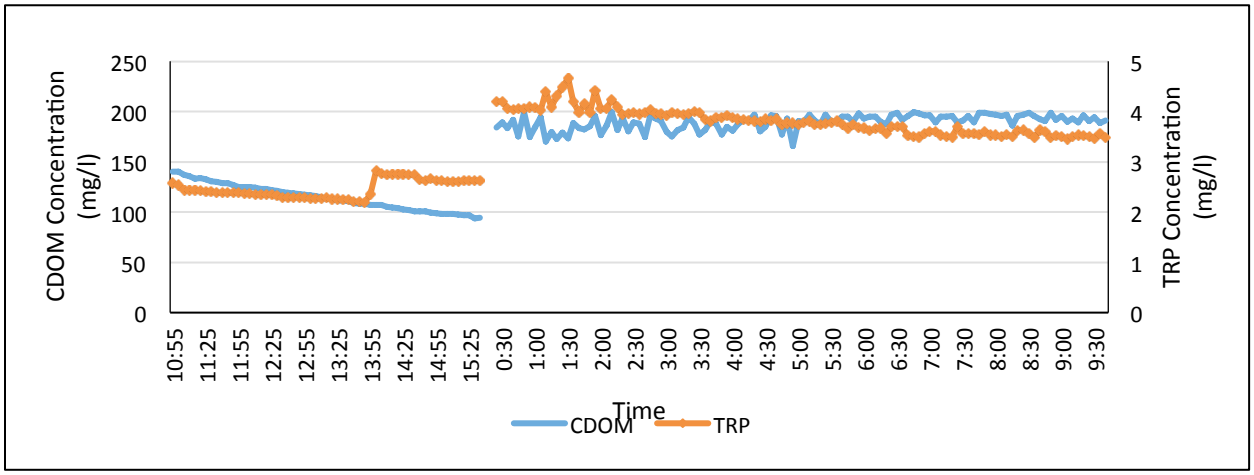

Figure 15. TRP-like and CDOM concentrations for 24-hour time lapse of ABR 4, Street 3. Measured at outlet, at 0.5m. An instrument malfunction caused a gap in data from 16:00 to 0:00 at which point no data was taken.

Last but not least, comparison measurements were taken of all three streets on July 17, 2015 at 9:00 and 11:00. The results can be seen in the following graph. Notice that Street 3 has consistently higher TRP concentrations than the other two streets at the outlet of the ABR and in AF2.

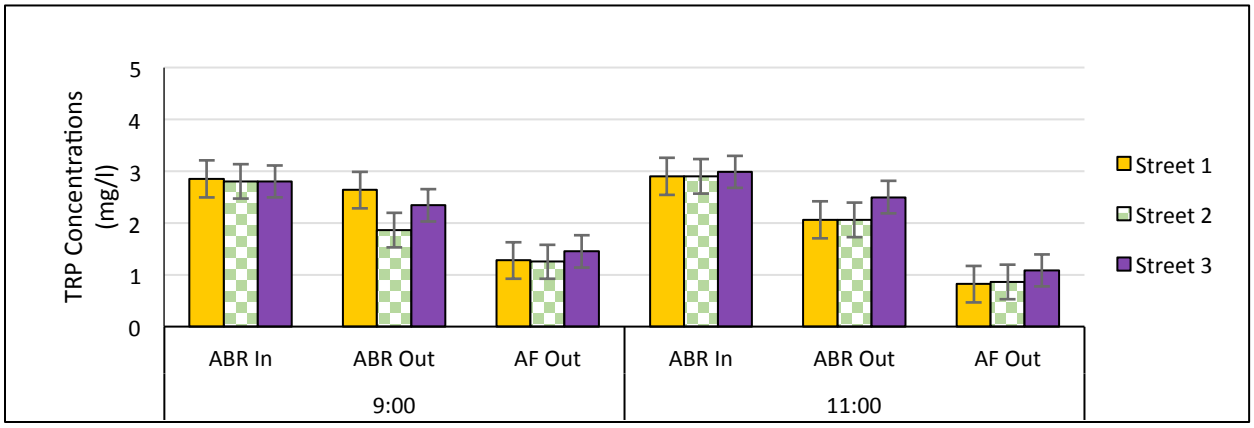

Figure 16. TRP-like concentrations for the inlet (chamber 1) and outlet (chamber 4/7) of the ABR in all three streets. Measured at outlet, at 0.5m.

