TREATMENT WETLAND REMEDIATION VIA IN SITU SOLIDS DIGESTION
USING NOVEL BLUE FROG CIRCULATORS

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ABSTRACT

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After 33 years of operation with little maintenance, the treatment wetlands at the Arcata Wastewater Treatment Facility (AWTF) suffer from reduced treatment capacity and internal loading issues, leading to reduced performance in recent years. The next NPDES permit for the facility will regulate discharge ammonia concentrations, and internal ammonia loads, associated with accumulated solids in the system, may lead to future regulation violations.

To begin addressing this problem, the City of Arcata entered into a rental agreement with Absolute Aeration to install two Blue Frog circulators in Treatment Wetland 3 (TW3). The goal of this project was to determine if the circulators could digest settled solids in-situ, restoring the treatment capacity and reducing the internal loads of nitrogen in TW3, without causing negative impacts to downstream water quality. These questions were addressed by four periodic sludge surveys, which occurred between April 2016 and November 2017, and weekly water quality sampling. The surveys tracked volumetric changes in the settled solids layer using cut and fill analysis with ArcGIS. Survey results showed that the Blue Frog System (BFS) reduced sludge depths and restored treatment capacity in its area-of-influence, but not across the entire wetland.
At the end of the project, there was a net increase in the volume of the settled solids layer of approximately 4800 ft\(^3\). The incoming volume of solids, estimated with TSS removal rates measured during the project, was approximately 6900 ft\(^3\) to 11700 ft\(^3\), meaning the estimated volumetric increase of the settled solids layer was 30\% to 59\% of the total volume of solids that entered the wetland. Due to the lack of a control solids survey on a wetland without the BFS in place, it is uncertain to what degree the BFS affected solids reduction compared to biochemical processes that naturally occur in wetlands. Weekly sampling of TSS, nitrogen, and BOD indicated there were no negative impacts to downstream water quality during the project. Results showed that the BFS did not reduce ammonia concentrations through TW3, seen by internal increases of ammonia over much of the project. Overall, results indicate that the BFS will not be the method to deal with the settled solids within the treatment wetlands but may be useful in restoring some capacity in open areas and potentially aid in reduction of incoming loads.
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# TABLE OF CONTENTS

ABSTRACT ....................................................................................................................... ii

ACKNOWLEDGEMENTS ................................................................................................. iv

TABLE OF CONTENTS ................................................................................................. vii

LIST OF TABLES ........................................................................................................... x

LIST OF FIGURES ........................................................................................................ xi

LIST OF ACRONYMS ...................................................................................................... xv

LIST OF APPENDICES .................................................................................................. xvii

INTRODUCTION ............................................................................................................. 1

  Project Objective ........................................................................................................ 7

REVIEW OF LITERATURE ............................................................................................ 9

  AWTF Treatment Wetlands Water Quality ............................................................. 9

  Sludge Characteristics ............................................................................................ 12

  Sludge Digestion ..................................................................................................... 14

    Hydrolysis ............................................................................................................... 17

    Acidogenesis ......................................................................................................... 19

    Acetogenesis ......................................................................................................... 20

    Methanogenesis .................................................................................................... 21

  Nitrogen Removal Pathways .................................................................................... 22

    Ammonia Volatilization ....................................................................................... 23

    Plant Uptake .......................................................................................................... 23
Internal Water Quality: Phase One ................................................................. 77
Internal Water Quality: Phase Two .............................................................. 80
Internal Mass Removal ............................................................................ 81
Comparison of TW3 and TW1 ................................................................. 82
Areal Mass Removal ................................................................................ 83
Settled Solids Survey ............................................................................... 85
CONCLUSIONS ......................................................................................... 89
REFERENCES ......................................................................................... 92
Appendix A: Additional Water Quality Data ............................................. 97
Appendix B: Additional Solids Survey Results ........................................ 105
LIST OF TABLES

Table 1: Standard Methods for utilized water quality procedures (American Public Health Association 2005) .................................................................................................................. 43

Table 2: Volumetric survey results across TW3 during the project. .............................. 73

Table 3: Volumetric survey results in the Mid Cutout. The asterisks indicate the presence of the BFS in the cutout. .................................................................................................................. 74

Table 4: Volumetric survey results in the Influent Cutout. The asterisks indicate the presence of the BFS in the cutout. .................................................................................................................. 75

Table 5: Parameters used in incoming volume estimation. ............................................. 76

Table 6: Collected pH samples within TW3 during Phase One. ................................. 102

Table 7: Collected pH samples within TW3 during Phase Two................................. 103

Table 8: ORP samples collected at the bottom of the water column for Phase One. .... 103

Table 9: ORP samples collected at the bottom of the water column for Phase Two.... 104

Table 10: Raw data for sludge depths collected during baseline survey (continued on next page). ........................................................................................................................................ 108

Table 11: Raw data for sludge depths collected during December 2016 survey (continued on next page). ........................................................................................................................................ 109

Table 12: Raw data for sludge depths collected during May 2017 survey (continued on next page). ........................................................................................................................................ 110

Table 13: Raw data for sludge depths collected during November 2017 survey (continued on next page). ........................................................................................................................................ 112
LIST OF FIGURES

Figure 1: The AWTF is located within and is part of the AMWS................................. 3

Figure 2: Treatment wetlands and oxidation ponds act as secondary treatment (Google Earth).......................................................... 5

Figure 3: Seasonal variation in the BOD and TSS concentrations of AWTF discharge showing out-of-compliance concentrations.......................................................... 6

Figure 4: Average water temperatures of the combined TW effluent............................. 11

Figure 5: Average monthly pH of TW influent................................................................. 12

Figure 6: Schematic of anaerobic digestion showing intermediate products and required bacterial groups (Adapted from Christy et al. 2014). ................................................................. 16

Figure 7: The nitrogen cycle in wetland ecosystems (Adopted from Inglett et al. 2011). .............................................................................................................................. 23

Figure 8: Basic BF circulator diagram showing discharge segments, lower lip, and water intake inlet (Adapted from Bettle 2012). ........................................................................ 26

Figure 9: Stratification induced by lateral surface mixing from BF circulators, showing minimum required depth for adequate stratification (Adapted from Bettle 2016). ........... 28

Figure 10: Flow diagram showing multiple steps of perimeter flow............................... 29

Figure 11: Lagoon sludge showing formed granules (Bettle 2012). ................................. 31

Figure 12: Stages of the BF sludge digestion process (Adopted from Bettle 2016). Showing the initial digestion of alluvial sludge (A), apparent increase in volume due to gas production (B), continued digestion of alluvial sludge leading to decreased depths (C), slower digestion of legacy solids (D), and equilibrium sludge depths (E). ............. 32

Figure 13: Sludge depth measurement for a seven-year application in a poultry processing plant treatment lagoon (Bettle 2012). ................................................................. 34

Figure 14: Sludge depth measurement from municipal sludge holding tanks in Lyon County, NV (Bettle 2012b)................................................................. 35
Figure 15: Three locations of vegetation removal in TW3. Areas of removed vegetation allowed for internal sample locations. Aerial photography provided by Dr. Brad Finney.

Figure 16: Circulators in the Mid Cutout showing the floating baffle installed around the southern circulator.

Figure 17: Sample site map of TW3 showing Phase One and Phase Two sample locations.

Figure 18: Sludge tube used during the first two sludge surveys showing measuring tape and shutoff valve to keep water and sludge in the tube.

Figure 19: Sample locations of the sludge surveys used to generate GIS surface plots.

Figure 20: Internal BOD concentrations during Phase One of the pilot project.

Figure 21: Phase One internal sBOD concentrations showing a release of soluble compounds as water enters the wetland during summer months.

Figure 22: BOD concentrations through TW3 on 9/23/2016 showing release of BOD from internal sources. Soluble BOD shows the largest increase within the wetland.

Figure 23: BOD concentrations through TW3 on 12/15/2016 showing less pronounced release of BOD from internal sources.

Figure 24: Internal ammonia concentrations for Phase One, showing release of ammonia through the wetland.

Figure 25: Internal nitrate concentrations for Phase One showing decreasing nitrate concentrations through TW3.

Figure 26: Internal TSS concentrations during Phase One of the project.

Figure 27: Internal BOD concentrations during Phase Two of the project.

Figure 28: Internal sBOD concentrations during Phase Two of the project.

Figure 29: Internal ammonia concentrations during Phase Two of the project.

Figure 30: Internal nitrate concentrations during Phase Two of the project.

Figure 31: Internal TSS concentrations during Phase Two, showing spikes in solids at the front section (Inf 1 and Inf 2) of TW3.
Figure 32: Internal variation in TSS concentration while in series or parallel operation.

Figure 33: Comparison of mass removal of TSS through areas with and without BF circulators. BFS located between west cutout and effluent during Phase One and influent and west cutout during Phase Two.

Figure 34: Removal of TIN through TW3 over the course of the project.

Figure 35: Effluent BOD concentrations of TW3 and TW1 showing the installation date of the BFS.

Figure 36: Effluent TSS concentrations of TW3 and TW1 showing the installation date of the BFS.

Figure 37: Effluent ammonia concentrations of TW3 and TW1 showing the installation date of the BFS.

Figure 38: Effluent nitrate concentrations of TW3 and TW1 showing the installation date of the BFS.

Figure 39: Areal BOD removals for TW3 and TW1 during the project (2016 – 2017).

Figure 40: BOD areal mass removal for TW3 and TW1 over the period of 2011 to 2012.

Figure 41: Areal TSS removals for TW3 and TW1 over the course of the pilot project.

Figure 42: Areal TSS removals for TW3 and TW1 from 2011 to 2012.

Figure 43: Distribution of the settled solids layer during the baseline survey conducted in April 2016.

Figure 44: Distribution of the settled solids layer during the December 2016 survey showing a reduction in sludge depth in the Mid Cutout and an accumulation of solids in the Influent Cutout.

Figure 45: Exceedance probability curve for the TSS removal rate seen in TW3 over the course of the project.

Figure 46: Seasonal variations in AWTF effluent ammonia concentrations that contribute to the seasonal spike in BOD as NBOD.

Figure 47: Water temperatures through TW3 over the course of the project indicating conditions suitable for psychrophilic anaerobic digestion.
Figure 48: CBOD concentration through TW3 during Phase One of the project........... 99

Figure 49: CBOD concentration through TW3 during Phase Two of the project........ 100

Figure 50: DO concentration at the top and bottom of the water column showing stratification effect caused by the BFS. ................................................................. 100

Figure 51: Snapshot of DO concentrations during Phase One of the project showing aeration by the BFS................................................................. 101

Figure 52: Snapshot of DO concentrations during Phase Two of the project showing aeration by the BFS................................................................. 101

Figure 53: Conductance at the top and bottom of the water column in the Mid Cutout and Influent Cutout................................................................. 102

Figure 54: Distribution of the settled solids layer during the May 2017 survey. ........ 106

Figure 55: Distribution of the settled solids layer during the November 2017 survey.. 107
LIST OF ACRONYMS

AMRI: Arcata Marsh Research Institute
AMWS: Arcata Marsh and Wildlife Sanctuary
AWTF: Arcata Wastewater Treatment Facility
BF: Blue Frog
BFS: Blue Frog System
BOD: Biochemical Oxygen Demand
CBOD: Carbonaceous Biochemical Oxygen Demand
DO: Dissolved Oxygen
DON: Dissolved Organic Nitrogen
GBSR: Granule Bed Sludge Reactor
HP: Horsepower
L: liter
lbs: pounds
mg: milligram
MGD: Million Gallons per Day
NBOD: Nitrogenous Biochemical Oxygen Demand
ORP: Oxidation-reduction Potential
PON: Particulate Organic Nitrogen
QGIS: Quantum Geographic Information System
sBOD: soluble Biochemical Oxygen Demand
TIN: Total Inorganic Nitrogen
TKN: Total Kjeldahl Nitrogen
TSS: Total Suspended Solids
TW: Treatment Wetland
VFAs: Volatile Fatty Acids
LIST OF APPENDICES

Appendix A: Additional Water Quality Data…………………………………………79
Appendix B: Additional Solids Survey Results and Raw Data.............................86
INTRODUCTION

Constructed treatment wetlands provide the opportunity to create wildlife habitat, treat wastewater, sequester carbon, and provide recreational opportunities to communities. In the early 1980s, the City of Arcata, a coastal community located in Humboldt County of Northern California, elected to employ this treatment process by adding treatment and enhancement wetlands to the Arcata Wastewater Treatment Facility (AWTF) and creating the Arcata Marsh Wildlife Sanctuary (AMWS). Located within the AMWS, the AWTF (Figure 1) treats wastewater produced within the City of Arcata before discharge to Humboldt Bay. Currently, the AWTF utilizes conventional pretreatment and primary treatment, oxidation ponds followed by six treatment wetlands (TWs) as secondary treatment, chlorination as disinfection, and additional biological treatment with three in-series enhancement wetlands. Water that receives polishing from the enhancement wetlands is mixed with disinfected secondary-treated water, dechlorinated using sulfur dioxide, and discharged into the Humboldt Bay. The City is currently undergoing a facility upgrade project that aims to replace aging infrastructure, increase the treatment capacity of the plant, and ensure reliability in meeting discharge regulations that will be implemented in future permits.

