Bioflocculation of Wastewater Treatment Pond Suspended Solids

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Abstract

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Wastewater treatment lagoons and high rate algae ponds (HRAPs) can provide cost effective wastewater treatment, but they commonly have high effluent concentrations of total suspended solids (TSS). In this thesis algae pond effluent was treated in a beaker testing apparatus (mixed and aerated) with various mixtures of activated sludge and primary effluent simulating differing activated sludge aeration basin compositions then was allowed to settle to assess settleability. Conventionally, microalgal suspended solids are removed by chemical coagulation followed by separation methods that often have a high cost relative to the low cost lagoon or HRAP system where the solids were produced. This separation step is often cost prohibitive or operationally complex for municipalities or too energy intensive for application in algae biofuels production. This research investigates using a small amount of activated sludge material to promote bioflocculation of algae in pond effluent. It was hoped that the findings may demonstrate a path for municipalities to keep their lagoons, while increasing capacity and improving treatment without excessive cost or complexity. Experiments were conducted on microalgae samples from a pilot-scale HRAP and activated sludge and primary effluent samples from a local municipal wastewater plant. The samples were placed in a mixing apparatus and allowed to settle for a given period of time, after which TSS was analyzed for settleability.

The experiments investigated the effect of various lab-scale activated sludge reactor operational schemes by varying the volumes (and masses) of activated sludge, algae-rich water, and activated sludge in the beaker. Results in the sorption test (tests with only activated sludge and algae-rich water) demonstrated algae pond effluent treated with activated sludge concentrations of 3000 mg/L or greater produced final effluent TSS concentrations near discharge requirements (40-50 mg/L) with only 30 minutes of settling and without addition of primary effluent. However, such high activated sludge concentrations are not feasible at full scale. Furthermore, beakers with activated sludge concentrations greater than 3000 mg/L reduced TSS concentrations by more than 150 mg/L with only 30 minutes of settling and without addition of primary effluent. Results in the aerobic beaker tests (tests with primary effluent, activated sludge, and algae-rich water) showed greater than 200 mg/L TSS removal and final effluent TSS concentration less than 30 mg/L was achieved using activated sludge to primary effluent volumetric ratios of 1:1 and greater which corresponded to activated sludge concentrations of 730 mg/L and greater. Activated sludge concentrations of 730 mg/L may not be feasible at full scale. This report shows that a PETRO-like process is effective in lowering wastewater pond suspended solids, however not to typical discharge standards.

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Introduction

Wastewater treatment pond systems are known for their low cost, but effluent suspended solids and nutrient concentrations are often higher than modern discharge limits allow (Gutzeit et al., 2005). As discharge limits for wastewater treatment facilities become stricter, and energy inputs become more expensive, municipalities and various industries will be looking to enhance their treatment efficiency and limit cost. Improving the sustainability of wastewater treatment facilities is another current emphasis of the wastewater treatment industry (Van De Hende et al., 2011). In conventional activated sludge treatment technology, 50% or more of plant energy consumption can be used for mechanical aeration, and their overall energy consumption is substantial (Tchobanoglous et al., 2003). When performing nitrification-denitrification, activated sludge and similar technologies also destroy fixed nitrogen, which under ideal conditions or with some pond technologies, would be captured and recycled as fertilizer to improve sustainability (Van De Hende et al., 2011). Additionally, Shaw and Barnard (2011) make the case that phosphorus removal and reuse is, above all other environmental impacts, the most important consideration in wastewater treatment because phosphorus is a non-renewable resource upon which the world's food supply is dependent as a fertilizer. Algal ponds represent a means of phosphorus recovery and reuse (Van De Hende et al., 2011).

One wastewater treatment design that promises to have lower cost and greater sustainability than conventional mechanical treatment technologies is high rate algal ponds (HRAPs), which are shallow, paddle wheel-mixed raceways for growing microalgae at high productivity (Craggs et al., 2011). Current full-scale HRAPs have similar suspended solids and nutrient removal deficiencies as conventional oxidation ponds. However, these deficiencies reportedly have been overcome by integration of HRAPs with activated sludge or trickling filter systems (Meiring and Oellerman, 1995; Gutzeit et al., 2005; Shipin et al., 1999).

HRAPs oxidize wastewater constituents and assimilate nutrients through photosynthesis performed by microalgae within the pond (Nurdogan and Oswald, 1995). Microalgae are considered a potential feedstock for the production of transportation fuels and other energy sources (Murphy and Allen, 2011), as well as other uses including animal feed supplements, fertilizers, soil conditioners, cosmetics, and health food supplements (Benemann, 2003; Ogbanna et al., 1997; Becker, 2007). However, the use of HRAP technology for wastewater treatment is restricted by land availability, evaporation, and climate (Benemann, 1997). Regardless of these resource limitations, simple gravity separation of microalgae remains difficult to achieve for large-scale HRAPs (Park et al., 2011a).

Separation of microalgae can be accomplished by centrifugation, flotation, filtration, and sedimentation (Murphy and Allen, 2011). These energy intensive (i.e. dissolved air flotation and centrifugation) and/or chemical intensive (i.e. chemical coagulation/flocculation) processes may defeat the goals of simplicity, economy, recycling of biomass for some uses, and low energy intensity of HRAPs systems (Christenson and Sims, 2011; Park et al., 2011b). Thus, to maintain a “clean” biomass product, high effluent quality, and low energy requirement, bioflocculation of algae followed by gravity sedimentation is the ideal separation methodology.

Microbial bioflocculation is often observed in the presence of extracellular polysaccharides (EPS) (Leppard, 1995; Liao et al., 2001; Sheng et al., 2010). Some algae and bacterial biomasses used in activated sludge systems are known to secrete sticky EPS (Medina and Neis, 2007; Gutzeit et al., 2005; Shipin et al., 1999a; Lundquist et al., 2011). These EPS have significant effect on the physicochemical properties of microbial aggregates including structure, surface charge, flocculation, settling properties, dewatering, and adsorptive capacity (Sheng et al., 2010).

The primary focus of the research herein is to investigate, at lab scale, the ability of activated sludge to promote the flocculation of colloidal algae with the longer-term goal of developing a full-scale process for pond effluent clarification. The

experiments measured the effect of blending various ratios of algae-rich pond effluent, primary clarifier effluent, and waste activated sludge. A secondary objective was to perform qualitative community analysis, comparing settleable communities with suspended-colloidal communities using microscopy.

Observations presented in this research may help guide municipal, agricultural, and industrial wastewater treatment works with managing their pond systems or provide insight into an alternative treatment process for systems needing upgrades. Similarly, this thesis has implications for the algae biofuel/biomass industry by developing methods to improve algal bioflocculation, a key step for competitive commercial algal biomass production.

Background

Pond systems are common wastewater treatment technologies, with around 7,000 publicly-owned systems in the United States alone (USEPA, 2008).

Because of their low capital cost, operational simplicity, and ability generally to meet oxygen demand discharge limits, more ponds are likely to be built in the future. Furthermore, many more ponds may be constructed if a substantial market for algae biofuels develops. However, many existing pond systems need upgrading, replacement, and/or expansion due to population growth, new discharge limits, desire for wastewater reuse, or poor treatment performance (Lundquist et al., 2011). A few simple measures can be taken to prolong the life of a pond system and increase performance. Typically this includes adding floating mechanical aeration to enhance biochemical oxygen demand (BOD) removal (Lundquist et al., 2011). Another way to enhance a pond system's effluent quality is to integrate a secondary biological treatment process downstream of the ponds.

Meiring and Oellerman (1995) demonstrated a high quality effluent using an integrated pond system with a trickling filter downstream of the pond system (Figure 1). This process is known as the Pond Enhanced Treatment and Operation (PETRO) process, or more specifically, the PETRO trickling filter (PETRO TF). Shipin et al., (1999b) operated multiple lab-scale PETRO TFs and concluded that high performance in the trickling filter was based on three factors:

(1) increased activity of predation on microalgae by protozoa and rotifers, (2) heterotrophy of both microalgae and bacteria, and (3) dissolved organics conversion to, and production of, extracellular polysaccharides (Shipin et al., 1999b). Meiring and Oellerman (1995) reported, for two operating schemes, that an integrated system effluent suspended solids and chemical oxygen demand (COD) were below 30 mg/L and 100 mg/L, respectively. The effluent sent to the trickling filter was pretreated in a facultative pond or anaerobic pond. A portion of the effluent from the facultative or anaerobic pond was diverted to an oxidation pond, while the remainder of the flow was sent to the trickling filter.

The primary differences between anaerobic, aerobic, and facultative ponds type are below (Birchall et al., 2008):

1. Anaerobic ponds use bacterial metabolism to decompose incoming organic matter in three stages (hydrolysis, fermentation, and methanogenesis). These processes breakdown particulate matter into soluble substrate (hydrolysis), the soluble substrate is degraded into volatile fatty acids (VFAs), CO_2 , and hydrogen (fermentation), and methanogenic bacteria produce methane from acetate or a combination hydrogen and CO_2 .
2. Aerobic ponds use aerobic bacterial metabolism to degrade incoming organic material into cell mass, CO_2 , and water using dissolved oxygen. Bacteria in naturally aerated ponds use oxygen produced by

phytoplankton and are better suited for dilute wastes. Cell growth is much quicker than in anaerobic ponds.

3. Facultative ponds are ponds in which anaerobic, aerobic, and facultative (metabolism of substrate with or without the presence of oxygen) bacteria decompose wastes.

In the PETRO system anaerobic pretreatment was done to increase the supply of readily available organic matter, which stimulated heterotrophic growth in the biofilms formed on the trickling filter media and markedly increased the biofilm's adsorptive capacity (Meiring and Oellerman, 1995). In Figure 1 there are two PETRO TF schemes depicted. The lower schematic uses an anaerobic pond to pre-treat raw waste before a portion of the total flow is diverted to the oxidation ponds and trickling filter. The upper schematic uses a facultative pond to pre-treat raw waste before flow is diverted to the oxidation ponds and the trickling filter. In both schematics a portion of effluent from the trickling filter is recycled to the anaerobic or facultative pond. Similarly in both schematics a portion of the flow leaving the anaerobic or facultative ponds is recycled back to the anaerobic or facultative pond before flowing downstream to either the trickling filter or the oxidation ponds.

Figure 1. PETRO integrated pond systems with trickling filters at two different locations, from Meiring and Oellermann (1999).

Another method in which a mechanically driven biological process is integrated into a pond system is by adding an activated sludge basin downstream of the oxidation pond, instead of a trickling filter. The secondary positioning of the activated sludge process (ASP) leads to increased flocculation and nitrification potential in the activated sludge flocs (Shipin et al., 1999a; Figure 2). Shipin et al. (1999a) suggested that the mechanism is a co-flocculation of algae with bacteria due to light-limiting, stress induced EPS production causing flocculation. Similarly, it was found that algae secrete EPS in light-limiting turbid natural

waters to flocculate particulate matter thus allowing increased light penetration (Avnimelech et al., 1982).

The PETRO activated sludge process (ASP) variant uses a biological nutrient removal (BNR) ASP, in which there are aerobic, anoxic, and anaerobic zones within the reactor. Figure 2 depicts a full scale PETRO ASP process. Raw sewage enters a primary facultative pond with a fermentation pit. A portion of the flow from the facultative pond flows to the activated sludge basin, while the other portion of the flow goes to the first secondary oxidation pond. Then a portion of the flow from the first secondary oxidation pond goes to the activated sludge basin while the remainder of flow is sent to the second two oxidation ponds. All flow through the secondary oxidation ponds is recycled back to the primary facultative pond. Flow from the activated sludge reactor is sent to two clarifiers where a portion of the underflow is returned back the activated sludge basin and the remainder is wasted. Supernatant from the clarifier is discharged to the nearby waterway.

