**Bioflocculation and CO₂ Supplementation in
Wastewater Treatment by Algal High-Rate Ponds**

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APPROVAL PAGE

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ABSTRACT

Bioflocculation and CO₂ Supplementation in Wastewater Treatment by Algal High-Rate Ponds

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High-rate ponds can treat wastewater by growing microalgae with a high areal productivity. The photosynthetic oxygen released to the water during growth contributes to the removal of oxygen-demanding substances. Assimilation of nitrogen and phosphorus by microalgae, followed by the separation of the algae from the wastewater, accomplishes nutrient removal, with recovery of the nutrients for reuse as fertilizer. However, reliable complete nutrient removal has yet to be accomplished with pilot high-rate ponds, and excessive suspended solids concentrations following sedimentation are another common problem of high-rate ponds.

A one-year outdoor pilot study was conducted on two modifications of conventional wastewater high-rate pond operations. The first modification was the addition of carbon dioxide to the ponds with the goal of improving algae productivity and nutrient removal. The second modification, studied independently of the CO₂ addition, was inoculation of the ponds with return activated sludge (RAS), meant to improve the bioflocculation of algal-bacterial biomass and the subsequent gravity clarification of pond effluent.

Two sets of duplicate 3-m² high-rate ponds were operated. The channelized ponds were 30-cm deep, with a volume of approximately 925 liters. A paddle wheel in each pond maintained a water velocity of 25 cm/s. Pond influent was effluent from a primary clarifier at a municipal wastewater reclamation facility in coastal central California. Water sampling and analysis was conducted weekly on influent feed and pond effluent.

Pure carbon dioxide was automatically sparged into the experimental ponds to maintain a pH near 8.4. The pH levels in control ponds were allowed to vary freely (often up to pH 9.5). Carbon dioxide supplementation did not affect biomass productivity or nitrogen removal. In the experimental ponds receiving CO₂, the algae were presumably grown to their light limit (reaching 50-250 mg/L depending on season), and then the effluent was discharged. As such, nutrient removal was incomplete and carbon limitation did not occur. The average biomass productivities for the CO₂-supplemented ponds and control ponds were 17.7 g/m²-d and 17.2 g/m²-d, respectively. Total ammonia nitrogen (TAN) removal was between 80-92% for all ponds. Alkalinity removal was 26-40%, and effluent alkalinity concentrations were approximately 250 mg/L in all ponds throughout CO₂ supplementation. Since inorganic carbon did not become limiting in the control ponds, supplemental carbon dioxide did not affect biomass productivity in the experimental ponds.

In the experiment with activated sludge inoculation, RAS was added daily to the ponds at 1.6% of the wastewater influent flow. The RAS was collected from a full-scale municipal nitrifying activated sludge system. The effluents from the ponds were settled in Imhoff cones, with supernatant sampling at 2 hours and 24 hours of settling. The ponds with RAS inoculation exhibited higher settling efficiency compared to the control ponds. The 2-hr and 24-hr TSS levels for the RAS-inoculated ponds were 34 and 22 mg/L, respectively, compared to 49 and 38 mg/L for the control ponds. On average, the RAS-inoculated ponds settled 25% more efficiently over 2 hours, and 21% more efficiently over 24 hours. TAN removal in the ponds receiving RAS was 11% higher

than in control ponds, which may have been due to nitrifying bacteria in daily RAS inoculations. Biomass productivity was similar in all ponds.

An ammonia volatilization study was conducted to corroborate volatilization rates surmised from nitrogen mass balances. The ponds were cleaned to remove biomass and then filled with tap water. Ammonium chloride was used to establish predetermined TAN concentrations in the ponds. Ammonia mass transfer coefficients (kLa) were determined experimentally at pH levels of 8.0, 8.4, and 9.4. A 24-hr monitoring study (diel study) of the ponds produced pH, temperature, and TAN concentration data for a typical day. Using experimental kLa values, ammonia volatilization estimates were calculated for the diel study. The calculated ammonia volatilization from the diel study predicted between 11-26% TAN loss due to volatilization. These results were then compared to nitrogen mass losses from the ponds during wastewater treatment. A nitrogen balance revealed nitrogen losses in the ponds between 16-20%. These results indicate that most or all of the observed nitrogen losses were due to ammonia volatilization. The major nitrogen transformation mechanism, nitrogen assimilation by biomass, accounted for 39-59% of the nitrogen removal.

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Chapter 1: Introduction

Although the majority of wastewater treatment plants in the United States use activated sludge or trickling filters as their secondary treatment process, pond systems are the third-most common treatment technology and one that offers the advantages of low energy consumption, few mechanical components, low capital cost, and low operations & maintenance costs (Frost 2008). Pond systems are usually comprised of a series of ponds that are 1-4 m deep and mixed only by wind or influent flow momentum. Rather than using mechanical blowers or pumps to facilitate transfer of atmospheric oxygen to a community of microorganisms (as in the activated sludge and trickling filter processes), microalgae in ponds photosynthesize and produce oxygen, which encourages the growth of bacteria and the oxidation of contaminants.

Although pond systems offer several advantages, many municipalities upgrade existing pond systems with mechanical technologies, such as activated sludge, because activated sludge systems tend to have more consistent treatment performance than ponds, while occupying less land area (Frost 2008). The most basic need for improvement in pond systems is controlling effluent suspended solids concentrations, which are often higher than desired discharge limits. Sedimentation of pond effluent usually does not clarify the water enough to allow for efficient use of disinfectant, and, instead, expensive coagulation and/or filtration may be needed (Shilton 2005).

Another capability generally lacking in treatment ponds is nutrient removal. Only heavily aerated ponds or lagoons are able to consistently remove nitrogen via nitrification-denitrification (Rich 1999).

A type of pond potentially able to produce both low effluent suspended solids and nutrients is algal high-rate ponds (HRPs). This rarely used technology employs mechanical mixing to promote microalgae production for wastewater treatment. HRPs consist of a raceway that is continuously mixed with a paddle wheel. The development of these systems began over 50 years ago (Oswald and Gotaas 1957). Continual mixing keeps biomass in suspension and aids in algal production, which accelerates aerobic treatment compared to unmixed conventional ponds and potentially increases the assimilation of nutrients by algae per volume treated (Oswald 1991).

The objective of this thesis project was to address two hypotheses regarding algal high-rate ponds treating municipal wastewater:

- (1) pH-regulated carbon dioxide supplementation to high-rate ponds increases algal productivity and nutrient assimilation.
- (2) The settleability of high-rate pond algal-bacterial flocs can be increased by the daily addition of return activated sludge from an external source.

To address these hypotheses, four pilot high-rate ponds were constructed and operated in two operational modes: (1) two experimental ponds were supplemented with CO₂ at a pH setpoint of 8.4, while two control ponds operated without CO₂ supplementation. This experiment was conducted in warmer months when algae productivity was expected to be higher, and, therefore, the potential for carbon limitation was higher; (2) Two experimental ponds were supplemented with 3.78 L of return activated sludge daily, while two control ponds operated without any supplementation. This experiment was conducted in colder months.

The addition of RAS in high-rate ponds has been explored in past Cal Poly master's thesis projects (Frost 2008, Podevin 2011). The addition of RAS has been hypothesized to encourage bioflocculation of algae and bacteria. RAS by itself settles well because it consists of large microbial agglomerations. In terms of the RAS addition, the main difference in the present study, compared to Frost and Podevin, is the depth of the ponds: the past experiments used 0.75-meter deep ponds, while this study used 0.3-meter deep ponds. Shallower HRPs increase the exposure of the biomass to sunlight and should increase the volumetric production of algae, with its concomitant production of dissolved oxygen and nutrient assimilation. In addition, the increased rate of exposure of water to the pond surface would be expected to increase ammonia volatilization (given equal hydraulic residence time, pH, temperature, etc.).

Algae require several main substrates for growth: nitrogen, phosphorus, trace metals and nutrients, water, sunlight, and carbon dioxide. Wastewater provides nitrogen, phosphorus and other nutrients, but the carbon content of domestic wastewater is usually too low to allow complete assimilation of wastewater nitrogen and phosphorus in algal biomass. Providing supplemental carbon dioxide to HRPs should allow assimilation of N and P up to the growth limitation imposed by sunlight. In practical application, flue gas from industrial processes would be used as the source of carbon dioxide. Eliminating the potential for inorganic carbon limitation has not been studied previously at pilot scale at Cal Poly.

Chapter 2: Background

2.1 Wastewater Treatment Overview

The major steps in wastewater treatment are preliminary, primary, secondary, tertiary (or advanced), and disinfection. Preliminary treatment involves the screening and removal of large debris, followed by the removal of grit. The main purpose of this step is to prevent clogging and protect downstream pumps and piping from physical damage. Conventional primary treatment removes particles from suspension through settling in clarifiers. Some treatment plants elect to use a chemical coagulant to get higher solids removal from their clarifiers. Secondary treatment is the main step for removal of oxygen demanding substances through growth of microorganisms, mainly aerobic heterotrophic bacteria. Tertiary, or sometimes called advanced treatment, can consist of further biological and/or chemical processes for additional removal of suspended solids and oxygen demand. Filtration is a prime example. Nutrient removal is another process that can be considered tertiary treatment or included with other tertiary treatment. Finally, a variety of disinfection processes can be used to prepare the water for final discharge or reuse. Depending on local conditions, many additional process steps may be needed, including dechlorination, cooling, and desalination.

The high-rate algal ponds used in this study represent a secondary treatment and nutrient removal step, which would be used in place of conventional aerated activated sludge or trickling filter systems.

2.2 Natural Biological Treatment Systems

Natural wastewater treatment systems use the symbiotic relationship of plants, algae, and bacteria to minimize the need for mechanical components and high energy use. Rather than constructing containments of concrete and steel, natural systems often consist of excavated earthen basins transformed into constructed wetlands or pond systems. In contrast, activated sludge systems require continuous oxygen supplementation, while natural systems can depend on algal photosynthesis for a source of oxygen. In addition to lower energy use, properly designed natural systems are also considered more aesthetically pleasing than activated sludge facilities. Since algae cannot produce oxygen levels comparable to those in continuously aerated basins, the land requirements in pond systems often range from 50-500 times that of activated sludge aeration (Frost 2008). However, the cost of constructing concrete and steel reactors can be orders of magnitude greater than the cost of earthen treatment basins of the same size (Oswald 1995).

Figure 2-1: Basic biological cycle contributing to the treatment of wastewater in pond systems. Source: Gutzeit et al. 2005.

The treatment mechanisms in algal ponds are direct and indirect (Oswald 1995). The direct treatment mechanisms involve the symbiotic relationship between algal and bacterial growth, in which algae provide oxygen through photosynthesis and bacteria provide carbon as a byproduct of their growth and nutrients through degradation of wastewater particulates.

The indirect treatment mechanisms are numerous. Algal ponds typically reach a slightly alkaline pH during the sunlight hours. A raised pH promotes the precipitation of metals and phosphates, ammonia volatilization, and disinfection.

2.2.1 Traditional Pond Treatment Systems

Traditional pond treatment systems use a series of ponds that do not typically receive mechanical mixing. Two common designs consist of the following ponds, in order: (1) a primary facultative pond and a series of maturation ponds, and (2) an anaerobic pond, a secondary facultative pond, and a series of maturation ponds (Shilton 2005). Facultative ponds operate with both aerobic and anaerobic conditions and are typically 1-2 meters deep. The top layer becomes oxygenated by algal activity, while the bottom functions like an anaerobic reactor. As mentioned above, some systems utilize a fully anaerobic pond before a secondary facultative pond. These ponds have a lower hydraulic residence time, have a smaller area requirement, and can reduce organics by 40-70% in several days (Shilton 2005). Maturation ponds are a final treatment step which are used to help reduce pathogens, but can also further reduce nutrient levels (Shilton 2005).

2.2.2 Advanced Integrated Wastewater Pond Systems (AIWPS™)

At a 1990 ASCE conference, William Oswald presented the AIWPS™, which was designed to maintain the benefits of a pond system, while mitigating some of their drawbacks. These systems require less land than typical pond systems, while also reducing odor potential and sludge accumulation. The following is a summary of Oswald's 1990 ASCE publication on the AIWPS™ system.

AIWPS™ consist of four ponds operated in series. The first level of treatment occurs in a facultative pond, which has an aerobic surface and an anoxic pit at the bottom. The anoxic pit receives the influent wastewater, where it settles and undergoes methane fermentation. Barriers constructed around this anoxic pit prevent cold, oxygenated water

from infiltrating. The overflow rate of these initial ponds is kept at 1-2 meters per day to achieve high removal of solids and parasite ova.

The second treatment step is an algal high-rate pond (HRP) with a shallow raceway and paddle wheel. Algae grow rapidly and produce oxygen, which is used by a diverse community of bacteria and protozoa. Nutrient assimilation into cells is a main dissolved nutrient removal mechanism in the pond. Recycling a portion of the HRP effluent back to the surface of the facultative pond creates an oxygen-rich environment in which odors are oxidized, and odor potential is reduced.

The effluent from the HRP is then sent to a sedimentation pond, where suspended algae and remaining parasite ova are allowed to settle, although algal sedimentation is not always reliable. Paddle-mixed raceways tend to select for more settleable algae than still ponds, so effluents from these sedimentation basins are often lower in total suspended solids (TSS) than traditional systems. The settled sludge is rich in nitrogen, phosphorus and potash, making it a candidate for use as fertilizer.

The last step in AIWPS™ is a maturation pond, which provides for additional pathogen removal and algae settling (Oswald 1990).

Like pond systems in general, a major drawback of the AIWPS™ system is the inability to produce consistently settleable effluents. Algae settling ponds usually operate with a hydraulic residence time of 1-2 days, achieving TSS settling efficiencies of 50-80% (Shilton 2005).

2.3 RAS Inoculation in HRPs

Consistently meeting suspended solids limits at algal wastewater pond facilities generally requires expensive coagulation and perhaps even filtration. Bioflocculation is a potential means of harvesting algae and producing effluents that meet regulatory limits without chemical coagulation or filtration. Bioflocculation is the spontaneous flocculation, without chemical addition, of colloids and other small particles into larger particles (flocs) that rapidly settle (Benemann et al. 1996).

The main factor in bioflocculation is thought to be bacterial production of extracellular polysaccharides (EPS), which allow bacteria and algae to stick together and form settleable flocs (Pavoni et al. 1972). By introducing RAS to the HRPs, a population of EPS-producing microorganisms should be consistently present. Furthermore, inoculation with RAS will provide daily addition of large flocs to which algal cells might agglomerate. The result was expected to be an effluent that is more settleable than the effluent of typical ponds or HRPs. One of the primary goals of this experiment was to continue acquiring data on the effects of adding activated sludge to algal ponds in terms of settleability and nutrient removal.

A thorough review of bioflocculation, its theorized mechanisms, and past research can be found in Frost (2008). No research into RAS effects on algal ponds has been published since then, so no further literature review on the subject is provided in this thesis.

2.4 Carbon Dioxide Supplementation in HRPs

Addition of CO₂ to HRPs theoretically allows near complete assimilation of nutrients and eliminates any limit on growth rate caused by insufficient inorganic carbon

concentrations (Heubeck et al. 2007). Carbon dioxide addition also promotes lower pond pH, which suppresses ammonia volatilization.

Supplementing algal growth operations with carbon dioxide has been studied extensively in the past, but only recently has research touched on CO₂ supplementation of wastewater HRPs. As mentioned in the introduction, laboratory studies found that augmenting inorganic carbon in municipal wastewater led to up to a two-fold increase in algal production (Feffer 2007). However, these experiments used diluted wastewater feed that had low inorganic carbon to simulate partially-treated wastewater. Recent studies in New Zealand have observed a greater than 30% increase in biomass productivity in pilot-scale HRPs receiving carbon dioxide supplementation (Park et al. 2011).

Carbon dioxide supplementation allows the carbon-to-nitrogen ratios in municipal wastewater to be increased to that typically found in algal biomass. Wastewater typically has a C:N ratio between 3-7, while algal biomass typically has a C:N ratio between 6-15 (Park et al. 2011). Thus, carbon is usually a limiting nutrient in wastewater-fed algal HRPs that achieve near-total nitrogen removal. The analysis is similar for the C:P ratio in municipal wastewater and algal biomass, with carbon addition needed to allow complete phosphorus assimilation.

Carbon dioxide supplementation may also provide a pH that is more conducive to algal and bacterial growth. In HRPs, pH can reach between 9-11 due to the consumption of CO₂ and HCO₃⁻ (Heubeck et al. 2007), but many freshwater algae have their maximum growth rates at a pH around 8.0 (Kong et al. 2010). However, studies have also shown that some species grow well at higher pHs (Park et al. 2011). pH considerations might be applicable only in cases where certain pH-sensitive algal species are to be promoted.

Aerobic heterotrophic bacteria in wastewater treatment HRP have an optimum pH of 8.3 (Craggs et al. 2005), so carbon dioxide supplementation at a pH setpoint around 8.3 should have a favorable effect on bacterial growth and associated nutrient removal. Heightened pH in combination with high total ammonia (common in wastewater) can cause ammonia toxicity in bacteria and some algae, thereby decreasing productivity potential (Heubeck et al. 2007).

2.5 Ammonia Volatilization

The main nitrogen removal mechanisms in well-mixed HRP are ammonia volatilization and biological uptake and conversion (Garcia et al. 2000). The importance of ammonia volatilization as a removal mechanism is dependent on pond conditions. In some studies, volatilization has been greater than biological uptake, while in others, volatilization was not determined to be an important factor (Garcia et al. 2000, Picot et al. 1991, Zhou et al. 2006).