The AWTF has an average dry weather design flow of 2.3 million gallons a day (MGD) and an average wet weather design flow of 5.0 MGD, but can experience peak instantaneous flows of up to 16.5 MGD (LACO and Carollo 2017). Based on AWTF inflow data averaged over the past 10 years, and considering the dry weather period as
May 1st to October 31st and the wet weather period as November 1st to April 30th, the average operating dry weather flow is approximately 1.3 MGD and the average operating wet weather flow is approximately 2.3 MGD. Influent flows above 5.0 MGD bypass primary treatment and are pumped, via the First Street Pump Station, to Oxidation Pond 1. The two oxidation ponds have a combined footprint of approximately 46 acres, an estimated treatment volume of 89 million gallons, and are operated in series. The six TWs have a footprint of approximately 9.7 acres, an estimated maximum treatment capacity of 3.3 MGD, and are typically operated in parallel (LACO and Carollo 2017).
Figure 1: The AWTF is located within and is part of the AMWS.

TWs 1-3 (Figure 2) of the AWTF were originally designed as emergent macrophyte systems and were planted with broad-leaved cattail (*Typhus latifolia*) and hardstem bulrush (*Schoenoplectus acutus*). After multiple years of operation, buoyant forces acting on the vegetation caused the mat to uproot and float at the top of the water column, causing the system to operate as a floating-plant wetland. TW4 is the original
pilot wetland and receives a constant flow rate of approximately 0.2 MGD. TW4 also operates as a floating-plant wetland and has a serpentine flow configuration. The flow configuration of TW4 is a result of the connection of the original pilot treatment wetlands. TW5 and TW6 were installed in 2012 and were designed to have settling zones interspaced by berms planted with *T. latifolia* and *S. acutus*; both wetlands were constructed in the footprint of Oxidation Pond 3 and are divided lengthwise by sheet piling. Open water areas of the six TWs are dominated by floating marsh pennywort (*Hydroctyle ranunculoides*), duckweed (*Lemna minor*), and water parsley (*Oenanthe sarmentosa*). The TWs are also host to various other flora species, both native and non-native, and provide forage, nesting, and spawning habitat to several avian and amphibian species.
The natural treatment components of the system result in seasonal variations in the biochemical oxygen demand (BOD), total suspended solid (TSS), and ammonia (NH₃) concentration of the facility’s effluent. These seasonal spikes cause the AWTF to occasionally exceed the National Pollution Discharge Elimination System (NPDES) monthly average limit of 30 mg/L for BOD and TSS. Water quality sampling results from the official City of Arcata records indicate that the discharge exceeded the BOD and TSS concentration limit 14 and 53 times, respectively, since 2010 (Figure 3). Under the current permit, there are no effluent ammonia concentration limits; however, an ammonia
limit will be included in the permit currently being written by the North Coast Water Quality Control Board.

![Graph showing seasonal variation in BOD and TSS concentrations of AWTF discharge](image)

**Figure 3:** Seasonal variation in the BOD and TSS concentrations of AWTF discharge showing out-of-compliance concentrations.

Over the 33 years of operation, the lack of maintenance on the accumulated solids in the TWs has resulted in treatment capacity and effectiveness reductions. The solids accumulation has resulted in large internal loads of organic nitrogen and organic carbon, which contribute to the seasonal fluctuations shown in Figure 3 (Burke 2011). The organic nitrogen within the accumulated solids can act as a significant source of ammonium. Organic nitrogen can be converted to ammonium through mineralization and has been noted to occur at higher rates in recently deposited materials (Inglett et al. 2011). Previous studies have shown that accumulated solids within the wetlands contain a
significant quantity of total nitrogen among the settled solids and accumulated peat layers, approximately 6,000 kg/ha (Burke 2011). Ammonium concentration in the system also shows a pattern of seasonal spikes (See Appendix A, Figure 46) that increase well above expected future concentration limits of 3-mg/L NH₃-N to 5-mg/L NH₃-N (Gearheart, personal communication, 2017). Along with the imminent regulation, ammonium contributes, as soluble nitrogenous BOD (NBOD), a significant portion of the seasonal spike in BOD concentrations.

As the City of Arcata implements their facility upgrade plans, the accumulated solids within the treatment wetlands and oxidation ponds must be treated in some manner to minimize the impacts of seasonal spikes in ammonia concentrations. To begin addressing this problem, the City of Arcata entered into a contract with Absolute Aeration, LLC to conduct a pilot run of two Blue Frog (BF) circulators in Treatment Wetland 3 (TW3). In a presentation to the City, Absolute Aeration, LLC outlined three goals of the pilot project. The goals were to show that 1) the circulators could act as an effective method to reduce the accumulated solids levels in TW3, 2) that their installation would reduce average effluent BOD concentrations relative to past years of operation, and 3) that their operation would reduce ammonia levels through TW3. These goals helped developed the objectives of this study.

Project Objective

The objectives of this project are to: 1) measure and provide estimates of volumetric changes to the accumulated solids in TW3 during the trial application of the
BF circulators, 2) track and assess the water quality impacts resulting from the implementation and operation of two BF circulators in TW3, and 3) provide recommendations to the City of Arcata on the effectiveness and potential use of BF circulators at the AWTF.
REVIEW OF LITERATURE

In order to understand the various processes that are involved in this project, it is important to cover the state of the wetlands in the AWTF, the sludge, how this sludge can be removed, potential mechanisms by which ammonia can be removed, and by what means the BFS can aid in the removal of sludge and nitrogen. This review compiles the available information on the AWTF treatment wetlands, the characterization of primary and secondary sludge, the biochemical processes of anaerobic digestion, and nitrogen removal pathways. AWTF treatment wetland water quality presented in the following section was obtained from data collected by the City of Arcata and the Arcata Marsh Research Institute (AMRI).

Also included is information on the Blue Frog circulators produced by Absolute Aeration, LLC. Information on the technology is sourced from the Blue Frog design manual and various presentations, articles, and design reviews published by the company. Case studies from previous applications of the Blue Frog System (BFS) at multiple locations are included. The BFS treatment processes are described by Absolute Aeration and are not verified by third party sources.

AWTF Treatment Wetlands Water Quality

The AWTF incorporates six treatment wetlands as part of secondary treatment, following the oxidation ponds. TW3, the study site, currently consists of open water zones,
dominated by *H. ranunculoides* and *O. sarmentosa*, and floating vegetated mats, dominated by *T. latifolia* and *S. acutus*. Approximately 42% of the wetland area is open water zone, with the remaining 58% covered by a floating vegetation mat. Prior to the study, TW3 was a fully planted wetland with a floating mat dominated by *T. latifolia* and *S. acutus*.

Effluent from Oxidation Pond 2, which has an average BOD and TSS concentration of 45 mg/L but can range from 10 to 130 mg/L, is the influent to the TWs (Burke 2011). Water temperatures, taken as the monthly average over the past five years, in the wetlands vary through the seasons and ranges from approximately 10 °C to approximately 20 °C (Figure 4). Influent water to the TWs has a relatively constant pH over the course of a year; average pH values based on data collected by AMRI from 2010 to 2014 show little variation, ranging from approximately 7.2 to approximately 7.5 (Figure 5). Data published in Garrison (2011) indicate that any dissolved oxygen (DO) in the influent water to the TWs is quickly utilized, leading to anaerobic conditions in the TWs. TSS and BOD removed by the TWs is incorporated into the sludge layer.

Studies conducted by Burke (2011) and Garrison (2011) showed that the treatment wetlands have an internal load of nitrogen. Data collected by both studies showed that the TWs release ammonia into the water column due to decay of settled solids and detrital material within the wetlands. Burke (2011) also determined that the settled solids and accumulated peat layers act as significant sources of total nitrogen, approximately 6,000 kg/ha, which can be converted to ammonia through the process of mineralization (Ingeltt et al. 2011). The buildup of internal nitrogen loads in the treatment
wetlands is a result of the uptake of inorganic nitrogen compounds by the wetland vegetation over the operational lives of the wetlands. Plant uptake during summer months is a potentially significant ammonia removal pathway during the growing season (Al-Shafie 2014). After senescing during fall and winter months, plant material is incorporated into the vegetative mat and, eventually, into the settled solids layer. This returns the nitrogen that was previously removed back into the treatment wetland as organic nitrogen in the vegetation mat and sludge layer. As indicated by previous studies, the sludge within TW3, like the sludge in other wetlands at the AWTF, should have large amounts of total carbon and total nitrogen. Due to the nature of the influent water and presence of a vegetated mat in TW3, the sludge layer should be composed of large amounts of algal and plant material.

Figure 4: Average water temperatures of the combined TW effluent.
Figure 5: Average monthly pH of TW influent.

Sludge Characteristics

Sludge characteristics vary at different points of the wastewater treatment process. Sewage sludge is a complex material that has characteristics that depend on the source of the material, previous treatment processes, chemical additives, and mechanical treatment processes (Baroutian et al. 2013). Parameters often used in characterizing sewage sludge for anaerobic stabilization include solids concentration, volatile solids, temperature, pH, volatile acids, fats and oils, and nutrients (Bresters et al. 1997). Ratios of volatile solids to total solids, known as a sludge stability index, can also be used for characterizing sewage sludge (Bresters et al. 1997).

Literature reviewed for this project recognizes differences between primary sludge and secondary sludge, also known as waste-activated. Primary sludge is typically
regarded as settled raw sewage from primary settling tanks and lagoons that receive raw sewage, while secondary sludge is considered the settled solids of wastewater that has received secondary treatment (Davis 2010). Primary sludge characteristics exhibit wide variation depending upon the origin of the material (municipal sewage, manure sludge, processing plant sludge). The solids concentration in typical municipal primary sludge ranges from 4% – 6%, while the solids concentration of secondary sludge is lower (Herwijn 1996).

One major difference between primary and secondary sludge is the percentage of bacterial biomass that composes the material. While primary sludge is dominantly colloidal and suspended organic matter that settles out during primary clarification, secondary sludge has a larger percentage of settled aerobic microorganisms that are produced during secondary treatment (Baroutian 2013, Mahdy et al. 2015). Multiple authors have observed decreased biodegradability of secondary sludge compared to primary sludge (Jones et al. 2008, Mahdy et al. 2015). Digestion efficacy is reduced in secondary sludge due to the difficulty in hydrolyzing bacterial cell membranes; efficacy is further reduced in secondary sludge that has larger microalgal content due to the thicker cell walls of microalgal cells and bacterial inhibition by ammonium that is released from digested algal cells (Sialve et al. 2008, Mahdy et al. 2015).

Algal sludge has been noted to be recalcitrant to biodegradation by Yen and Brune (2007), who proposed that pretreatment is required to obtain efficient anaerobic digestion; this conclusion has also been suggested by Chen and Oswald (1998). Mahdy et al (2015) notes that the biodegradability of a microalgal substrate also depends upon the
species of algae present in the substrate; this is due to variations in the proportion of proteins, carbohydrates, and lipids that are present in different species (Sialve et al. 2008). Further, Yen and Brune (2007) states that the carbon to nitrogen ratio, approximately 6:1, of algal sludge is too low for adequate anaerobic digestion.

Due to the importance of substrate type to the anaerobic digestion process, the efficacy of the BFS will depend on the characteristics of the settled solids in TW3. Wastewater entering TW3 has undergone secondary treatment in the oxidation ponds, with the main source of oxygen being algae. Because of prior treatment processes, the settled solids in TW3 should have characteristics similar to secondary sludge with a large algal content. Another significant component of the settled solids in TW3 is plant detritus generated within the wetland. This indicates that the sludge may be recalcitrant to biodegradation.

Sludge Digestion

Sludge digestion in municipal wastewater treatment plants is generally attained through anaerobic digestion, although aerobic digestion of sludge is also possible. Anaerobic digestion is a multi-stage reaction process (Figure 6) that requires many trophic groups of bacteria (McCarty 1964, Parkin and Owen 1986, O’Reilly et al. 2009, Christy et al. 2014). Complete anaerobic digestion can be broken into three main steps: 1) hydrolysis, liquefaction, and fermentation, 2) hydrogen and acetic acid formation (acidogenesis and acetogenesis), and 3) methane formation (methanogenesis) (Parkin and
Owen 1986). Each of these steps requires different groups of bacteria, such as hydrolytic, acid forming, acetogenic, and methanogenic bacteria (Shah et al. 2014).