Figure 2. Flow diagram of the Soshanguve PETRO ASP variant plant. (A) Primary facultative pond, broken line square- fermentation pit, (B±D) secondary oxidation ponds, (E) BNR reactor, (F) clarifiers, (G) chlorination facility, (a) stream containing VFA for P-removal in the BNR reactor, (b) primary pond effluent, (c) oxidation pond recycle; recycle rate= $c:(a+e)=1.5$, (d) algae-rich recycle, (e) BNR aerobic zone feed, (g) return activated sludge, (h) waste activated sludge to the aerobic sludge digester, (j) effluent to river, (k) to the sludge drying beds from Shipin et al., (1999a).

Alternatively, Gutzeit et al. (2005) followed by Medina and Neis (2007) found that algae and activated sludge, when cultured together work symbiotically to form settleable flocs. In their experiments, a reactor with a mixed liquor of microalgae and activated sludge bacteria was fed a synthetic wastewater and operated in a sequencing batch reactor (SBR) mode as well as continuous flow mode. After 6 weeks of operation in SBR mode, the culture was assumed to be stable. In these studies Gutzeit et al. (2005) and Medina and Neis (2007) focused on the effects of hydraulic and organic loading as well as biomass recycling on general treatment performance and floc formation. Gutzeit et al. (2005) saw that the system could remove %65 of total nitrogen and achieve a sludge volume index (SVI) of 118 mL/g. Similarly, Medina and Neis (2007) found that food to

microorganism ratio (F/M) was important for stabilizing algae once incorporated into biomass and also effected the removal efficacy of total nitrogen. While SVI is an indicator of settleability, neither stated effluent TSS concentrations were in their respective tests.

In addition to biological and operational characteristics that aided in successful operation of wastewater treatment methods described by Gutzeit et al. (2005), Medina and Neis (2007), Shipin et al. (1999a), and Meiring and Oellerman (1999), physical characteristics of activated sludge were found to have an effect of effluent quality (Sheng et al., 2010; Subramanian et al., 2010). Fuchs and Staudinger (1999) found that activated sludges with a “cross-linked matrix” had clearer effluents than sludges without a cross-linked matrix. After 30 minutes of settling with sludge possessing a well cross-linked matrix relative transparency was nearly restored where the “disperse” sludge required more time to regain a lower level of relative transparency (Fuchs and Staudinger, 1999). Results from Fuchs and Staudinger (1999) extrapolate on the “mass action” effect of a blanket of activated sludge moving down through the water column, clarifying the supernatant.

Based on knowledge found in the literature review, this thesis research was conducted to investigate the effect on bioflocculation of similar methods on algae-rich pond effluents using activated sludge. Experiments were carried out in

lab scale reactors with typical reaction times of 4 hours, settling times of 30 minutes, and with aerobic and anaerobic conditions.

Methods

Three independent types of experiments were completed (Table 1). In the Aerobic Beaker Tests volumes (and subsequently suspended solids masses) of algae-rich water, primary effluent, and activated sludge were varied. In the Anaerobic Preconditioning Beaker Test the same three constituents were varied however an additional variable, anaerobic conditioning of algae-rich water and primary effluent, was added. Lastly, in the Bacterial-Algal Sorption Beaker Test only algae-rich water and activated sludge volumes (and subsequently suspended solids masses) were varied.

Table 1. An overview of the three independent test and the constituents that were varied in each test.

Aerobic Beaker Tests	Anaerobic Preconditioning Beaker Test	Bacterial-Algal Sorption Test
1. Algae-rich water	1. Algae-rich water	1. Algae-rich water
2. Primary effluent	2. Primary effluent	2. Activated sludge
3. Activated sludge	3. Activated sludge	

The Aerobic Beaker Tests and Anaerobic Preconditioning Beaker Test used similar mixtures of primary effluent, algae-rich water, and activated sludge inoculum. The difference between the two tests was that in the Aerobic Beaker Tests, all of the mixture constituents (primary effluent, algae-rich water, and activated sludge) were collected the day of the experiment and were considered “fresh,” while in the Anaerobic Beaker Test, the primary effluent mixed with algae-rich water was aged anaerobically for more than 12 hours prior to experimentation and the other constituents were “fresh.” The Bacterial-Algal Sorption Test was different from the Aerobic Beaker Tests and the Anaerobic

Preconditioning Beaker Test because no primary effluent was used in any of the mixtures.

The mixtures in the three test types were placed into 2-L rectangular beakers (Phipps & Bird, Richmond, Va.), then mixed/contacted for a specified period using a 1.5-in² paddle with a rotational speed of 15 RPM using a mixing apparatus (also Phipps & Bird, Richmond, Va., Model PB 700 and Model 7790-400; Figure 3). After the allotted mixing/contact time, the mixing apparatus was turned off and the mixture was allowed to settle.

The beakers were aerated with lab compressed air, with the flow split to each of the beakers that required aeration using a simple air manifold. Valves on the manifold allowed for manual adjusting to achieve approximately equal air flow to each beaker.

Figure 3. Phipps & Bird, Richmond, Va. mixing apparatus used in all experimentation.

The primary output variable of concern for all three tests was TSS at the end of the settling step (measured as described below). The input variables for the various tests were the initial TSS, the anaerobic period (for the Anaerobic Preconditioning Beaker Test only), and the volumetric ratio of the components added to the beakers. Additionally, in the Anaerobic Preconditioning Beaker Test, primary effluent dissolved oxygen concentration was measured with a dissolved oxygen meter (YSI Inc., Yellow Springs, Ohio, Model 58) prior to commencing the contact period of the test and while the constituents were contacting in the mixing apparatus. Controls without activated sludge, and in some cases without primary effluent, were used to simulate the environments of typical HRAPs and wastewater treatment ponds.

The primary effluent and activated sludge used in the experiments were collected at the San Luis Obispo Wastewater Reclamation Facility (SLOWRF) in San Luis

Obispo, Calif. The primary effluent was collected from the effluent channel of the #2 primary clarifier, and the activated sludge came from the return activated sludge influent channel of the activated sludge basins.

Prior to running any beaker test, the influent TSS and volatile suspended solids (VSS) were determined for each of the three constituents (i.e., algae-rich water, primary effluent, and activated sludge) added to a given beaker.

Culturing Microalgae: Aerobic Beaker and Bacterial-Algal Sorption Tests

In preparation for the Aerobic Beaker Test and the Bacterial-Algal Sorption Test, suspended microalgae were collected from a pond system treating municipal wastewater from the community of San Jerardo near Salinas, California. These algae were used for inoculation to grow larger quantities of algae in the lab.

Culturing of the algae-rich water used in the Aerobic Beaker Tests and the Bacterial-Algal Sorption Test began by adding 5 gallons of the San Jerardo inoculum and 20 gallons of primary effluent to a transparent 40-gallon fish tank. A small circulation pump (Hydor, Sacramento, Ca., Model Koralia Evolution) was placed in the fish tank, the tank was then covered with transparent plexiglass with an air gap, and was allowed to acclimatize for one week outside. After one week, the tank was brought to a volume of 40 gallons with 15 more gallons of primary effluent. Every day thereafter, 10 gallons of the algae-wastewater mixture was removed and replaced with 10 gallons of primary effluent that was

collected daily. Replacing 10 gallons of the water inside the fish tank with freshly collected primary effluent daily provided a hydraulic residence time (HRT) of 4 days, similar to that of typical HRAPs. After two weeks of operating the fish tank on a 4-day HRT, samples were collected for beaker testing.

Culturing Microalgae: Anaerobic Preconditioning Beaker Test

The algae culture used in the Anaerobic Preconditioning Beaker Test was grown in HRAPs at the Cal Poly Algae Field Station (AFS) located at the SLOWRF. The plastic-lined HRAPs at the Cal Poly AFS had a surface area 30 m^2 and a nominal depth of 30 cm. Paddle wheels continuously mixed the ponds and imparted an average flow velocity of 30 cm/s. No chemical or gas addition was used in culturing the algae in the ponds. This algae culture initially grew, in batch mode, in SLOWRF reclaimed effluent circulating in the HRAPs, but then primary effluent was added continuously to grow the algae with a 4-day HRT. After two weeks of 4-day HRT operation, the culture in Pond 5 was used in the Anaerobic Preconditioning Beaker Test.

Aerobic Beaker Tests – Experimental Procedure

Beakers in the Aerobic Beaker Tests were mixed and aerated to simulate conditions in a full scale activated sludge basin or the gentle mixing in HRAPs. Each beaker was placed in the mixing apparatus unless the beaker was an unmixed control or otherwise specified. After placing the beakers in the mixing

apparatus, they were brought to a volume of 2 liters according to each tests' respective beaker plan (Table 3 and Table 4). Immediately after being brought to a 2-L volume, the beakers started to receive aeration and mixing as called for in their respective beaker plan.

Figure 4. A diagram of the Aerobic Beaker Tests, associated abbreviations for each test in the series, and a brief explanation of the waters tested.

Once the constituents were added to the beakers, and mixing and aeration began, a given amount of time was allotted for any reaction to take place and achieve appropriate contact. In the case of AE-2, the beakers were allowed a

contact/reaction time of 4 hours before they received a 30-minute quiescent period for settling. After 30 minutes of quiescent settling, a sample of supernatant was taken from each beaker and analyzed for TSS and VSS. The supernatant sample was drawn using a micropipette with the tip of the pipette placed one inch below the water surface. The process spelled out above is shown in Table 2 below.

Table 2. A process summary for Aerobic Beaker Tests AE-2, AE-3, and AE-4.

Step Number	Step Description	When/Duration
1	Take samples for TSS analysis from primary effluent, algae-rich water, & activated sludge	1 hour prior to test start
2	Measure appropriate volumes of beaker constituents in separate vessels	30 minutes prior to test start
3	Pour measured constituents into appropriate beakers	2 minutes before test start
4	Turn on mixer	0 minutes before test start
5	Turn on aeration	0 minutes before test start
6	Cover with double layer felt	0 minutes before test start
7	Contact (mix and aerate)	for 4 hours
8	Turn off mixer	4 hours after test start
9	Turn off aeration	4 hours after test start
10	Settle	for 30 minutes
11	Supernatant sample draw	4 hours and 30 minutes after test start

For all three experiments in the Aerobic Beaker Test Series, the mixers were set to a speed of 15 RPM. The air flow rate into the beakers was approximately 2 L/min. Simple aquarium air stones were used to diffuse the air. All beakers were covered by a double layer of black felt fabric to provide a dark environment and limit any photosynthetic growth by the algae potentially caused by the lights in the lab and indirect sunlight coming in from windows.

Test AE-2 Beaker Plan

The volumes of the constituents used in Experiment AE-2 are described in Table 3. Beakers labeled C1 and C2 were for algae-rich water controls (Table 3). C1 received no aeration and no mixing, while C2 received only mixing and no aeration. These control treatments were chosen to mimic the relative stagnation typical in treatment ponds (C1) and the gentle mixing induced in flocculation basins and in HRAPs (C2). Furthermore, the volumetric ratios chosen for AE-2, and the subsequent Aerobic Beaker Tests (AE-3 and AE-4) and Anaerobic Preconditioning Beaker Test were based on the typical mixed liquor suspended solid (MLSS) concentrations of conventional return activated sludge plant aeration basins. Using conventional aeration basin MLSS concentrations would yield results more applicable to municipalities considering attaching an activated sludge system downstream of treatment ponds. No duplicate beakers were tested in AE-2.