Pond pH controls the fraction of total ammonia nitrogen that exists as dissolved ammonia gas (Figure 3-17). Municipal wastewater is high in total ammonia nitrogen (TAN), which exists primarily as ammonium ions (NH_4^+) at a neutral pH (6.5-7.5), but algal ponds often operate at elevated pH levels. As pH rises, ammonium ions lose a hydrogen atom and become ammonia gas (NH_3), which can leave the solution at the air-water interface. The higher the pH, the more volatilization is likely to occur.

Ammonia volatilization should be promoted by higher pH levels and longer HRTs. However, one study indicated that algal uptake can occur before volatilization. Therefore, influent wastewater with low total nitrogen concentrations and short HRT can suppress ammonia volatilization (Garcia et al. 2000). In another study, ammonia

volatilization was found to be a minor nitrogen removal mechanism (Zhou et al. 2006). In Zhou et al., a flux chamber was used to capture pond off-gassing, which allowed for ammonia volatilization rates to be estimated. Two ponds were tested with pHs of 7.7 and 8.07, and ammonia volatilization data indicated that volatilization was not a major factor in nitrogen removal. Unfortunately, the study did not reveal daily trends in pH. At the low pH values of 7.7 and 8.07 used, volatilization was rather low, so a pond with these conditions would not be anticipated to lose much ammonia nitrogen to the atmosphere.

Chapter 3: Materials and Methods

This chapter describes the outdoor experiments conducted with algal HRP (which will be referred to as ponds for the remainder of this thesis) and the accompanying laboratory analyses undertaken to evaluate the experimental objectives. First, the project site is described with respect to the pond design, construction, and operation. Next, the various systems conveying primary influent and waste streams, carbon dioxide, and return activated sludge are introduced. Following project site description, operational parameters are described for each experiment. Finally, an overview of laboratory analyses is presented prior to laboratory procedures.

Experiments were run in four identical pilot-scale ponds (Figure 3-1). The reactors were hand-built in the spring of 2009 and transported to the San Luis Obispo Water Reclamation Facility (SLOWRF), located on Prado Road in San Luis Obispo. The ponds were fully operational by the end of July 2009.

Figure 3-1: Simplified pilot plant process diagram. Note: P = pump, PW = paddle wheel, M = motor, 1° WW = primary clarifier effluent wastewater.

The ponds were constructed to analyze the effects of experimental variables on algal growth, nutrient removal, and settleability. The SLOWRF provided for a continuous supply of municipal wastewater, as well as a source of return activated sludge. The research site was already occupied by four high-rate ponds that were used to conduct experiments in past years.

3.1 Construction and Layout

The gathering of materials and initial construction began in the spring of 2009 on the second floor balcony of Building 13 at the California Polytechnic State University in San Luis Obispo. The fabrication of pond frames and paddle wheels was completed prior to moving to the SLOWRF to have a more convenient workspace.

3.1.1 Pond Installation

The pond frames were constructed using several pieces of 2.4-m long 2"x8" pine lumber (38 x 184 mm), Simpson[®] metal straps, and heavy-duty hinges. The frames needed to allow a resting water depth of 30 cm, while also containing the water during mixing. This was achieved by stacking two 2"x8"s and fastening them together with metal straps, resulting in an approximate height of 37 cm. Each pond was constructed using eight 2"x8" stacks: two 2.4-m lengths, four 46-cm lengths, and two 51-cm lengths. The pond frame design is shown in Figure 3-2 and Figure 3-3.

Before attaching the stacked 2"x8" components, each section was primed and painted to ensure the integrity of the frame through adverse weather. Once painted, the sections were transported to the SLOWRF. At the project site, the components were assembled. Each section connection consisted of two hinges, one on each of the stacked 2"x8"s.

Figure 3-2: Plan view of symmetrical pond frame design. Note: The actual dimensions of the ponds are slightly larger due to the spacing created by connecting hinges.

Figure 3-3: Three-dimensional representation of pond frame design.

Prior to pond placement, the site had to be graded to create a uniform surface that promoted drainage towards the planned sump area. Using surveying equipment, the site's initial slope was measured and noted. A plant operator then used a front loader to grade the site. The planned locations of the pump house and ponds were built up manually using shovels. An overhead view of the site can be seen in Figure 3-4.

Figure 3-4: Plan view of the SLOWRF, including the operations relative to the pond pilot-scale project. Google Earth captured the image before any construction had taken place in the area.

The ponds were placed in secondary containment to prevent any spilled wastewater from escaping the site. A large roll of pond liner was used for both the secondary containment area and each pond. Several 2"x12" wooden planks (38 x 286 mm) were used to create a wall to contain the pond liner. The planks were held up by positioning rebar on both sides and driving them into the ground. Prior to placing the pond liner into the containment area, a hole was dug in the shape of a large, circular trash can to make space for the sump. A trashcan was cut in half and placed in the hole. The pond liner was then laid over the containment area.

The pond wooden frames were then placed on the leveled portion of the containment area. The pond liner was cut into four identical sections and laid into the frames. Metal C channels (shelf standard tracks) were attached to the frames to provide stabilization.

This allowed a dual channel width of 97 cm to be maintained. These tracks also allowed the baffle to be installed by fastening hinges onto the precut holes.

3.1.2 Paddle Wheels

The baffles and paddle wheels were both constructed using 0.64-cm thick white, high-density polyethylene (HDPE). The central baffles were roughly 2.4-m long and 37-cm tall and were attached to the shelf standards. Each paddle consisted of two circular pieces and five blades. The five blades were connected to both circular pieces using elbow brackets. Holes were drilled into the large circular sections to insert the drive shaft.

The paddle wheels were positioned at the end of the straight sections of the ponds. Each pond had its own drive shaft, but all were connected using universal joint shaft couplers. The drive shafts were 316 stainless steel and had a diameter of 2.5 cm. The paddle wheels were installed in series to a single waterproof motor (Leeson[®] Model M1125262, maximum 31 RPM), which was powered by a variable speed unit (Leeson[®] Washguard[®] Model 174107). The motor was bolted onto a custom-made wooden table that was placed at the proper height to ensure the motor and drive shafts were level. Operating with a single motor ensured uniform channel velocity in all ponds. Figure 3-5 details the final placement of the paddle wheels and motor assembly.

Figure 3-5: Post-construction photograph of ponds and paddle wheel motor assembly.

The ponds were spaced equally apart from one another to achieve uniform shading, and a mock paddle wheel was installed next to the last pond to simulate the shading the other pond received from the functional paddle wheels. The mock paddle wheel was constructed with a single HDPE circular piece attached to a wooden support (Figure 3-6).

Figure 3-6: Mock paddle wheel designed to simulate shading of functional paddle wheel.

3.1.3 Pond Infrastructure

Important considerations for plant design were the systems for supplying wastewater, carbon dioxide, and return activated sludge, and the drainage system. The systems for supplying carbon dioxide and return activated sludge to the ponds were considered auxiliary systems and are presented after the details on influent and effluent systems. A simplified process diagram for wastewater supply and effluent discharge is shown below in Figure 3-7.

Figure 3-7: Wastewater supply system process diagram. Wastewater was drawn from the primary clarifier with a submersible pump and transferred to a constant head tank. Peristaltic pumps drew water from the constant head tank, through a manifold system, and conveyed it to the ponds. Effluent from the ponds left via overflow pipes, which connected to a central drainage pipe that emptied into a sump hole. A sump pump was triggered by a float valve once the sump water level reached approximately 0.5 m. The effluent was transferred back into the SLOWRF head works for treatment. Note: Crossed circles = valves, P = peristaltic pump with dual channel pump heads.

The pilot plant received wastewater from the SLOWRF primary clarifier. An influent supply line was constructed to convey the primary wastewater to the pilot plant site. The line was approximately 35 meters in length and consisted of plastic 1.3-cm irrigation line. To prevent spillage in the event of a line rupture, hard PVC secondary containment piping was installed over the primary influent line. The influent line connected directly to a constant head tank located at the pilot plant site. The constant head tank is described shortly hereafter.

A submersible pump was installed at the supply side of the influent line to move wastewater from the primary clarifier to the constant head tank. A Flotec® Tempest™ utility pump (Model FP0S1300X, maximum 5500 L/hr) was used for this application. Particulate matter accumulated on the pump screen during operation and required scrubbing every 7-10 days, or in the event that the constant head tank water level was low.

Figure 3-8: Flotec® Tempest™ water removal utility pump used to supply water from the primary clarifier to the pilot plant site. Model FP0S1300X, this pump was rated to deliver a maximum flow of 5500 L/hr. Particulate matter accumulated on the pump screen, requiring scrubbing every 7-10 days, or when the water level in the constant head tank was low.

Following transfer through the influent piping system, wastewater was collected in a constant head tank prior to being supplied to the ponds. The head tank ensured that peristaltic feed pumps leading to each pond had equal head pressure. The head tank was designed for continuous recirculation of wastewater from and back to the primary clarifier to prevent stagnation in the head tank and limit biological growth. The head tank had a 46-cm x 46-cm base, was 76 cm tall, and was made of white plastic. Primary influent entered the head tank 8 cm from the tank base. An overflow standpipe was installed to allow water to gravity flow back to the primary clarifier and maintain constant recirculation of primary influent. This standpipe was constructed to draw water from 13 cm below the water surface to prevent floating scum from entering the drain line

and causing clogging. To achieve gravity flow, an air gap was installed that protruded from the head tank water level (Figure 3-10, side view 2). In anticipation of an overflow pipe clog, a second overflow pipe was constructed to drain the head tank directly to the on-site sump. The inlet to this overflow pipe was above the resting water level, but below the air gap height. Primary influent was drawn from the head tanks through a simple piping structure. The inlet was located 20 cm from the base of the tank to prevent settled solids from becoming resuspended. Detailed sketches of the head tank design are provided below in Figure 3-9 and Figure 3-10.

Figure 3-9: Plan view of the constant head tank showing all influent flows through four bulkhead fittings (F1-F4). F1 was the over flow standpipe, which was designed to allow the head tank to continuously drain. F2 was the inlet of primary wastewater supplied from the SLOWRF primary clarifier. F3 is the auxiliary overflow pipe which allowed the head tank to drain directly to the sump in the event that the overflow to the primary clarifier became clogged. F4 was the supply piping that fed the peristaltic pumps, which conveyed wastewater to the ponds. The arrows indicate the direction of flow. Side views 1 and 2, labeled on the above drawing, are shown in Figure 3-10.

Figure 3-10: Side views 1 and 2 of the constant head tank used to hold supply primary wastewater. Refer to the caption in Figure 3-9 above for explanations for each fitting (F1-F4). Water level and direction of flows are indicated.

The drainage line from the head tank back to the primary clarifier was a 1.9-cm PVC line, with a 3.2-cm PVC secondary containment. Like the influent supply line, this drainage line was approximately 35 m in length. Occasionally, debris would buildup in the drainage line and prevent proper drainage. The head tank would then overflow directly to the sump. In this event, the head tank would be drained and a pump would be used to force tap water back through the drainage line until the effluent exiting into the primary clarifier was free of debris.

From the head tank, influent wastewater entered a manifold consisting of nine valves (Figure 3-11). The influent wastewater supply was shared by this project's four ponds and an adjacent project's four ponds. Each pond had a valve which allowed individual influent supply lines to be shut off for maintenance. A ninth valve was installed as a flush or bypass valve, which was commonly used to drain the head tank or clear debris from the supply line.

Figure 3-11: Manifold system leading to peristaltic pumps supplying ponds with primary influent. The four grouped valves on the left were used to feed the ponds in this project. The bypass valve was used to flush debris from the manifold system and partially drain the head tank.

Peristaltic pumps were used to control the flow of influent wastewater from the head tank to the ponds. Two pump motors were used to simplify pump adjustments, each motor using a dual-channel pump head (Figure 3-12). This system allowed two ponds to be controlled with one motor, ensuring more uniform flow rates. The peristaltic pumps were operated between 160-215 mL/min depending on the hydraulic retention time. The pumps used were Cole Parmer[®] Masterflex[®] L/S[®] precision variable-speed drives (Model WU-07521-40). These drives had operational speeds between 6-600 rpm and available flow rates between 0.36-3400 mL/min. The pump heads used were Masterflex[®] L/S[®] two channel Easy-Load[®] II pump heads (Model 77202-60).

Figure 3-12: Cole-Parmer® Masterflex® variable-speed drive and dual-channel pump head used to supply influent wastewater to the ponds. Images from www.cole-parmer.com.

Two types of tubing were used between the manifold and ponds. Plastic drip irrigation tubing (0.64-cm inner diameter) was used from the manifold to the pumps and from the pumps to the ponds. The pump heads required special tubing designed for use in peristaltic pumping applications. Short lengths of this tubing were used (just enough to fit in the channel with space to insert the irrigation tubing). The special pump head tubing used was Masterflex® Norprene® L/S® 17 tubing (product number WU06404-15), which had an inner diameter of 0.64 cm. The drip irrigation tubing was inserted into the Norprene® tubing and secured using zip ties. The outside diameter of the drip irrigation tubing was slightly bigger than 0.64 cm, so the resulting connection was snug. The drip irrigation lines supplying influent wastewater to the ponds were attached to the cross bracing just downstream of the overflow pipe (described next) to prevent short-circuiting (Figure 3-13).

Figure 3-13: Location of influent tubing and effluent standpipes which were placed to avoid flow short-circuiting. The influent entered downstream of the effluent standpipe.

Standpipes were installed in each pond to maintain a depth of approximately 30 cm. The standpipes consisted of 1.3-cm PVC piping and connected to a 1.9-cm drainage PVC line, which collected effluent from all four ponds. The effluent drainage line terminated at a 46-cm deep sump (Figure 3-14). A Flotec® submersible sump pump (model FP0S1800A, maximum 11000 L/hr) was placed in the sump hole. A tethered on/off float switch attachment turned the pump on once the water level exceeded 30 cm, and shut off once the water level dropped below 6 cm. A 1.3-cm plastic irrigation line delivered the effluent from the sump back into the SLOWRF treatment system. A 2.5-cm plastic irrigation line was used as a secondary containment for the effluent line.

Figure 3-14: Pilot plant effluent sump setup. A Flotec® sump pump rested in the sump and transferred effluent from the ponds and occasional overflow from the head tank back into the SLOWRF treatment system. A wooden frame was installed to prevent the piping connected to the sump pump from tilting and interfering with float switch operation. Sump pump photo: www.flotecpump.com.

The CO₂ experiment used industrial-grade pure carbon dioxide supplied by the local Airgas Inc. distributor. The 23-kg cylinders were stored in a shed and fitted with a pressure regulator. A flexible supply line was run from the tank to the containment area, where it was teed-off with a three-way fitting to supply the two experimental ponds with CO₂. Smaller supply lines ran from the three-way fitting to solenoid valves, and then to diffusers placed in the ponds. The supply lines for each run were kept the same length to provide for equal gas flow during simultaneous operation.

The supply system was designed to deliver carbon dioxide to the ponds at a pH setpoint of 8.4 (Figure 3-15). A pH controller was used to actively monitor pond pH and open a

solenoid valve once the setpoint was reached. The pH controllers were calibrated with a two-point calibration. pH standards of 7 and 10 were used for the calibration since the expected pH ranges in the ponds were 7.5 to 9.5. Each controller and valve unit was kept in a sealed Tupperware[®] container to lessen exposure to moisture. A 30-cm x 1.3-cm diffuser strip, typically used to aerate home aquaria, was placed in each of two experimental ponds. To keep the diffuser units from moving with pond water flow, a small portion of PVC pipe was filled with sand, capped and glued, and attached to the strips with zip ties.

Figure 3-15: Carbon dioxide supply system diagram. Industrial-grade CO₂ flowed from the 23-kg supply tank through flexible supply line. Each experimental pond had an individual pH controller, which opened the solenoid valve once a pH of 8.4 was registered. Note: Crossed circles = solenoid valves, C = pH controller.

An automated supply system was not practical for return activated sludge additions. Daily supplementation was achieved by manually collecting 7.56 L of RAS into a bucket. To achieve uniform additions to the ponds, the RAS was mixed vigorously and divided evenly between two buckets prior to being poured into the ponds. The RAS was added directly upstream of the paddle wheels to achieve mixing.

Figure 3-16: Completed, operational pilot-scale HRP plant. The tube settlers on the left and the deep HRPs in the background were not used in this study.

3.2 Operational Variables

Channel velocity, hydraulic residence time, and pH setpoint for CO₂ addition were chosen prior to experimentation and are described below.

3.2.1 Channel Velocity

A channel velocity of 25 cm/s was used to keep the algal flocs in suspension and make the exposure of algal cells to sunlight more uniform. This velocity was achieved by adjusting the variable speed motor controller measuring the channel velocity using the “orange test.” An orange fruit was placed into the center of a pond, and a stopwatch was used to record its travel time over a one meter distance. A channel velocity of 25 cm/s corresponded with a paddle wheel speed of approximately 4 rpm.

3.2.2 Hydraulic Residence Time

A hydraulic residence time (HRT) of 4 days was chosen, corresponding to a wastewater influent flow of 160 mL/min. During the last two months of experimentation, the hydraulic residence time was shifted to 3 days, which corresponded to an influent flow rate of 215 mL/min.

3.2.3 pH Setpoint for CO₂ Addition

A pH setpoint of 8.4 is optimal for ponds to stimulate aerobic bacterial degradation and avoid ammonia gas toxicity (Heubeck et al. 2007), while a more practical reason to use this setpoint is to improve CO₂ dissolution efficiency because lower pH levels lead to excessive offgassing and inefficient CO₂ use (Weissman et al. 1988). During the CO₂ experiments, a pH setpoint of 8.4 was programmed into the solenoid valve controllers. Once the pH of the ponds reached a level several points above 8.4, the solenoid valve opened and pure CO₂ gas bubbled into the ponds. pH generally fluctuated between 8.4 and 8.5 during peak sunlight hours.