Temperature is an important parameter of the anaerobic digestion process in that it affects bacterial activity rates and selects which bacterial groups are participating in digestion. Anaerobic digestion is typically defined by three temperature ranges: thermophilic, mesophilic, and psychrophilic (Chen and Neibling 2014). Thermophilic anaerobic digestion occurs at temperatures of 49 °C to 60 °C, mesophilic anaerobic digestion occurs at temperatures of 32 °C to 43 °C and psychrophilic anaerobic digestion occurs at temperatures below 32 °C (Chen and Neibling 2014). In general, bacterial processes involved in anaerobic digestion become inactive at temperatures less than 15 °C (Chen and Neibling 2014). Zhu et al. (2014) notes that psychrophilic digestion is not as efficient as mesophilic or thermophilic digestion but can continue at water temperatures as low as 15 °C if cold water adapted microbial communities are present. At this time, there is no available literature on the microbial community of the AWTF treatment wetlands; however, based on water temperature provided by the AMRI database, psychrophilic anaerobic digestion conditions should be present in TW3.
Figure 6: Schematic of anaerobic digestion showing intermediate products and required bacterial groups (Adapted from Christy et al. 2014).
Hydrolysis

The first step in anaerobic digestion is hydrolysis; this stage also constitutes the rate-limiting step of anaerobic digestion when hydrolyzing solid, lignocellulosic compounds, such as those that would exist in algal and plant detrital matter (Eastman and Ferguson 1981, Parkin and Owen 1986, Mata-Alvarez and Llabrés 2000, Shah et al. 2014, Christy et al. 2014). The rate of hydrolysis is affected by the pH, particle geometry, and the available surface area of the particle (Vavilin et al. 1996, Ziemiński and Fraç 2012). Inhibition of hydrolysis has been shown to occur where free ammonia concentrations are high; high concentrations of free ammonia are also known to be toxic to methanogenic bacteria (Vavilin et al. 1996, Hansen et al. 1998). Vavilin et al. (1996) noted inhibition at a free ammonia concentration of 990 mg NH₃-N/L while Hansen et al. (1998) noted inhibition at concentrations of 1100 mg NH₃-N/L.

Hydrolysis relies on enzyme-mediated transformation of insoluble organic materials and complex compounds, such as lipids, proteins, and polysaccharides, into soluble organic materials, such as simple sugars, amino acids, and long-chain fatty acids (Figure 11) (Adekunle and Okolie 2015). This process occurs through extracellular enzymes, from the hydrolases group, secreted by bacteria that “cut” larger insoluble compounds into smaller compounds that are accessible to these and other bacterial groups (Parkin and Owen 1986, Schink 1997, Shah et al. 2014). Both facultative bacteria, such as *Streptococcus* and *Enterobacterium*, and obligate anaerobes, such as *Bacteroides* and
*Clostridia*, contribute the hydrolytic enzymes necessary for this stage (Shah et al. 2014, Adekunle and Okolie 2015).

Christy et al. (2014) states that hydrolysis occurs in two phases. In the first phase, bacteria colonize the substrate surface and begin to release enzymes that produce monomers. In the second phase, the colony begins to degrade the sludge surface, reducing the depth at a constant rate. The two-phase model is discussed in detail in Vavilin et al. (1996). Their work showed that hydrolysis was more accurately modeled using a two-phase model or a model based on the Contois function, a bacterial growth model in which the specific growth rate is a function of population density and the concentration of the limiting nutrient, when compared to a single-phase model or Monod equations (Contois 1959, Vavilin et al. 1996).

A representative hydrolysis reaction can be presented by an approximate chemical formula for a mixture of organic waste (Equation 2) being converted into glucose and hydrogen (Christy et al. 2014).

\[
C_6H_{10}O_4 + 2H_2O + Enzymes \rightarrow C_6H_{12}O_6 + H_2 \quad (2)
\]

Parkin and Owen (1986) states that not all organic matter can be hydrolyzed due to the structure of the compounds, inaccessibility of the substrate, and complex linkages. The paper also reported that the non-biodegradable fraction of municipal sludge ranges from 35% up to 80% of the volatile solids, depending on the nature of the substrate. As previously noted, the nature of the settled solids in TW3 has the potential to slow hydrolysis.
**Acidogenesis**

Acidogenesis is typically the fastest stage of anaerobic digestion (Christy et al. 2014). Growing at a rate ten times faster than methanogens, acidogenic organisms, such as *Lactobacillus*, *Bacillus*, *Escherichia coli*, and *Salmonella*, make use of the hydrolysis products (simple sugars, long-chain fatty acids, and amino acids) as substrate. Products of the reaction include acetic acid, propionic acid, butyric acid, alcohols, gaseous hydrogen, and carbon dioxide (Christy et al. 2014, Shah et al. 2014). Short chain fatty acids produced in this step are collectively known as volatile fatty acids (VFAs). Studies have shown acetic acid and propionic acid to be the dominant VFAs that are formed during this stage, regardless of solids retention time (McCarty 1964, Elefsiniotis and Oldham 1993).

Shah et al. (2014) discussed two pathways of product creation: dehydrogenation and hydrogenation. Dehydrogenation results in the production of hydrogen, carbon dioxide, and acetate: these products are directly utilizable by methanogenic bacteria and do not need to undergo acetogenesis. Hydrogenation occurs when the hydrogen concentrations are high and the reaction favors the production of VFAs. Higher VFAs (propionic acid, butyric acid) must be converted into substrates via acetogenesis that can be utilized by methanogens (Ziemiński and Fraç 2012, Shah et al. 2014). On average, acidogenesis favors the production of VFAs. Christy et al. (2014) provides the three typical common reactions of acidogenesis (Equation 3-5). These reactions are the conversion of glucose to alcohol (Equation 3), to propionic acid (Equation 4), and to acetic acid (Equation 5). The production of VFAs during acidogenesis leads to a decrease
in pH in the system (Christy et al. 2014). Excess acidogenic activity can drop the pH to lethal limits for methanogens, leading to digestion failure (Ostrem 2004).

\[
C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O
\]  
(4)

\[
C_6H_{12}O_6 \rightarrow 3CH_3COOH
\]  
(5)

**Acetogenesis**

The next step in the anaerobic digestion process is acetogenesis. This stage converts VFAs, produced during acidogenesis, into methogenic substrates, such as carbon dioxide, hydrogen and acetate (Adekunle and Okolie 2015). Acetogenic bacteria, such as *Syntrophomonas* and *Syntrophobacter*, are slow-growing, obligate anaerobes that have a syntrophic relationship with methanogens (Amani et al. 2011, Christy et al. 2014, Adekunle and Okolie 2014). McCarty (1964) compared this relationship between acetogens and methanogens to a factory assembly line. Acetogenic bacteria produce hydrogen from the oxidation of higher VFAs into acetate; hydrogen produced from this step acts as substrate for methanogens. Acetogenic activity is inhibited by high concentrations of hydrogen and the utilization of hydrogen as a substrate by methanogens is vital to keep the hydrogen partial pressure low, allowing acetogenic activity to continue (Amani et al. 2011, Christy et al. 2014). Without methanogens, the hydrogen produced from acetogenic activity would eventually reach toxic concentrations to the acetogens; without the acetogens, methanogenic activity would be inhibited by the build-up of VFAs (McCarty 1964, Schink 1997, Amani et al. 2011).
Three representative acetogenic reactions are described in Christy et al. (2014); these include the conversion of propionate into acetate (Equation 6) which can only occur at low hydrogen partial pressure, direct conversion of glucose into acetate (Equation 7), and conversion of ethanol into acetate (Equation 8).

$$CH_3CH_2OO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$$  \hspace{1cm} (6)

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2$$  \hspace{1cm} (7)

$$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 2H_2 + H^+$$  \hspace{1cm} (8)

**Methanogenesis**

Methanogenesis, or methane fermentation, is the final stage of the anaerobic digestion process in which the products of acidogenesis and acetogenesis are converted to methane under anaerobic conditions (Shah et al. 2014). Two types of methanogenesis occur: hydrogenotrophic methanogenesis and aceticlastic methanogenesis (Christy et al. 2014). Hydrogenotrophic methanogens, such as *Methanobacteria*, *Methanococci*, and *Methanomicrobia*, use dihydrogen and carbon dioxide as a substrate to produce methane and water (Equation 9) (Demirel and Scherer 2008, Christy et al. 2014). Aceticlastic methanogens, such as *Methanosarcinales*, use acetate as a substrate and produce methane and carbon dioxide (Equation 10) (Demirel and Scherer 2008, Christy et al. 2014). Other substrates that can be utilized by methanogens include formate, methanol, methylamine, and dimethyl sulfide (Shah et al. 2014). Approximately 28% of the methane produced during anaerobic digestion is a product of carbon dioxide reduction (Equation 9), with the remaining 72% resulting from acetate cleavage (Equation 10) (Parkin and Owens 1986).
\[ CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \]  \hspace{1cm} (9)

\[ CH_3COOH \rightarrow CH_4 + CO_2 \]  \hspace{1cm} (10)

Alongside potential inhibitions by VFA buildup discussed in the previous section, methanogenesis can also be inhibited by the pH of the substrate, usually due to decreased pH caused by excess acidogenic activity. Christy et al. (2014) noted that methanogens cannot survive at pH < 6.0, while Ostrem (2004) states that the optimum pH range for methanogens is 7.0 – 7.2.

Nitrogen Removal Pathways

Nitrogen removal in a wetland system occurs via a series of chemical and physical reactions known as the nitrogen cycle (Figure 7). Removal pathways shown in Figure 12 include volatilization of ammonia, nitrification and denitrification of ammonia and nitrate, respectively, and plant uptake of ammonium. These reactions represent the commonly known removal pathways of the nitrogen cycle; however, the relatively recent discovery of anaerobic ammonia oxidation, known as annamox, has added another known removal pathway to the nitrogen cycle that is not shown in Figure 7.
Ammonia Volatilization

Ammonia volatilization is a significant source of nitrogen removal under environmental conditions that favor gaseous ammonia to ammonium. Gaseous ammonia occurs in waters with a pH greater than 8.5 (Inglett et al. 2011). In such cases, soluble ammonium is converted to ammonia gas, which exits the water column through volatilization.

Plant Uptake

Nitrogen, in the form of soluble ammonium, is available to wetland plants that uptake and assimilate the nitrogen into plant tissue. This uptake acts as a temporary displacement of nitrogen rather than a true removal pathway due to the re-introduction of nitrogen to the water column, as particulate organic nitrogen (PON) and dissolved
organic nitrogen (DON), when the plant tissue dies and decays (Reddy et al. 2010). This process occurs as plants grow throughout summer and senesce in the fall.

Nitrification and Denitrification

Nitrification is a well known, two-stage, aerobic reaction that occurs in aquatic systems. The first stage involves the oxidation of ammonia to nitrite and the second stage involves the oxidation of nitrite to nitrate, as summarized by Equation 12 and 13, respectively (Ward 2013).

\[
\text{NH}_3 + 1.5\text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} + \text{H}^+ \quad (12)
\]

\[
\text{NO}_2^- + 0.5\text{O}_2 \rightarrow \text{NO}_3^- \quad (13)
\]

Denitrification of nitrate into gaseous nitrogen and nitrous oxide occurs under anaerobic conditions that have an ample carbon source. Shah and Coulman (1978) describe the reaction with glucose as the carbon source (Equation 14); other sources of carbon include methanol or acetic acid. Equation 14 represents a simplified chemical reaction that involves many intermediate reactions. Due to the anaerobic conditions and high organic carbon loads present in wastewater treatment wetlands, denitrification is considered a major nitrogen removal mechanism in most constructed wetlands (Vymazal 2005).

\[
24\text{NO}_3^- + 5\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 12\text{N}_2 + 24\text{HCO}_3^- + 6\text{CO}_2 + 18\text{H}_2\text{O} \quad (14)
\]

Annamox

Annamox is the anaerobic oxidation of ammonium coupled with nitrite reduction with gaseous nitrogen as the end product (Equation 15) (Zhu et al. 2010). This reaction is carried out by a distinct phylogenetic order, the \textit{Brocadiales}, which has been observed in
wastewater treatment plants, marine sediments, and some anoxic freshwater habitats. Zhu et al. (2010) noted that wetland systems showed the highest degree of biodiversity of annamox bacteria. Annamox bacteria have very slow growth rates and require an approximate 50:50 mixture of ammonium to nitrite to carry out the process (Kuenen 2008). Nitrite concentrations can be a limiting factor for annamox to occur in treatment wetlands due to fast metabolism of nitrite to nitrate (Russow et al. 2009).

\[ \text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O} \]  

(15)

Mineralization

Nitrogen mineralization, also known as ammonification, has been noted as a significant source of nitrogen within wetland systems dominated by macrophytes. Mineralization is the process of converting organic nitrogen into ammonium and DON compounds through aerial decay and leaching (Inglett et al. 2011). Higher rates of mineralization occur in recently deposited litter and detrital floc material compared to heavily decomposed material (Inglett et al. 2011). This indicates that recently senesced plant material and recently deposited algal cells can act as a significant nitrogen source in treatment wetlands, as was shown by Burke (2011).