Beakers 1 and 4 did not receive any activated sludge but did have a primary effluent component and were the only beakers in AE-2 that did not receive activated sludge, besides the controls. All beakers, except the two controls, received aeration. The aeration of the beakers was performed in attempt to imitate the oxic, growth-promoting conditions of an activated sludge basin.

Beaker 11 had a significantly higher volumetric ratio of algae-rich water to primary effluent and subsequently higher algal TSS in the lab reactor. This high algal solids concentration in the reactor was used to see if an “algal-overloading” effect as described in Shipin et al., (1999b) and Meiring and Oellermann (1999), was observed. For all experiments, the suspended solids concentration was determined in each ingredient of the mixtures. These data are reported in the Results section under the “Beaker Constituents - Initial Total Suspended Solids Concentrations” subheading.

Table 3. Plan for Aerobic Beaker Tests AE-2.

Beaker Label	Algae-rich Water (mL)	Primary Effluent (mL)	Activated Sludge (mL)	Total (mL)	Volumetric Ratio of Algae-rich Water to Primary Effluent	Volumetric Ratio of Primary Effluent to Activated	Mixing and Aeration
C1	2000	0	0	2000	n/a	n/a	Not Mixed, Not Aerated
C2	2000	0	0	2000	n/a	n/a	Mixed, Not Aerated
1	1600	400	0	2000	4:1	n/a	Mixed, Aerated
2	1456	364	182	2002	4:1	2:1	Mixed, Aerated
3	1332	333	333	1998	4:1	1:1	Mixed, Aerated
4	1332	666	0	1998	2:1	n/a	Mixed, Aerated
5	1144	572	286	2002	2:1	2:1	Mixed, Aerated
6	1000	500	500	2000	2:1	1:1	Mixed, Aerated
7	1000	400	600	2000	5:2	2:3	Mixed, Aerated
8	1200	300	500	2000	4:1	3:4	Mixed, Aerated
9	1000	800	200	2000	5:4	4:1	Mixed, Aerated
10	1200	600	200	2000	2:1	3:1	Mixed, Aerated
11	1400	200	400	2000	7:1	1:2	Mixed, Aerated

Tests AE-3 and AE-4 Beaker Plan

The beaker plan for Tests AE-3 and AE-4 were the same as test AE-2 except that beakers 7 through 11 were omitted. Beaker 11 was omitted because of a lack of substantial difference relative to other beakers in the prior experiment, AE2. Beakers 7 through 10 were omitted largely to simplify the experiment and because there was no reason to trial intermediate volumetric ratios without first substantiating differences of the more elementary volumetric ratios (e.g. 2:1 algae-rich water to primary effluent, 1:1 primary effluent to activated sludge, 1:1 algae-rich water to primary effluent).

Duplicate beakers were tested in AE-3 and AE-4. In Test AE-3 beakers C1, C2, 1, 2, and 6 used duplicates (Table 4). In Test AE-4 beakers C1, C2, and 6 used duplicates (Table 4).

Table 4. Plan for Aerobic Beaker Tests AE-3 and AE-4.

Beaker Label	Algae-rich Water (mL)	Primary Effluent (mL)	Activated Sludge (mL)	Total (mL)	Volumetric Ratio of Algae-rich Water to Primary Effluent	Volumetric Ratio of Primary Effluent to Activated Sludge	Mixing and Aeration
C1	2000	0	0	2000	n/a	n/a	Not Mixed, Not Aerated
C2	2000	0	0	2000	n/a	n/a	Mixed, Not Aerated
1	1600	400	0	2000	4:1	n/a	Mixed, Aerated
2	1456	364	182	2002	4:1	2:1	Mixed, Aerated
3	1332	333	333	1998	4:1	1:1	Mixed, Aerated
4	1332	666	0	1998	2:1	n/a	Mixed, Aerated
5	1144	572	286	2002	2:1	2:1	Mixed, Aerated
6	1000	500	500	2000	2:1	1:1	Mixed, Aerated

Bacterial-Algal Sorption Beaker Test – Experimental Procedure

The Bacterial-Algal Sorption Beaker Test was used to determine how much TSS could be sorbed to activated sludge flocs during a short time that allowed for only minor bacterial growth. The Bacterial-Algal Sorption Beaker Test used the same equipment as the Aerobic Beaker Tests and was generally conducted in the same fashion. The primary difference was that the Bacterial-Algal Sorption Beaker Test used only mixtures of algae-rich water and activated sludge. Table 5 summarizes the process followed in the Bacterial-Algal Sorption Beaker Test.

After the beakers were placed under the mixing apparatus the specified volumes of activated sludge and primary effluent were added. Immediately after bringing the beakers to a volume of 2 liters with algae-rich water and activated sludge, aeration and mixing commenced. The mixers were set to 15 RPM. Equal air flow to the beakers set at approximately 2 L/min. A contact time of only 30 minutes was allowed for any reaction to take place, followed by a 30-minute quiescent settling period. After 30 minutes of settling, supernatant samples were drawn from one inch below the water surface using a micropipette for TSS analysis. All the beakers were covered with cloth as described previously to ensure a dark environment and reduce photosynthetic algae growth.

Table 5. A summary of when the steps take place and/or their duration for Bacterial-Algal Sorption Beaker Test.

Step Number	Step Description	When/Duration
1	Take samples for TSS analysis from algae-rich water and activated sludge	1 hour prior to test start
2	Measure appropriate volumes of beaker constituents in separate vessels	30 minutes prior to test start
3	Pour measured constituents into appropriate beakers	2 minutes before test start
4	Turn on mixer	0 minutes before test start
5	Turn on aeration	0 minutes before test start
6	Cover with double layer felt	0 minutes before test start
7	Contact (mix and aerate)	for 30 minutes
8	Turn off mixer	30 minutes after test start
9	Turn off aeration	30 minutes after test start
10	Settle	for 30 minutes
11	Supernatant sample draw	30 minutes after test start

Bacterial-Algal Sorption Beaker Plan

The Bacterial-Algal Sorption Test tested ten concentrations of activated sludge and algae-rich water (Table 6). The activated sludge concentrations used in this experiment ranged from no activated sludge to typical activated sludge system aeration basin mixed liquor suspended solids (MLSS) concentration (~3000 mg/L).

Table 6. Plan for the Bacterial-Algal Sorption experiment.

Beaker Label	Algae-rich Water (mL)	Activated Sludge (mL)	Total (mL)	Mixing and Aeration
1	2000	0	2000	Mixed, Aerated
2	1900	100	2000	Mixed, Aerated
3	1800	200	2000	Mixed, Aerated
4	1700	300	2000	Mixed, Aerated
5	1600	400	2000	Mixed, Aerated
6	1500	500	2000	Mixed, Aerated
7	1400	600	2000	Mixed, Aerated
8	1300	700	2000	Mixed, Aerated
9	1200	800	2000	Mixed, Aerated
10	1100	900	2000	Mixed, Aerated

Anaerobic Preconditioning Beaker Test – Experimental

Procedure

The Anaerobic Preconditioning Beaker Test was largely the same as the Aerobic Beaker Tests: the main difference was in constituent conditioning and repetition of the contact operation, as explained later. The algae and primary effluent were subjected to an anaerobic period prior to contact with the activated sludge, with the intention of generating volatile fatty acids (and other “readily available organic matter”) and/or stress that might promote flocculation. Before adding the constituents to the beakers, algae-rich water, activated sludge, and primary effluent were collected the day before the experiment. The algae-rich water and primary effluent were then added to a clean 5-gallon bucket at a volumetric ratio specified by the beaker plan. This mixture was then agitated vigorously 3 seconds. Immediately after agitation, the mixture was poured into two 2-L Erlenmeyer flasks. The flasks were filled such that there was one-quarter inch of headspace. The flask mouths were then covered with Parafilm. These measures promoted anaerobic conditions. Filling flasks in this manner was repeated for all specified volumetric ratios of algae-rich water and primary effluent.

After intermixing of algae water and primary effluent, the flasks were stored at room temperature in the dark, with no agitation for a period of 12 to 14 hours. The dissolved oxygen concentrations of each mixture before and after the 12-hour period were determined, and confirmed anaerobic conditions were

achieved. The dissolved oxygen concentration of freshly collected algae-rich water and primary effluent was also determined prior to intermixing. In addition to preparing the anaerobic flasks the day before the experiment, TSS analysis was carried out on the “fresh” algae-rich water, primary effluent, activated sludge, and the freshly prepared mixtures (of algae-rich water and primary effluent) in the flasks. After the sample of fresh activated sludge was taken for solids analysis, the remainder was stored overnight at a temperature of 5⁰C for use in the experiment the following day.

Solids analyses were conducted at intermediate points in the experimental process to allow for comparison to “no-treatment” results (Table 7). The day of the experiment, another solids analysis was carried out on the 12-hour aged constituents. This solids analysis was performed on the (1) supernatant of the quiescent anaerobic flask mixtures, (2) mixed anaerobic flask mixtures, (3) “pure” aged quiescent algae-rich water, and (4) “pure” aged mixed algae-rich water.

Using the solids data from the day before the experiment, the appropriate amount of activated sludge “seed” could be determined. An approximate 20% (by mass) seed was used. The 20% (by mass) seed was based on the mass of the algae-rich water and primary effluent suspended solids in the beaker. For example, if the beaker contained an algae-rich suspended solids mass of 50 mg

and a primary effluent suspended solids mass of 50 mg (a total of 100 mg/L TSS), then 20 mg of activated sludge mass was added to the beaker.

Once the beakers were brought to a volume of 2-L with the specified volumetric ratio of algae-rich water to primary effluent and the activated sludge “seed,” the beakers were promptly placed in the stirring apparatus. Immediately, the stirrers were turned on and aeration commenced. The stirrers were set at 15 RPM, and to approximately 2 L/min. Dissolved oxygen concentration readings were taken periodically to ensure that the beakers were receiving adequate air flow. The aeration and mixing lasted for 4 hours and was followed by 30 minutes of quiescent settling, after which time supernatant samples were drawn from each beaker one inch below the water surface using a micropipette and analyzed for solids. This first contact operation was referred to as “Cycle 1.”

The settled activated sludge and other solids were recycled into subsequent cycles to simulate a continuous activated sludge-like process, possibly with some change (adaptation) in the characteristics of this seed material. Following sample draw, the contents in the beakers that contained activated sludge seed were manually decanted leaving only the solids that settled during the quiescent period. This was done by removing the beakers from the mixing apparatus and slowly tilting the beaker to not re-suspend the settled material.

Finally, after the beakers were decanted, another set of anaerobic flask mixtures, exactly same as the previous mixtures, were added to the beakers. When the beakers were refilled, they were placed under the mixing apparatus, and aeration and mixing at 15 RPM were applied for 4 hours. The 4 hours of mixing and aeration were followed by 30 minutes of settling, after which supernatant samples were drawn from all beakers. This second operation was referred to as “Cycle 2.” Time constraints inhibited additional decant and biomass recycling operations, where further adaptations may have been observed.

This experiment can be summarized in terms of stages that correspond to sampling point and to the intermediate points of treatment. The stages were defined as follows:

Stage 1. TSS concentrations reported in Stage 1 were for samples that were freshly prepared (e.g., the resulting TSS obtained immediately after mixing a 2:1 ratio of algae-rich water with primary effluent).