3.3 Pilot Plant Operation & Maintenance

Experimentation was directly preceded by the startup process and the development of a daily monitoring routine. The daily monitoring routine was made to ensure that plant operations were running properly and to alert operators to any mechanical or electrical issues that needed routine maintenance.

3.3.1 Pilot Plant Start-Up

The pilot plant became operational on July 15, 2009, when the ponds were filled with 40 L of algal inoculum and fed primary wastewater. The inoculum was obtained from

neighboring experimental HRP's being run by student Michael Podevin. The ponds were initially partially filled, but over several days were up to capacity and overflowing into the effluent standpipes. During this period, the paddle wheels were operating at 4 rpm. Once the ponds were full and green, the pH controllers were calibrated and carbon dioxide was injected into Ponds 1 and 3 when pH levels reached 8.4.

3.3.2 Monitoring and Maintenance

Daily monitoring ensured that the ponds were running according to the experimental plan. Generally, a student visited the plant once a day to conduct routine monitoring and undertake maintenance work as needed. All important observations and monitoring data were recorded in a field log.

The typical monitoring routine consisted of several tasks: (1) checking and adjusting wastewater influent flows, (2) calibrating pH controllers, (3) monitoring pond pH, temperature and dissolved oxygen (DO) levels, (4) monitoring and removing filamentous growth on the pond sidewalls and standpipe, (5) recording general pond conditions and noting any observed presence of zooplankton, (6) checking water level in the head tank, and (7) checking all pumps and electrical equipment.

Wastewater influent flows were checked with a 250-mL graduated cylinder and stopwatch. The influent wastewater was collected in the graduated cylinder over a period of 30 seconds, and the volumetric flow rate was recorded in mL/min. If the flows had strayed substantially from the desired 160 mL/min, the peristaltic pump drives were adjusted. The pump drives were adjusted in the event that flow had strayed by 4 mL/min or more. In the event that flows became routinely erratic, the supply lines were removed and replaced because of fouling (the buildup of slime in the tubing). The ponds were

operated with two peristaltic pumps with dual-channel pump heads, so two ponds received wastewater with the same pump (Ponds 1 & 2 in one grouping, Ponds 3 & 4 in the other; Figure 3-15). In this configuration, fouling in one line could be identified by observing differences in flow between ponds being supplied by the same pump. The specialty MasterFlex[®] tubing used in the peristaltic pump heads also needed to be monitored for tears or a loss of resiliency. It was common practice to replace both the drip irrigation tubing and MasterFlex[®] tubing simultaneously to assume a “fresh start.”

The pH controllers were calibrated using a two-point calibration. The two calibration points should bracket the expected operational range. The typical pH range in algal ponds is 8-11, so the controllers were calibrated using pH solutions of 7.0 and 10.0. The probes were cleaned by rinsing with tap water and scrubbing lightly with a soft, clean rag. This procedure was repeated every few days, or when there was visible algal buildup on the probe.

The temperature and pH of each pond was measured using a hand probe (separate from the pH controller associated probe), which was calibrated daily using a three-point calibration consisting of pH solutions of 4.0, 7.0, and 10.0. Temperature and pH were recorded every day that a full plant monitoring routine took place. Dissolved oxygen readings were not available in the field until the end of Experiment III. Once the DO meter was available in the field, readings were taken 2-3 times a week.

Especially in warmer months, filamentous growth (algal and bacterial) on the pond sidewalls and standpipe became an issue. The experiment was concerned with the nutrient removal performance of suspended algae, so filamentous organisms needed to be controlled. The filamentous growth also presented a clogging hazard to the standpipes.

Filamentous growth was removed using a stiff-bristle hand brush whenever it was observed. Future experiments may benefit from removal and quantitative analysis of filamentous mass growth. This may indicate the level of hindrance filamentous growth had on suspended algal productivity. This experiment did not investigate such considerations.

During routine site monitoring, the color of the ponds and visible presence of zooplankton was noted. The presence and color of flocs was also noted. Using beakers, the presence of large zooplankton, such as *Daphnia*, ostracods, or red worms, could also be determined. A sample from each pond would be collected and all samples would be set side-by-side to compare color and zooplankton presence.

A final critical step in daily monitoring was checking on pump operation, the water level in the head tank, and all other electrical devices. When the head tank water level was low, the submersible pump in the primary clarifier had stopped functioning properly or the electrical outlet had experienced a power failure. The issue was quickly diagnosed by grabbing the wastewater supply line near the connection to the submersible pump. If a vibration could be felt, the pump impeller was still spinning, but the pump screen was likely clogged with debris. To clean the screen, the pump was lifted from the clarifier and scrubbed with a long-handled brush. If the supply line was not vibrating, the circuit breaker supplying electricity to the pump had likely popped and needed resetting. Loss of power was common when it rained, including several occasions where a breaker would trip and require almost continuous resetting.

3.4 Experimental Procedures

Over the course of a year, the ponds were run in two primary operational modes. During one operational mode, the ponds received CO₂ to observe its effect on productivity, nutrient removal, and bioflocculation. The other operational mode involved the addition of return activated sludge (RAS) to observe its effect on the same performance factors. A third setup included diluting the primary influent with tap water to simulate recycling of algae-free effluent to the ponds, although this mode did not result in sustainable growth. Such recycling would produce a wastewater media with lower nitrogen and inorganic carbon concentrations and presumably higher pH in ponds not provided with CO₂. The following section outlines the dates and experimental procedures for each experiment. Any data analyses mentioned are further explained in Section 3.5.

3.4.1 Experiment I

The first experiment conducted on the newly constructed ponds began on July 21, 2009. The ponds had been filled with primary influent and inoculated one week earlier and were green by this date. Ponds 1 and 3 were automatically supplemented with CO₂ when the pH rose above 8.4. During the first several weeks of operation (until August 13th, 2009), the ponds were tested only for total suspended and total volatile solids (TSS and VSS, respectively) to observe the relative growth rates of the CO₂-supplemented ponds and control ponds. The hydraulic residence time during this experiment was 4 days (feed rate of 160 mL/min) and the channel velocity was approximately 25 cm/s per the “orange test.” As with all experiments, the ponds were fed wastewater from the SLOWRF primary clarifiers.

On August 13, more extensive analysis began on the collected samples. Nutrient removal efficiency and settleability were evaluated in the lab using samples from the ponds and influent lines. The first experiment concluded on September 28, 2009.

3.4.2 Experiment II

The second experiment, which began October 3, 2009, aimed to reduce the carbon load in the ponds, thereby increasing the effect of CO₂ supplementation. The HRT remained at 4 days, and the channel velocity was maintained at 25 cm/s. The wastewater feed rate was decreased to 80 mL/min and tap water hoses were run to the ponds. The tap water feed was set to 80 mL/min to achieve the correct HRT. The dilution with tap water cut the influent carbon load in half.

After two weeks, including a rainstorm, the ponds turned light tan and had no algal presence. The dilution experiment was abandoned on October 20.

3.4.3 Experiment III

The third experiment began on October 22, 2009 and involved adding RAS to the experimental ponds to study its effect on effluent settling and nutrient removal. Prior to the start of the experiment, the ponds were intermixed and allowed to become green before beginning the RAS additions. On November 12, Ponds 1 and 2 began receiving 3.78 L of RAS per day. The weekly testing procedure of past experiments was continued. The experiment was suspended on December 20 due to the Christmas holiday break.

During the Christmas holiday break, pond mixing continued, but the influent supply and RAS additions were halted. On January 9, 2010, the influent supply was re-started and

RAS was again added to Ponds 1 and 2. Data were steadily collected until Pond 1 experienced a culture crash and became clear. The ponds were shut down on March 15 to remix and restart them in order to reestablish equivalent cultures in all ponds.

After remixing the ponds, the ponds were operated with RAS additions to Ponds 1 and 2 until May 17. The paddle wheel motor was accidentally turned up, resulting in a period of higher intensity mixing. Sludge was resuspended from the pond bottoms and resulted in three weeks of high solids data. Two weeks of data were collected after the spike in solids. The ponds were then intermixed to prepare for another carbon dioxide supplementation experiment.

3.4.4 Experiment IV

On May 18, 2010, carbon dioxide supplementation began in Ponds 1 and 3. RAS was no longer added to any of the ponds. The operational setup was identical to the initial experiment the previous summer. On July 7, the ponds were switched from a 4-d HRT to a 3-d HRT to take advantage of the summer weather, which allows higher dilution rates. Data collection for this analysis ceased on September 1, 2010.

3.4.5 Experiment V

An ammonia volatilization study was conducted using three of the ponds in mid-September. The ponds were drained, scrubbed, and rinsed out using tap water prior to experimentation. The goal of this experiment was to determine a volatilization mass transfer coefficient for ammonia at several pH levels and ammonia concentrations. The experiment spanned four days and used a different pH value each day: 7.4, 8.0, 8.4, and 9.4. Using ammonium chloride and tap water, the ponds were filled each day and set to

ammonia concentrations of approximately 3, 10, and 17 mg/L TAN (refer to Table 3-1). For example, on day 1, all three ponds used were set to a pH of 7.4. Pond 1 started at 3 mg/L TAN, Pond 2 at 10 mg/L TAN, and Pond 3 at 17 mg/L TAN.

Table 3-1: Ammonia volatilization experimental plan. The TAN concentrations shown were the planned initial values for each pond.

Day	pH	Pond 1, mg/L TAN	Pond 2, mg/L TAN	Pond 3, mg/L TAN
1	7.4	3	10	17
2	8	3	10	17
3	8.4	3	10	17
4	9.4	3	10	17

The appropriate amounts of ammonium chloride to attain 3, 10 and 17 mg/L TAN were calculated using the molecular weight of ammonium chloride and the approximate volume of the ponds. The ammonium chloride was dried in the oven at 200°C for 24-hours prior to use to ensure accurate knowledge of the N mass. The ammonium chloride was completely dissolved in tap water before being added to the ponds. After each day, small amounts of ammonium chloride were added to the ponds to raise levels back to 3, 10 and 17 mg/L TAN.

Pond pH control was achieved by adding small increments of sodium hydroxide. Concentrated sodium hydroxide and sulfuric acid were used to fine-tune the pH. The pH of each pond was monitored every 30 minutes using a hand-probe which was calibrated prior to each set of measurements. If pH levels had strayed by 0.05 or more from the target, concentrated acid or base was added in 1-mL increments until the appropriate pH was reached.

Samples were collected from each pond every two hours from 10 AM to 6 PM for every day of the experiment. The samples were acidified and stored on ice on-site until transport to campus could be arranged. The following day, a sample was collected to complete the analysis for that pH. After collecting the sample, the ponds were prepared for the next pH level by adding sodium hydroxide and additional ammonium chloride.

Ammonia volatilization was expected to be greater as the pH of the ponds increased. As pH increases, the ratio of ammonium concentration to dissolved ammonia gas concentration decreases (Figure 3-17). The greater the concentration of ammonia gas in the water, the greater the rate that ammonia gas escapes to the atmosphere.

pH	Temperature													
	42.0 (°F)	46.4	50.0	53.6	57.2	60.8	64.4	68.0	71.6	75.2	78.8	82.4	86.0	89.6
	6 (°C)	8	10	12	14	16	18	20	22	24	26	28	30	32
7.0	.0013	.0016	.0018	.0022	.0025	.0029	.0034	.0039	.0046	.0052	.0060	.0069	.0080	.0093
7.2	.0021	.0025	.0029	.0034	.0040	.0046	.0054	.0062	.0072	.0083	.0096	.0110	.0126	.0150
7.4	.0034	.0040	.0046	.0054	.0063	.0073	.0085	.0098	.0114	.0131	.0150	.0173	.0198	.0236
7.6	.0053	.0063	.0073	.0086	.0100	.0116	.0134	.0155	.0179	.0206	.0236	.0271	.0310	.0369
7.8	.0084	.0099	.0116	.0135	.0157	.0182	.0211	.0244	.0281	.0322	.0370	.0423	.0482	.0572
8.0	.0133	.0156	.0182	.0212	.0247	.0286	.0330	.0381	.0438	.0502	.0574	.0654	.0743	.0877
8.2	.0210	.0245	.0286	.0332	.0385	.0445	.0514	.0590	.0676	.0772	.0880	.0998	.1129	.1322
8.4	.0328	.0383	.0445	.0517	.0597	.0688	.0790	.0904	.1031	.1171	.1326	.1495	.1678	.1948
8.6	.0510	.0593	.0688	.0795	.0914	.1048	.1197	.1361	.1541	.1737	.1950	.2178	.2422	.2768
8.8	.0785	.0909	.1048	.1204	.1376	.1566	.1773	.1998	.2241	.2500	.2774	.3062	.3362	.3776
9.0	.1190	.1368	.1565	.1782	.2018	.2273	.2546	.2836	.3140	.3456	.3783	.4116	.4453	.4902
9.2	.1763	.2008	.2273	.2558	.2861	.3180	.3512	.3855	.4204	.4557	.4909	.5258	.5599	.6038
9.4	.2533	.2847	.3180	.3526	.3884	.4249	.4618	.4985	.5348	.5702	.6045	.6373	.6685	.7072
9.6	.3496	.3868	.4249	.4633	.5016	.5394	.5762	.6117	.6456	.6777	.7078	.7358	.7617	.7929
9.8	.4600	.5000	.5394	.5778	.6147	.6499	.6831	.7140	.7428	.7692	.7933	.8153	.8351	.8585
10.0	.5745	.6131	.6498	.6844	.7166	.7463	.7735	.7983	.8207	.8408	.8588	.8749	.8892	.9058
10.2	.6815	.7152	.7463	.7746	.8003	.8234	.8441	.8625	.8788	.8933	.9060	.9173	.9271	.9389

Figure 3-17: Fraction of total ammonia existing as ammonia gas at varying temperatures and pH values. The pond temperature ranged from 14°C to 21°C during the experiment. Table from Emerson et al. (1975).

3.5 Water Quality Sampling and Analysis

Water quality analysis was conducted weekly, except during severe storms, power outages, or pond shutdowns. In addition to weekly analysis, routine field measurements were taken daily to monitor for significant changes in pond conditions. Each experiment incorporated similar data. This section first states the methods used for water quality analyses for both weekly sampling and daily monitoring. Diel pond monitoring, weather data acquisition and statistical analysis methods are then described. The sampling schedules for each experiment are outlined at the end of this section (Table 3-2 through Table 3-4).

During each experiment, a day of the week was chosen as the designated main sample day, while another day was chosen as the semiweekly sample day. The main sample day included all the analytical tests and required large sample volumes (approximately 3000 mL). The semiweekly sample day included TSS/VSS and ammonia tests, and required no more than 150 mL sample volumes.

Four-liter LDPE milk jugs and two-liter Nalgene[®] bottles were used to collect samples on the main sample day. If phosphorus testing was planned, the two-liter bottles were acid-washed to remove any residual phosphorus from the bottle sidewalls. On semiweekly sample days, 250 mL sample containers were used. Pond samples were obtained at the center of the ponds, directly upstream of the influent supply line to avoid short-circuiting of raw influent into the sample bottles. A primary influent sample was obtained by placing a sample bottle below the influent supply lines of each pond. Prior to filling any of the sample bottles, they were each rinsed with the water to be sampled. After sampling, the bottles were immediately rinsed and set upside down to dry until the next

sampling period. If growth was present on a sample bottle, it was brushed with a light glass-soap solution until clean.

3.5.1 Total and Volatile Suspended Solids

Total and volatile suspended solids (TSS/VSS) testing was performed according to APHA Method 2540 D. The experiments utilized Fisher Scientific G4 glass fiber filters, with a nominal pore diameter of 1.2 microns. Prior to use, the filters were rinsed with approximately 60 mL of deionized water and ashed in an oven at 550°C to remove any loose glass fibers or dust from packaging. When necessary, filtrate from the TSS/VSS procedure was used for subsequent testing. TSS/VSS testing was conducted on the pond effluent and primary influent samples on both the main and semi-weekly sampling days. Settled pond supernatant was only tested for TSS.

3.5.2 Total Ammonia Nitrogen

The total ammonia content of each sample was determined using the Ammonia-Selective Electrode Method, APHA Method 4500D. Due to the temperature sensitivity of the electrode, samples were allowed to warm to room temperature prior to conducting the test. The Orion 95-12 Ammonia Gas Sensing Electrode used for analysis had consistent readings at or above 0.5 mg/L TAN. The pH of the samples was raised using Orion Ammonia pH-adjusting ISA (#1310-73-2). A calibration curve was prepared prior to each run with a series of dilute standards made from 2500 mg/L stock solution. The calibration curve consisted of four ammonium concentrations: 1.0, 10, 25, and 50 mg/L. The ammonia-selective electrode probe and pH probe read concurrently and were rinsed with deionized water between each standard and sample. If the calibration curve did not

have an R^2 value of 0.99 or greater, one or more standard was rerun prior to testing any of the experimental samples. During testing, three samples were chosen randomly for spike matrix analysis, and duplicates were run for every pond sample. If the samples were not within 1°C of each other, sampling was redone after temperatures equalized. Matrix spikes resulted in recoveries between 95-105%, well within the range established in QA/QC standards. Total ammonia was measured for both pond effluent and primary influent samples.

3.5.3 Nitrate & Nitrite

Nitrate and nitrite levels were determined using a Dionex[®] Ion Chromatograph (IC), as referenced in Feffer (2007). The calibration curves created for these experiments utilized stand-alone nitrate and nitrite samples, rather than 7-anion standards, after Experiment III. Nitrate standards of 0.1, 1, 5, 10, and 25 mg/L $\text{NO}_3\text{-N}$, and nitrite standards of 0.1, 1, 2, 5, and 10 mg/L $\text{NO}_2\text{-N}$ were used to create the more recent calibration curves. Much of the IC data from the first and second experiment could not be used because of poor quality control procedure pertaining to the calibration standards. Beginning with Experiment III, more rigorous QA/QC methods were put into place.