Blue Frog Circulators

The basic unit of the Absolute Aeration BFS implemented in TW3 is the Blue Frog circulator (Figure 8). BF circulators are designed to create aerobic and anaerobic conditions at different depths in the water column that stabilize and sink suspended solids.
in the aerobic zone and digest the settled solids layer in the anaerobic zone. The circulator weighs 520 pounds, has an 8-ft diameter and 12 discharge segments. Each unit circulates 7-MGD using a 144 rpm impeller driven by a 3-HP electric motor. Water is drawn into the bottom of the unit and radially discharged over the lower lip of the circulator. Water flows laterally from the discharge segments until encountering a baffle, hydraulic wall, or bank. A float depth of four feet or greater is required for optimal operation (Bettle 2016).

**Figure 8:** Basic BF circulator diagram showing discharge segments, lower lip, and water intake inlet (Adapted from Bettle 2012).

**Induced Processes**

BF circulators work to mimic stratification of water layers that occurs in natural deep-water bodies (Bettle 2016). Stratification of the water column depends on a balance between air temperatures, surface turbulence, and buoyant forces. Thermal differences
between surface and deeper water lead to varying density throughout the water column, with denser, cooler water at the bottom. This difference in density results in two distinct layers, the epilimnion and the hypolimnion, with a third mixed layer, the metalimnion, between them (Woolway et al. 2014). The epilimnion is characterized by warmer temperatures and mixing of gases by air currents leading to aerobic conditions. The hypolimnion is characterized by cooler temperatures and undisturbed conditions, caused by a lack of transport of water and dissolved gases from the surface layer, leading to anaerobic conditions (Boehrer and Schultz 2008). Presence of these distinct layers results in different biochemical processes occurring throughout the water column (Boehrer and Schultz 2008, Bettle 2012, Woolway et al. 2014).

The BF circulators mechanically induce this process in shallow water treatment ponds through lateral mixing of the surface layer. According to Bettle (2012), the horizontal mixing by the BFS creates a pycnocline, a high-density gradient, between the upper and lower portions of the water column leading to stratification. Stratification results in a surface aerobic layer, a middle facultative layer, and a bottom anaerobic layer (Figure 9).
**Figure 9:** Stratification induced by lateral surface mixing from BF circulators, showing minimum required depth for adequate stratification (Adapted from Bettle 2016).

**Perimeter Flow**

According to Bettle (2012), BF circulators make use of a novel concept known as perimeter flow (Figure 10) to circulate water through the different zones of the water column. The idea of perimeter flow is different from plug flow or well-mixed flow. A thin layer of aerated water flows radially from the BF discharge segments until encountering a baffle. This baffle could be a physical baffle supplied with the BFS, a hydraulic wall created by lateral flow from nearby BF units, or a physical wall created by a pond bank or vegetative strip. The baffle redirects the flow straight down until the flow ricochets off the bottom of the water column and is pulled across the settled solids layer.
by the BF inlet (Bettle 2012). The effect of perimeter flow is to transport low amounts of DO to the surface of the sludge blanket. This small amount of DO is too high for strict anaerobes and too low for strict aerobes, creating favorable conditions for facultative bacteria.

This is another key aspect of the BFS; BF circulators create conditions that select toward facultative bacteria, which enhance sludge digestion (Bettle 2012). Facultative anaerobes, such as *Streptococcus* and *Enterobacterium*, play an important role in hydrolysis of substrate (Shah et al. 2014). Selection of facultative anaerobes enhances hydrolysis of the settled solids layer, which is the rate-limiting step of anaerobic digestion of solids (Mata-Alvarez and Llabrés 2000, Bettle 2012, Shah et al. 2014). According to Absolute Aeration, different processes occur at various stages of the perimeter flow “circuit” (Figure 10) that increase the anaerobic digestion capacity of the circulators (Bettle 2016)

**Figure 10:** Flow diagram showing multiple steps of perimeter flow.
According to information provided by Absolute Aeration, intentional cavitation, caused by the impeller in the BF chassis, of the water stream occurs before discharge to Stage 1 (Figure 10) (Bettle 2012). Intentional cavitation serves multiple purposes. One purpose is to lyse facultative bacteria in the return flow to release intracellular enzymes; the release of these enzymes allows extracellular hydrolysis to occur (Bettle 2016). Cavitation also converts fats into soaps and initiates formation, when pH is above 7.5, of calcium carbonate granules that serve as surface for biofilm growth (Bettle 2016). The lateral surface flow reaches a baffle or hydraulic wall at Stage 2 (Figure 10). Downward flow caused by the baffle brings lysed bacteria, intracellular enzymes, and low concentrations of DO down to the sludge blanket. The low concentrations of DO selects toward facultative bacteria that form a biofilm on the surface of the sludge layer. Benthic detrivores such as worms, rotifers, and ciliates also benefit from low DO concentrations at the sludge surface and aid in biosolids digestion (Bettle 2016). At Stage 3 (Figure 10), flow travels horizontally through and above the sludge layer. Calcium carbonate seeds that were initiated at Stage 1 settle onto the sludge layer and acquire a biofilm of facultative bacteria, leading to granule formation (Bettle 2016). As bacteria and calcium carbonate seeds spread, granules form a granule bed sludge reactor (GBSR) on the pond bottom. According to Bettle (2016), digestion of incoming and legacy solids is focused in the GBSR. Formed granules (Figure 11) can range from a few millimeters up to a centimeter in diameter. The texture of the granules will vary depending on the mineral content of the water (Bettle 2016).
After granule structures have been initiated, further carbonate seeds propagate from available calcium ions and dissolved carbon dioxide using gaseous ammonia as an electron donor (Equation 16). This chemical reaction occurs at pH ≥ 6.5. If the bulk pH is greater than 7.5, CaCO$_3$ precipitates from the solution and the granules are inactivated (Bettle 2016).

\[
[2NH_3 + CO_2]_{gas} + [H_2O + Ca^{2+}]_{liquid} \rightarrow 2NH_4^+ + CaCO_3
\]

At Stage 4 (Figure 10), horizontal bottom flow reaches the vertical inlet of the BF; bottom flow and bacterial cells are brought into the circulator as return activated sludge (Bettle 2016). At Stage 5 (Figure 10), the process starts over.

**Blue Frog Sludge Digestion Stages**

According to Absolute Aeration representatives, sludge digestion associated with the BF circulators occurs in five stages (Figure 12). The process begins with digestion of
easily accessible “alluvial sludge”, recently deposited that has a low solids content (< 2.5% Total Solids). As the alluvial sludge is digested and biological activity is increased, gas is produced within the sludge layer. At this stage, there is an apparent increase in sludge layer volume, due to decreased sludge density from the gas production (Bettle 2016). Decreased sludge density due to gas production can lead to pond turnover, similar to turnover caused by decreased density from surface temperature increases described in Boehrer and Schultz (2008).

**Figure 12:** Stages of the BF sludge digestion process (Adopted from Bettle 2016). Showing the initial digestion of alluvial sludge (A), apparent increase in volume due to gas production (B), continued digestion of alluvial sludge leading to decreased depths (C), slower digestion of legacy solids (D), and equilibrium sludge depths (E).
After biological activity ramps up and biofilms become prominent, alluvial sludge is digested at a linear rate until the “easy” substrate source is depleted. After digestion of the alluvial sludge, microbes begin to utilize the legacy sludge substrate. This substrate has a higher solids content (> 2.5% Total Solids) and a more stable structure compared to the alluvial sludge. As a result, digestion of this substrate occurs at a slower rate. Digestion of this substrate occurs until the legacy sludge is gone and an equilibrium point is reached. The depth at this equilibrium point is the depth of active granules that continue to digest incoming solids (Bettle 2016).

Previous applications of the BFS found that the digestion efficiency of the system is highly dependent on the characteristics of the substrate and the difficulty in selecting for particular bacteria that specialize in hydrolyzing the original substrate (Bettle 2012). Bettle (2016) describes alluvial sludge as sludge that behaves like a fluid and hard sludge as sludge that behaves like a solid. Efficiency of the system depends on the difficulty of hydrolyzing the original substrate into soluble compounds (Bettle 2012). Data collected by Absolute Aeration shows the BFS operating more efficiently when treating municipal waste sludge compared to more complex solids, such as those found in winery wastes (Bettle 2012).

Previous Applications

The BFS has been applied at many wastewater facilities across the country including facilities in Texas, Georgia, California, and Nevada. The BFS has also been applied to dairy manure lagoons, hog farm lagoons, poultry processing plants, and
wineries. In most of the municipal applications, the BFS was incorporated into primary treatment lagoons and digested primary sludge (Bettle 2012).

Application of the BFS in a poultry processing plant treatment lagoon in Marshville, North Carolina over a seven-year period saw a sludge depth reduction rate of approximately 0.66 ft/yr over the first two years of operation. Settled sludge for this lagoon had a total solids content of approximately 2.4 %, with 46% volatile solids. Over the final five years of the project, the sludge depth stayed relatively constant at approximately 1.3 ft (Figure 13), indicating the solids were being digested at a rate approximately equal to the loading rate of the system (Bettle 2012). Information on solids loading rates or changes in solids loading rates over the course of the case study was not provided by Absolute Aeration for this case study.

Figure 13: Sludge depth measurement for a seven-year application in a poultry processing plant treatment lagoon (Bettle 2012).
Application of the BFS in a municipal sludge holding pond in Lyon County, Nevada resulted in a decrease in the average sludge depth by one foot over a two-year period (Figure 14). The data shows an initial linear decrease in sludge depth until an equilibrium point is reached, similar to the results from the Marshville, NC application. The initial linear decrease occurs while bacteria digest the alluvial sludge layer; the new equilibrium depth occurs after this layer has been digested and the remaining solids are recalcitrant (Bettle 2012).

![Sludge depth measurement](image)

**Figure 14:** Sludge depth measurement from municipal sludge holding tanks in Lyon County, NV (Bettle 2012b).

**Summary**

In summary, this review of literature covers the available information on the water quality of the AWTF treatment wetlands; the characteristics of municipal solids,
including primary solids, secondary solids, and algal-dominated solids; anaerobic
digestion and each intermediary stage of the process; nitrogen removal pathways, along
with mineralization as a potential source of nitrogen, within wetland systems; and
information regarding the BF circulators provided by Absolute Aeration, LLC. Water
temperatures in the AWTF treatment wetlands indicate that psychrophilic digestion will
be the dominant mode of anaerobic digestion in TW3. Burke (2011) showed that the
settled solids and decomposing plant material layers act as a significant internal source of
total nitrogen. Both Burke (2011) and Garrison (2011) showed that the TWs release
ammonia into the water column from stored organic nitrogen sources, and this should
hold true for TW3.

Based on previous treatment processes, the settled solids present in TW3 consist
of settled algae cells, plant material, and microbial material that can be better described as
secondary or algal-dominated solids. Several studies have noted that algal-dominated
solids are generally recalcitrant to anaerobic digestion. This indicates that digestion rates
in TW3 are likely to be lower than previous applications of the BFS in lagoons
dominated by primary solids.

Review of the three types of anaerobic digestion processes, thermophilic,
mesophilic, and psychrophilic, indicates that psychrophilic anaerobic digestion is
expected to be the main sludge digestion process in TW3 based on water temperatures
attained from the AMRI database. Zhu et al. (2014) notes that psychrophilic digestion is
not as efficient as the other anaerobic digestion processes but can continue at water
temperatures below 15 °C if cold water adapted microbial communities are present.
Consistent with most constructed wetlands, as stated by Vymazal (2005), denitrification is expected to be the primary nitrogen removal pathway in TW3, while mineralization of organic nitrogen is expected to be the primary internal source of nitrogen. The internal source of organic nitrogen is a result of the assimilation of inorganic nitrogen compounds in the wastewater by the wetland vegetation over the operational lives of the treatment wetlands.