Stage 2. TSS concentrations reported in Stage 2 were for samples that underwent 12-hour settling along with their precondition/pretreatment (e.g., the TSS resulting after 12-hr settling of “pure” algae-rich water and “pure” anaerobic algae-rich water).

Stage 3. TSS concentrations reported in Stage 3 were for samples from the supernatant of beakers that had undergone its prescribed treatment in Cycle 1.

Stage 4. TSS concentrations reported in Stage 4 were for samples from the supernatant of beakers that had undergone its prescribed treatment in Cycle 2.

Table 7 below further describes the experimental process of the Anaerobic Preconditioning Test.

Table 7. A summary of the experimental process for the Anaerobic Preconditioning Beaker Test demonstrating when each step occurred within a given stage.

Stage Number	Step Number	Step Description	When/Duration
1	1	Take samples for TSS analysis from algae-rich water, primary effluent, and activated sludge	1 day prior to test start
1	2	Measure appropriate volumes of algae-rich water and primary effluent for 12-hour aging	12-14 hours prior to test start
1	3	Pour measured algae-rich water and primary effluent into Erlenmeyer flasks, cover (for darkness) and promote anaerobicity	12-14 hours prior to test start
2	4	Take samples for TSS analysis from 12-14 hour aged (settled) flasks	1 hour prior to test start
2	6	Measure dissolved oxygen in flasks	45 minutes prior to test start
2	5	Pour flask constituents into square beakers	30 minutes before test start
2	6	Measure appropriate volume of activated sludge seed volume based on prior day's TSS analysis for each beaker	20 minutes before test start
2	7	Add appropriate volume of activated sludge seed to respective beaker	5 minutes before test start
2	8	Turn on mixer	0 minutes before test start
2	9	Turn on aeration	0 minutes before test start
2	10	Contact (mix and aerate), record dissolved oxygen every 30 minutes	for 4 hours
2	11	Turn off mixer	4 hours after test start
2	12	Turn off aeration	4 hours after test start
2	13	Settle	4 hours after test start
3	14	Take supernatant samples from all beakers for TSS analysis	4.5 hours after test start
3	15	Decant supernatant from all beakers that received an activated sludge seed. Leave only settled biomass.	4.75 hours after test start
3	16	Measure dissolved oxygen in second set of flasks	5 hours after test start
3	17	Pour second set of flask constituents in respective square beakers with settled (return) biomass	5.25 hours after test start
3	18	Turn on mixer	5.25 hours after test start
3		Turn on aeration	5.25 hours after test start
3	19	Contact (mix and aerate), record dissolved oxygen every 30 minutes	5.25 hours after test start and for 4 hours
3	20	Turn off mixer	9.25 hours after test start
3	21	Turn off aeration	9.25 hours after test start
3	22	Settle	9.25 hours after test start and for 30 minutes
4	23	Take supernatant samples from all beakers for TSS analysis	9.75 hours after test start

Anaerobic Preconditioning Beaker Plan

The Anaerobic Preconditioning Beaker Test used eight different mixtures of algae-rich water, primary effluent, and activated sludge (Table 8). Two of the mixtures were controls termed algae-rich water (ARW) and did not receive activated sludge seed. One of the controls received no aeration, no mixing, and no anaerobic preconditioning, thus it was labeled ARW No Treatment. The second control received anaerobic preconditioning (achieved through 12-14 hour aging), and mixing, but did not receive aeration (Mixed ARW Anaerobic Control). The remaining six beakers received differing volumetric ratios of anaerobically preconditioned algae-rich water and primary effluent. Beakers that received activated sludge seed are labeled – “w/ seed.”

Table 8. Plan for Anaerobic Preconditioning Beaker Test.

Beaker Label	Volumetric Ratio of Primary Effluent to Algae-rich Water	Activated Sludge Seed (mL)	Total (mL)	Mixing and Aeration
ARW Control No Treatment	n/a	0	2000	Not Mixed, Not Aerated
ARW Anaerobic Control No Seed	n/a	0	2000	Mixed, Not Aerated
2:1 Anaerobic Feed	2:1	0	1999	Mixed, Aerated
1:1 Anaerobic Feed	1:1	0	2000	Mixed, Aerated
1:2 Anaerobic Feed	1:2	0	1999	Mixed, Aerated
2:1 Anaerobic Feed w/ Seed	2:1	9	2008	Mixed, Aerated
1:1 Anaerobic Feed w/ Seed	1:1	10	2010	Mixed, Aerated
1:2 Anaerobic Feed w/ Seed	1:2	11	2010	Mixed, Aerated

Beaker Constituents – Solids analysis

TSS and VSS concentrations were determined using standard methods using Fisher Scientific G4 filters (nominal particle retention size 1.2 μm) in accordance

with Method 2500 (APHA, 1995). No set sample volume was passed through the filters. A sample volume was poured until the filter became nearly clogged and flow passed through in a drop-by-drop fashion.

Microscopy

Culture observations were carried out using a phase contrast microscope (Olympus, Lapu-Lapu City, Cebu, Philippines, Model: CX41RF) and camera (Lumenera, Ottawa, Ontario, Canada, Model: Infinity 2-1C). Identification of algal genus and diatom taxa was assisted by use of Prescott et al. (1978) and Spaulding et al. (2012) respectively. Species and morphological dominances were determined qualitatively at 400X and 1000X magnifications.

Results and Discussion

This section describes in detail the results of the five successful experiments conducted. First, the algae cultures subjected to the experiments are described.

Culture Observations

Chlorella, *Scenedesmus*, and *Chlorococcum* were the dominant algal genera in the algae stock culture used in the Aerobic Beaker Tests and the Bacterial-Algal Sorption Test. However, there was a minor presence of filament-forming algae tentatively identified as *Anabaena* and *Oscillatoria*. A significant diatom fraction was observed, with morphologies including nitzschoids, biraphids, and monoraphids.

The algae culture from the high rate ponds used in the Anaerobic Preconditioning Beaker Test was dominated by *Micractinium*, *Golenkinia*, and other spherical unicellular algae such as *Chlorella*. In this culture, many sub-dominant *Euglena*-like and *Synedra*-like algae were present. Filamentous algae were noticeably more populous in the anaerobic preconditioning test algae-rich water than the algae-rich water used in Aerobic Beaker and Bacterial-Algal Sorption experiments. *Oscillatoria*-type algae appeared to exclusively comprise the filamentous population.

Activated Sludge Observations

Activated sludge flora and fauna appeared to be relatively constant in terms of microfauna composition over the duration of the experiments. The most populous microfauna were various forms of protozoa. Stalked, crawling, and free swimming ciliates appeared nearly without exception in all flocs. Rotifers were numerous in the periphery of the activated sludge flocs and in the supernatant. In the experiments, after the activated sludge was contacted with algae, significant amounts of algae were observed within the matrix of many flocs.

Suspended Solids Removal

The TSS of the original algae-rich water from the fish tank or pond (A_{raw}) was diluted by the addition of primary effluent and activated sludge. This dilution needed to be considered in TSS removal calculations.

The relationship between A_{raw} and the pre-reaction diluted algae-rich water TSS concentration (A_{dil}) was proportional to the ratio of raw algae-rich water volume (V_{raw}) to the final volume of the entire mixture in the beaker (V_{beaker}):

$$A_{dil} = \frac{A_{raw} (V_{raw})}{V_{beaker}} \quad (\text{Eq. 3})$$

The TSS change was calculated two ways—one used A_{raw} and one used A_{dil} . Both used the settled beaker supernatant TSS (A_{fin}) as the final concentration.

The TSS concentration change that used the initial diluted TSS (A_{dil}) was termed ΔA_{true} , indicating true net removal of TSS, corrected and not overstated due to dilution.

$$\Delta A_{true} = A_{dil} - A_{fin} \quad \text{True removal, corrected for dilution of algae-rich water (Eq. 1.)}$$

The second method determined the observed TSS change (ΔA_{obs}) based on the undiluted raw algal TSS concentration (A_{raw}) in the original algae-rich water from the fish tank or pond.

$$\Delta A_{obs} = A_{raw} - A_{fin} \quad \text{TSS change observed due to dilution and removal (Eq. 2.)}$$

Where:

A_{raw} = original raw algae-rich water TSS concentration in the tank or pond

A_{dil} = pre-reaction diluted algae-rich water TSS concentration

A_{fin} = final settled supernatant TSS concentration after treatment. A_{fin}

was a combination algae-rich, activated sludge, and primary effluent TSS concentration remaining in supernatant after treatment.

ΔA_{true} = TSS removal based on pre-reaction diluted algae-rich water TSS

ΔA_{obs} = TSS change observed between raw undiluted algae-rich water
final settled supernatant TSS

The increase of TSS due to the addition of primary effluent and activated sludge is not considered in the equations. To simplify, this added TSS is assumed to be completely removed in the reaction and settling process.

The TSS removed (calculated using both Eq. 1 and Eq. 2) contains algal solids, primary effluent solids and activated sludge solids.

Constituent Total Suspended Solids Concentrations

Before any test was run, the initial total suspended solids concentration of each constituent added to the mixture was measured (Table 9).

Table 9. Total suspended solids concentrations of the constituents used in all tests prior to testing. (Algae-rich water values are TSS in the “raw” algae-rich water samples, A_{raw} .)

Test	Constituent	TSS (mg/L)
AE-2	Algae-rich Water	269
	Primary Effluent	55
	Activated Sludge	2836
AE-3	Algae-rich Water	276
	Primary Effluent	83
	Activated Sludge	2918
AE-4	Algae-rich Water	253
	Primary Effluent	55
	Activated Sludge	5265
Bacterial-Algal Sorption	Algae-rich Water	285
	Primary Effluent	n/a
	Activated Sludge	4125
Anaerobic Preconditiong	Algae-rich Water	96
	Primary Effluent	56
	Activated Sludge	5971

Aerobic Beaker Tests

The three Aerobic Beaker Tests used volumetric ratios defined in Table 3 (AE-2) and Table 4 (AE-3 and AE-4). A summary of change in TSS concentration, based on Eq. 1 and Eq. 2, as a result of the Aerobic Beaker Tests (AE-2, AE-3, and AE-4) accompanies the beaker plans in Tables 10 and 11.

Table 10. Aerobic Beaker Test AE-2 data including change in supernatant TSS based on Eq. 1 and Eq. 2. A_{fin} and solids ratios are provided in Table B-1.

Beaker Label	Algae-rich Water (mL)	Primary Effluent (mL)	Activated Sludge (mL)	Total (mL)	Volumetric Ratio of Algae-rich Water to Primary Effluent	Volumetric Ratio of Primary Effluent to Activated Sludge	Mixing and Aeration	Eq. 1 ΔA_{true} (mg/L)	Eq. 2 ΔA_{obs} (mg/L)
C1	2000	0	0	2000	n/a	n/a	Not Mixed, Not Aerated	109	109
C2	2000	0	0	2000	n/a	n/a	Mixed, Not Aerated	79	79
1	1600	400	0	2000	4:1	n/a	Mixed, Aerated	45	99
2	1456	364	182	2002	4:1	2:1	Mixed, Aerated	76	149
3	1332	333	333	1998	4:1	1:1	Mixed, Aerated	54	144
4	1332	666	0	1998	2:1	n/a	Mixed, Aerated	-1	89
5	1144	572	286	2002	2:1	2:1	Mixed, Aerated	59	174
6	1000	500	500	2000	2:1	1:1	Mixed, Aerated	85	219
7	1000	400	600	2000	5:2	2:3	Mixed, Aerated	80	214
8	1200	300	500	2000	4:1	3:4	Mixed, Aerated	86	194
9	1000	800	200	2000	5:4	4:1	Mixed, Aerated	70	204
10	1200	600	200	2000	2:1	3:1	Mixed, Aerated	51	159
11	1400	200	400	2000	7:1	1:2	Mixed, Aerated	108	189

Table 11. Plan for Aerobic Beaker Tests AE-3 and AE-4 and the change in supernatant TSS based on Eq. 1 and Eq. 2. Solids ratios are provided in Tables B-2 and B-3 in Appendix B.