3.5.4 Total Kjeldahl Nitrogen

Total Kjeldahl nitrogen was used in conjunction with total ammonia nitrogen, nitrate, and nitrite data to determine total nitrogen (TN), and organic nitrogen (ON). APHA Method 4500-N_{org} Macro-Kjeldahl Method was used.

3.5.5 Dissolved Reactive Phosphorus

Dissolved reactive phosphorus (DRP) concentrations were determined used APHA Method 4500-P C, the vanadomolybdophosphoric acid colorimetric method. Acid-washed containers were used for collection and transport of samples for DRP testing.

3.5.6 Alkalinity

APHA Method 2320B was used to measure alkalinity concentrations. This test was conducted immediately after samples were brought into the lab.

3.5.7 Biochemical Oxygen Demand

Total and soluble carbonaceous 5-day biochemical oxygen demand (TBOD₅ and scBOD₅) was determined using APHA Method 5210 B. TBOD₅ data were collected on 24-hour settled supernatant from Imhoff cones and primary influent. scBOD₅ data were collected on the filtrate pond water and primary influent that had passed through 1.2-micron Fisher G4 glass fiber filters. Nutrient buffer solution was prepared using Hach BOD Nutrient Buffer Pillows and deionized water. When testing for scBOD₅, Hach nitrification inhibitor (Formula 2533TM) was used. Standards and blanks were analyzed during each weekly test period.

3.5.8 Settling Tests

Settling tests were conducted weekly using 1-liter Imhoff cones, which were filled with pond sample water. After two hours, supernatant samples were collected from each cone for TSS analysis. After 24 hours, supernatant samples were again collected for TSS and TBOD analysis. Imhoff cones are routinely used as a standard device for determining settleable solids in wastewater laboratories, as referenced in APHA Method 2540 A.

Prior to filling the Imhoff cones, sample bottles were gently shaken to keep all solids suspended. After 2 and 24 hours, supernatant was collected from each cone using a pipette. To avoid collecting any floating debris, the pipette tip was submerged approximately one centimeter below the water surface.

3.5.9 Quality Control & Quality Assurance

Data obtained during laboratory analyses were deemed acceptable if the analytical batch generating the data passed established quality control and quality assurance (QA/QC) standards. Matrix spikes were used to confirm that analytical devices were properly registering water quality constituent concentrations, and ensure that there were no potential analytical interferences. To pass QA/QC, the matrix spike must have resulted in a concentration within 85-115% of the expected value. Duplicate samples were run on selected samples and standards to ensure that results were reproducible. To qualify as an acceptable result, the duplicates had to be within 90-110% of the concentration of the original sample.

3.5.10 Zooplankton Counts

Zooplankton counts were conducted throughout Experiment III and occasionally during Experiment IV. Prior to analysis, a 10-mL sample was filtered through a 100- μ m nylon screen to isolate zooplankton from algae and other microorganisms. Captured zooplankton was rinsed into a 4-chambered petri dish with 10 mL of deionized water. One drop of Rose Bengal dye was added to the sample to make zooplankton more visible. Using a dissecting microscope, ostracods, *Daphnia*, and rotifers were counted. Counts

were repeated five times and the average was reported for each organism. A detailed procedure for zooplankton counts is located in Appendix C.

3.5.11 Daily Temperature and pH

Daily temperature and pH measurements were obtained using a portable Oakton Acorn® Ion 6 meter. The meter was calibrated daily using 4.0, 7.0, and 10.0 pH solutions. The measurements were made to monitor daily changes in pH and temperature. Relative pH changes between experimental and control ponds were observed. Daily pH readings are available for review in Appendix E.

3.5.12 Dissolved Oxygen

Dissolved oxygen (DO) field readings were obtained using a portable YSI 58 DO meter. The meter was calibrated prior to each use according to the manufacturer's instructions. When measuring pond DO, the probe was gently swirled in the pond to prevent localized DO depletion. Once a DO measurement was obtained, the probe was rinsed with deionized water and replaced in a clean BOD bottle filled halfway with water. DO measurements were taken daily beginning on April 8, 2010. Prior to this date, the DO meter located in the field did not function properly. Daily DO readings are available for review in Appendix E.

3.5.13 Diel Pond Monitoring

Diel variations in temperature, pH, DO, TSS/VSS, and total ammonia nitrogen were analyzed during two 24-hour sampling days. The first analysis occurred on June 17, 2010, and the second occurred on July 21, 2010.

During the first analysis, temperature, pH, and DO were monitored every hour from 5 PM to 12 PM and 4 PM to 10 PM. These measurements were also taken at 2 PM, 4 PM and at 2-hour intervals from 12 AM to 6 AM the following day. Samples were collected every 4 hours for TSS/VSS and TAN analysis starting at 6 AM on the June 17, and ending at 2 AM on June 18. The ponds were operated at a 4-day hydraulic residence time during the first diel study.

The second diel analysis measured temperature, pH, and DO every hour from 6 AM to 11 AM and 1 PM to 11 PM. These measurements were also taken at 2, 5, and 6 AM the following morning. TSS/VSS and TAN samples were drawn every five hours, beginning at 8 AM and ending at 11 PM. The ponds were operated at a 3-day residence time during the second diel study.

3.5.14 Weather Data

Daily weather data were obtained from the California Irrigation Management Information System (CIMIS) through the Department of Water Resources Office of Water Use Efficiency online database. The data were recorded at San Luis Obispo Station No. 52, which is located on the Cal Poly Campus, approximately 3 miles from the pilot plant. The data include evapotranspiration, precipitation, insolation, maximum and minimum air temperature, relative humidity, and wind speed.

3.6 Statistical Analysis

All water quality and weather data were organized in a Microsoft Excel spreadsheet. Graphs and statistical analyses were also completed using Microsoft Excel.

3.7 Experimental Sampling Schedules

The following sampling schedules detail the laboratory analyses conducted throughout each experiment.

Table 3-2: Experiment I Sampling Schedule

Parameter	Sample Location	Measurement Frequency
TSS/VSS	INF, Ponds	Semiweekly*
2-hour Settled TSS	Ponds	Weekly
24-hour Settled TSS	Ponds	Weekly
Ammonia Nitrogen	INF, Ponds	Semiweekly*
Nitrate & Nitrite	INF, Ponds	Weekly**
DRP	INF, Ponds	Weekly***
Alkalinity	INF, Ponds	Weekly
Settled TBOD ₅	Ponds	Weekly
scBOD ₅	INF, Ponds	Weekly
Influent Flow	Ponds	Daily****
Pond pH	Ponds	Daily****
Pond Temperature	Ponds	Daily****

* Semiweekly TSS/VSS and TAN testing was initiated September 14, 2010

** Nitrate & Nitrite data could not be used for the first experiment due to QA/QC failure

*** DRP analysis was conducted weekly over a 4-week period

**** Measurements performed on a near-daily basis

Table 3-3: Experiment III Sampling Schedule

Parameter	Sample Location	Measurement Frequency
TSS/VSS	INF, Ponds	Semiweekly
2-hour Settled TSS	Ponds	Weekly
24-hour Settled TSS	Ponds	Weekly
Ammonia Nitrogen	INF, Ponds	Semiweekly
Nitrate & Nitrite	INF, Ponds	Weekly*
DOC	INF, Ponds	Weekly
Alkalinity	INF, Ponds	Weekly
Settled TBOD ₅	Ponds	Occasional
Zooplankton Counts	Ponds	Weekly
Influent Flow	Ponds	Daily
Pond pH	Ponds	Daily
Pond Temperature	Ponds	Daily

* Reliable nitrate & nitrite data began in January 2010

Table 3-4: Experiment IV Sampling Schedule

Parameter	Sample Location	Measurement Frequency
TSS/VSS	INF, Ponds	Semiweekly
2-hour Settled TSS	Ponds	Weekly*
24-hour Settled TSS	Ponds	Weekly
Ammonia Nitrogen	INF, Ponds	Semiweekly
Nitrate & Nitrite	INF, Ponds	Weekly
TKN	INF, Ponds	Weekly
DRP	INF, Ponds	Weekly
Alkalinity	INF, Ponds	Weekly
Settled TBOD	Ponds	Weekly*
Influent Flow	Ponds	Daily
Pond pH	Ponds	Daily
Pond Temperature	Ponds	Daily

* 2-hour settling and supernatant BOD tests were abandoned halfway through the experiment because of unchanging results

Chapter 4: Results and Discussion

This chapter includes the data analysis and observations from Experiments I-V. The experimental phase of this thesis project ran from July 21, 2009 to September 18, 2010. Hydraulic residence time, pond depth, and channel velocity were the variables controlled throughout the experiments. RAS inoculation and CO₂ addition were the experimental variables. Influent water quality, RAS characteristics, and weather were all uncontrolled variables. This chapter first presents the weather conditions during experimentation and then chronologically presents the results of each experiment.

Table 4-1: Experiments conducted during this thesis study. Experiment II did not result in the collection of substantial data.

Exp	Experimental Description
I	CO ₂ supplementation
II	CO ₂ supplementation with influent dilution
III	Return activated sludge inoculation
IV	CO ₂ supplementation
V	Ammonia volatilization experiment

4.1 Weather Conditions

The main weather conditions that affect algal growth rate are temperature and insolation. In addition, algae productivity can be affected by wash-out due to heavy rainfall. Up to a point, the warmer the temperature, the faster algae grow and the quicker nutrients are assimilated into biomass. Similarly, growth rate typically correlates with average daily insolation. Figures 4-1 through 4-3 show weather condition data over the entire project timeline.

Figure 4-3: Maximum air temperature throughout the entire project

Clear day insolation ranged from about 120 to 350 W/m², punctuated with frequent cloudy days throughout the year. The temperature data are fairly similar during winter and summer months due to the marine influence in San Luis Obispo. The average insolation and temperature data for each of the successful experiments are listed below in Table 4-2.

Table 4-2: Average insolation and air temperature data for each experiment.

Parameter	Experiment I	Experiment III	Experiment IV
Avg Insolation, W/m ²	249	157	284
Avg Temperature, °C	17.1	12.5	15.2
Avg Max Temperature, °C	25.7	18.7	22.2
Avg Min Temperature, °C	11.6	7.0	10.7

4.2 Experiment I – CO₂ Supplementation

This section describes water quality results for the first experiment. All ponds were operated at a 4-day hydraulic residence time, and Ponds 1 and 3 received supplemental CO₂ with a pH setpoint of 8.4. All ponds were fed from the same source of primary clarifier effluent. An overview of water quality results for Experiment I is located at the end of Section 4.2.1 (Table 4-3). Pond labels are shortened in graph legends to save space: P1 = Pond 1, P2 = Pond 2, and so forth.

4.2.1 Treatment Performance – Experiment I

The main parameters of concern for the CO₂ supplementation experiments were VSS and nitrogen data (particularly TAN). VSS data can be used to calculate the estimated algal and bacterial productivity. TAN removal is the first objective in nutrient removal. Settling data were also gathered through 2-hour and 24-hour Imhoff settling tests, even though bioflocculation was not expected to be affected by CO₂ addition. Other relevant water quality data included scBOD₅, TBOD₅ of settled pond effluent, alkalinity, and DRP. DRP removal was not expected to be extensive because only a single cycle of algae growth and harvesting was used.

Effluent VSS concentrations did not substantially differ between the control and CO₂-supplemented ponds during Experiment I (Figure 4-4). In early August the CO₂ supplemented ponds had heightened VSS concentrations. However, during the rest of Experiment I, VSS in all ponds experienced simultaneous decreases, and the final VSS data showed no correlation between CO₂ supplementation and biomass productivity (Figure 4-4).

Figure 4-4: VSS data for the influent and pond effluents during Experiment I.

Although VSS data did not indicate that CO₂ supplementation consistently increased biomass productivity, closer inspection of the insolation data during Experiment I may reveal an insolation growth limitation (Figure 4-5). During the short period where insolation was consistently over 280 W/m² (August 1-August 8), VSS levels in the ponds with CO₂ briefly rose to about 300 mg/L, while control ponds hovered around 230-260 mg/L. The high insolation might have allowed sufficient productivity for carbon to become limiting. However, this brief period is only long enough to be suggestive and there are other periods of high insolation without this effect. During Experiment IV (also CO₂ supplementation), a period of heightened insolation, consistently over 300 W/m², did not support increased productivity in the CO₂-supplemented ponds.

Figure 4-5: VSS data for pond effluents plotted with insolation data during Experiment I. Insolation is reported in units of W/m^2 .

Biomass productivity ($g/m^2\text{-d}$) is calculated as VSS concentration (mg/L or g/m^3) $\times$ Depth (m) / HRT (days). It is a useful means of comparing algal high-rate pond systems in terms of seasonal growth. The average productivity for the CO_2 -supplemented and control ponds was $17.7 g/m^2\text{-day}$ and $17.0 g/m^2\text{-day}$, respectively. During Experiment I, productivity ranged from 11.4 to $22.6 g/m^2\text{-day}$ in the ponds receiving and 11.8 to $21.2 g/m^2\text{-day}$ in the control ponds. Productivity was not significantly higher in the CO_2 -supplemented ponds.

Figure 4-6: Box and whisker plots of biomass productivity during Experiment I. The central line is the median, the extremes of the boxes are the 25th and 75th percentiles, and the whiskers are the minimums and maximums of the time-series data.

During Experiment I, all ponds experienced decreased TAN concentrations from an influent concentration of approximately 40 mg/L to between 3-4 mg/L in pond effluents. The overall removal efficiencies for TAN were approximately 90-92% during Experiment I (Figure 4-7 and Table 4-3). Carbon dioxide supplementation was expected to increase productivity in the experimental ponds, which would also increase nitrogen uptake and conversion into biomass. This effect was not seen, however, as the ammonia removal was almost identical in all ponds. A full evaluation of nitrogen removal mechanisms could not be conducted during this experiment due to lack of NO_2^- & NO_3^- or TN data.

Figure 4-7: Total ammonia nitrogen levels for influent and pond effluents during Experiment I.

Figure 4-8: Total ammonia nitrogen in pond effluents during Experiment I, not showing primary influent.

During Experiment I, no differences in settleability were expected between the control and CO₂-supplemented ponds. However, the average settled TSS levels for the CO₂-supplemented ponds were lower than those of the control ponds, but the settled TSS levels of the CO₂-supplemented ponds were not consistent with one another (Figure 4-9 and Figure 4-11). The CO₂-supplemented Ponds 1 and 3 averaged 151 and 98 mg/L for 2-hr TSS, and 69 and 47 mg/L for 24-hour TSS, respectively. The control Ponds 2 and 4 averaged 165 and 192 mg/L for 2-hr TSS, and 95 and 92 mg/L for 24-hr TSS, respectively (Table 4-3).

The weekly settling data indicate that settling was generally more efficient for the ponds receiving CO₂ (Figure 4-10 and Figure 4-12). The CO₂-supplemented ponds had average two-hour settling efficiencies of 42% and 61%, while the control ponds had average two-hour settling efficiencies of 28% and 24% (Table 4-3). After 24 hours of settling, the CO₂-supplemented ponds had average settling efficiencies of 74% and 81%, while the control ponds had average settling efficiencies of 58% and 63% (Table 4-3). However, having only five sampling points to consider does not allow confidence in any relationship between CO₂ supplementation and settling efficiency.

Figure 4-9: TSS concentration after 2 hours settling of pond effluents during Experiment I.

Figure 4-10: Two-hour settling efficiency during Experiment I.

Figure 4-11: TSS concentration after 24 hours of settling of pond effluents during Experiment I.

Figure 4-12: Twenty-four hour settling efficiency during Experiment I.

Settled TBOD₅ and unsettled scBOD₅ results indicated that the CO₂-supplemented and control ponds exhibited nearly equal performance in organics removal (Figure 4-13 and Figure 4-14). Only four reliable BOD results were reported during Experiment I due to out of range dilutions.

Dissolved reactive phosphorus (DRP) and alkalinity were also measured during Experiment I (Table 4-3). DRP removal in the ponds was relatively consistent for all ponds, averaging 47-56 % removal. Alkalinity removal was also relatively consistent, averaging 26-29 % removal (Figure 4-15 and Table 4-3).

Figure 4-13: TBOD₅ for influent and 24-hour settled pond effluents during Experiment I.

Figure 4-14: scBOD₅ for influent and unsettled pond effluents during Experiment I.

Figure 4-15: Alkalinity data for influent and pond effluents during Experiment I.

Table 4-3: Water quality data during Experiment I (7/21/2009 – 9/28/2009). Samples were collected between 9-10 AM. Daily pH and DO readings are available in Appendix E.