The application of the BFS is designed to increase the reaction rate of hydrolysis by creating conditions favorable for facultative bacteria at the sludge-water interface. In previous applications, the main substrate has been primary solids; this is not the case for this project as the settled solids of TW3 are dominated by detrital and algal material. Due to the recalcitrant nature of the detrital and algal dominated solids in TW3, it is unclear how the BFS will affect solids digestion in the wetland.
MATERIALS AND METHODS

To meet the project objectives, the BFS was monitored via weekly water quality sampling and periodic solids surveys of TW3. Removal of vegetation was required for the BFS to operate and allowed for internal measurement of several water quality parameters. Water quality sampling included collection of field measurements and measurements made in lab following *Standard Methods* procedures (American Public Health Association 2005). Solids surveys were conducted using a measurement device developed by AMRI, with help from the Environmental Resources Engineering (ERE) department lab technicians Marty Reed and Colin Wingfield, to collect data on the depth of solids throughout TW3. These measurements were used to develop estimates of volumetric changes of the settled solids layer over the course of the project using Quantum GIS (QGIS) and ArcMap.

Installation and Project Phases

Prior to the installation of the BFS, two sections of the floating vegetation mat were removed from TW3 in April 2016. The western section is referred to as the West Cutout and the eastern section is referred to as the Mid Cutout. In January 2017, a portion of the floating mat was removed at the front end of TW3; this area is referred to as the Influent Cutout (Figure 15).
**Figure 15:** Three locations of vegetation removal in TW3. Areas of removed vegetation allowed for internal sample locations. Aerial photography provided by Dr. Brad Finney.

Data collection for the project was separated into two phases. Phase One began with the installation of two BF units in the Mid Cutout on May 3\textsuperscript{rd}, 2016 and continued until the circulators were shut off on January 16\textsuperscript{th}, 2017. Phase Two of the project refers to all sampling that takes place after the circulators were moved into the Influent Cutout on February 10\textsuperscript{th}, 2017. During both phases, circulators were operated continuously, except when water samples were being collected and from 7/17/2017 to 7/25/2017 due to system malfunction. During Phase One, the southern circulator was equipped with a floating baffle (Figure 16) until September 5\textsuperscript{th}, 2016.
Figure 16: Circulators in the Mid Cutout showing the floating baffle installed around the southern circulator.

Water Quality Data Collection and Analysis

During Phase One and a portion of Phase Two, water quality samples and measurements were taken on a weekly basis during AMRI work hours on Fridays. Lab measurements were conducted the same day as sample collection. Sample locations include the influent and effluent of TW3 and multiple internal samples: two samples in the Influent Cutout (Inf 1 and Inf 2), a sample in the West Cutout (West), and three samples in the Mid Cutout (Mid, Mid 1, and Mid 2) (Figure 17). During the second half
of Phase Two, sampling was reduced to every other week and the “Mid” sample location was eliminated as the project ramped down.

Depending on the operation of the wastewater plant, influent to TW3 was either effluent from Oxidation Pond 2 or mixed effluent from TW5 and TW6. Influent to TW3 was distributed across multiple orifices on the western edge of the marsh during Phase One of the project. During vegetation removal just prior to Phase Two of the project, the inlet structure was broken, causing all flow to enter TW3 from the northernmost orifice.

![Figure 17: Sample site map of TW3 showing Phase One and Phase Two sample locations.](image)

Several water quality parameters were measured in the AMRI lab in accordance to *Standard Methods* (Table 1) (American Public Health Association 2005). These
include total suspended solids (TSS), total biochemical oxygen demand (BOD),
carbonaceous BOD (CBOD), soluble BOD (sBOD), ammonia (NH₃), nitrate (NO₃), and
turbidity. Carbonaceous BOD tests included the addition of a nitrification inhibitor to
each sample bottle. Soluble BOD samples were filtered through 1.5 µm filters before
being diluted. Samples of settled solids from TW3 and TW1 were analyzed for Total
Kjeldahl Nitrogen (TKN). TKN was measured by the Alpha Analytics Lab in Ukiah,
California.

BOD, CBOD, and sBOD tests were conducted using standard 300 mL BOD
bottles and a YSI Model 58 DO probe. TSS was measured using 1.5 µm filters that were
weighed on a Sartorius precision weighing balance. Turbidity was measured using a
HANNA HI 93703 Microprocessor Turbidity Meter. Ammonia and nitrate were
measured using a MA130 Ion Meter with USA Bluebook Ion Selective Electrodes.

Field measurements include water temperature, DO concentration, pH,
conductivity, and oxidation-reduction potential (ORP). Water temperature, DO
concentration, and conductivity were measured using a YSI Model 85 probe. ORP was
measured using a Pinpoint ORP/Redox Monitor and pH was measured using an EcoTestr
pH2.
Table 1: Standard Methods for utilized water quality procedures (American Public Health Association 2005).

<table>
<thead>
<tr>
<th>Water Quality Test</th>
<th>Method Used</th>
<th>Measurement Location</th>
</tr>
</thead>
<tbody>
<tr>
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<td>AMRI water quality lab</td>
</tr>
<tr>
<td>BOD</td>
<td>Standard Method 5210A</td>
<td>AMRI water quality lab</td>
</tr>
<tr>
<td>CBOD</td>
<td>Standard Method 5210A</td>
<td>AMRI water quality lab</td>
</tr>
<tr>
<td>Nitrate</td>
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</tr>
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<tr>
<td>pH</td>
<td>-</td>
<td>AMRI water quality lab</td>
</tr>
</tbody>
</table>

Water Quality Data Analysis

Due to the heterogeneity of the natural treatment wetlands at the AWTF and the size of the wetlands involved in the project, no “true” control could be applied to the water quality analysis. To work around this drawback, two “pseudo-controls” were applied: TW1 and historic data from TW3. TW1 was chosen as a pseudo-control because both wetlands receive Oxidation Pond 2 effluent as influent, under most conditions. Both wetlands have a relatively similar species distribution in the floating vegetation mats,
have been in operation for similar amounts of time, and experience the same climatic conditions.

Differences in the flow rate through each wetland and the total area of each wetland are accounted for by comparing areal mass removals through each marsh (Equation 17), rather than just comparing concentrations, which can be misleading due to varying degrees of dilution caused by flow rate differences. Concentration comparisons were used to look at changes in water quality parameters at internal sample locations of TW3. Data collected over the course of the project was compared to historic values for TW3 via areal mass removal. The years of 2011 and 2012 were chosen as they were late enough in the system’s life to express the internal loading currently seen in the wetlands. This could provide a way to compare TW3 with the BFS in place to how it would theoretically perform without the system in place. Areal mass removal through TW3 and TW1 was compared to determine if there was an increase in water treatment efficacy in TW3 due to the installation of the BFS; student’s t-test ($\alpha = 0.05$) was employed to determine if any significant differences between the datasets existed.

$$\text{Areal Mass Removal (lbs/acre/day)} = \frac{k(C_{in}Q - C_{out}Q)}{A} \quad (17)$$

where:

$C_{in}$ = Influent concentration of parameter of interest (mg/L)

$Q$ = Flow through the treatment marsh (MGD)

$k$ = Conversion factor

$A$ = Treatment wetland area (acres)
TSS mass removal rates within TW3 were calculated by slightly altering Equation 16 with removal of the treatment wetland area term (Equation 17). Due to the lack of available outflow data, it was assumed that the flow exiting TW3 was equal to the flow entering TW3. Equation 18 was implemented to calculate removal rates of TSS through areas of TW3 where the BFS was present and areas that did not have the BFS in order to ascertain if the BFS removes significantly more solids than the rest of the treatment wetland.

\[
\text{Mass Removal (lbs/day) = } k(C_{in}Q - C_{out}Q) \tag{18}
\]

Settled Solids Survey and Analysis

Four settled solid surveys were conducted over the course of the project. The baseline survey, conducted by Chuck Swanson, was completed in April 2016 prior to the installation of the BFS. The second survey was conducted at the end of December 2016 after seven months of operation. The third survey at the end of May 2017 occurred after approximately one year of operation. The fourth, and final, survey was conducted in the beginning of November 2017 after approximately one year and four months of operation.

As stated in the introduction of this section, settled solids surveys were conducted using a sludge depth measurement device (Figure 18) developed by AMRI with the help of ERE lab technicians Marty Reed and Colin Wingfield. The measurement device is an 8-ft, clear, PVC tube that is capped at one end by a rubber stopper on a hinge; measuring tape is attached to the side of the tube so that depth measurements could be recorded.
Figure 18: Sludge tube used during the first two sludge surveys showing measuring tape and shutoff valve to keep water and sludge in the tube.

The device was inserted down through the water column to the bottom of the settled solids layer, total depth was measured, and the rubber stopper hinge was closed. After removing the device from the water, solids were allowed to settle and the depth of settled solids was recorded. Measurements were taken at 122 sample locations spread across TW3 (Figure 19). Data collected during the surveys was used to develop solids distribution maps with QGIS and volumetric change estimates were made using cut and fill analysis with ArcMap.
Figure 19: Sample locations of the sludge surveys used to generate GIS surface plots.

Distribution and Volumetric Change Analysis

Solids depth data for each solids survey was cataloged in an Excel spreadsheet and uploaded to QGIS as a discrete, point shapefile. Shapefiles were uploaded to QGIS under the Universal Transverse Mercator coordinate system, Zone 10 (EPSG: 26910). Extra points were included along the boundary of TW3 to act as a zero boundary condition for interpolation of the discrete, sludge depth data. Interpolated one-meter by one-meter cell size raster surfaces, representing the settled solids layer, were created using Delauney triangulation. Raster surfaces were used to make distribution maps of the solids layer for each survey. After developing the raster surfaces in QGIS, they were
exported to ArcMap to perform cut and fill analyses. Cut and fill analysis takes the difference of the z-value, or sludge depth value in this application, of each raster cell for two raster surfaces representing different surveys. The difference is multiplied by the cell area to determine a change in volume for each cell; the sum of the incremental volume changes is taken as the overall volume estimate (Equation 19). Using cut and fill analysis, the volumetric change between each solids survey was estimated, as well as the total change in volume relative to the baseline.

\[ \Delta V = \sum_{i=1}^{n} A_n \Delta z_n \]  

(19)

Where:

\( \Delta V \) = Total change in volume between \( (ft^3) \)  
\( n \) = Number of cells \( n \) interpolated raster  
\( A_n \) = Area of the \( n \)th raster cell \( (ft^2) \)  
\( \Delta z_n \) = Change in z-value between “before” and “after” raster \( (ft) \)  

The final change in volume predicted by the cut and fill analysis was compared to the estimated total volume of solids that entered TW3 over the course of the pilot project. The incoming solids volume was estimated using Equation 20. Removal rates used in Equation 18 were determined by a probability exceedence curve developed from data collected during the project.

\[ V = \frac{\dot{m} t}{\rho_s (1 - \%MC)} \]  

(20)

where:
\[ V = \text{Volume of added solids (ft}^3) \]
\[ \dot{m} = \text{The median, first, or third quartile TSS mass removal rate (lbs/day)} \]
\[ t = \text{Total number of days within project period (days)} \]
\[ \rho_s = \text{Density of dried settled solids (lbs/day)} \]
\[ \%MC = \text{Volumetric moisture content of solids within TW3} \]

The three removal rates were chosen to provide a range of incoming volume estimates. Considering that mass estimates were based on TSS samples, Equation 18 assumes a density for dried settled solids of 87 lbs/ft\(^3\) or 1400 kg/m\(^3\) (O’Kelly 2006). The total project time was the time between the installation of the BFS on 5/13/2016 and the completion of the final solids survey on 11/15/2017.

The volumetric moisture content of the solids within TW3 was determined by measuring the reduction in volume that occurred after drying solids collected from the influent zone of TW3. The volume of the wet solids was estimated by measuring the height of the sludge while in a beaker of a known diameter. Solids were dried in a 105 °C oven for a 24-hour period; following drying, the mass of the dried solids was measured and converted to a volume using the density value reported in O’Kelly (2006).
RESULTS

This section presents the internal water quality of TW3 for both phases of the project, internal mass removals, comparisons of water quality between TW3 and TW1, comparisons of the water quality of TW3 over the pilot project to previous years, and the results of volumetric solids surveys. Lines connecting water quality data are not meant to imply a functional relationship between data points and are included to make trends more-easily visible. Discontinuities in the lines are a result of missing data points.

Internal Water Quality: Phase One

Evaluating internal BOD concentrations of TW3 during Phase One of the project shows a typical pattern of decreasing BOD as water flows through the treatment wetland (Figure 20). Figure 20 also shows the seasonal fluctuations of BOD within the system, with a spike in BOD concentrations at all sampling locations occurring in late October.
**Figure 20:** Internal BOD concentrations during Phase One of the pilot project.