Beaker Label	Algae-rich Water (mL)	Primary Effluent (mL)	Activated Sludge (mL)	Total (mL)	Volumetric Ratio of Algae-rich Water to Primary Effluent	Volumetric Ratio of Primary Effluent to Activated Sludge	Mixing and Aeration	AE-3 Eq. 1 ΔA_{true} (mg/L)	AE-3 Eq. 2 ΔA_{obs} (mg/L)	AE-4 Eq. 1 ΔA_{true} (mg/L)	AE-4 Eq. 2 ΔA_{obs} (mg/L)
C1	2000	0	0	2000	n/a	n/a	Not Mixed, Not Aerated	128	128	128	128
C2	2000	0	0	2000	n/a	n/a	Mixed, Not Aerated	62	62	113	113
1	1600	400	0	2000	4:1	n/a	Mixed, Aerated	73	128	57	108
2	1456	364	182	2002	4:1	2:1	Mixed, Aerated	101	176	69	138
3	1332	333	333	1998	4:1	1:1	Mixed, Aerated	104	196	83	168
4	1332	666	0	1998	2:1	n/a	Mixed, Aerated	-1	91	48	133
5	1144	572	286	2002	2:1	2:1	Mixed, Aerated	98	216	64	173
6	1000	500	500	2000	2:1	1:1	Mixed, Aerated	118	256	66	193

Control beakers that did not have activated sludge or aeration were used to mimic the relative stagnation typical in treatment ponds (C1) and the gentle mixing used in flocculation basins and in HRAPs (C2). Beakers with higher initial diluted algae-rich water solids concentrations tended to have higher TSS concentrations after the aerobic beaker treatment was performed (Figure 5). Part of this effect is due to the lesser volume of dilution water from primary effluent and activated sludge liquid for beakers with higher initial algae-rich water TSS concentrations.

Figure 5. Aerobic beaker test initial algae-rich water TSS concentration, A_{dil} , and final TSS concentrations, A_{fin} .

The tests also showed that the higher the activated sludge concentration in the beaker at the start of the test, the lower the final TSS in the supernatant (Figure 6). Of course, most of this effect is due to the higher dilution of algae-rich water TSS by the greater volumes of activated sludge liquid added. In later figures, this dilution effect is factored out.

Figure 6. Final TSS concentrations at different activated sludge concentrations in lab reactors for tests AE-2, AE-3, and AE-4.

The average slope of the three curves in Figure 6 is -0.13 suggesting that for every milligram of activated sludge added to the reactor, 0.13 fewer milligrams of TSS will remain in suspension after treatment.

Figure 6 shows the range of initial activated sludge concentrations was 0-1200 mg/L for experiments AE-2, AE-3, and AE-4. However, typical mixed liquor suspended solids (MLSS) concentrations are 1000-3000 mg/L in complete-mix aeration basins and 3000-5000 mg/L in plug flow activated sludge processes and nitrification processes, respectively (Tchobanoglous et al., 2003). The higher

activated sludge concentrations used in the present thesis research would not be practical in most situations because generating sufficient activated sludge would mean most of the wastewater would be receiving activated sludge treatment and the ponds would be only marginally useful. .

In spite of demonstrating an obvious relationship between activated sludge concentration in the lab reactor and final TSS, Figure 6 does not demonstrate any TSS removal. Figure 7 shows relationship between initial activated sludge concentration and TSS concentration removed based on Eq. 1 for “true” removal correcting for the initial dilution.

Figure 7. TSS removed, based on Eq. 1, at different activated sludge concentrations in lab reactors. Control reactors (reactors with no activated sludge) were omitted from this graph.

Though the correlation is not strong, as demonstrated by low R^2 values, regressions from AE-2 and AE-3 show a trend of higher activated sludge concentrations resulting in increased supernatant solids removal, however, AE-4 does not. The flatness of slope observed in the AE-4 regression was likely a result of the high TSS concentration of the activated sludge used in test AE-4 (Table 9). Nevertheless the high TSS concentration of the activated sludge does not explain the poor TSS removal, resulting in the negatively sloped regression, observed in test AE-4. The relatively flat slopes demonstrate that activated sludge concentration did not have a large effect on TSS removed.

Figure 8, below, is based on Eq. 2 and demonstrates substantive weak relationship between the TSS removal capacity by activated sludge; demonstrated by the relatively flat slope of the three curves. The regression for test AE-3 had the highest slope (0.17). This suggests approximately, an additional 450 mg/L of activated sludge solids concentration is required remove an extra 75 mg/L from the beaker supernatant.

Figure 8. TSS removed, based on Eq. 2, after treatment with different activated sludge concentrations. Control reactors (reactors with no activated sludge) were omitted from this graph.

The effect of initial activated sludge concentration on TSS removed was strongest for test AE-3 (greatest slope) and the R^2 values were greater in Figure 8, than Figure 7. This stronger correlation is due to dilution.

Dilution would also occur in this process at full scale. The algae-rich water would be mixed with activated sludge and be diluted, just as in the beaker tests. Such dilution also occurs in conventional activated sludge operations where primary effluent is diluted with return activated sludge upon entering the aeration basin. The Eq. 1 “true” removal is presented to help with the understanding of

bioflocculation, but the Eq. 2 change in TSS is equivalent to what would be observed at a full-scale plant.

Activated Sludge to Primary Effluent Volumetric Ratio

Figure 9 displays the TSS removed (calculated using Eq. 2) as a result of treatment with the various activated sludge to primary effluent volumetric ratios used in the Aerobic Beaker Tests. The average slope for the TSS removed for activated sludge to primary effluent volumetric ratio relationship in the Aerobic Beaker Tests is 73. This suggests for every one unit increase in the activated sludge to primary effluent volumetric ratio will result in 73 mg/L less TSS in the final supernatant.

Figure 9. TSS removed, based on Eq. 2, after treatment with specified activated sludge to primary effluent volumetric ratios (Table 3 and Table 4) for aerobic beaker tests AE-2, AE-3, and AE-4. The zero ratio points refer to mixtures with no activated sludge or to control mixtures that had only algae-rich water.

The correlation in Figure 9 was best represented with a linear regression. This suggests that the sorptive capacity or suspended solids removal capacity per volume (and consequently mass) of activated sludge was being fully used. Nonetheless, if the solids removal capacity is fixed per mass of activated sludge, then the test with the highest activated sludge concentration (Table 9) should have exhibited the greatest solids removal. There was a more substantial effect, demonstrated by greater slopes, on TSS removed when analyzed in this fashion (Figure 9) as opposed to the analysis of initial activated sludge concentration on TSS removed (e.g. Figure 8). However, this stronger trend was not observed when the effect of algae-rich water to primary effluent volumetric ratio was analyzed (Figure 10).

Algae-rich Water to Primary Effluent Volumetric Ratio

Because there was a small range of algae-rich water to primary effluent ratios any trend observed in Figure 10 may be misleading.

Figure 10. TSS removed, based on Eq. 2, after treatment different algae-rich water to primary effluent volumetric ratios (Table 3 and Table 4) for aerobic beaker tests AE-2, AE-3, and AE-4.

The volumetric ratio of algae-rich water to primary effluent was important because it represents two important variables in the performance of a PETRO-like system; algae-rich water and primary effluent. Drawing a relationship between the relative volumes of algae-rich water and primary effluent in the beaker was desired. Shipin et al. (1999a) saw flocculation benefits and increased effluent quality (lower TSS and other pollutants) in similar experiments. However, Figure 10 shows a generally negative slope of the algae-rich water to primary effluent volumetric ratio regression. This suggests the increase in primary effluent relative to algae-rich water may improve the ability of activated sludge to remove suspended solids. The increase in primary effluent relative to algae-rich water was represented by a decrease in the algae-rich water to primary effluent volumetric ratio; it was at these lower ratios where greater TSS

removal was observed. If there were a synergistic flocculating effect, which would have been observed via increased TSS removal for both of the ratios (activated sludge to primary effluent ratio and algae-rich water to primary effluent ratio) tested, a non-linear relationship would have resulted.

Activated Sludge Solids to Algae-rich Solids Mass Ratio

The following three figures (Figures 11, 12, and 13) display the TSS removal for the three Aerobic Beaker Tests as a function of activated sludge solids to algae-rich solids mass ratio. All three tests showed increased TSS removal with increasing activated sludge solids to algae-rich solids mass ratio.

Figure 11. TSS removed observed in test AE-2, calculated using Eq. 2, at different activated sludge solids to algae-rich solids mass ratios. The zero ratio points refer to mixtures with no activated sludge in the mixture or control mixtures that had only algae-rich water.

Figure 12. TSS removed observed in test AE-3, calculated using Eq. 2, at different activated sludge to algae-rich solids mass ratios. The zero ratio points refer to mixtures with no activated sludge in the mixture or control mixtures that had only algae-rich water.

Figure 13. TSS removed observed in test AE-4, calculated using Eq. 2, at different activated sludge to algae-rich solids mass ratios. The zero ratio points refer to mixtures with no activated sludge in the mixture or control mixtures that had only algae-rich water.

The slope in Figures 11, 12, and 13 represents the TSS removed per unit increase (i.e. 1:1 to 2:1) of mass ratio of activated sludge solids to algae-rich solids. The average slope of the curves in Figure 12, 13 and 14 is approximately 18.6. Thus the average describes the ability for a one unit increase in the mass ratio of activated sludge solids to algae-rich solids (i.e. 1:1 to 2:1) to remove 18.6 mg/L of TSS. AE-4 had a flatter slope (Figure 14) than in the other figures (Figure 12 and 13). This was not expected. One plausible explanation for the flat slope was the much higher activated sludge concentration (Table 8). There was much more activated sludge mass used in test AE-4 because of the higher initial activated sludge concentration (Table 8). The greater activated sludge solids mass effected the slope in Figure 13 by lowering the TSS removed per unit increase in the activated sludge solids to algae-rich solids mass ratio. If there was a sorptive effect to be observed it would have been seen in Figure 14. The increased sludge mass in AE-4 provided more potential sorption sites and additional solids contact relative to AE-2 and AE-3 resulting in an increase in TSS removal; but this did not happen.

Bacterial-Algal Sorption Beaker Test

In this experiment, primary effluent was not used, but a broader range of activated sludge and algae-rich water concentrations in the lab reactors was used than in the Aerobic Beaker Tests. Additionally, less reaction time was allotted in this experiment. Unlike in the Aerobic Beaker Tests with 4-hours of reaction time, only 30 minutes of reaction time in the Bacterial-Algal Sorption

Test was used. The settling time was 30 minutes in both experiments. A shorter reaction time was used because the goal of the experiment was to observe any mass action effect or spontaneous sorption of suspended solids onto the activated sludge.

The TSS concentration remaining in suspension after treatment decreased dramatically with increasing activated sludge concentration (Figure 14). Also shown in Figure 14 is a theoretical curve, termed the dilution curve, demonstrating the final TSS concentration if clean water was used in place of activated sludge. This dilution curve demonstrates the theoretical effect that solely diluting algae-rich water with non-reactive water would have on TSS removed.