Constituent	Influent	Pond 1 (w/ CO2)	Pond 2 (control)	Pond 3 (w/ CO2)	Pond 4 (control)
TSS (mg/L)	88	262	229	250	251
Std. Error	5.1	7.2	7.6	12.6	8.6
# of samples	14	20	20	20	20
VSS (mg/L)	83	244	219	234	239
Std. Error	4.7	6.6	6.6	10.9	7.0
# of samples	14	20	20	20	20
2-hr TSS (mg/L)	n/a	151	165	98	192
Std. Error	n/a	13.0	14.9	27.8	17.6
# of samples	n/a	5	5	5	5
% settled	n/a	42%	28%	61%	24%
24-hr TSS (mg/L)	n/a	69	95	47	92
Std. Error	n/a	10.2	18.6	12.2	12.7
# of samples	n/a	5	5	5	5
% settled	n/a	74%	58%	81%	63%
NHx-N (mg/L)	38	3.2	2.9	4.0	3.1
Std. Error	2.2	1.0	0.2	0.9	0.5
# of samples	70	7	7	7	7
% removed	n/a	92%	92%	90%	92%
Alkalinity (mg/L)	349	257.5	248.0	254.0	251.5
Std. Error	12.3	11.2	3.7	6.2	6.0
# of samples	4	5	5	5	5
% removed	n/a	26%	29%	27%	28%
Supernatant TBOD, mg/L	162.8	53.9	50.6	34.7	54.7
Std. Error	21.9	13.4	4.0	9.0	6.6
# of samples	4	5	4	4	4
% removed	n/a	67%	69%	79%	66%
scBOD, mg/L	74.1	9.3	12.4	10.9	9.9
Std. Error	8.6	1.3	4.3	1.8	2.6
# of samples	4	4	4	4	4
% removed	n/a	87%	83%	85%	87%
DRP, mg/L	6.2	3.2	3.1	3.2	2.7
Std. Error	0.5	0.2	0.1	0.1	0.4
# of samples	4	4	4	4	4
% removed	n/a	48%	49%	47%	56%

4.2.2 General Observations – Experiment I

The control ponds experienced much higher pH levels than the CO₂-supplemented ponds receiving carbon dioxide. Since carbon dioxide addition was regulated by pH, the maximum pH level experienced in the experimental ponds was around 8.5. Since the experiment was conducted during the sunny summer months, the control ponds regularly reached pH levels above 9.5, with a maximum-recorded pH of 9.87. The control ponds typically reached pH levels higher than the CO₂-supplemented ponds during the early morning (around 9-10 AM). The diel study presented in Section 4.5.4.1 showed that pH in the control ponds was above 9.0 for approximately 10 hours.

Throughout a typical day, pond temperature did not vary more than 0.2-0.3°C among the ponds. High-low thermometers became available at the end of Experiment I. The maximum-recorded water temperature was 24°C, while the lowest temperature was 12°C.

4.2.3 Discussion of Results – Experiment I

The carbon dioxide supplementation in Ponds 1 and 3 was expected to increase algal productivity and lead to greater nutrient assimilation. If algal productivity were to increase, suspended solids concentrations would be higher and nutrients, primarily ammonia and BOD, would be lower than in the control ponds. This is based on the assumption that increased algal productivity would boost O₂ production and result in higher organics removal. Effluent alkalinity levels were above 250 mg/L as CaCO₃, indicating that a substantial portion of inorganic carbon was still present in the ponds after treatment. This indicated that inorganic carbon was not a limiting nutrient.

However, pH regularly exceeded a level of 9.0, which has been considered to indicate carbon limitation (Weissman et al. 1988).

Overall, algal productivity was not distinguishably higher in Ponds 1 and 3, which received CO₂. While one of the control ponds had substantially lower productivity than all the other ponds, the other had productivity that was bracketed by the experimental ponds receiving CO₂. In early August 2009, the CO₂-supplemented ponds did show a brief period of increased productivity, outgrowing the control ponds 16-25% for a two-week period. However, the productivity in all ponds eventually equalized and then appeared to fluctuate randomly throughout the remaining 1.5 months of experimentation. Averaging all data points, the TSS and VSS concentrations in the CO₂-supplemented ponds were 6% and 4% higher than the control ponds, respectively. Although these data indicate a slightly higher overall productivity, this result does not stem from a consistent pattern of higher productivity.

Table 4-4: Mean TSS and VSS data for CO₂-supplemented and control ponds during Experiment I. Although the CO₂-supplemented ponds had 6% higher average TSS and 4% higher average VSS, CO₂ injection did not consistently increase productivity (Figure 4-4).

	CO ₂ -supplemented Ponds	Control Ponds
TSS, mg/L	256	240
Standard Deviation	45.6	37.4
VSS, mg/L	239	229
Standard Deviation	40.1	31.9

Table 4-5: Biomass productivity for the CO₂-supplemented and control ponds during Experiment I. The ponds receiving CO₂ had slightly higher average productivity. However, trends in VSS throughout the experiment did not establish consistently higher productivity.

	CO ₂ -supplemented Ponds	Control Ponds
Min. Productivity, g/m ² -d	10.0	11.7
Max. Productivity, g/m ² -d	22.6	22.7
Avg. Productivity, g/m ² -d	17.7	17.0
Standard Deviation	3.0	2.4

A possible inhibitor to biomass productivity may have been zooplankton or grazer blooms. Aquatic organisms such as *Daphnia*, ostracods, rotifers, and fungi can grow in large quantities and consume algal-bacterial biomass. Zooplankton counts did not show any major pond productivity decreases coinciding with zooplankton blooms. More continuous zooplankton counting in future experiments may help to better understand the effects of grazers on pond productivity.

The average total ammonia removal in all the ponds was between 91-92% (Table 4-6). Given the regulated pH of the CO₂-supplemented ponds (which reached a maximum of approximately 8.4), ammonia volatilization might have occurred to a lesser degree than in the control ponds, which reached a maximum pH over 9.5. As discussed in Section 4.6, ammonia mass transfer coefficients at a pH of 9.4 were between 1.6-2.6 times those at a pH of 8.4. At similar ammonia concentrations, volatilization rate should be higher in the control ponds when their pH is high. However, the observed similarity in total ammonia removal among all ponds suggests that ammonia removal via volatilization may not have been significantly higher in the control ponds. The bubbling action of the diffusion stone may have produced a periodic stripping action, aiding in the removal of NH₃ from the

CO₂-supplemented ponds. The effect of sparging on ammonia volatilization was outside the scope of this experiment.

The suppressed pH in the ponds receiving CO₂ may have also produced favorable conditions for bacterial nitrification. Nitrate levels in CO₂ supplemented ponds in Experiment IV were higher than in control ponds (Section 4.5).

Table 4-6: Total ammonia data for the CO₂-supplemented and control ponds during Experiment I. Both achieved similar total ammonia removal efficiencies.

	CO ₂ -supplemented Ponds	Control Ponds
TAN, mg/L	3.6	3.0
TAN Removal	91%	92%
Standard Deviation	6.6%	3.0%

Other treatment indicators, such as BOD, DRP and alkalinity, did not clearly indicate any removal advantages for either the control or CO₂-supplemented ponds.

Analysis of 2-hour and 24-hour settling data revealed increased settling efficiencies in the ponds receiving carbon dioxide (Table 4-7). Data from Experiment IV (another CO₂-supplementation experiment) did not support this conclusion, however, as discussed in Section 4.5.5.

Table 4-7: Average settling efficiencies during Experiment I.

	CO ₂ -supplemented Ponds	Control Ponds
2-hr Settling Efficiency	45%	16%
Standard Deviation	23.9%	6.4%
24-hr Settling Efficiency	74%	56%
Standard Deviation	11.8%	10.9%

4.3 Experiment II – Influent Dilution & CO₂ Supplementation

During Experiment II, the primary influent feed was diluted with tap water (50% dilution by volume) to decrease the influent carbon load and simulate ponds with settled effluent recirculation. With a decreased influent carbon load, CO₂ supplementation should have had a more pronounced effect on algal growth. With tap water dilution, the algae cultures in three ponds experienced culture crashes, and no substantial results were gathered before the experiment was terminated (Figure 4-16).

Figure 4-16: TSS data during Experiment II with tap water dilution of wastewater feed.

4.4 Experiment III – RAS Inoculation

This section describes water quality results for the third experiment in which RAS was added to two of the four ponds. All ponds were operated at a 4-day residence time, as in Experiment I. Due to winter weather conditions, algae productivity was expected to be light- and temperature-limited rather than carbon-limited. Therefore, carbon dioxide supplementation was ceased. Instead, an experiment on bioflocculation and nitrification was conducted. Each pond received 3.78 L of RAS (properties of which are described in Section 4.4.1) once per day for the duration of the experiment. This volume of RAS was 1.6% of the inflow to each pond. All ponds were fed primary clarifier effluent from a common constant head tank. Channel velocities were once again 25 cm/s.

This experiment consisted of three extended testing periods, which were separated by the Cal Poly winter break and an early spring pond culture crash. The first segment of experimentation was conducted between October 22, 2009 and December 20, 2009. During the three-week break between sampling segments, the influent lines were shut off, but the paddle wheels were kept on to maintain the suspension of biological material. A sheet of plastic was fitted over the top of Pond 1 in an attempt to maintain a higher temperature to explore any effect on bioflocculation. During the beginning of January, 2010, the plastic sheet was removed (no marked temperature increase had been noticed), and the ponds were prepped for the continuation of the RAS experiment. The configuration of control and experimental ponds remained the same, and all RAS addition procedures were maintained. This second segment of Experiment III occurred between January 9 and March 15, 2010.

In early March, Pond 1 experienced an extreme decrease in productivity and eventually reached VSS concentrations as low as 34 mg/L. Pond 1 was drained, and half of the Pond 2 culture was transferred to Pond 1. Pond operations began again on March 20, 2010, but the paddle-wheel motor speed controller knob was accidentally bumped and turned up for some time, resulting in a re-suspension of settled sludge. This resulted in a four-week period with extremely high solids concentrations. After allowing the ponds to settle and returning the channel velocity to 25 cm/s, water quality data were collected for a two-week period until the experiment concluded on May 17, 2010. The resulting data were not included in the summary analysis of Experiment III because of its variability.

The data from the first two periods of Experiment III are presented separately in the following section to account for the interruptions incurred.

4.4.1 RAS Properties

During Experiment III, 3.78 L of return activated sludge (RAS) was added to Ponds 1 and 2 daily. Each pond had an approximate volume of 940 L. The SLOWRF staff supplied water quality data for the return activated sludge. The suspended solids of the RAS ranged from 3000 to 14000 mg/L during the experiment. The average solids concentration was roughly 7000 mg/L and is used for the following mass balance calculations to estimate the concentration changes directly caused by RAS addition. The daily dose of RAS contributed an average equivalent of 122 mg/L TSS to the experimental ponds. The RAS also contributed a small amount of nitrogen to the ponds. The concentration of TAN, nitrite-N, and nitrate-N in the RAS averaged 0.6, 0.6, and 25.2 mg/L, respectively. The 3.78 L/d RAS dose contributed an equivalent of 0.02 mg/L

TAN, 0.02 mg/L NO₂-N, and 0.39 mg/L NO₃-N to the experimental ponds. The RAS TSS and nitrogen species data are available in graphical form in Appendix D.

4.4.2 Treatment Performance – Experiment III

The goal of Experiment III was to determine if daily RAS inoculation affected nutrient removal and biomass settleability. As referenced earlier, this experiment encountered two data analysis breaks. The water quality constituents measured were different during each segment due to the availability of student laboratory technicians.

During the first experimental segment of Experiment III, the water quality constituents analyzed included initial TSS and VSS, 2-hr and 24-hr TSS, TAN and alkalinity. Nitrate and nitrite data are not available due to ion chromatograph malfunction. During the second and third segments of Experiment III, all of the above water quality constituents were analyzed, with the addition of nitrate and nitrite. Average constituent levels for each segment of Experiment III are located at the conclusion of each segment's subsection.

4.4.2.1 Experiment III, Segment One

The addition of RAS to the experimental ponds was expected to encourage biomass flocculation within the ponds, resulting in improved settling. The ponds receiving RAS might also have a higher suspended solids level due to the addition of the concentrated RAS. During the first segment of Experiment III, both ponds receiving RAS had higher initial TSS and VSS concentrations than the control ponds, as would be expected (Figure 4-17). The ponds receiving RAS averaged 95 mg/L TSS and 90 mg/L VSS, while the control ponds averaged 71 mg/L TSS and 69 mg/L VSS.

Figure 4-17: VSS data for the influent and pond effluents during the first segment of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

The effect of RAS on settling was much more pertinent during Experiment III. The 2-hour and 24-hour Imhoff cone supernatant TSS concentrations in the ponds receiving RAS were expected to be lower than in the control ponds because of increased bioflocculation. During the first segment of Experiment III, the ponds receiving RAS did not settle consistently better over 2 hours than the control ponds (Figure 4-18). Pond 1 (RAS-inoculated) and Pond 3 (control) had the lowest 2-hr TSS.

The 2-hr settling efficiencies in the ponds receiving RAS were higher by the end of the first segment of Experiment III (Figure 4-19). On all but one sampling day, the RAS-inoculated Pond 1 supernatant had higher settling efficiency than all other ponds. Pond 2, the other RAS-inoculated pond, did not achieve settling efficiencies higher than the control ponds until the last two sampling days (Figure 4-19).

Figure 4-18: TSS data for pond effluents settled 2 hours during segment one of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

Figure 4-19: Two-hour TSS settling efficiency of supernatant pond effluent during segment one of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

The 24-hour settling data also indicated no effect of RAS in the experimental ponds. The control ponds averaged 41 mg/L TSS after a full day of settling. The first pond receiving RAS (Pond 1) averaged 27 mg/L, indicating better settling performance. However, the other pond receiving RAS, Pond 2, averaged 49 mg/L, a higher settled TSS than the control ponds.

RAS-inoculated Pond 1 had consistently higher 24-hr settling efficiencies for the entire first segment of Experiment III (Figure 4-21). The settling efficiency of the other RAS-inoculated pond was higher than the control ponds only on the last day of 24-hour settling tests. Since both ponds receiving RAS did not have consistently higher settling efficiencies than the control ponds, a strong conclusion could not be reached on the effectiveness of RAS on improving settling.

Figure 4-20: TSS data for pond effluents settled 24 hours during segment one of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

Figure 4-21: Twenty-four hour TSS settling efficiency of supernatant pond effluent during segment one of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

The effects of RAS on TAN removal were also analyzed. The activated sludge basins that were the RAS source were operated to promote nitrification. The daily addition of RAS to the experimental ponds was expected to establish both flocculating and nitrifying communities. Thus, the resultant TAN levels in the ponds receiving RAS were expected to be lower than in the control ponds.

The ponds receiving RAS both exhibited higher TAN removal efficiencies than the control ponds (Figure 4-22 and Figure 4-23). Pond 1 and Pond 2 (with RAS) had average removal efficiencies of 74% and 54%, respectively, while Pond 3 and Pond 4 averaged 45% and 49% removal, respectively. Pond 1 (with RAS) performed at a much higher level than the control ponds, in terms of TAN removal, while Pond 2 performed only slightly better than the control ponds (Figure 4-23). The increase in ammonia removal in Pond 1 was not attributable to raised pH levels, as the pH in all ponds were similar, with

Pond 1 at times being lower in pH. The addition of RAS may have established a strong community of nitrifying organisms that resulted in increased ammonia consumption in Pond 1. However, the TAN removal in the second pond receiving RAS did not exhibit increased ammonia removal to the same degree.

Figure 4-22: Total ammonia nitrogen levels of influent and pond effluents during segment one of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily. Influent TAN data were provided by the San Luis Obispo WRF.

Figure 4-23: TAN removal efficiencies in pond effluents during segment one of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

Alkalinity was also monitored throughout the first segment of Experiment III. The alkalinity removal in Pond 1, which received RAS was over 50% higher than in the control ponds and the other pond receiving RAS (Table 4-8). Alkalinity removal is an indicator of nitrification, with approximately 7 grams of alkalinity consumption (as CaCO_3) being associated with each gram of ammonia-N converted to nitrate-N (Frost, 2008). The average alkalinity difference between Pond 1 and the control ponds was 51 mg/L. Assuming the differences in alkalinity consumption were due to increased nitrification in Pond 1, the ammonia levels should differ by approximately 7.3 mg/L. The average ammonia concentration in Pond 1 was roughly 10.5 mg/L lower than levels in the control ponds. Based on these numbers, the decreased total ammonia concentration in Pond 1 was likely due to nitrification.

Table 4-8: Water quality data for the first segment of Experiment III, 10/22/2009-12/20/2009. Samples were collected between 9-10 AM. Daily pH and DO readings are available in Appendix E.

Constituent	Influent	Pond 1 (w/ RAS)	Pond 2 (w/ RAS)	Pond 3 (control)	Pond 4 (control)
TSS (mg/L)	75	94	96	66	75
Std. Error	7.6	9.8	11.5	4.0	4.3
# of samples	10	10	10	10	10
VSS (mg/L)	68	90	89	64	73
Std. Error	6.1	8.9	10.0	3.9	4.5
# of samples	10	10	10	10	10
2-hr TSS (mg/L)	n/a	45	54	47	64
Std. Error	n/a	8.9	9.9	3.1	7.8
# of samples	n/a	7	7	7	7
% settled	n/a	52%	44%	28%	15%
24-hr TSS (mg/L)	n/a	27	49	41	41
Std. Error	n/a	3.9	8.9	4.8	9.5
# of samples	n/a	5	5	5	5
% settled	n/a	72%	49%	38%	45%
NHx-N (mg/L)	38	9.8	17.6	20.9	19.3
Std. Error	1.8	1.2	1.7	1.1	1.6
# of samples	60	10	10	10	10
% removed	n/a	74%	54%	45%	49%
Alkalinity (mg/L)	359	246.4	287.6	298.2	295.1
Std. Error	14.1	12.0	18.1	9.7	12.5
# of samples	8	7	7	7	7
% removed	n/a	31%	20%	17%	18%

4.4.2.2 Experiment III, Segment Two

The second segment of Experiment III began in January 2010 after the Cal Poly winter break. After a three-week hiatus, the influent supply was re-started, and the experiment was continued.