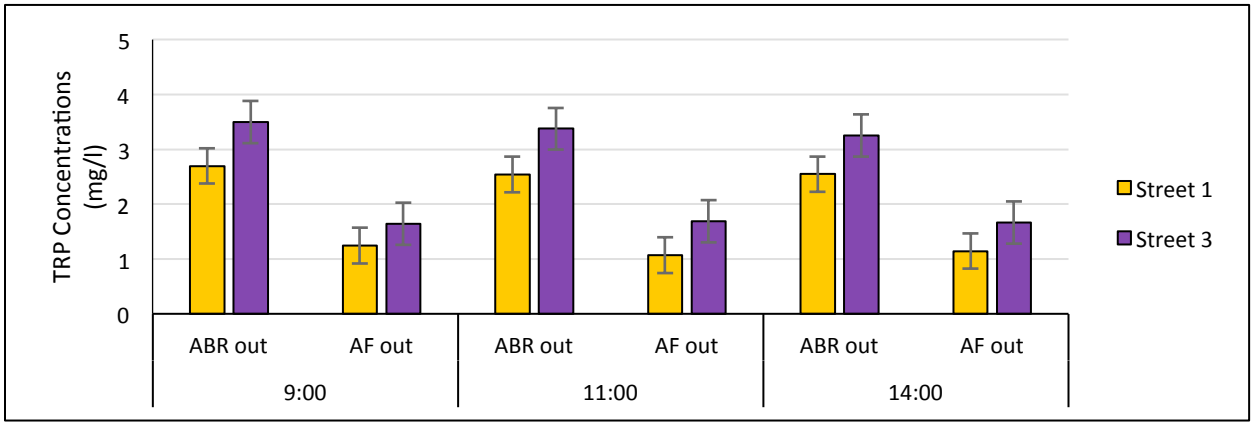

Figure 17. TRP-like concentrations for the ABR outlet (chamber 7/4) and AF outlet (AF 2) of Streets 1 and 3. Measured at outlet, at 0.5m.

Results from the first week of sampling compared TRP-like concentrations for varying depths and locations within each chamber. A t-test was performed on the data for ABR 3 and ABR4 (t-test values can be seen in the table below) which did show a significant difference in both depth and location with a 95% confidence interval, however, based on the graphs seen in the results section above, it was determined that there were no practical differences found within the chamber for either depth or location. With no practical differences observed, the chamber could be considered one unit with a constant biodegradation rate throughout the entire chamber.

Table 1. ABR 3 Location Comparison T-Test

	Inlet – Middle	Inlet – Outlet	Middle – Outlet
0.5 m	1.049 E-19	5.180 E-86	4.312 E-10
1.0 m	0.918 *	2.732 E-07	0.459 *
1.5 m	1.924 E-15	0.001	9.538 E-07
ABR 3 Depth Comparison T-Test			
	0.5 m – 1.0 m	0.5 - 1.5 m	1.0 – 1.5 m
Inlet	0.001	3.430 E-13	0.0001
Middle	8.254 E-17	1.159 E-16	0.137*
Outlet	2.735 E-95	2.810 E-92	0.056*

*P-value <0.05 indicates a significant difference. Only four areas in ABR 3 showed no significant difference.

Table 2. ABR 4 Location Comparison T-Test P-values

	Inlet – Middle	Inlet – Outlet	Middle – Outlet
0.5 m	3.228 E-24	0.004	2.722 E-27
1.0 m	1.769 E-146	1.122 E-39	2.318 E-42
1.5 m	1.652 E-125	3.341 E-32	3.621 E-46
ABR 4 Depth Comparison T-Test			
	0.5 m – 1.0 m	0.5 - 1.5 m	1.0 – 1.5 m
Inlet	2.395 E-117	1.418 E-120	8.297 E-31
Middle	1.581 E-09	1.512 E-15	3.961 E-48
Outlet	1.739 E-13	7.769 E-39	1.472 E-14

With a constant biodegradation rate throughout the entire chamber, the subsequent measurements after week one were only done at 0.5m at the outlet of each chamber. Time trials done on Street 1 at 9:00, 11:00, and 13:00 at this location in each chamber indicated a general decrease in TRP-like concentrations as the wastewater went through the treatment train. The steepest decrease, however, was seen in the anaerobic filter chambers. Based on the correlation of TRP-like concentration and E. coli presence found in other research, this would suggest that there was a gradual removal of E. coli or bacteria within the ABR with the largest removal in the AF chambers (Baker *et al.* 2015). Percent removal as compared to the concentrations in the previous chamber can be seen in the following graphs.

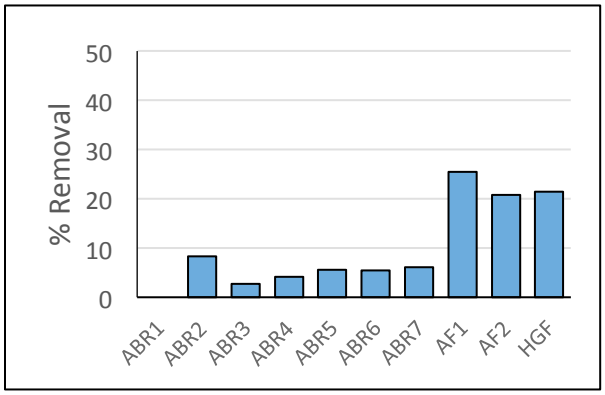

Figure 18. Percent removal of TRP-like concentrations from the previous chamber within Street 1.