Figure 21 shows the release of sBOD through TW3, primarily during summer months, indicated by higher concentrations at internal sample points relative to the influent concentration. Like BOD concentrations, Figure 21 shows a spike in sBOD concentrations throughout TW3 in November.
**Figure 21**: Phase One internal sBOD concentrations showing a release of soluble compounds as water enters the wetland during summer months.

The influence of internal BOD sources is highlighted in Figure 22 and 23, where Figure 22 shows the BOD concentrations through TW3 in late summer and Figure 23 shows concentrations through TW3 during early winter.
**Figure 22:** BOD concentrations through TW3 on 9/23/2016 showing release of BOD from internal sources. Soluble BOD shows the largest increase within the wetland.

**Figure 23:** BOD concentrations through TW3 on 12/15/2016 showing less pronounced release of BOD from internal sources.
Ammonia concentrations increased through TW3 (Figure 24) as internal loads were released through diffusion of soluble ammonium and mineralization of organic nitrogen. As with BOD, there are also the seasonal ammonia spikes that occur throughout the system in spring and fall.

![Ammonia Concentration Graph](image)

**Figure 24:** Internal ammonia concentrations for Phase One, showing release of ammonia through the wetland.

Overall, nitrate concentrations were observed to decrease through TW3 (Figure 25). There was a small increase in nitrate concentrations in the Mid Cutout, potentially from nitrification of ammonia caused by aerobic conditions. TSS concentrations decreased through TW3, following typical patterns seen in treatment wetland systems. Overall, TSS concentrations were highest during the summer months, coinciding with large algae blooms in the oxidation ponds (Figure 26).
Figure 25: Internal nitrate concentrations for Phase One showing decreasing nitrate concentrations through TW3.

Figure 26: Internal TSS concentrations during Phase One of the project.
During Phase Two, the BFS was moved to the Influent Cutout; half way through this phase influent water changed from oxidation pond effluent to treatment wetland effluent. During this phase, there was a decrease in BOD between the inlet and outlet of TW3 (Figure 27). Increases in BOD within TW3 were less pronounced than those seen during Phase One and did not occur over the summer months. This same pattern was observed in sBOD concentrations (Figure 28). During spring months of Phase Two, there were small increases in sBOD concentrations between the influent water and Influent Cutout sample locations; after the small increase, sBOD concentrations decreased through the remainder of the wetland. After flow was reconfigured so that TW3 was operated in series with TW5 and TW6, no internal increases above influent concentrations were observed.
Figure 27: Internal BOD concentrations during Phase Two of the project.

Figure 28: Internal sBOD concentrations during Phase Two of the project.
Ammonia concentrations during Phase Two followed similar trends as observed during Phase One (Figure 29). Concentrations gradually increased as water moved through TW3 and there were generally lower ammonia concentrations within the system during the summer months. During the second half of Phase Two, ammonia concentrations stayed relatively constant or decreased slightly across TW3. Nitrate concentrations increased in the Influent Cutout during Phase Two (Figure 30). After the initial increase in the Influent Cutout, nitrate concentrations dropped through the remainder of the wetland.

**Figure 29:** Internal ammonia concentrations during Phase Two of the project.
Re-suspension of solids by the BFS had a larger effect on TSS concentrations during Phase Two of the project, with TSS concentrations in the Influent Cutout generally higher than influent concentrations (Figure 31). After this initial spike in TSS, concentrations decreased through the rest of the wetland. This was particularly apparent during the summer of 2017 while TW3 was operated in series with TW5 and TW6; TSS concentrations spike well above influent values at the Influent Cutout but dropped in concentration before reaching the effluent of the wetland. This effect was also observed while TW3 operated in parallel with TW5/TW6 and received influent water from the Oxidation Pond 2 (Figure 32).
Figure 31: Internal TSS concentrations during Phase Two, showing spikes in solids at the front section (Inf 1 and Inf 2) of TW3.

Figure 32: Internal variation in TSS concentration while in series or parallel operation.

Internal Mass Removal
The effect of the BFS on TSS removal was analyzed by comparing the mass removal through the portion of TW3 that contained the circulators to the portions that did not contain circulators. Mass removals were calculated between the West Cutout and the effluent, where the BFS were located during Phase One, and between the influent and West Cutout, where the BFS were located during Phase Two. As stated in the Methods section of this document, the student’s t-test was used to determine if a statistically significant difference between the means of the two datasets was present. The mass removal through sections with the BFS may be slightly greater than in areas without the circulators (Figure 33); however, the differences were not shown to be significant during Phase One ($df = 49, t = -0.59, P = 0.56$) or Phase Two ($df = 29, t = -0.003, P = 0.997$).

**Figure 33:** Comparison of mass removal of TSS through areas with and without BF circulators. BFS located between west cutout and effluent during Phase One and influent and west cutout during Phase Two.
Looking at mass removal of total inorganic nitrogen (TIN) through TW3 shows that, for much of the project, mass removal was negative (Figure 34). This indicates that nitrogen stored in plant and settled solids biomass was being released into the water column. This trend was also observed in the internal ammonia sampling, where concentrations increased as water moved through TW3 (Figure 24). This analysis assumes that nitrite is a small component of the TIN and is negligible.

Figure 34: Removal rates of TIN through TW3 over the course of the project.

Comparison of TW3 and TW1

Comparison of effluent BOD concentrations, while both wetlands operated in the same configuration, shows that TW3 typically had lower concentrations than TW1 (Figure 35). Statistical analysis of the effluent concentration of both wetlands indicates that the difference is significant ($df = 107$, $t = -2.45$, $P = 0.016$); however, concentrations
can be misleading as flow per unit area, which can differ between TW1 and TW3, is not considered.

**Figure 35:** Effluent BOD concentrations of TW3 and TW1 showing the installation date of the BFS.

TSS concentrations were also generally higher in TW1 effluent than TW3 effluent (Figure 36). These differences in concentrations between the two wetlands were also statistically significant ($df = 96, t = -2.89, P = 0.005$). Comparison of ammonia concentrations in TW3 and TW1 effluent shows no significant observable difference (Figure 37). Statistical analysis of the effluent concentrations also shows no significant differences ($df = 95, t = -0.01, P = 0.99$).
**Figure 36:** Effluent TSS concentrations of TW3 and TW1 showing the installation date of the BFS.

**Figure 37:** Effluent ammonia concentrations of TW3 and TW1 showing the installation date of the BFS.
Comparisons of nitrate concentrations in TW3 and TW1 effluent indicate no major differences, with the exception of a couple nitrate spikes in TW1 effluent during Spring 2016 and Summer 2017 (Figure 38). Statistical analysis shows that, even with the spikes, there is no significant difference between the effluent nitrate concentrations of the wetlands ($df = 68, t = -1.04, P = 0.30$).

![Figure 38: Effluent nitrate concentrations of TW3 and TW1 showing the installation date of the BFS.](image)

**Figure 38:** Effluent nitrate concentrations of TW3 and TW1 showing the installation date of the BFS.

**Areal Mass Removal**

Areal mass removals were calculated to account for differences in the footprint and flowrates of TW1 and TW3. Overall, TW3 showed greater areal mass removal of BOD (Figure 39), when compared to TW1, throughout the project. Statistical analysis showed these differences to be significant ($df = 57, t = 4.12, P < 0.001$). BOD removal rates for TW3 from 2011 to 2012, four years prior to the installation of the BFS were
slightly higher than the areal removal rate for TW1 although the differences were not statistically significant \((df = 122, t = 1.26, P = 0.105, \text{Figure 40})\). Comparing the BOD areal mass removal rate of TW3 during the project (2016 – 2017) to the 2011 – 2012 period indicates that the BOD removal rate was significantly higher during the project \((df = 94, t = 2.80, P = 0.003)\), with a mean removal rate of 43 lbs/acre/day and 28 lbs/acre/day during the project and during the the 2011 – 2012 period, respectively.

![Figure 39: Areal BOD removals for TW3 and TW1 during the project (2016 – 2017).](image)
Comparing areal mass removal of TSS through both wetlands also showed greater amounts of removal through TW3 compared to TW1 (Figure 41). Statistical analysis showed these differences to be significant ($df = 85, t = 3.59, P < 0.001$). Although TSS removals were significantly greater in TW3 than TW1, this cannot necessarily be attributed to the BFS. Comparing the areal TSS removals for the two wetlands between the year 2011 and 2012 (Figure 42) shows that TW3 has historically removed a significantly larger amount of TSS ($df = 104, t = 2.166, P = 0.03$). It should be noted that the differences in TSS removals between the two wetlands were much more significant during the project compared to removals from 2010 to 2012, with a reduction in P-values by an order of magnitude. Comparing the TSS removal rate of TW3 during the project to the 2011-2012 year indicates that the TSS removal rate was significantly lower during the
project \((df = 101, t = -3.44, P < 0.001)\), with a mean removal rate of 51 lbs/acre/day during the project and a mean removal rate of 85 lbs/day/acre during the 2011 – 2012 period.

**Figure 41:** Areal TSS removals for TW3 and TW1 over the course of the pilot project.
Figure 42: Areal TSS removals for TW3 and TW1 from 2011 to 2012.

Settled Solids Survey

As stated in the Methods section of this document, settled solids surveys were conducted four times over the course of the project in order to track changes in the settled solids layer and provide estimates of volumetric changes of the settled solids layer. The depths of the settled solids layer varied over the course of the project. Figure 43 and 44 show the distribution of solids during the baseline survey (April 2016) and the survey conducted in December of 2016, respectively. For the distribution maps of the May 2017 and November 2017 solids surveys, see Appendix B. Comparing the distribution of solids in December to April shows that there was a reduction of solids depth in the Mid Cutout,
where the BFS was located during this phase of the project, and an accumulation of solids in the Influent Cutout.
Figure 43: Distribution of the settled solids layer during the baseline survey conducted in April 2016
Figure 44: Distribution of the settled solids layer during the December 2016 survey showing a reduction in sludge depth in the Mid Cutout and an accumulation of solids in the Influent Cutout.
Results of the solids survey show fluctuating volumes of the settled solids layer within TW3 over the course of the project (Table 2). The net change in volume over the course of the project showed an increase of approximately 4800 ft$^3$ of solids.

**Table 2:** Volumetric survey results across TW3 during the project.

<table>
<thead>
<tr>
<th></th>
<th>Estimated Solids Volume (ft$^3$)</th>
<th>Change From Baseline (ft$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>75,856</td>
<td>-</td>
</tr>
<tr>
<td>December 2016</td>
<td>73,137</td>
<td>-2,719</td>
</tr>
<tr>
<td>May 2017</td>
<td>71,795</td>
<td>-4,061</td>
</tr>
<tr>
<td>November 2017</td>
<td>80,694</td>
<td>4,838</td>
</tr>
</tbody>
</table>

Performing a cut and fill analysis on just the Mid Cutout and Influent Cutout, isolates the effects of the BFS on the cutouts where the system was installed. Table 3 shows a reduction in the settled solids layer volume of approximately 3,100 ft$^3$ that occurred in the Mid Cutout during Phase One of the project; after the BFS was moved to the Influent Cutout during Phase Two of the project, solids began to accumulate in the Mid Cutout. Overall, at the end of the project, the volume of the settled solids layer in the Mid Cutout was approximately 2,000 ft$^3$ less than the baseline volume.
Table 3: Volumetric survey results in the Mid Cutout. The asterisks indicate the presence of the BFS in the cutout.

<table>
<thead>
<tr>
<th></th>
<th>Estimated Solids Volume (ft³)</th>
<th>Change From Baseline (ft³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline *</td>
<td>8,663</td>
<td>-</td>
</tr>
<tr>
<td>December 2016 *</td>
<td>5,749</td>
<td>-2,913</td>
</tr>
<tr>
<td>May 2017</td>
<td>5,537</td>
<td>-3,125</td>
</tr>
<tr>
<td>November 2017</td>
<td>6,618</td>
<td>-2,045</td>
</tr>
</tbody>
</table>

Table 4 shows the changes in the settled solids layer in the Influent Cutout over the course of the project. Unlike the Mid Cutout, the Influent Cutout saw large, seasonal fluctuations in the settled solids layer volume over the course of the project. During Phase One of the project, the settled solids layer volume was estimated to increase by approximately 5,500 ft³. After moving the BFS to the Influent Cutout at the beginning of Phase Two, there was an estimated decrease in volume of approximately 1,700 ft³; however, by the end of the project, the settled solids layer volume was estimated to have increased by approximately 1,400 ft³. Due to error involved in sampling solids depth and having multiple surveyors over the course of the project, the estimates should not be taken as their exact numbers but as rough estimates.
Table 4: Volumetric survey results in the Influent Cutout. The asterisks indicate the presence of the BFS in the cutout.