The suspended solids data during the second segment of Experiment III was not similar to the data acquired in the first segment. The average TSS and VSS values for the ponds receiving RAS and control Pond 3 were similar, while Pond 4 TSS and VSS values were initially significantly lower (Figure 4-24). Ponds 1, 2, and 3 had TSS/VSS values of 129/112, 138/122, and 137/122 mg/L respectively, while Pond 4 averaged 108/87 mg/L. The addition of RAS did not produce a marked increase in VSS.

Figure 4-24: VSS data for influent and pond effluents during segment two of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

The effect of RAS on settling ability of the experimental ponds was more consistent during segment two of Experiment III. During the majority of sampling dates, the RAS-inoculated ponds had lower 2-hr and 24-hr TSS (Figure 4-25 and Figure 4-27). The average 2-hr TSS for the RAS-inoculated and control ponds was 26 and 44 mg/L, respectively. The average 24-hr TSS for the RAS-inoculated and control ponds was 18 and 37 mg/L, respectively. Over 2-hours, the RAS-inoculated ponds TSS dropped 79% compared to the initial concentration (Figure 4-26). Over the same period, the control ponds TSS dropped 64%. Over 24-hours, the TSS in the ponds receiving RAS dropped 87%, while the TSS in the control ponds dropped 70% (Figure 4-28).

Pond 1 (with RAS inoculation) had higher 2-hour and 24-hour settling efficiency on every sampling date. Pond 2 (with RAS inoculation) had higher settling efficiencies than the control ponds until a sharp decrease on the fifth sampling date (Figure 4-28). The control ponds reached settling efficiencies similar to those in the RAS-inoculated ponds on the fourth sampling date (Figure 4-28).

Figure 4-25: TSS data for pond effluents settled 2 hours during segment two of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

Figure 4-26: Two-hour TSS settling efficiency of pond effluent during segment two of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

Figure 4-27: TSS data for pond effluents settled 24 hours during segment two of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

Figure 4-28: Twenty-four hour TSS settling efficiency of pond effluent during segment two of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

During the first half of sampling in segment two of Experiment III, the ponds receiving RAS had higher ammonia removal efficiencies (Figure 4-29 and Figure 4-30). During the second half of sampling, however, all four ponds appeared to develop arbitrary removal efficiencies. On average, ammonia removal was slightly higher in the ponds receiving RAS, averaging 84% removal, while control ponds averaged 78% removal.

Figure 4-29: TAN data for influent and pond effluents during segment two of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

Figure 4-30: TAN removal efficiencies in pond effluents during segment two of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

To determine if RAS contributed to nitrification, nitrate and nitrite concentrations were determined using an ion chromatograph. The control ponds served as a baseline to compare the RAS-inoculated ponds in terms of nitrate production.

The nitrate levels in Pond 1 (with RAS) were consistently higher than the other ponds (Figure 4-31). During the first segment of Experiment, Pond 1 presumably experienced more nitrification based on reduced alkalinity and TAN levels. The culture in Pond 1 likely maintained a similar community of microorganisms during the break in operations over the winter holiday. Pond 1 experienced a large spike in nitrate concentration in the beginning of February (over 17 mg/L). Pond 3, a control pond, had a large spike in nitrate concentration the next sampling date (over 11 mg/L). The other RAS-inoculated pond, Pond 2, had nitrate levels similar to the control ponds (Figure 4-31). A positive relationship between RAS inoculation and nitrification in the ponds cannot be established since the ponds receiving RAS had significantly different levels of nitrate.

Figure 4-31: Nitrate-N concentrations in influent and pond effluent during the second segment of Experiment III. Ponds 1 and 2 received 3.78 L of RAS daily.

Table 4-9: Water quality data for the second segment of Experiment III, 1/9/2010-3/15/2010. Samples were collected between 9-10 AM. Daily pH and DO readings are available in Appendix E.

Constituent	Influent	Pond 1 (w/ RAS)	Pond 2 (w/ RAS)	Pond 3 (control)	Pond 4 (control)
TSS (mg/L)	54	129	138	137	108
Std. Error	7.2	20.0	26.9	23.0	17.8
# of samples	12	12	12	12	12
VSS (mg/L)	46	112	122	111	87
Std. Error	4.6	17.6	23.7	18.7	11.8
# of samples	11	11	11	11	11
2-hr TSS (mg/L)	n/a	31	20	38	50
Std. Error	n/a	4.6	4.4	8.5	11.1
# of samples	n/a	7	7	7	7
% settled	n/a	76%	86%	72%	53%
24-hr TSS (mg/L)	n/a	22	14	33	40
Std. Error	n/a	3.5	5.7	9.8	10.1
# of samples	n/a	7	7	7	7
% settled	n/a	83%	90%	76%	63%
NH _x -N (mg/L)	30	4.2	5.1	6.4	6.7
Std. Error	0.7	1.2	0.9	0.8	1.0
# of samples	76	9	9	9	9
% removed	n/a	86%	83%	79%	78%
NO ₃ -N (mg/L)	0.8	7.4	3.3	3.8	2.3
Std. Error	0.4	2.1	1.1	1.4	0.5
# of samples	7	7	7	7	7
NO ₂ -N (mg/L)	0.3	1.8	1.5	1.0	0.7
Std. Error	0.1	0.5	0.4	0.3	0.1
# of samples	7	7	7	7	7
Alkalinity (mg/L)	614	431.3	454.4	470.5	471.4
Std. Error	79.4	57.1	56.0	61.2	58.3
# of samples	6	6	6	6	6
% removed	n/a	30%	26%	23%	23%

4.4.2.3 Experiment III, Segment Three

During the third segment of Experiment III, the paddle-wheel motor was accidentally turned up during maintenance, which resulted in a temporary spike in suspended solids, as sludge was kicked up from the bottom of the pond. After the disruption, the ponds

behaved erratically and the data produced was not applied to the long-term analysis of RAS inoculation (Table 4-10).

Table 4-10: Water quality data for the third segment of Experiment III, 3/20/2010-5/17/2010. Samples were collected between 9-10 AM. Daily pH and DO readings are available in Appendix E.

Constituent	Influent	Pond 1 (w/ RAS)	Pond 2 (w/ RAS)	Pond 3 (control)	Pond 4 (control)
TSS (mg/L)	63	119	129	195	198
Std. Error	5.8	8.7	10.6	6.3	10.6
# of samples	11	4	4	4	4
VSS (mg/L)	60	110	114	168	171
Std. Error	5.8	10.3	9.6	7.7	10.2
# of samples	11	4	4	4	4
2-hr TSS (mg/L)	n/a	39	20	32	30
Std. Error	n/a	9.0	7.2	6.5	3.3
# of samples	n/a	6	6	6	6
% settled	n/a	67%	85%	84%	85%
24-hr TSS (mg/L)	n/a	28	16	26	19
Std. Error	n/a	9.0	4.6	4.6	5.6
# of samples	n/a	6	6	6	6
% settled	n/a	77%	88%	87%	90%
NHx-N (mg/L)	37	6.5	3.9	6.1	6.7
Std. Error	0.8	2.3	1.1	1.5	1.6
# of samples	52	11	11	11	11
% removed	n/a	83%	89%	84%	82%
NO3-N (mg/L)	n/a	6.7	8.1	6.5	9.3
Std. Error	n/a	2.3	1.9	1.9	2.2
# of samples	n/a	5	5	5	5
NO2-N (mg/L)	n/a	2.3	1.2	1.1	1.4
Std. Error	n/a	0.5	0.3	0.3	0.3
# of samples	n/a	5	5	5	5
Alkalinity (mg/L)	367	247.5	223.3	225.0	228.3
Std. Error	20.2	21.7	16.8	14.7	14.3
# of samples	6	6	6	6	6
% removed	n/a	33%	39%	39%	38%
DRP, mg/L	5.2	4.3	4.7	3.5	3.4
Std. Error	0.5	0.3	0.4	0.4	0.4
# of samples	4	4	4	4	4
% removed	n/a	18%	9%	32%	34%

4.4.3 General Observations – Experiment III

During Experiment III on RAS inoculation a high-low thermometer was placed in Pond 1 to record fluctuations in daily temperature. Since the ponds were observed to all have similar temperatures within 0.2°C of each other during Experiment I, there was no need to install more than one high-low thermometer. During Experiment III, zooplankton were observed, mostly ostracods (seed shrimp).

The influent supply rate during Experiment III was not as consistent as Experiment I. Although the hydraulic residence times were generally between 3.7 and 4.3 days, the supply pumps required frequent adjustment. Over time, biofilm and debris built up in the drip irrigation supply lines. The repeated action of the peristaltic pump eventually wore the soft line and reduced its resilience. The level of biofilm and wear on each line was not always consistent, so periodic replacement of all lines and pump head tubing was necessary.

The cool weather during most of Experiment III apparently inhibited algal growth and resulted in lower pH levels than Experiment I. During daily pond monitoring, between 8 AM and 3 PM, the average pH levels were lower in the RAS-inoculated ponds. The average pH level in these ponds receiving RAS was 7.82, while the average pH level in the control ponds was 8.01.

The addition of RAS introduced bacteria and microorganisms that might not normally be present in algal ponds in large numbers. However, larger zooplankton were observed during the second and third segment of Experiment III that were not present in the activated sludge. Ostracods, sometimes referred to as seed shrimp, were observed

attached to filamentous growth on the sidewalls of the pond. They were also observed grazing upon the settled sludge during the periodic draining of the ponds. *Daphnia* were also observed on the surface of the ponds, but they did not dominate the ponds and only appeared briefly.

The earliest significant appearance of either species occurred during March, when small numbers of *Daphnia* were observed in the ponds receiving RAS. Several days after they appeared in Ponds 1 and 2, *Daphnia* also began to be observed in the control ponds. The appearance of *Daphnia* was observed after the pond cultures had already begun crashing. Zooplankton counts during this time were unavailable. The appearance of *Daphnia* was relatively short-lived, as they were not observed in the ponds in significant numbers since March.

In early November, ostracods began showing up in Pond 1 only. After restarting the ponds in January, ostracods were observed in all ponds. Significant numbers of ostracods appeared in the ponds during late April when the weather became warmer and the paddle wheel speed was higher than normal. Once the paddle wheels were slowed to the appropriate speed, ostracods were observed in limited numbers on pond filament. They were found in large numbers at the end of the experiment once the ponds were drained and the sludge layer was revealed. All ponds had large numbers of ostracods, but the RAS-inoculated ponds appeared to have even higher numbers (based solely on observation during pond draining).

Pond micrographs from a culture observation on December 21, 2009 are located in Appendix F.

4.4.4 Zooplankton Counts – Experiment III

Periodic zooplankton counts were conducted during Experiment III. Ostracods, rotifers, *Daphnia*, and stalked ciliates were organisms of interest. However, ostracods and rotifers were the only organisms that were consistently observed during the counts.

In November, ostracods were found in low to moderate levels in Pond 1. In four zooplankton counts for the month, Pond 1 had an average of approximately 200 ostracods per liter of pond water. In December, two zooplankton counts revealed an average concentration of approximately 915 ostracods per liter of pond water in Pond 1. During this two-month stretch ostracods were observed in Pond 2 on two dates, with a mere 50 organisms per liter each sampling session. On the last sampling day before the winter break, ostracods appeared in Pond 4 at approximately 150 organisms per liter. Ostracods were not found in Pond 3 during any of the counts between November and December.

During January, Ponds 1-4 all had a measured presence of ostracods. One count was conducted in both February and March. In February, Ponds 1 and 3 had moderate levels of ostracods (350 and 580 per liter, respectively), while in March, ostracods only showed up in Pond 1 at lower levels (200 per liter).

During the period in April when the paddle wheel was sped up, ostracod levels in Ponds 1 and 2 were extremely high. On April 16, the ostracod levels in Ponds 1 and 2 were 2500 and 2300 organisms per liter of pond water, respectively. On the same date, Ponds 3 and 4 registered 900 and 400 organisms per liter, respectively. Over the next week, ostracod levels dropped into the low hundreds.

The ostracod counts may not have been entirely consistent and may not be representative of the general concentration in the ponds. As noted earlier, ostracods were observed in the settled sludge and in the sidewall filamentous growth. Ostracods could also be seen being kicked up just upstream of the paddle wheel, presumably from grazing on the sludge. When collecting samples, the proximity of the sample bottle opening to the sidewall might have influenced the number of ostracods captured. Large changes in ostracod numbers during zooplankton counting probably were indicative of increased presence, but small changes may not have been. Since the counts used only 10 milliliters of pond water, a difference of 100 organisms per liter would be due to counting only one more ostracod.

Rotifers were also counted during routine zooplankton counts. Rotifers pose a risk to algal productivity if populations are extremely large, due to their tendency to consume microalgae. However, rotifers tend to consume smaller algae, which may select for larger, more settleable algal species (Schluter 1987). Pond 1 contained large numbers of rotifers from October to December. On November 17, Pond 1 rotifer numbers peaked at 3490 organisms per liter of pond water. Rotifers were counted in moderate numbers in the remaining ponds. The presence of large numbers of rotifers in Pond 1 may have been due to the addition of recycled activated sludge.

Zooplankton counts for the entire thesis project are available for review in Appendix A.

4.4.5 Discussion of Results – Experiment III

The goal of Experiment III was to determine whether inoculation with return activated sludge could help in floc formation and settling in algal ponds. Nutrient removal was also analyzed.

After adding RAS to Ponds 1 and 2 in mid-November, several months of data were gathered through March 11. The RAS-inoculated ponds settled 25% more efficiently in two hours, and 21% more efficiently in 24 hours with respect to the control ponds (Table 4-11). These numbers do not include the data collected in April and May, when the excessive mixing velocity scoured-up sludge from the bottom of the ponds.

A previous Cal Poly experiment explored the effects of RAS addition on bioflocculation and settling. This earlier experiment observed 24-hour TSS removal efficiencies of 92% in RAS-inoculated ponds, and 74% in control ponds (Frost 2008).

Table 4-11: Simultaneously-collected mean settling data for the ponds receiving RAS and control ponds during Experiment III. The settling tests were conducted using 1-L Imhoff cones. The ponds receiving RAS settled more efficiently than the control ponds. After 24 hours, the average TSS concentration in the experimental ponds was less than 30 mg/L.

	RAS-inoculated Ponds	Control Ponds
2-hr TSS, mg/L	34	48
24-hr TSS, mg/L	23	38
2-hr TSS Removal	66%	41%
24-hr TSS Removal	80%	59%
24-hr TSS Removal (Frost 2008)	92%	74%

Biomass productivity was much lower during the winter months due to low levels of insolation and cool temperatures (Table 4-12). The experimental ponds did not exhibit significantly higher productivity, especially considering that 3.78 L/d addition of RAS would typically add solids equivalent to a productivity of 8.5 g/m²-d.

Table 4-12: Biomass productivity data for the ponds receiving RAS and control ponds during Experiment III (10/22/09 – 3/11/10). Productivity was much lower than in the summer months due to low insolation and cool temperatures.

	RAS-inoculated Ponds	Control Ponds
Min. Productivity, g/m ² -d	2.5	1.4
Max. Productivity, g/m ² -d	19.3	15
Avg. Productivity, g/m ² -d	7.6	6.5
Standard Deviation	4.0	3.2

The ponds receiving RAS had higher TAN removal efficiencies during the first segment of Experiment III, but Pond 1 outperformed all other ponds (Table 4-13). The superior TAN removal efficiency in Pond 1 was attributed to nitrification, but there was no direct data to support this conclusion. Alkalinity removal, an indirect indicator of nitrification, was much higher in Pond 1, leading to the conclusion that nitrification was the likely factor in the observed increase in TAN removal efficiency during winter.

Table 4-13: TAN data from November 19, 2009 to December 16, 2009. The experimental ponds (receiving RAS) had higher removal efficiency, but Pond 1 significantly outperformed the other experimental pond. The control ponds performed almost identically.

	RAS-inoculated Ponds		Control Ponds
	Pond 1	Pond 2	Ponds 3 & 4
NHx-N, mg/L	8.6	16.3	19.0
Standard Deviation	3.6	2.7	2.8
NHx-N Removal	78%	59%	52%
Standard Deviation	11.3%	10.2%	8.6%

From January 28 to March 11, 2010, the TAN removal efficiencies were similar for each pair of ponds (Table 4-14). The ponds receiving RAS had slightly higher removal efficiencies than the control ponds.

Table 4-14: Average TAN data from January 28 to March 11, 2010. RAS-inoculated ponds had a 6% increase in ammonia removal.

	RAS-inoculated Ponds	Control Ponds
NH _x -N, mg/L	4.6	6.5
Standard Deviation	3.1	2.7
NH _x -N Removal	84%	78%
Standard Deviation	10.6%	8.3%

The increased ammonia removal efficiency in the experimental ponds was likely caused by the daily additions of RAS. The RAS is rich in nitrifying organisms, which have already proved to be efficient in removing ammonia in the SLOWRF aeration basin. Each day, the ponds received enough RAS to increase the pond TSS by 122 mg/L on average, a substantial portion of which was presumably nitrifying organisms. Nitrification, however, occurred at higher levels in only Pond 1, which averaged approximately two-times the nitrate-N concentration (7 mg/L) compared to the remaining three ponds (Table 4-9). Control Ponds 3 and 4, and RAS-inoculated Pond 2 averaged 2.3-3.5 mg/L nitrate-N.

4.5 Experiment IV – CO₂ Supplementation

As in Experiment I, the goal of Experiment IV was to observe any difference in biomass productivity and treatment performance between ponds receiving carbon dioxide and a control group. The main water quality constituents analyzed during this experiment were TSS/VSS, 2-hour and 24-hour settled TSS, TAN, nitrate, and nitrite. Total Kjeldahl nitrogen (TKN) measurements were also obtained for several weekly testing dates, which allowed for in-depth nitrogen balances for several sample dates.