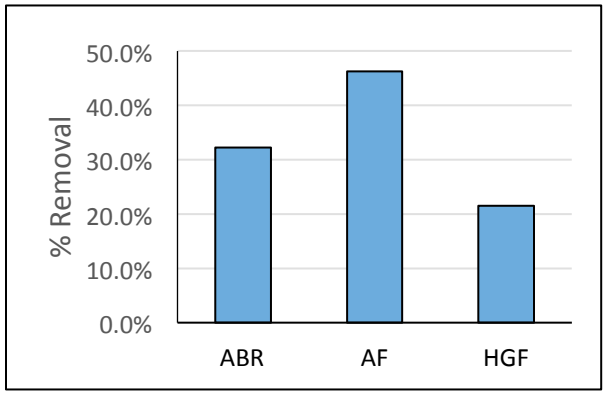

Figure 19. Percent removal of TRP-like concentrations by treatment section in Street 1: ABR, AF, and HF.

It is also important to note that the concentrations in ABR 1 (the inlet of the whole system) were typically at 3 mg/l with ABR 7 (the outlet) usually at 2 mg/l, AF 2 at <1 mg/l, and HGF at or near 0 mg/l. Comparing these concentrations to those found by Baker *et al,* these concentrations are much higher even in the AF. They measured TRP-like concentrations ranging from only 0.006-0.196 mg/l which correlated to an E. coli concentration of 146-9,204,500 CFU/100 ml. It must be added, however, that their research was performed on sewage contaminated freshwater while this research was on municipal wastewater which, after treatment, reached a level similar to or better than contaminated fresh water.

24-Hour time lapse trials on Street 1 and 3 were inconclusive as they were not indicative of any daily cyclical pattern. It was assumed that TRP-like concentrations would follow the same daily pattern as flow rates, a large peak in the morning, a smaller peak in the evening, and then decreasing overnight. This was not seen through Cyclops sampling, however. Differences in TRP-like concentrations were seen between different chambers between the different streets. Both ABR 1 chambers from Streets 1 and 3 only showed a gradual decrease in concentrations while ABR 7/4 showed sudden changes in concentrations. It is unclear if this was a function of differences in daily loads, chambers, instrument malfunction, or another unknown factor. Further research, possibly leaving the Cyclops in one chamber for an entire week, would be needed to fully understand the patterns in TRP-like concentrations.

Different treatment streets showed different TRP-like concentrations as well. In time trials done at 9:00 and 11:00 of all three streets, a clear difference was found in Street 3. All three streets started at the same concentration at the ABR inlet (ABR 1). By the time the water exited the ABR at ABR 7/4, however, concentrations were noticeably higher in Street 3 than the other two streets. Similarly, a comparison of only Streets 1 and 3 ABR and AF outlets show that Street 3 had consistently higher TRP concentrations than Street 1. This would also imply that Street 3 has higher concentrations of E. coli than Street 1. This data could be indicating that Street 3, with only four ABR chambers, is less efficient at removing bacteria than the other two streets. Higher concentrations may have been a function of a lower flow rate through Street 3 however. Flow patterns and their effect on TRP concentrations would need to be analyzed further to confirm this.

Conclusion

Altogether the biodegradability rates via TRP-like fluorescence were able to be estimated using in-situ fluorescence instrumentation. Individual chambers within the DEWATS treatment streets were found to have a uniform biodegradation regardless of depth or location within a chamber. Biodegradation decreased throughout the treatment street with the largest decrease occurring in the anaerobic filters. Further research is needed to establish microbial treatment patterns as 24-hour time lapses did not reveal any daily cyclical patterns. In addition to this, based on these results it is recommended not to use four ABR chambers instead of seven as Street 3 with only four chambers seemed to be less efficient for biodegradation.

All results from the Cyclops, however, need to be used in conjunction with standard biodegradability tests such as use of a respirometer, 5-day Biochemical Oxygen Demand tests, and E. coli enumeration. Used alongside these traditional tests, in-situ fluorescence using TRP-like peak intensities gives a quick snapshot of overall biodegradability. This method could then be extremely useful in evaluating other DEWATS plants in an effort to provide a solution for sustainable sanitation around the world.

References

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Hudson, N., Baker, A., Ward, D., Reynolds, D., Brunsdon, C., Carliell-Marquet, C., Browning, and S. Can fluorescence be used as a surrogate for the Biochemical Oxygen (BOD) test in water quality assessment? An example from South West England. *Science Direct*. 2008, 391: 149-158.

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