<table>
<thead>
<tr>
<th></th>
<th>Estimated Solids Volume (ft³)</th>
<th>Change From Baseline (ft³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>18,400</td>
<td>-</td>
</tr>
<tr>
<td>December 2016</td>
<td>23,950</td>
<td>5,550</td>
</tr>
<tr>
<td>May 2017*</td>
<td>16,700</td>
<td>-1,700</td>
</tr>
<tr>
<td>November 2017*</td>
<td>19,790</td>
<td>1,390</td>
</tr>
</tbody>
</table>

The volume of solids that entered TW3 over the course of the project was estimated to compare to the overall volume change seen in TW3. The median, first, and third quartile TSS removal rates were extracted from a probability exceedence curve developed from weekly sampling (Figure 45). The parameters used to calculate the incoming volume are outlined in Table 5.

Figure 45: Exceedance probability curve for the TSS removal rate seen in TW3 over the course of the project.
Table 5: Parameters used in incoming volume estimation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median TSS Removal Rate</td>
<td>71 lbs/day</td>
</tr>
<tr>
<td>First Quartile TSS Removal Rate</td>
<td>54 lbs/day</td>
</tr>
<tr>
<td>Third Quartile TSS Removal Rate</td>
<td>91 lbs/day</td>
</tr>
<tr>
<td>Project Time</td>
<td>561 days</td>
</tr>
<tr>
<td>Dry Settled Solids Density(^1)</td>
<td>87 lbs/ft(^3)</td>
</tr>
<tr>
<td>Volumetric Moisture Content</td>
<td>95%</td>
</tr>
</tbody>
</table>

\(^1\)O’Kelley 2006

Considering the parameters shown in Table 5, the estimated incoming solids volumes are approximately 6,900 ft\(^3\) when using the first quartile removal rate, 9,100 ft\(^3\) when applying the median removal rate, or 11,700 ft\(^3\) when considering the third quartile removal rate. By these estimates, the net increase in solids volume was 59\%, 47\%, or 30\%, respectively, of the influent sludge volume.
This section of the document includes a discussion of the results presented in the previous section as follows: water quality results for both phases of the project, internal mass removal, comparison of TW3 and TW1 concentrations, historical comparisons of areal mass removal rates of TW3, and estimates of volumetric change in the settled solids layer. Phase One of the project is the period when the BFS was located in the Mid Cutout, while Phase Two refers to the period when the BFS was located in the Influent Cutout. Water entering TW3 as influent from oxidation pond 2 or TW5 and TW6, moves through the Influent Cutout (Inf 1 and Inf 2), the West Cutout (West), then the Mid Cutout (Mid 1, Mid 2, Mid), and exits as effluent on the eastern end of TW3.

Internal Water Quality: Phase One

During summer months, BOD concentrations in the West Cutout increased above influent concentrations, as shown by Figure 20, primarily due to the release of soluble compounds (Figure 21 and Figure 22) caused by increased biological activity associated with higher water temperatures that occur during the summer (see Figure 4). Concentrations of BOD began dropping at the Mid Cutout sample points after the initial spike at the West sample point due to aerobic conditions created in the top layer of the water column by the BFS as evidenced by DO concentrations measured across TW3 (see Appendix A, Figure 50 and Figure 51). Higher amounts of biological activity, including aerobic and anaerobic digestion, likely resulted in the release of, primarily soluble,
oxygen-demanding compounds from accumulated internal loads in the settled solids layer and plant debris within the treatment wetland. This would coincide with results observed by Al-Shafie (2014) which noted internal releases of NBOD from settled material in the wetlands. The dominance of sBOD released from the internal loads is indicated in Figure 22 by the larger increase in concentration between the influent water samples and samples from the West Cutout seen in the sBOD as compared to the BOD. The increase in sBOD through TW3 was less pronounced during colder months due to decreased biological activity caused by colder water temperatures. Although internal releases still occurred, due to the accumulated internal loads of carbon and nitrogen in the wetland as discussed by Burke (2011), they were less pronounced than internal releases that occurred over summer (Figure 22 and 23). During winter, larger concentrations of sBOD were coupled with larger concentrations of NBOD in the influent to TW3. This is likely showing the role that algae play in nutrient removal in the oxidation ponds. During winter months, algae are less active and uptake less soluble nitrogen, which resulted in the higher concentrations of sBOD and NBOD. During winter months, the increase in sBOD occurred in the area of TW3 where the BFS system was located, instead of the Influent Cutout, and may be a result of mixing by the circulators rather than biological activity.

The presence of a legacy internal load of organic carbon and organic nitrogen is a characteristic of the treatment wetlands at this stage of their operational lives and has been reported and quantified in previous studies (Burke 2011, MacFarlane 2014). Burke (2011) found that the settled solids layer acts as a significant source of total carbon and total nitrogen, 26,160 kg/ha and 2,820 kg/ha, respectively. The decomposing plant
material that forms the floating vegetation mat was shown to have even larger amounts of total carbon and total nitrogen, 48,280 kg/ha and 3,180 kg/ha, respectively (Burke 2011).

As shown by Figure 24, ammonia is released from internal sources as water moves through TW3, which matches trends observed in Burke (2011). This release is likely due to diffusion of soluble ammonium and mineralization of organic nitrogen within the settled solids layer. Ammonia releases were most prominent during summer months when warmer water temperatures were present. Increases of ammonia through the wetland, particularly through the areas with the BFS, indicated that the BFS did not have an observable effect on ammonia reduction. This matches conclusions presented in MacFarlane (2014) which stated that internal carbon and nitrogen loading could hinder aeration assisted nitrification. Nitrate concentrations through TW3 followed predictable patterns; concentrations slightly decreased through TW3, likely via denitrification (Figure 25).

As seen in Figure 26, there were no large spikes in TSS concentrations in the Mid Cutout where the BFS was operating. Any re-suspension of solids in the Mid Cutout did not have a large effect on the TSS in that section and did not cause any abnormal spikes of TSS concentrations in the effluent. This indicates that the BFS was able to operate in TW3 without suspending and exporting legacy solids from the system. Overall, the results of the water quality analysis over Phase One of the project show that the BFS can be operated in the treatment wetlands without negatively impacting the effluent water quality.
Internal Water Quality: Phase Two

Trends seen during Phase Two of the project were similar to those seen during Phase One. BOD and sBOD increases during Phase Two were less pronounced than increases seen during Phase One (Figure 27 and Figure 28). This is likely due to aerobic conditions created at the Influent Cutout of the wetland by the BFS (Appendix A, Figure 52). Aerobic conditions at the front end allowed for the degradation of released internal loads, dampening observable internal increases. As was the case during Phase One, no abnormal spikes in BOD concentrations in the effluent were observed while the BFS was in operation. While being operated in series with TW5 and TW6, seen in Figure 27 after June 2017, BOD concentrations in TW3 effluent fell below 30 mg/L. This is likely due to the lower concentrations of BOD entering TW3, along with aerobic conditions in the Influent Cutout allowing for increased BOD degradation.

Ammonia concentrations gradually increased through TW3, although increases were not as pronounced as compared to Phase One (Figure 29). The lower overall increase in ammonia concentration could be due to the aerobic conditions created in the front end of TW3, allowing nitrification to occur earlier in the system which could reduce observable increases in ammonia concentration. Again, no observable decreases in ammonia concentration were seen in the section where the BFS was located, indicating that the system was not playing an observable role in ammonia concentration reduction. The lower concentrations of ammonia entering TW3 during summer months were a result of increased algal activity in the oxidation ponds. Nitrate concentrations were shown to
increase in the Influent Cutout during Phase Two of the project (Figure 30). This is likely caused by nitrification of ammonia resulting from the aerobic conditions created by the BFS in the Influent Cutout.

Increases in internal TSS concentrations were more pronounced during Phase Two (Figure 31). During a majority of sampling occasions, TSS concentrations were higher in the Influent Cutout than in the influent water; this was caused by re-suspension of legacy solids by the BFS. Suspension of legacy solids by the BFS seemed to have a larger affect during Phase Two due to the larger volume of legacy solids in the Influent Cutout, as documented by settled solids surveys (Figure 44). Even with the spike in TSS concentrations in the Influent Cutout, effluent TSS concentrations were often below 30 mg/L, indicating that any re-suspended solids have sufficient time to settle before reaching the discharge weirs of TW3. Figure 32 shows that internal spikes in TSS occurred while TW3 was operated in series and in parallel, thus indicating that the spike is due to internal processes.

As was the case in Phase One of the project, water quality analysis results show the operation of the BFS did not negatively impact the effluent water quality of TW3. This suggests that the BFS can be moved and operated in the other TWs of the AWTF without causing negative water impacts to those units.

Internal Mass Removal

Results and statistical analysis of the internal mass removal show that the BFS system may lead to slight increases in TSS removal rates compared to areas in the
wetland without the BFS, but these increases in removal rates were not shown to be significantly greater during either phase of the project (Figure 33). This indicates that the BFS did not increase TSS removal rates compared to the removal rates in portions of TW3 that did not contain the BFS. Re-suspension of legacy solids, as seen in Figures 26 and 29, could be reducing the measured removal rates in the wetland.

Analysis of TIN (Figure 34) shows that, for much of the year, the mass of TIN in the water column was increasing as water moved through TW3. This indicates that nitrogen that was stored in the plant and settled solids biomass was being released. Plant and algal assimilation of ammonia and nitrate over the system’s lifespan acted as removal pathway in which ammonia and nitrate was removed from the wastewater and converted to organic nitrogen. Over time, the settled solids and vegetation mat layers accumulated material in TW3 and have become a significant internal source of organic nitrogen, as found in Burke (2011). Results of this study show that the internal source is releasing inorganic forms of nitrogen into the water column via ammonification of the stored organic nitrogen, causing an increase in ammonia concentrations and TIN mass between the influent and effluent of TW3. This trend is likely to continue unless the accumulated internal load is removed.

Comparison of TW3 and TW1

Effluent from TW3 typically had statistically significant lower BOD and TSS concentrations than TW1, as shown in Figures 35 and 36, respectively. As stated in the results, this does not necessarily mean that TW3 is removing more of either parameter
than TW1 because flow magnitude is not considered. No significant differences were observed in effluent ammonia and nitrate concentrations between both wetlands. As seen in Figure 37, the effluent ammonia concentrations of both wetlands closely followed each other, indicating potential underlying processes present in both wetlands leading to ammonia releases. Overall, Figures 35 through 38 indicate that the implementation of the BFS in TW3 did not result in major improvements in effluent water quality compared to TW1.

Areal Mass Removal

The areal removal rates of TW3 and TW1 were compared over two time periods: the years of 2011 and 2012, prior to the installation of the BFS, and over the course of the project with the BFS in place. Although this analysis assumes a constant flow, which is not a completely valid assumption, as flow rates are controlled by manually altered weirs, no major water surface elevation changes were observed, indicating that the wetland was operating at a steady state. The results show that, over the course of the project (May 2016 to October 2017), TW3 had a significantly larger BOD mass removal rate compared to TW1 (Figure 39); this relationship between removal rates in TW1 and TW3 did not hold over the period of 2011 to 2012 (Figure 40). TW3 was also shown to have a significantly higher BOD removal rate during the project then it did over the 2011 to 2012 period. These results were expected due to changes in the configuration of TW3. From 2011 through 2012, both wetlands operated as completely planted, floating mat wetlands that received influent from the same source, thus it is not surprising that the two wetlands had similar removal rates. With the addition of the BFS and removal of
vegetation strips in preparation for this project, multiple sub-regions of aerobic conditions were created in TW3. The BFS acts as horizontal flow surface aerators and encourage aerobic conditions in the top layer of the water column (Appendix A, Figure 50). Open water areas also provided the potential for surface mixing of oxygen into the water column. More dissolved oxygen in TW3 would allow for larger BOD removal rates compared to TW1, which did not have any sources of dissolved oxygen other than what was brought in with the influent.