By May, the start of this Experiment, average air temperatures and insolation were rising to high levels. The ponds operated initially at a 4-day HRT and then were switched to a 3-day HRT for summer. The data in this section are notated to include this change in operation.

During Experiment IV, two diel studies were conducted to observe changes in DO, pH, temperature, TAN, and TSS/VSS over a 24-hour period. These studies are included after the discussion of weekly sampling data.

4.5.1 Treatment Performance – Experiment IV

CO₂ supplementation did not result in consistent increases in biomass productivity during Experiment IV. The VSS levels in the CO₂-supplemented ponds were never consistently higher than the VSS levels in the control ponds (Figure 4-32).

During the 4-day HRT, the average biomass productivity was 16.6 g/m²-day. During the 3-day HRT, the average biomass productivity was 18.2 g/m²-day. Thus, productivity was slightly higher once the ponds were operated at a 3-day HRT. No significant effect of CO₂ supplementation on pond productivity was observed (Figure 4-33).

Figure 4-32: VSS data for influent and pond effluents during Experiment IV.

Figure 4-33: Box and whisker plot of productivity in individual ponds for both the 4-day and 3-day HRT periods of Experiment IV. Both operational HRT periods are included. As noted, P1 and P3 received CO_2 supplementation. The central line is the median, the extremes of the boxes are the 25th and 75th percentiles, and the whiskers are the minimums and maximums of the time-series data.

Insolation during May and June was over 300 W/m^2 . Unlike data from Experiment I, fluctuations in biomass productivity did not closely follow trends in insolation levels (Figure 4-34). VSS concentrations decreased after implementing a 3-day HRT, as would be expected from the higher flushing rate, but this period also corresponded with a period of decreased insolation. Even with yearly highs in insolation during May and June, CO_2 supplementation did not appear to increase biomass productivity. The presumption formed after Experiment I that insolation over 280 W/m^2 may produce conditions in which CO_2 supplementation becomes beneficial, was not confirmed during Experiment IV.

Figure 4-34: VSS data for pond effluents coupled with insolation data. Insolation data are presented in units of W/m^2 .

The shift in hydraulic residence time from 4 days to 3 days was expected to reduce ammonia volatilization. Using a longer HRT increases the likelihood that free ammonia gas will volatilize to the atmosphere (Garcia et al. 2000), which may be a drawback in

some air pollution control districts. Monitoring of total ammonia nitrogen revealed greater levels in the ponds during the 3-d HRT (Figure 4-36). Analysis of total nitrogen was necessary to determine whether the fraction of nitrogen lost (presumably to the atmosphere, although a small portion may be retained in sludge) was greater during the 3-day or 4-day HRT period of the experiment.

Figure 4-35: Total ammonia nitrogen data for influent and pond effluents during Experiment IV.

The ponds receiving carbon dioxide did not exhibit any substantial difference in total ammonia removal compared to the control ponds (Figure 4-36). During the 4-day HRT, the average ammonia removal in all ponds was 91%. This average removal was identical to the ammonia removal efficiency in Experiment I. During the 3-day HRT, the average ammonia removal in all ponds was 82% (Figure 4-36).

Figure 4-36: Box and whisker plot of total ammonia removal efficiency in individual ponds for both the 4-day and 3-day HRT periods of Experiment IV. As noted, Ponds 1 and 3 received CO₂ supplementation. The central line is the median, the extremes of the boxes are the 25th and 75th percentiles, and the whiskers are the minimums and maximums of the time-series data.

The ponds receiving CO₂ had higher nitrate concentrations than the control ponds during both the 4-day and 3-day HRTs (Figure 4-37). The average concentrations of nitrate-N in the CO₂-supplemented and control ponds during the 4-day HRT were 6.3 and 4.2 mg/L, respectively (Table 4-19). During the 3-day HRT, nitrate-N concentrations were 5.3 and 3.3 mg/L, respectively, although there was variance between individual control and CO₂-supplemented ponds (Table 4-20).

Figure 4-37: Nitrate-N concentrations in pond effluents during Experiment IV. Influent nitrate-N concentrations were approximately zero.

Nitrite levels were similar in the CO₂ supplemented ponds and control ponds during Experiment IV. During the 4-day and 3-day HRTs, the average nitrite-N levels in the ponds receiving CO₂ and control ponds were 1.1 and 1.5 mg/L, respectively (Table 4-19 and Table 4-20).

During Experiment IV, TKN testing was performed to develop nitrogen balances. Two nitrogen balances were developed while the ponds were operating at a 4-day HRT, and two nitrogen balances were developed during the 3-day HRT. TAN, nitrate, nitrite, TKN, and VSS values were used to develop the balances. These numbers were used to determine total nitrogen (TN) and organic nitrogen (ON) values. Total nitrogen is the sum of TKN, NO₂⁻ and NO₃⁻, while organic nitrogen is TKN less TAN. An approximation for the percentage of nitrogen present in algal and bacterial cells was calculated by the ratio of organic nitrogen to total organic mass (VSS). The percentage

of ammonia volatilized to the atmosphere was approximated by observing the difference in total nitrogen between the ponds and the influent. For example, on May 24, the influent contained 52.8 mg/L TN, while Pond 1 contained 36.4 mg/L TN. The difference in TN, 16.4 mg/L, was assumed to have mainly volatilized to the atmosphere, with a negligible fraction settling. This loss represents 31% of the original TN level in the influent; therefore, Pond 1 lost 31% of its nitrogen to the atmosphere. Denitrification was assumed to be negligible because the ponds had overnight dissolved oxygen levels of approximately 1.0 mg/L (Figure 4-43 and Figure 4-51), and denitrification typically occurs in anoxic conditions.

The nitrogen balances indicated that ammonia volatilization was higher when operating the ponds at a 4-day HRT, as expected. However, the control ponds did not experience greater volatilization, even though their pH was not controlled (experimental ponds were kept at or below a pH of 8.4 with carbon dioxide supplementation). Field measurements confirmed that afternoon pH levels in control ponds were generally between 9-9.3. During the 4-day HRT, the ponds averaged 32% ammonia volatilization, while during the 3-day HRT, they averaged 15% ammonia volatilization. Nitrogen balance data are presented below in table and bar graph form.

Table 4-15: Nitrogen balance for sampling date 5/24/2010 (4-d HRT). All concentrations are reported in units of mg/L.

	Type	NH _x -N	NO ₂ -N	NO ₃ -N	TKN	TN	ON	% Biomass	N Lost	VSS	%N cell	% volat.
Influent	n/a	36.8	0.0	0.1	52.6	52.8	15.8	n/a	n/a	69	n/a	n/a
Pond 1	CO ₂	7.2	1.4	7.0	28.0	36.4	20.8	39%	16.4	191	10.9%	31%
Pond 3	CO ₂	7.2	0.6	6.7	30.2	37.5	23.0	44%	15.2	214	10.8%	29%
Pond 2	Control	10.1	0.6	2.9	31.4	34.8	21.3	40%	18.0	190	11.2%	34%
Pond 4	Control	6.3	0.8	5.7	31.4	37.8	25.1	47%	14.9	252	9.9%	28%

Figure 4-38: Nitrogen balances for influent and pond effluents on 5/24/2010 (4-day HRT).

Table 4-16: Nitrogen balance for sampling date 6/2/2010 (4-d HRT). All concentrations are reported in units of mg/L.

	Type	NH _x -N	NO ₂ -N	NO ₃ -N	TKN	TN	ON	% Biomass	N Lost	VSS	%N cell	% volat.
Influent	n/a	30.5	0.0	0.0	49.3	49.3	18.8	n/a	n/a	73	n/a	n/a
Pond 1	CO ₂	3.2	1.6	7.9	24.1	33.5	20.9	42%	15.8	180	11.6%	32%
Pond 3	CO ₂	3.3	0.9	6.2	26.9	34.0	23.6	48%	15.3	218	10.8%	31%
Pond 2	Control	3.6	0.8	3.8	29.1	33.7	25.5	52%	15.6	227	11.2%	32%
Pond 4	Control	3.1	0.9	5.5	25.2	31.6	22.1	45%	17.7	208	10.6%	36%

Figure 4-39: Nitrogen balances for influent and pond effluents on 6/2/2010 (4-d HRT).

Table 4-17: Nitrogen balance for sampling date 7/19/2010 (3-d HRT). All concentrations are reported in units of mg/L.

	Type	NH _x -N	NO ₂ -N	NO ₃ -N	TKN	TN	ON	% Biomass	N Lost	VSS	%N cell	% volat.
Influent	n/a	39.2	0.0	0.0	39.2	39.2	0.0	n/a	n/a	76	n/a	n/a
Pond 1	CO ₂	3.1	1.1	5.4	26.3	32.8	23.2	59%	6.4	209	11.1%	16%
Pond 3	CO ₂	2.6	1.2	6.8	24.1	32.1	21.5	55%	7.1	198	10.8%	18%
Pond 2	Control	5.2	1.9	5.2	24.1	31.2	18.9	48%	8.0	158	11.9%	20%
Pond 4	Control	4.8	1.8	4.6	25.8	32.2	21.0	53%	7.0	181	11.6%	18%

Figure 4-40: Nitrogen balances for influent and pond effluents on 7/19/2010 (3-d HRT).

Table 4-18: Nitrogen balance for sampling date 8/9/2010 (3-d HRT). Ponds 1 and 3 received CO₂ supplementation.

	Type	NH _x -N	NO ₂ -N	NO ₃ -N	TKN	TN	ON	% Biomass	N Lost	VSS	%N cell	% volat.
Influent	n/a	33.2	0.0	0.0	36.4	36.4	3.2	n/a	n/a	50	n/a	n/a
Pond 1	CO ₂	4.3	1.4	8.4	23.0	32.8	18.7	51%	3.6	192	9.7%	10%
Pond 3	CO ₂	9.2	1.1	3.2	26.9	31.2	17.7	49%	5.2	166	10.7%	14%
Pond 2	Control	8.9	1.2	3.9	28.6	33.7	19.7	54%	2.7	190	10.3%	8%
Pond 4	Control	7.8	1.3	1.1	28.6	31.0	20.8	57%	5.4	165	12.6%	15%

Figure 4-41: Nitrogen balances for influent and pond effluents on 8/9/2010 (3-day HRT).

CO₂ addition had no observable effect on settling, as determined by 2-hr and 24-hr settled TSS concentrations (Table 4-19 and Table 4-20). During the 4-day HRT portion of Experiment IV, 2-hr and 24-hr settling efficiencies were similar among all ponds, ranging between 48-60% and 70-75%, respectively (Table 4-19). Since settling tests were not producing any telling results, 2-hr settling tests were halted shortly after switching to a 3-day HRT. The 24-hr settling efficiency ranged between 54-65% for all ponds (Table 4-20). During Experiment I, settling efficiency was higher in the ponds receiving CO₂ supplementation. In Experiment IV, however, settling efficiencies were very similar, showing only a slight, but insignificant increase in settling among the control ponds.

Table 4-19: Water quality data for the 4-d HRT section of Experiment IV (5/18/2010 – 7/7/2010). Samples were collected between 9-10 AM. Daily pH and DO readings are available in Appendix E.

Constituent	Influent	Pond 1 (w/ CO2)	Pond 2 (control)	Pond 3 (w/ CO2)	Pond 4 (control)
TSS (mg/L)	67	233	251	264	277
Std. Error	3.0	14.5	22.2	16.2	14.9
# of samples	8	7	7	7	7
VSS (mg/L)	61	207	217	239	230
Std. Error	3.4	16.3	19.9	19.4	13.0
# of samples	8	7	7	7	7
2-hr TSS (mg/L)	n/a	121	126	125	110
Std. Error	n/a	9.1	8.0	17.8	13.2
# of samples	n/a	5	5	5	5
% settled	n/a	48%	50%	53%	60%
24-hr TSS (mg/L)	n/a	70	76	73	70
Std. Error	n/a	10.2	3.5	6.0	5.9
# of samples	n/a	5	5	5	5
% settled	n/a	70%	70%	73%	75%
NHx-N (mg/L)	37	4.7	5.3	4.3	4.2
Std. Error	1.0	0.7	1.2	0.8	0.7
# of samples	52	7	7	7	7
% removed	n/a	90%	89%	92%	90%
NO3-N (mg/L)	n/a	6.6	3.7	6.0	4.6
Std. Error	n/a	0.5	0.3	0.3	0.7
# of samples	n/a	4	4	4	4
NO2-N (mg/L)	n/a	1.4	1.0	0.9	1.0
Std. Error	n/a	0.1	0.2	0.1	0.1
# of samples	n/a	4	4	4	4
Alkalinity (mg/L)	396	262	252	239	246
Std. Error	11.7	21.9	12.9	13.1	7.6
# of samples	4	4	4	4	4
% removed	n/a	34%	36%	40%	38%
DRP, mg/L	5.9	3.8	4.2	4.0	4.3
Std. Error	0.8	0.5	0.5	0.4	0.6
# of samples	3	3	3	3	3
% removed	n/a	26%	19%	22%	17%

Table 4-20: Water quality data for the 3-day HRT section of Experiment IV (7/8/2010 – 9/1/2010). Samples were collected between 9-10 AM. Daily pH and DO readings are available in Appendix E.

Constituent	Influent	Pond 1 (w/ CO2)	Pond 2 (control)	Pond 3 (w/ CO2)	Pond 4 (control)
TSS (mg/L)	73	226	205	217	227
Std. Error	7.1	21.6	9.7	14.0	13.0
# of samples	7	7	7	7	7
VSS (mg/L)	64	204	185	198	197
Std. Error	6.6	16.6	10.5	11.2	11.9
# of samples	7	7	7	7	7
2-hr TSS (mg/L)	n/a	142	103	132	122
Std. Error	n/a	8.0	10.5	10.0	14.0
# of samples	n/a	2	2	2	2
% settled	n/a	37%	50%	39%	46%
24-hr TSS (mg/L)	n/a	83	71	99	79
Std. Error	n/a	13.1	6.6	3.4	6.4
# of samples	n/a	6	6	6	6
% settled	n/a	63%	65%	54%	65%
NHx-N (mg/L)	35	4.7	5.6	5.0	5.1
Std. Error	1.0	0.9	0.7	1.1	0.6
# of samples	55	8	8	8	8
% removed	n/a	81%	82%	80%	83%
NO3-N (mg/L)	n/a	5.8	4.3	4.7	2.6
Std. Error	n/a	1.0	0.4	0.7	0.7
# of samples	n/a	5	5	5	5
NO2-N (mg/L)	n/a	1.3	1.6	1.3	1.6
Std. Error	n/a	0.1	0.1	0.1	0.1
# of samples	n/a	5	5	5	5
Alkalinity (mg/L)	368	249	255	255	267
Std. Error	2.3	6.6	5.8	5.3	6.7
# of samples	6	6	6	6	6
% removed	n/a	32%	31%	31%	27%

4.5.2 General Observations – Experiment IV

As the weather warmed during late spring and into the summer, the pH of the control ponds rose above the 8.4 threshold of the ponds receiving CO₂. Daily monitoring registered maximum pH values of 9.28 during August. During the first diel study, Pond 2 reached a maximum pH of 9.31 during mid-afternoon on June 17, 2010.

Since the experiment ranged from late spring to summer, the ponds were warmer than the previous experiment. In late May and June, the average temperature range was 12.1 to 22.9°C. In July, the average temperature range was 14.3 to 24.3°C, while in August, the average temperature ranged from 14.8 to 23.6°C.

Throughout the experiment, ostracods were observed in all ponds. As in previous experiments, ostracods were observed in higher numbers among filamentous growth on the pond sidewalls and at areas of high turbulence near the paddle wheels.

4.5.3 Zooplankton Counts – Experiment IV

Three zooplankton counts were taken throughout Experiment IV. Two were conducted during the 4-day hydraulic residence time on May 24 and June 14. The last count was conducted on July 28 when the ponds were operating at a 3-day hydraulic residence time.

Ostracods were observed in all ponds during each of the three zooplankton counts. Ponds 1 and 3, which received CO₂, experienced large increases in ostracod numbers between the two 4-day HRT counts, increasing by 750 and 880 organisms per liter, respectively. Large numbers of ostracods were observed in the last count during the 3-day hydraulic residence time. The samples for Ponds 1, 2 and 4 contained over 1500 ostracods per liter of pond water, while Pond 3 contained 750 ostracods per liter.

Rotifers were observed in Ponds 1, 3 and 4 during Experiment IV. During the 4-day HRT, Ponds 1, 3, and 4 averaged 275, 515 and 275 rotifers per liter, respectively. The presence of rotifers declined during the 3-day HRT. No rotifers were observed in Ponds 1 and 2, while Ponds 3 and 4 only contained 150 and 50 rotifers per liter, respectively.

On July 28, large numbers of paramecia were observed in the pond samples. The paramecia were observed containing consumed algae cells. The presence of paramecia and rotifers did not correspond with a marked decrease in productivity.

Zooplankton counts for the entire thesis project are available for review in Appendix A.

4.5.4 Diel Studies – Experiment IV

Diel studies were conducted during both the 4-day HRT and 3-day HRT portion of Experiment IV. The goal of these studies was to observe changes in pH, dissolved oxygen, temperature, suspended solids, and TAN during a 24-hour period. Nitrate and nitrite analysis were planned for both the studies, but the ion chromatograph did not produce results that passed QC requirements.

During the first diel study, conducted on June 17, 2010, monitoring was conducted in two groupings. Hourly and bi-hourly checks were conducted to measure and record pH, DO, and temperature in each pond. Samples were collected at four-hour intervals for transport to the Cal Poly labs where TSS/VSS and total ammonia testing was performed. Six samples were collected, beginning at 6 AM. pH, DO and temperature were recorded hourly from 5 AM to 12 PM, and 4 PM to 10 PM. Bi-hourly measurements were recorded from 12 PM to 4 PM, and 10 PM to 6 AM the following morning. During the second diel study on July 21, monitoring was conducted in a similar fashion.