Calculating final supernatant TSS for the dilution curve was similar to Eq. 2, stated previously. However, because this thesis did not experiment using only water to dilute the algae-rich water, the final supernatant TSS concentration (A_f) was not determined from a water sample. Instead final supernatant TSS was calculated (Eq. 4).

$$\Delta A_{obs} = A_{raw} - A_{fin} \quad \text{TSS change observed due to dilution and removal (Eq. 2a)}$$

$$A_{fin} = \frac{A_{raw}(V_w)}{V_{jar}} \quad \text{theoretical final TSS concentration (Eq. 4)}$$

Where:

ΔA_{obs} = change in TSS based on raw algae – rich water TSS

A_{fin} = final supernatant TSS concentration (Eq. 4)

A_{raw} = raw algae – rich ater TSS concentration

V_w = theoretical volume of dilution water used

V_{jar} = the final volume of the entire mixture in the jar

Clarifying further, V_w , was the theoretical volume of water used to obtain the final TSS concentration in Eq. 2a. This theoretical volume of water was equal to the different volumes of activated sludge in the experiment. Therefore the x-axis in Figure 14 is not valid for the dilution curve (no activated sludge was used).

Figure 14. Final TSS concentration after treatment at different activated sludge concentrations in the beaker. The “clean water curve” represents theoretical final TSS

concentration if water was used in place of activated sludge. Average activated sludge solids to algae-rich solids mass ratio was 3.3.

The Bacterial-Algal Sorption Test demonstrated a strong linear correlation between the final TSS and the activated sludge concentration ($r^2 = 0.96$). The linear relationship suggests some of this decrease in TSS could be attributed to dilution of the algae-rich water by the activated sludge or that the number of sorption sites provided by the activated sludge bacterial consortium is a constant property of the activated sludge sample used and that all the sites become “immediately” occupied by suspended microalgae and other solids:

$$(constant = \frac{\text{number of sorption sites}}{\frac{\text{mg}}{\text{L}} \text{ of activated sludge}})$$

The two curves in Figure 14 diverge as a result of differing slopes. However, the dilution has a flatter slope than the slope observed with activated sludge. This suggests that the difference in the final TSS concentration is due to the presence of activated sludge.

Because the final TSS concentration was not determined over a range of settling times it is difficult to infer a discrete sludge blanket-supernatant interface separation process as described by Fuchs and Staudinger (1999). After 30 minutes of batch settling, no significant increase in supernatant clarity (defined by % transparency) occurs for a cross-linked settling activated sludge (Fuchs and Staudinger, 1999).

The slope of the activated sludge regression line in Figure 14 is -0.11; this implies that 0.11 less milligrams of TSS will remain in suspension for every milligram of activated sludge increase in the reactor. If the algae-rich water was solely diluted with clean water, .06 less milligrams of TSS would remain in suspension for every milliliter of clean water; represented by the slope of the dilution curve

The relationship in Figure 14 provides a means to predict effluent TSS of a similarly operated activated sludge reactor with a similar algae-rich influent.

Figure 15 and Figure 16 display the amount of TSS removed due to treatment in the Bacterial-Algal Sorption Test. Results were not substantially different compared to results in the Aerobic Beaker Tests.

Figure 15. TSS removed from suspension after treatment with different activated sludge concentrations, using Eq. 1.

Figure 16. TSS removed from suspension after treatment with different activated sludge concentrations, using Eq. 2.

Once more it is shown that the higher the activated sludge concentration in the reactor, the higher TSS removal observed. Figures 15 and 16 above do demonstrate a significant reduction in TSS and perhaps more importantly do so within a range of conventional aeration basin activated sludge concentrations (1500-4000 mg/L). It is important to note that the shape of the curve in Figure 15 and Figure 16 is identical the curve produced when the x-axis is changed to the mass ratio of activated sludge solids to algae-rich solids mass. The curves in Figure 15 and Figure 16 have a slight sorption isotherm appearance when the linear regression line is removed. This observation may suggest that Figure 15 and 16 are demonstrating the initial and more linear portion of a sorption isotherm. Nevertheless, the inverse of the regression line slope is the sorption ratio: 18 mg activated sludge were able to sorb 1 mg of microalgal cells (Figure 16).

Anaerobic Preconditioning Beaker Test

The Anaerobic Preconditioning Beaker Test was similar to the Aerobic Beaker Tests, however there were two main differences. One new variable was introducing the algae-rich water and primary effluent to anaerobic conditions for twelve hours prior to use in the test. Secondly, recycling settled biomass was completed in attempt to develop a settleable culture over the course of the test. Another difference in the Anaerobic Preconditioning Beaker Test was that at intermittent points in the experimental process TSS samples were taken and analyzed. This allowed for comparison of treatment quality during the course of

the test. Figure 18, below, displays the results from the Anaerobic Preconditioning Beaker Test.

Figure 17. TSS concentrations after each of four sequential repetitions of treatment in the Anaerobic Preconditioning Beaker Test. TSS concentrations reported in Stage 1 were of a sample of the specified volumetric ratio while still “fresh”. TSS concentrations reported in Stage 2 were of a sample that underwent 12 hour–overnight anaerobic settling. TSS concentrations reported in Stage 3 were from the supernatant of a mixture after Cycle 1. TSS concentrations reported in Stage 4 were from the supernatant of a mixture that after Cycle 2. The seed was 20% (m/m) dose of activated sludge. Refer to Table 8 for the beaker plan. ARW stands for algae-rich water. The ratios specified in the legend are primary effluent to algae-rich water volumetric ratios. The stages are defined in Table 7.

As in the previous two experiments, the Anaerobic Preconditioning Test showed the pattern of higher final TSS concentration after running the test with higher initial algae-rich water TSS. In this experiment less TSS remained in the supernatant of beakers without the addition of activated sludge compared to

beakers that added activated sludge. Furthermore, through the determination of solids over the course of this experiment, it was discovered that TSS in the supernatant of all 12-hour aged quiescent beakers was less than in the beakers that underwent the prescribed treatments, which included mixing.

ARW labeled beakers that were not aerated, or mixed (ARW Control No Treatment) had lower TSS concentrations at nearly all stages of treatment (Figure 17). Conversely, Figure 17 showed that the beaker with 1:2 anaerobic feed with activated sludge seed had the highest TSS concentration at nearly all stages of treatment. This observation, however, was expected for the 1:2 anaerobic feed beakers because these two beakers had the most initial solids, mainly in the form of diluted algae-rich water solids. All the beakers, except the control beaker, which received no treatment, demonstrated an increase in TSS after receiving activated sludge seed, aeration, and mixing relative to the TSS concentrations observed due to overnight settling.

Since supernatant TSS was higher for all beakers after 12-14 hours of settling than after Stage 2, simple overnight settling of the waters tested was more effective than any of the treatments tried in this test. Lower TSS concentration observed after 12-14 hours (for all beakers) compared to TSS concentrations measured after mixing/aeration with or without activated sludge, suggests a

longer period of settling may be required to show substantive differences in the treatments' ability to produce an effluent with low TSS concentration.

The seed (a 20% (m/m) dose of activated sludge) had the greatest effect on the beakers with 1:2 anaerobic feed. At the end of Stage 4 the beaker with 1:2 anaerobic feed and activated sludge seed had a final TSS concentration 30 mg/L greater than the identically treated beaker with no seed. All beakers that received activated sludge seed demonstrated higher TSS concentrations at the end of Stage 4 compared to their "no-seed" counterparts. Because 30-minute settled TSS concentrations were not measured on "pure" activated sludge, an increase in final TSS in the seeded beakers may be blamed on the addition of suspended solids from the activated sludge seed. Approximately 10 mL of activated sludge seed was added to the beakers that required seed (specified by beaker plan Table 8). The activated sludge used in the Anaerobic Preconditioning Test had a TSS concentration of 5971 mg/L (Table 8). Therefore a 10-mL seed only added 60 mg of readily settleable solids to the beaker (settleable, relative to solids in algae-rich water and primary effluent).

Lastly, the ARW control beaker that did not receive any treatment (ARW Control No Treatment) had the lowest TSS concentrations at Stages 3 and 4. This observation shows that algae-rich water is more settleable if left quiescent than if gently mixed followed by 30 minutes of settling (ARW Anaerobic Control No

Seed). After Stage 2, the controls performed nearly identical to each other. It was only after two 4-hour periods of gentle mixing followed by 30-minute settling that the TSS concentration increased in the ARW Anaerobic Control No Seed beaker (Stages 3 and 4). This indicates that a settling time longer than 30-minutes may be required to highlight substantive differences in TSS among the treatments.

Relating the activated sludge solids to algae-rich solids mass ratio to TSS removed in the Anaerobic Preconditioning Test is only possible for beakers that received activated sludge seed. Analyzing the relationship between the activated sludge solids to algae-rich solids mass ratio showed a stronger trend in Stage 4 results compared to Stage 3 results (Figure 18).

Figure 18. TSS removed observed in Stages 3 and 4 of the Anaerobic Preconditioning Test, calculated using Eq. 2, at different activated sludge to algae-rich solids mass ratios. Control beakers and beakers that received no activated sludge seed were omitted from this analysis.

The primary difference between Stages 3 and 4 was the reuse of settled biomass from Stage 3 in Stage 4. This allowed activated sludge bacteria another 4 hours of growth, contact time, and adaptation to the conditions imparted in the beaker. Additionally there was more settleable biomass (activated sludge bacteria, bacterial-algal flocs, and algae flocs) to sorb the suspended solids poured into the beaker during Stage 4.

In Figure 18, Stage 4 demonstrated a stronger correlation (greater slope) in this analysis than Stage 3. While TSS removed during Stage 3 was higher at activated sludge solids to algae-rich solids mass ratios equal to and less than 1, the greater slope observed by the Stage 4 curve shows promise for increased removal at higher ratios.

General Discussion

The most valuable pattern observed in the course of experimentation was the pattern of higher activated sludge to primary effluent volumetric ratios resulting in higher TSS removal. For all experiments, except the Anaerobic Preconditioning Beaker Test, higher activated sludge concentrations (activated sludge to primary effluent volumetric ratios) always demonstrated greater TSS removal than beakers with less (or no) activated sludge. Beakers in the Anaerobic Preconditioning Beaker Test that had activated sludge seed showed higher final TSS concentrations than beakers that did not receive activated sludge seed. Increased final TSS in beakers with activated sludge seed was not expected in the Anaerobic Preconditioning Beaker Test. Though some unexpected observations were made in the Anaerobic Precondition Beaker Test, the observations were important. For example, it was observed that the control beaker that was not mixed produced a final TSS concentration lower than the same control beaker that was mixed. Furthermore, the Anaerobic Preconditioning Beaker Test showed that all mixtures of algae-rich water and primary effluent had lower TSS concentrations after a 12-hour settling period than after being mixed with or without an activated sludge seed followed by 30-minute settling.

There were, however, two primary concerns regarding the experiments in this thesis:

1. Limited settling time

2. Inability to culture a settleable activated sludge-algal biomass

Allowing colloidal microalgae only 4 hours of contact time with activated sludge was meant to simulate the hydraulic residence time of activated sludge aeration basins. The sequencing batch mode of operation during the Anaerobic Preconditioning Beaker Test required a filling, reaction, sedimentation, and decant cycle time of about 5 hours, which is typical for treatment of domestic wastewater with a semi-batch reactor (SBR) activated sludge system. The equivalent filling, reaction, sedimentation and decanting processes described in Medina and Neis (2007) and Gutzeit et al. (2005) took 2 days. This allowed ample time for reaction and build-up of settleable biomass to occur. Furthermore, Medina and Neis (2007) and Gutzeit et al. (2005), cultured their symbiotic algal-bacteria broth for 6 weeks. This method of culturing recycled settled biomass would have likely encouraged the buildup of a more settleable culture.