Results of the same analysis conducted on TSS areal mass removal rates indicated that TW3 removed significantly more TSS than TW1 over the course of the project (Figure 41) and during the 2011 to 2012 period (Figure 42). Although the differences were more significant during the project, P < 0.0001 compared to P = 0.03, the significantly higher removal rate cannot be attributed to the BFS due to the historically higher removal rates seen in TW3. Comparing the TSS removal rates of TW3 during the project to the 2011-2012 year showed that TW3 removed significantly less solids during the project. This could be due to re-suspension of solids by the BFS in combination with low influent TSS concentrations while TW3 was being operated in series with TW5 and TW6. The previously mentioned morphological changes to TW3 mainly aided in increasing aerobic conditions in TW3; this change would be expected to alter BOD removal rates but not TSS removal rates, as TSS removal is not a function of available dissolved oxygen. Please note that there is potential of climatic variables affecting differences in TSS removal rates between the two time periods that were not analyzed in this project.
Settled Solids Survey

The results of the solids survey show seasonal fluctuations in the volume of the settled solids layer throughout the project, particularly in the Influent Cutout. This is likely due to the large influx of solids into TW3 in the form of algal solids, during summer algal blooms, and senescing plant material during fall and winter months. It was noted during the final solids survey (November 2017) that the top of the sludge column was composed of minimally decomposed plant material. This fresh plant material added to the overall volume of sludge, which may have led to the observed increase in volume compared to the previous survey. This seasonal fluctuation is expected to occur in the other treatment wetlands of the system as well. Even with the seasonal fluctuations in the volume of solids within TW3, the BFS was shown to be effective in restoring storage volume within the area-of-influence of the circulators, as shown by the distribution maps (Figure 43 and Figure 44). It should be noted that there is a potential that the percentage of solids within the sludge could have been altered by the BFS leading to reductions in the total mass of solids that may not be seen from volumetric analysis. Any future studies should include percent solids analysis.

Comparing the overall increase in the volume of the settled solids layer in TW3, to the estimated volume of incoming solids shows that the net increase in solids volume was 30% to 59% of the total volume of incoming solids. However, due to the lack of a similar analysis in a wetland without the BFS in operation, this estimated reduction of the incoming solids volume cannot be solely attributed to the BFS, as natural anaerobic
digestion rates were also at work. Conducting periodic sludge surveys in a wetland without the BFS, such as TW1, would provide insight into the percentage of the observed reduction that could be attributed to the BFS.

The combination of these results suggest that the AWTF can apply the BFS to restore treatment capacity in sections of the other wetlands at the treatment plant without causing negative impacts on the water quality of the system. The BFS may also act as a means of prolonging the functional life of the wetlands by reducing incoming volumetric solid loads. However, the BFS cannot act as a means to fully restore the treatment capacities of the treatment wetlands, as open water is a requirement for operation of the BFS. This constraint would require large swaths of vegetation to be removed from the treatment wetlands, negating the basic operational functionality of the treatment wetland stage of the overall AWTF treatment train. Secondly, there was no overall volumetric decrease in the settled solids layer at the end of the project, indicating that the system is unlikely to be the primary means of removing the solids throughout the treatment wetlands. Although some restored capacity and potentially load dampening was observed, the primary goal of the BF pilot project was to reduce the volume of the settled solids layer; this was not observed so it cannot be concluded that the BFS can act as a means of removing solids in the TWs.

The results of this project do not match results of previous studies conducted by Absolute Aeration, LLC, in which large-scale volume reductions were accomplished over two years. It is hypothesized that this is due to three primary reasons: depths in TW3 were generally shallower than design depths of the BFS, conditions in TW3 were not
ideal for fast anaerobic digestion, and the settled solids that exist in treatment wetland systems have very different characteristics compared to previous BFS applications.

Water depths in TW3 were generally lower or near the minimum 4-ft engineered depth required by the BFS. It is unclear how this would affect the hydraulics of the BFS but it is hypothesized that stratification of the water column and perimeter flow, as described by Absolute Aeration, may not properly develop. Absolute Aeration claims that these processes help create preferential conditions for facultative bacteria which causes the enhanced sludge digestion by the system; if these processes did not develop, then enhanced sludge digestion would not be expected to occur.

Since the AWTF is located in a coastal region with a moderate climate, water temperatures within TW3 were below 32 °C; this indicates that psychrophilic anaerobic digestion would be the dominant sludge digestion process (see Appendix A, Figure 47). Zhu et al. (2014), noted that psychrophilic digestion is not very efficient and Chen and Niebling (2014) stated that bacterial processes involved in anaerobic digestion generally did not occur at temperatures below 15 °C. Temperatures below 15 °C occur in TW3 from late fall to early winter (see Appendix A, Figure 47). Another condition in TW3 that could hinder efficient anaerobic digestion was the pH level near the settled solids layer. Christy et al. (2014) stated that methanogens cannot survive at pH level below 6.0 and Ostrem (2004) suggest an optimum range of 7.0 – 7.2. Measured pH levels near the settled solids layer in TW3 were not typically in the optimal range for methanogens, ranging from 6.2 to 7.1 (Appendix A, Table 6 and Table 7).
Previous BFS studies occurred in treatment lagoons for poultry processing factories, waste lagoons in swine farms, or lagoons that received raw influent. No previous study conducted by Absolute Aeration has taken place in treatment wetlands that were part of a wastewater treatment plant. The composition of the solids within the TWs is much different from the primary sludge treated in previous studies. The solids within the TWs are similar to secondary sludge and have a high percentage of algal mass, microbial mass, and plant fibers. Literature reviewed for this project indicated that solids with these characteristics are more recalcitrant to anaerobic digestion and this project’s results are consistent with performance limitations that would result from recalcitrant solids.
CONCLUSIONS

Over the course of the project, many insights were gained regarding the applicability of the BFS at the AWTF and several conclusions can be made. The BFS may not be the solution for removing all the legacy solids that have accumulated within the treatment wetlands over their 33 years of operation. There are several reasons for this conclusion. One is the functional changes that would be required to apply the system to the wetlands. Removal of vegetation that occurs during installation of the system would hinder the basic mechanisms that occur in the wetlands that lower algae concentrations. Another is the algal nature of the solids within the wetland which has been noted to be recalcitrant to anaerobic digestion by multiple literature sources. With this in mind, it appears that the system can have a role in restoring treatment capacity to the wetlands, as was seen by depth reductions in the Mid Cutout and Influent Cutout while the BFS was present. Based on the depth reductions seen in the distribution maps and the overall decrease in solids volume in the Mid Cutout, it can be concluded that the BFS can play a role in restoring capacity to the influent zones of the other TWs at the AWTF, where strips of vegetation have already been removed. Although the BFS may play a role in restoring some treatment capacity to the TWs, it will not act as a means of removing all the internal solids throughout the wetlands, as evidenced by the volumetric increase of the settled solids layer in TW3.

Although the circulators were shown to reduce the depth of solids in the Mid Cutout and portions of the Influent Cutout, there was a net increase in the settled solids
volume throughout TW3 at the end of the project. Overall, the net increase in the volume of the settled solids layer in TW3 is hypothesized to be a product of the seasonal solid loads and inefficient anaerobic digestion due to aforementioned conditions. The seasonal fluctuations are due to large algal blooms in the system and senescing vegetation and are expected to occur in all the treatment wetlands at the AWTF. The observed increase in volume of the settled solids may have been due to the fresh material observed in TW3 during the final survey. It is recommended that a concluding solids survey be conducted in summer of 2018 to compare the settled solids volume in a similar season as the original baseline survey.

Survey results and TSS removal data suggest that the BFS may have played a role in reducing potential increases in the settled solids volume within TW3; however, this cannot be quantified without knowing the reduction rates of a wetland without the BFS in place. It is recommended that periodic solids surveys be undertaken in a treatment wetland without the BFS, such as TW1 or TW2, to gain a better understanding on the role of the BFS in reducing incoming solids volumes. It is suggested that surveys be conducted once per season to provide better comparisons between surveys. These surveys should be accompanied by TSS sampling at the influent and effluent so that removal rates and incoming volumes can be estimated. Conducting percent solids analysis would also allow for change in solids to be compared on a mass basis alongside the volumetric comparisons; it is recommended that this be done in conjunction with volumetric analyses in any future solids surveying projects. Results did show that the BFS was able to restore treatment capacity in the Mid Cutout and portions of the Influent Cutout
without negatively affecting the water quality of TW3 effluent. This shows that any potential positive effects of the BFS did not occur at the cost of water quality. A final recommendation is to conduct a nitrogen budget where TKN is measured alongside ammonia and nitrate so that changes to organic nitrogen, and thus total nitrogen, by the BFS can be quantified.

The system may have applications in reducing volume of solids that have been extracted from other parts of the system and are being stored in external sludge retention ponds, as has been proposed as a potential use of a couple of the aquaculture ponds near TW6. Given the BFS performance reported in other case studies, this application would be most successful if primary sludge from Oxidation Pond 1 is to be stored in these retention basins. Literature reviewed for this project suggests that primary sludge is much less recalcitrant to anaerobic digestion than the algal-dominated sludge present in the treatment wetlands.

A final conclusion is that the BFS could be used to help remove BOD in particularly troublesome wetlands during the seasonal BOD spikes; this conclusion is based on the higher BOD removal rates seen in TW3 that resulted from aerobic conditions created by the BFS.
REFERENCES


APPENDIX A: ADDITIONAL WATER QUALITY DATA

Appendix A contains additional water quality data collected during the project. Data presented in this appendix was either briefly mentioned in the main document or was not considered pertinent enough to the main objectives the project to be included in the main document.

Figure 46 shows the seasonal spikes in ammonia concentrations that contribute to the seasonal BOD spikes as NBOD. Figure 47 shows the water temperatures in TW3 during the project, indicating conditions suitable for psychrophilic anaerobic digestion. Figure 48 shows CBOD concentration through TW3 during Phase One of the project. CBOD values typically dropped as water moved through TW3 although there were occasions where the CBOD concentration spiked above influent values after entering the wetland. CBOD concentrations followed similar trends during Phase Two of the project (Figure 49).

Figure 50 shows DO concentrations at the top and bottom of the water column in the Mid Cutout and Influent Cutout, where the BFS was located during Phase One and Phase Two of the project, respectively. Stratification was more prominent in the Influent Cutout due to the higher water depth. Figure 51 and 52 are a snapshot of DO concentration through TW3 during Phase One and Phase Two of the project, respectively, showing the aeration by the BFS in the Mid Cutout during Phase One and the Influent Cutout during Phase Two. Figure 53 shows conductance, which remained relatively constant through TW3, at the Mid and Influent Cutout. An apparent cyclical trend shows
that conductance, and, thus, soluble compounds, within the system increase over summer months and decrease over winter months. Table 6 and Table 7 shows pH for the various internal sample locations for Phase One and Phase Two of the project, respectively. Table 8 and Table 9 show ORP data collected at the bottom of the water column at each sample location for Phase One and Phase Two, respectively.

Figure 46: Seasonal variations in AWTF effluent ammonia concentrations that contribute to the seasonal spike in BOD as NBOD.
Figure 47: Water temperatures through TW3 over the course of the project indicating conditions suitable for psychrophilic anaerobic digestion.

Figure 48: CBOD concentration through TW3 during Phase One of the project.
Figure 49: CBOD concentration through TW3 during Phase Two of the project.

Figure 50: DO concentration at the top and bottom of the water column showing stratification effect caused by the BFS.
Figure 51: Snapshot of DO concentrations during Phase One of the project showing aeration by the BFS.

Figure 52: Snapshot of DO concentrations during Phase Two of the project showing aeration by the BFS.
Figure 53: Conductance at the top and bottom of the water column in the Mid Cutout and Influent Cutout.

Table 6: Collected pH samples within TW3 during Phase One.

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Table 7: Collected pH samples within TW3 during Phase Two.

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Table 8: ORP samples collected at the bottom of the water column for Phase One.

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Table 9: ORP samples collected at the bottom of the water column for Phase Two.

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APPENDIX B: ADDITIONAL SOLIDS SURVEY RESULTS

This appendix contains solids distribution maps for the May 2017 solids survey (Figure 54) and the November 2017 solids survey (Figure 55). Figure 54 shows small depth increases that have occurred in the Mid Cutout since the BFS was moved to the Influent Cutout; the figure also shows reductions in the solids layer depth in the Influent Cutout relative to the December survey. Figure 55 shows the continued increase in solids depth in the Mid Cutout and a large depth increase in portions of the Influent Cutout likely due settled of incoming algal solids through summer and fall. Table 10 provides the raw depth data collected during the baseline survey. Table 11 provides the raw depth data collected during the December 2016 survey. Table 12 provides the raw depth data collected during the May 2017 survey. Table 13 provides the raw depth data collected during the November 2017 survey.
Figure 54: Distribution of the settled solids layer during the May 2017 survey.
Figure 55: Distribution of the settled solids layer during the November 2017 survey
Table 10: Raw data for sludge depths collected during baseline survey (continued on next page).

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Table 12: Raw data for sludge depths collected during May 2017 survey (continued on next page).

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