In this thesis research, only 30 minutes of settling were allowed so that positive effects on settling might be more distinct. Longer settling periods (2-hour, 12-hour, and 24-hour) should result in improved supernatant clarity. This improved clarity was suggested in the Anaerobic Preconditioning Beaker Test, where the supernatant in all beakers had lowest TSS concentrations after 12 hours of settling. Typical sedimentation tanks allow 2 hours of detention time (Tchobanoglous et al., 2003), 4-times greater than was allowed for settling

herein. Thus, the results may underestimate the TSS removal capabilities of the trialed treatments.

Even when allowed a retention time of 1-2 days in an algal settling pond only 50-80% of algal biomass from a HRAP was observed to be removed (Park et al., 2011b). After performing algal biomass recycling and maintaining a near 90% species dominance of *Pediastrum* sp. in the HRAPs studied, Park et al. (2011b) saw settling efficiencies near 90%, an improvement of 30% over the settling efficiency of the control. Despite high removal efficiencies with biomass recycling and the maintenance of a settleable, multicellular algal species, removal of TSS to permissible levels was still difficult. Lastly, the case of Nurdogan and Oswald (1995) a removal of 86% of TSS was observed but only when a stable culture of *Micractinium* sp. was present and using a modest settler over flow rate of $0.15 \frac{gpm}{ft^2}$. Despite high removal efficiencies nowhere in these studies do the authors, Park et al. (2011b) and Nurdogan and Oswald (1995) disclose effluent TSS.

However, the build-up of a stable settleable bacterial-algal culture was not the purpose of this effort. Instead, the goal was to determine the ability for a conventional activated sludge to remove a colloidal culture from suspension. “Mass action” or rapid bio-sorptive mechanisms were sought rather than a languid bioflocculating mechanism.

The efforts in this study largely attempted to recreate the efforts of Shipin et al. (1999a & 1999b) and Meiring and Oellermann (1995) who saw high quality effluents from minutes of contact time (PETRO TF) to hours of contact time (PETRO ASP). This study was relatively successful in demonstrating improved final TSS concentrations in a PETRO-like fashion.

The “mass action” mechanism was described, by Fuchs and Staudinger (1999), briefly mentioned earlier. They describe the ability of two different sludges that have similar settling rates to produce entirely different effluent quality. It was found that sludges that produced a higher quality (as defined by transparency of the supernatant) formed a cross-linked matrix between sludge flocs, which in turn created a sharp interface between the settling sludge blanket and the supernatant (Fuchs and Staudinger, 1999). On the other hand, a disperse sludge blanket-supernatant interface produced a poorer quality effluent (Fuchs and Staudinger, 1999). Development of adequate cross linkage depended on activated sludge concentration (thus limiting the distance between the sludge flocs) and the individual flocculation properties of the sludge bacterial consortia (Fuchs and Staudinger, 1999). Well cross-linked sludges described in Fuchs and Staudinger (1999) demonstrated a maximum effluent quality after 30 minutes of batch settling. If the sludge used in this research project were of the cross-linking sort, then the best achievable supernatant (effluent) may have been observed.

On the other hand, if the sludge used in the present report was the disperse settling sort, then the best achievable supernatant (effluent) may not have been observed.

Furthermore, EPS may have a crucial role binding bacterial cells together forming flocs and adsorbing soluble and particulate matter in bulk fluid (Sheng et al. 2010; Subramanian et al. 2010). Sheng et al. (2010) and Wingender (1999) go further in saying, that EPS is responsible for forming a substantial net-like structure binding cells to cells, have many sites for adsorption, and can even be described by the Freundlich sorption equation in some instances. However, because no significant sorptive removal mechanism was observed, EPS concentrations in the activated sludge from SLOWRF may have been low. If EPS concentrations were significant enough to have an effect, they should have been observed as the time allotted for mixing in all experiments was more than adequate for typical flocculation processes in wastewater (Tchobanoglous et al., 2003).

Alternatively, significant quantities of EPS may have been in the activated sludge, but the characteristics and composition of the activated sludge may have not had any effect on the suspended algal matrix. The lack of multivalent cations has proved to reduce activated sludge flocculation and subsequent settling by bridging negatively charged particles (i.e. anionic EPS or negatively charged

bacteria/microalgae) (Subramanian et al. 2010). Therefore, a potential lack of multivalent cations in the waters tested in this research may have led to poor suspended algae removal.

Many flocs observed under the microscope demonstrated the tendency for algae to be at the center of flocs when the floc contained activated sludge organisms (Appendix – A Micrographs 11, 12, and 13). It appeared that ciliates and other components of activated sludge entrapped or encircled algae, forming a floc. Ciliates and other activated sludge components were almost always on the periphery of floc (with algae in the center) (Appendix- A). This suggested that algae may not be sorbing to the surface of the activated sludge floc. From the micrographs it is difficult to decipher the origin of the floc. Origin of algal-bacterial flocs observed include the following possibilities:

1. Colloidal microalgae began to flocculate forming a larger floc followed by a microbial envelopment
2. The algae floc was a pin floc and EPS (bacterial and algal) secretion sorbed surrounding algal pin floc and bacterial components
3. Stalked ciliates possess a flocculating mechanism near the end of their stalk to form the “ciliate rings”.

Other researchers show that EPS may even inhibit activated sludge performance. There is reported to be a threshold quantity of EPS that is

beneficial, after which EPS will inhibit mass transfer (Liao et al., 2001; Sheng et al., 2010). Inhibition of mass transfer is caused by the “clogging” of potential sorption site by the EPS (Sheng et al., 2010) and by increasing the net negative charge of the negatively charged microorganism (Mikkelsen & Nielsen, 2001). In any event, the complexity of the role of EPS on the flocculation of activated sludge, and its effect on a recalcitrant suspension of algae, warrants caution declaring relationships and drawing conclusions based on EPS concentration or composition.

Conclusions

In general, higher activated sludge concentrations led to higher TSS removal and subsequently lower final TSS concentration of algal suspensions. Conversely, greater concentrations of algae-rich water led to higher final TSS concentrations and less TSS removal.

When concentrations of activated sludge were nearly equal to those typical of activated sludge MLSS concentrations, supernatant TSS concentration approached regulatory limits (for example, <40 mg/L). However, only one reactor during the course of experimentation produced an effluent TSS concentration less than 30 mg/L. However, this beaker in the Anaerobic Preconditioning Beaker Test (ARW Control No Treatment) experienced a long settling time (>12 hours) which is likely to blame for low final TSS concentration.

Overnight quiescence proved to leave less TSS in the supernatant than a 4-hour aerobic reaction time with activated sludge and mixing. In fact, in the Anaerobic Preconditioning Beaker Test, activated sludge was observed to inhibit TSS removal from the supernatant compared to beakers that received no activated sludge seed. Only two 4-hour cycles of SBR-like operation in the Anaerobic Preconditioning Beaker Test likely did not build up a settleable microbial consortium acclimatized to algae-rich influents. Conversely, the PETRO ASP is a

continuously operating reactor that returns settled biomass, inevitably building a microbial stock accustomed to settling solids in algae-rich waters.

No predominance of a specific algae species was observed in any of the experiments. Species predominance can be affected by biomass recycling. Longer reaction times and biomass recycling, effectively increasing the mean cell residence time (MCRT) should be considered in future experimentation. Furthermore, studies explicitly on the effect of EPS on settleability of algae should be performed.

Results of this study specifically show:

1. >12 hours of anaerobic settling can lower HRAP TSS effluent concentration to 30 mg/L (Figure 18).
2. Addition of > 3000 mg/L activated sludge concentrations can produce final effluents near discharge requirements (40-50 mg/L) with only 30 minutes of settling and without the addition of any primary effluent (Figure 17).
3. A 1 unit increase in the activated sludge to primary effluent volumetric ratio (i.e. 1:1 to 2:1) results in approximately 73 mg/L less TSS in the supernatant (Figures 9, 10, and 11)
4. A 1 unit increase in the activated sludge solids to algae-rich solids mass ratio (i.e. 1:1 to 2:1) in the beaker resulted in approximately 19 mg/L less TSS in the supernatant (Figures 12, 13, and 14).

5. >200 mg/L TSS removal was achieved using activated sludge to primary effluent ratios equal to, and greater than 1:1 (Figures 9 and 10).
6. >250 mg/L TSS removal was achieved using an activated sludge concentration of 730 mg/L with the addition of primary effluent (Figure 8).

The work presented herein reports the difficulty of removing algae from suspension using typical activated sludge processes. Moreover, the results add to the library of activated sludge performance and algae removal from wastewater treatment lagoons and HRAPs.

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Appendix A Photo Micrographs

Figure A-1. Golenkinia attached to larger particle with spiral-like microorganism. From HRAP used in the Anaerobic Preconditioning Test before treatment.

Figure A-4. Micractinium colony in untreated HRAP sample used in Aerobic Beaker Test 4.

Figure A-2. Micractinium attached to a floc of bacteria and smaller spherical Chlorella-like algae cells.

Figure A-3. Micractinium colony in untreated "control" culturing fish tank water sample used in Aerobic Beaker Test 4.

Figure A-5. Large floc of stalked ciliates in an activated sludge sample. Demonstrates typical composition of activated sludge throughout experimentation.

Figure A-6. Activated sludge sample with typical consortia throughout testing. Rotifers and stalked ciliates appear in significant quantities.

Figure A-7. Periphery of large algal-bacterial floc. Discernible algae and diatom species are spherical Chlorella-like and Synedra type species respectively. Stalked ciliates were found near floc peripheries nearly without exception.

Figure A-8. Filamentous algae with algal-bacterial flocs attached.

Figure A-9. Symmetrically braided filamentous algae with unicellular microalgae adjacent.

Figure A-10. Floc of Micractinium-like algae with extracellular projections and colonial coating.

Figure A-11. Ring or torus shaped floc with stalked ciliates provide structure for algal aggregation. Sample from settled biomass in Anaerobic Preconditioning Test.

Figure A-12. Stalked ciliate at the periphery of algal-bacterial flocs.

Figure A-13. Filamentous algae with floc formed at the center of a stalked ciliate ring and colloidal algae in bulk water.

Figure A-14. Colloidal Chlamydomonas-like algae species with a Synedra species also present. Sample from HRAP in Anaerobic Conditioning Test.

Figure A-15. Oscillatoria-type filamentous algae next to large flow, sample from controlled fish tank used in Aerobic Beaker Tests and Bacterial-Algal Sorption Test.

Figure A-16. Large algae floc and rotifers in a sample from the “controlled” culturing fish tank.

Figure A-17. A finger of a large algae floc from the culturing fish tank.

Figure A-18. Large algae floc with free non-colonial Oscillatoria-like algae adjacent and rotifer. Notice hair-like structures on floc periphery.

Appendix B Data Tables

Table B-1. Aerobic Beaker Test AE-2 volumetric ratios, solids mass ratios, and results. Raw algae-rich water TSS concentration, Ar, for AE-2 was 269 mg/L.

Beaker Label	Algae-rich Water (mL)	Primary Effluent (mL)	Activated Sludge (mL)	Total (mL)	Volumetric Ratio of Algae-rich Water to Primary Effluent	Volumetric Ratio of Algae-rich Water to Activated Sludge	Volumetric Ratio of Activated Sludge to Primary Effluent	Mixing and Aeration	Activated Sludge TSS in Beaker (mg/L)	Diluted Algae-rich Water TSS in Beaker (mg/L)	Activated Sludge Solids to Algae-rich Solids Mass Ratio	Final TSS (mg/L)	Eq. 1 ΔAp (mg/L)	Eq. 2 ΔAt (mg/L)
C1	2000	0	0	2000	n/a	n/a	n/a	Not Mixed, Not Aerated	0	269	0.00	160	109.00	109.00
C2	2000	0	0	2000	n/a	n/a	n/a	Mixed, Not Aerated	0	269	0.00	190	79.00	79.00
1	1600	400	0	2000	4:1	n/a	0	Mixed, Aerated	0	215	0.00	170	45.20	99.00
2	1456	364	182	2002	4:1	8:1	1:2	Mixed, Aerated	258	196	1.32	120	75.83	149.00
3	1332	333	333	1998	4:1	4:1	1:1	Mixed, Aerated	472	179	2.64	125	54.15	144.00
4	1332	666	0	1998	2:1	n/a	0	Mixed, Aerated	0	179	0.00	180	-0.85	89.00
5	1144	572	286	2002	2:1	4:1	1:2	Mixed, Aerated	406	154	2.64	95	58.87	174.00
6	1000	500	500	2000	2:1	2:1	1:1	Mixed, Aerated	709	135	5.27	50	84.50	219.00
7	1000	400	600	2000	5:2	5:3	3:2	Mixed, Aerated	851	135	6.33	55	79.50	214.00
8	1200	300	500	2000	4:1	12:5	4:3	Mixed, Aerated	709	161	4.39	75	86.40	194.00
9	1000	800	200	2000	5:4	5:1	1:4	Mixed, Aerated	284	135	2.11	65	69.50	204.00
10	1200	600	200	2000	2:1	6:1	1:3	Mixed, Aerated	284	161	1.76	110	51.40	159.00
11	1400	200	400	2000	7:1	7:2	2:1	Mixed, Aerated	567	188	3.01	80	108.30	189.00

Table B-2. Aerobic Beaker Test AE-3 volumetric ratios, solids mass ratios, and results. Beakers DC1 and DC2 were duplicates of beakers C1 and C2, respectively. Beakers 1a, 2a, and 6a were duplicates of beakers 1, 2, 6, respectively. Raw algae-rich water TSS concentration, Ar, for AE-3 was 276 mg/L.

Beaker Label	Algae-rich Water (mL)	Primary Effluent (mL)	Activated Sludge (mL)	Total (mL)	Volumetric Ratio of Algae-rich Water to Primary Effluent	Volumetric Ratio of Algae-rich Water to Activated Sludge	Volumetric Ratio of Primary Effluent to Activated Sludge	Volumetric ratio of Activated Sludge to Primary Effluent	Mixing and Aeration	Activated Sludge TSS in Beaker (mg/L)	Diluted Algae-rich Water TSS in Beaker (mg/L)	Activated Sludge Solids to Algae-rich Solids Mass Ratio	Final TSS (mg/L)	Eq. 1 ΔAp (mg/L)
C1	2000	0	0	2000	n/a	n/a	n/a	n/a	Not Mixed, Not Aerated	0	276	0.00	148	128.00
DC1	2000	0	0	2000	n/a	n/a	n/a	n/a	Not Mixed, Not Aerated	0	276	0.00	152	124.00
C2	2000	0	0	2000	n/a	n/a	n/a	n/a	Mixed, Not Aerated	0	276	0.00	214	62.00
DC2	2000	0	0	2000	n/a	n/a	n/a	n/a	Mixed, Not Aerated	0	276	0.00	176	100.00
1	1600	400	0	2000	4:1	n/a	n/a	0	Mixed, Aerated	0	221	0.00	148	72.80
1a	1600	400	0	2000	4:2	n/a	n/a	0	Mixed, Aerated	0	221	0.00	176	44.80
2	1456	364	182	2002	4:1	8:1	2:1	1:2	Mixed, Aerated	266	201	1.32	100	100.93
2a	1456	364	182	2002	4:1	8:1	2:1	1:2	Mixed, Aerated	266	201	1.32	138	62.93
3	1332	333	333	1998	4:1	4:1	1:1	1:1	Mixed, Aerated	486	184	2.64	80	103.82
4	1332	666	0	1998	2:1	n/a	n/a	0	Mixed, Aerated	0	184	0.00	185	-1.18
5	1144	572	286	2002	2:1	4:1	2:1	1:2	Mixed, Aerated	417	158	2.64	60	97.87
6	1000	500	500	2000	2:1	2:1	1:1	1:1	Mixed, Aerated	730	138	5.29	20	118.00
6a	1000	500	500	2000	2:1	2:1	1:1	1:1	Mixed, Aerated	730	138	5.29	55	83.00

Table B-3. Aerobic Beaker Test AE-4 volumetric ratios, solids mass ratios, and results. Beakers labeled DC1 and 6a were duplicates of beakers C1 and 6a respectively. Raw algae-rich water TSS concentration, Ar, for AE-4 was 253 mg/L.

Beaker Label	Algae-rich Water (mL)	Primary Effluent (mL)	Activated Sludge (mL)	Total (mL)	Volumetric Ratio of Algae-rich Water to Primary Effluent	Volumetric Ratio of Algae-rich Water to Activated Sludge	Volumetric Ratio of Primary Effluent to Activated Sludge	Volumetric ratio of Activated Sludge to Primary Effluent	Mixing and Aeration	Activated Sludge TSS in Beaker (mg/L)	Diluted Algae-rich Water TSS in Beaker (mg/L)	Activated Sludge Solids to Algae-rich Solids Mass Ratio	Final TSS (mg/L)	Eq. 1 ΔAp (mg/L)
C1	2000	0	0	2000	n/a	n/a	n/a	n/a	Not Mixed, Not Aerated	0	253	0.00	125	127.50
DC1	2000	0	0	2000	n/a	n/a	n/a	n/a	Not Mixed, Not Aerated	0	253	0.00	140	112.50
C2	2000	0	0	2000	n/a	n/a	n/a	n/a	Mixed, Not Aerated	0	253	0.00	140	112.50
DC2	2000	0	0	2000	n/a	n/a	n/a	n/a	Mixed, Not Aerated	0	253	0.00	150	102.50
1	1600	400	0	2000	4:1	n/a	n/a	0	Mixed, Aerated	0	202	0.00	145	57.00
2	1456	364	182	2002	4:1	8:1	2:1	1:2	Mixed, Aerated	479	184	2.60	115	68.82
3	1332	333	333	1998	4:1	4:1	1:1	1:1	Mixed, Aerated	877	168	5.20	85	83.17
4	1332	666	0	1998	2:1	n/a	n/a	0	Mixed, Aerated	0	168	0.00	120	48.17
5	1144	572	286	2002	2:1	4:1	2:1	1:2	Mixed, Aerated	753	145	5.20	80	64.43
6	1000	500	500	2000	2:1	2:1	1:1	1:1	Mixed, Aerated	1316	127	10.41	60	66.25
6a	1000	500	500	2000	2:1	2:1	1:1	1:1	Mixed, Aerated	1316	127	10.41	65	61.25

Table B-4. Bacterial-Algal Sorption Test solids mass ratios, and results. Beakers labeled 3a and 8a were duplicates of beakers 3 and 8, respectively. All beakers were mixed and aerated. Raw algae-rich water TSS concentration, Ar, for the Bacterial-Algal Sorption test was 285 mg/L.

Beaker Label	Algae-rich Water (mL)	Activated Sludge (mL)	Total (mL)	Activated Sludge TSS in Beaker (mg/L)	Diluted Algae-rich Water TSS in Beaker (mg/L)	Activated Sludge Solids to Algae-rich Solids Mass Ratio	Final TSS (mg/L)	Eq. 1 ΔAp (mg/L)	Eq. 2 ΔAt (mg/L)
1	2000	0	2000	0	285	0.00	240	45.00	45.00
2	1900	100	2000	206	271	0.76	240	30.75	45.00
3	1800	200	2000	1533	257	5.98	225	31.50	60.00
3a	1800	200	2000	1534	257	5.98	223	33.50	62.00
4	1700	300	2000	619	242	2.55	198	44.25	87.00
5	1600	400	2000	825	228	3.62	150	78.00	135.00
6	1500	500	2000	1031	214	4.82	125	88.75	160.00
7	1400	600	2000	1238	200	6.20	100	99.50	185.00
8	1300	700	2000	1444	185	7.79	113	72.25	172.00
8a	1300	700	2000	1444	185	7.79	103	82.25	182.00
9	1200	800	2000	1650	171	9.65	67	104	218.00
10	1100	900	2000	1856	157	11.84	53	103.75	232.00

Table B-5. Anaerobic Preconditioning Test solids mass ratios, and results. (ARW is algae-rich water.) Raw algae-rich water TSS concentration, Ar, for the Anaerobic Preconditioning Test was 96 mg/L.

Beaker Label	Algae-rich Water (mL)	Primary Effluent (mL)	Activated Sludge Seed (mL)	Total (mL)	Volumetric Ratio of Primary Effluent to Algae-rich Water	Mixing and Aeration	Activated Sludge TSS in Beaker (mg/L)	Diluted Algae-rich Water TSS in Beaker (mg/L)	Stage 3 Activated Sludge Solids to Algae-rich Solids Mass Ratio	Stage 4 Activated Sludge Solids to Algae-rich Solids Mass Ratio	Stage 1 TSS (mg/L)	Stage 2 TSS (mg/L)	Stage 3 TSS (mg/L)	Stage 4 TSS (mg/L)	Stage 3 Eq. 1 ΔAp (mg/L)	Stage 3 Eq. 2 ΔAt (mg/L)	Stage 4 Eq. 1 ΔAp (mg/L)
ARW Control No Treatment	2000	0	0	2000	n/a	Not Mixed, Not Aerated	0	96	0.00	0.00	96	29	28	27	67.87	69.37	69.37
ARW Anaerobic Control No Seed	2000	0	0	2000	n/a	Mixed, Not Aerated	0	96	0.00	0.00	96	28	34	48	62.50	48.00	48.00
2:1 Anaerobic Feed	666	1333	0	1999	2:1	Mixed, Aerated	0	32	0.00	0.00	86	39	60	58	-28.03	38.25	-25.78
1:1 Anaerobic Feed	1000	1000	0	2000	1:1	Mixed, Aerated	0	48	0.00	0.00	89	39	59	58	-11.00	38.50	-9.50
1:2 Anaerobic Feed	1333	666	0	1999	1:2	Mixed, Aerated	0	64	0.00	0.00	101	43	59	56	5.48	39.83	7.81
2:1 Anaerobic Feed w/ Seed	666	1333	9	2008	2:1	Mixed, Aerated	27	32	0.42	1.64	86	39	57	62	-24.91	34.50	-29.53
1:1 Anaerobic Feed w/ Seed	1000	1000	10	2010	1:1	Mixed, Aerated	30	48	0.62	1.07	89	39	53	68	-5.13	28.50	-19.50
1:2 Anaerobic Feed w/ Seed	1333	666	11	2010	1:2	Mixed, Aerated	33	64	1.03	0.64	101	43	68	90	-3.52	6.50	-25.52