4.5.4.1 First Diel Study: June 17, 2010

On June 17, 2010, the ponds were operating at a 4-day hydraulic residence time and Ponds 1 and 3 received carbon dioxide supplementation once pH 8.4 was reached.

Pond 1 began receiving carbon dioxide shortly after 10 AM, while supplementation began around 11 AM in Pond 3. CO₂ supplementation occurred until 6 PM in both CO₂-supplemented ponds (7-8 hours of supplementation). The control ponds maintained a pH level 0.5 or greater than the CO₂-supplemented ponds from 2 PM to 4 AM (14 hours).

Figure 4-42: Pond pH during the June 17, 2010 diel study. Ponds 1 and 3 leveled off at approximately 8.5, when carbon dioxide injection began. Ponds 2 and 4 experienced higher pH levels, peaking just below 9.5.

Dissolved oxygen levels were similar in all ponds throughout the day (Figure 4-43). Maximum dissolved oxygen levels of 20 mg/L were observed at 2:00 PM. Overnight dissolved oxygen levels did not drop below 1 mg/L.

Figure 4-43: Pond dissolved oxygen during the June 17, 2010 diel study. Maximum dissolved oxygen levels occurred between 12 and 2 for all ponds. Dissolved oxygen reached a minimum at 2 AM for all ponds.

Pond temperatures did not vary widely between any of the ponds (Figure 4-44). On average Ponds 1 and 2 were slightly warmer than Ponds 3 and 4. Ponds 1 and 2 averaged 16.9 and 16.8°C, respectively, while Ponds 3 and 4 averaged 16.6°C. Pond temperature may have varied due to slight shading differences. The peak temperature of 22.7°C occurred at 4 PM, while the minimum temperature of 12.4°C occurred at 7 AM in the beginning of the study.

Figure 4-44: Pond temperature during the June 17, 2010 diel study. The maximum temperature in all ponds occurred at 4 PM. The minimum temperature was recorded at 7 AM during the beginning of the study.

Six sets of samples were taken throughout the day to measure TSS/VSS and TAN. TSS and VSS were measured directly after retrieving the samples from the field, while 100 mL of each sample was acidified (to less than 2 pH) and saved for future total ammonia testing.

TSS and VSS varied significantly throughout the diel study (Figure 4-45 and Figure 4-46). Maximum solids levels were measured during mid-afternoon. The ponds supplemented with CO₂ did not exhibit consistent increased algal biomass growth throughout the day, and the control ponds both had higher maximum VSS concentrations (Table 4-21). Pond 3, receiving CO₂, had a higher overall percent increase in VSS than the other ponds. However, the other pond receiving CO₂ had a lower overall percent increase in VSS than the other ponds (Table 4-21).

Table 4-21: Maximum and minimum TSS/VSS values during the June 17, 2010 diel study. Maximum solids levels were recorded at either 2 PM or 6 PM, while minimums were recorded at either 6 AM or 10 AM. All solids values reported as mg/L.

	P1 - CO2		P3 - CO2		P2 - Control		P4 - Control	
	TSS	VSS	TSS	VSS	TSS	VSS	TSS	VSS
Maximum, mg/L	332	290	314	287	377	358	324	298
Minimum, mg/L	264	229	221	196	301	264	269	231
% Difference	25.8%	26.6%	42.1%	46.4%	25.2%	35.6%	20.4%	29.0%

Figure 4-45: TSS in the ponds during the June 17, 2010 diel study. TSS levels were highest during the warmest, sunniest portion of the day, while solids levels decreased as the ponds cooled at night and early morning.

Figure 4-46: VSS in the ponds during the June 17, 2010 diel study. Variability between maximum and minimum VSS was greater than the variability of TSS.

Diel TAN levels decreased as TSS and VSS levels increased during the afternoon (Figure 4-47). TAN removal was greatest during the mid to late afternoon, while removal was lowest during the early morning hours (Figure 4-48). The average influent TAN concentration during the diel study was 33 mg/L (Figure 4-49). Influent TAN levels were lowest during the nighttime when household water use decreases.

Figure 4-47: TAN levels in the ponds during the June 17, 2010 diel study. The average influent TAN concentration during the day was 33 mg/L.

Figure 4-48: TAN removal efficiencies in the ponds during the June 17, 2010 diel study.

Figure 4-49: Influent TAN during the June 17, 2010 diel study. Influent ammonia was lowest at nighttime hours. Peaks in influent ammonia were observed at 10 AM and 6 PM.

4.5.4.2 Second Diel Study: July 21, 2010

On July 21, 2010, the ponds were operating at a 3-day hydraulic residence time and Ponds 1 and 3 received CO₂ supplementation once pH 8.4 was reached.

CO₂-supplemented Ponds 1 and 3 reached maximum pH at 1 PM, when supplementation began. Carbon supplementation continued in Ponds 1 and 3 until 6 PM and 5 PM, respectively (4-5 hours of CO₂ supplementation). The pH during supplementation spanned between 8.4 and 8.5. Pond pH was lowest during the early morning hours. pH levels rose slowly during the morning hours between 6 AM and 10 AM, until the morning fog burned off and temperatures began rising. Unlike the first diel study, pH levels in the control ponds did not maintain levels 0.5 or greater than those observed in the CO₂-supplemented ponds (Figure 4-50).

Figure 4-50: Pond pH levels during the July 21 diel study. Ponds 1 and 3 reached a pH of 8.5 by 1 PM, and maintained carbon dioxide supplementation until 5 PM. Ponds 2 and 4 surpassed 8.5 shortly after 1 PM, eventually reaching maximum values of 8.69 and 8.84, respectively.

Dissolved oxygen levels were not as similar at peak sunlight conditions as during the first diel study (Figure 4-51). All ponds reached maximum dissolved oxygen concentrations at 3 PM. Pond 1, which received CO₂, had the highest maximum dissolved oxygen concentration. However, the other CO₂-supplemented pond did not attain a maximum dissolved oxygen concentration greater than the control ponds. The ponds maintained overnight dissolved oxygen concentrations above 1 mg/L.

Figure 4-51: Dissolved oxygen levels in the ponds during the July 21, 2010 diel study. Dissolved oxygen levels peaked at 3 PM in all ponds. Overnight lows were recorded at 2 AM the following morning. Pond 1 dissolved oxygen levels were higher than the other ponds.

Pond temperatures did not vary widely between any of the ponds (Figure 4-52). On average Ponds 1 and 2 were slightly warmer than Ponds 3 and 4. Ponds 1 and 2 averaged 18.5°C, respectively, while Ponds 3 and 4 averaged 18.3°C. The slight variation, similar to that from the first diel study, could have been due to slight differences in shading. The peak temperature of 23.6°C occurred at 4 PM, while the overnight low of 15.1°C occurred at 6 AM the following morning.

Figure 4-52: Pond temperature during the July 21, 2010 diel study. The maximum temperature in all ponds occurred at 4 PM. The minimum temperature was recorded at 6 AM the following morning.

Four sets of samples were taken throughout the day to measure TSS/VSS and total ammonia nitrogen. TSS and VSS were measured directly after retrieving the samples from the field, while 100 mL of each sample was acidified (to less than 2 pH) and saved for future ammonia testing.

Similar to the first diel study, TSS and VSS varied substantially throughout the day (Figure 4-53 and Figure 4-54). Pond 1, receiving CO₂, had a higher overall percent increase in VSS than the other ponds. However, the other pond receiving CO₂ had a lower overall percent increase in VSS than the other ponds (Table 4-22).

Table 4-22: Maximum and minimum TSS/VSS values during the July 21, 2010 diel study. Maximum solids levels were recorded at 6 PM, while minimums were recorded at 8 AM. All solids data reported in mg/L.

	P1 - CO2		P3 - CO2		P2 - Control		P4 - Control	
	TSS	VSS	TSS	VSS	TSS	VSS	TSS	VSS
Maximum, mg/L	267	239	223	195	237	207	253	217
Minimum, mg/L	173	148	165	151	168	152	191	162
% Difference	54.3%	61.5%	35.2%	29.1%	41.1%	36.2%	32.5%	34.0%

Figure 4-53: TSS concentrations in ponds during the July 21, 2010 diel study. TSS levels were highest during the warmest, sunniest portion of the day, while solids levels decreased as the ponds cooled at night and early morning.

Figure 4-54: VSS concentrations in ponds during the July 21, 2010 diel study. VSS variability was similar to that of TSS during the study.

Diel TAN levels decreased as TSS and VSS levels increased during the afternoon (Figure 4-55). TAN removal was greatest during the mid to late afternoon, while removal was lowest during the early morning hours (Figure 4-56). The average influent TAN concentration during the diel study was 24 mg/L (Figure 4-57). Influent TAN levels were lowest during the nighttime when household water use decreases.

Figure 4-55: Total ammonia nitrogen in ponds during the July 21, 2010 diel study.

Figure 4-56: TAN removal efficiency in ponds during the July 21, 2010 diel study.

Figure 4-57: Influent total ammonia nitrogen during the July 21, 2010 diel study. Influent ammonia was lowest in the morning and nighttime.

4.5.5 Discussion of Results – Experiment IV

Experiment IV produced the most comprehensive set of data of all the experiments. In addition to the usual water quality testing (TSS/VSS, TAN, alkalinity, NO_2^- , NO_3^-), several total Kjeldahl nitrogen analyses and two diel studies were completed.

The ponds were also operated at both a 4-day HRT and 3-day HRT. Lowering the hydraulic residence time was expected to reduce ammonia volatilization due to a lower contact time.

The average TSS and VSS concentrations were similar in both the ponds receiving CO_2 and control ponds during Experiment IV (Table 4-23). VSS concentrations dropped after switching the ponds to a 3-day HRT. CO_2 supplementation did not have any beneficial impact on algal growth.

Table 4-23: TSS and VSS data for the CO₂-supplemented and control ponds during Experiment IV. TSS and VSS concentrations decreased when the ponds were operated at a 3-day HRT, and carbon dioxide supplementation did not appear to have any beneficial impact on productivity.

	CO ₂ -supplemented Ponds		Control Ponds	
	TSS, mg/L	VSS, mg/L	TSS, mg/L	VSS, mg/L
4-day HRT	248	221	257	218
Standard Deviation	39.6	45.3	51.0	43.5
3-day HRT	213	193	218	194
Standard Deviation	44.4	40.6	29.1	27.9
% Reduction	14%	13%	15%	11%

Although VSS concentrations decreased after switching to a 3-day HRT, the productivity increased (Table 4-24).

Table 4-24: Biomass productivity data for the CO₂-supplemented and control ponds during Experiment IV. Productivity increased after switching from 4-d to 3-d.

	CO ₂ -supplemented Ponds		Control Ponds	
	4-d HRT	3-d HRT	4-d HRT	3-d HRT
Min. Productivity, g/m ² -d	11.7	13.5	11.6	11.7
Max. Productivity, g/m ² -d	23.3	21.2	23.1	23.7
Avg. Productivity, g/m ² -d	16.4	19.1	16.2	19.2
Standard Deviation	3.4	4.0	3.2	2.8

Total ammonia nitrogen removal efficiencies were similar in the ponds with and without CO₂ supplementation. Removal efficiency decreased after shifting the ponds to a 3-day HRT (Table 4-25). During the 4-day HRT, the average removal efficiency in the ponds was 91%. After achieving a 3-day HRT, total ammonia removal dropped approximately 10%, resulting in an average TAN removal of 81%. Similar to Experiment I, no

substantial difference in total ammonia was observed between the CO₂-supplemented and control ponds, even though the control ponds reached higher pH levels. A future ammonia experiment should include air sparging to mimic carbon dioxide injection and determine if the stripping effect was substantial.

The CO₂-supplemented ponds had higher average levels of nitrate than the control ponds (Table 4-25). During the 4-day and 3-day HRT, the CO₂-supplemented ponds measured 35% and 33% greater nitrate concentrations than the control ponds, respectively. The increase in nitrification indicates greater nitrifier bacteria presence in the CO₂-supplemented Ponds 1 and 3. Suppressed pond pH appeared to result in greater bacterial activity.

Table 4-25: Total ammonia, nitrate, and nitrite data for the CO₂-supplemented and control ponds during Experiment IV. Total ammonia removal efficiency was similar in all ponds. Ammonia removal decreased with the shorter residence time. The CO₂-supplemented ponds had higher nitrate levels, indicating increased nitrification.

	CO ₂ -supplemented Ponds		Control Ponds	
	4-d HRT	3-d HRT	4-d HRT	3-d HRT
NH _x -N, mg/L	4.1	6.9	4.6	6.3
NH _x -N Removal	91%	81%	90%	82%
NO ₂ ⁻ , mg/L	1.2	1.3	1.2	1.6
NO ₃ ⁻ , mg/L	6.0	5.4	3.9	3.6

In-depth nitrogen removal analysis was made available with the addition of TKN data. Values for total and organic nitrogen, as well as nitrogen assimilation into biomass and pond ammonia volatilization, were calculated by combining TAN, nitrate, nitrite, TKN, and VSS data. Organic nitrogen and VSS data were combined to produce biomass

nitrogen, while the TN differential between the influent wastewater and pond samples was used to determine ammonia volatilization.

No substantial changes in biomass nitrogen or ammonia volatilization were observed between the CO₂-supplemented and control ponds. Biomass nitrogen was similar in all ponds, averaging 10.9% during the 4-day HRT, and 11.1% during the 3-day HRT (Table 4-26). These percentages fall within the typical range for algae: 0.6-16%, with an average of 8% (Shilton 2005).

As expected, ammonia volatilization decreased when the residence time was reduced from four days to three days. During the 4-day HRT, the ponds averaged 32% ammonia volatilization, while during the 3-day HRT, the ponds averaged 15% ammonia volatilization (Table 4-26). Several factors may have contributed to the decrease in volatilization, including the decrease in productivity and the shorter residence time. With less algal productivity, the pH of the ponds did not rise as high as with the lower residence time. During the diel studies, the maximum pH in the control ponds during the 4-day HRT and 3-day HRT was 9.30 and 8.84, respectively. Additionally, the ponds maintained peak pH for five hours during the 4-day HRT, while the ponds maintained peak pH for three hours during the 3-day HRT. Since the control ponds maintained a higher pH, more ammonia gas was available for volatilization. Longer residence times give ammonia nitrogen more opportunity to volatilize as well. The fact that the control and CO₂-supplemented ponds experienced similar volatilization while having different pH levels indicates that carbon dioxide supplementation may produce an ammonia stripping effect, leading to similar volatilization.

Table 4-26: Biomass nitrogen and ammonia volatilization data during Experiment IV. The control ponds had slightly higher volatilization percentages. Biomass nitrogen was similar in both CO₂-supplemented and control ponds.

	CO ₂ -supplemented Ponds		Control Ponds	
	4-d HRT	3-d HRT	4-d HRT	3-d HRT
% N of Cell Mass	11.0%	10.6%	10.8%	11.6%
NH ₃ Volatilization	30.7%	14.6%	32.5%	15.2%

In practical application, the effect of stripping may be much greater. This experiment used tanks of pure carbon dioxide for supplementation. Proposed practical applications have included injecting flue gas, which has only between 3% and 15% in CO₂ content (Packer 2009), or higher if biogas is combusted. The throughput of flue gas would have to be approximately 7-15 times more than required using pure carbon dioxide. This would lead to an even greater stripping effect. Future research endeavors are needed to determine ammonia volatilization due to gas sparging.

4.6 Experiment V – Ammonia Volatilization

After completing Experiment IV, the ponds were drained and cleaned in preparation for an ammonia volatilization experiment. Three of the ponds were filled with tap water and predetermined concentrations of dehydrated ammonium chloride. The experiment consisted of four days of data collection at four pH points: 7.4, 8.0, 8.4, and 9.4. Each day, the ponds received amounts of ammonium chloride to begin experimentation at approximately 3, 10, and 17 mg/L in Ponds 1, 2 and 3, respectively (refer to Table 3-1 for experimental schedule). Samples were drawn every two hours from 10 AM to 6 PM and transported to Cal Poly for lab analysis. Total ammonia nitrogen was the only constituent analyzed in the lab.

During the ammonia volatilization experiment, pond pH and temperature were recorded at each sampling point. Total ammonia concentrations were gathered and a spreadsheet was used to calculate rates of TAN loss and mass transfer coefficients. The raw data are available for review in Appendix B.

The following equation was used to determine the mass transfer coefficient. dC/dt is the rate of TAN loss between two sample points (between 10 AM and 12 PM, for example). kLa is the mass transfer coefficient, which has units of days^{-1} . C_p is the concentration of ammonia in the pond at a given time. C_A^* is the concentration of dissolved ammonia gas in equilibrium with air, which was assumed zero.

$$\frac{dC}{dt} = kLa(C_p - C_A^*)$$

To determine volatilization rates, the change in TAN concentration was divided by the time period between the samples (converted into days). This process yielded a volatilization rate reported in mg/L-d . The mass transfer coefficient, kLa , was then calculated by dividing the volatilization rate by the average TAN concentration in the pond between sampling points.

TAN concentration, $\text{NH}_x\text{-N}$, was used in the equation rather than $\text{NH}_3\text{-N}$ concentration out of convenience. However, using $\text{NH}_3\text{-N}$ concentration is theoretically correct since NH_3 is the compound actually volatilizing.

The cumulative drop in TAN concentration was similar for each pH, regardless of the starting concentration. However, mass transfer coefficients for the ponds were not uniform and did not always match with expectations. For instance, calculated mass

transfer coefficients varied significantly between individual ponds during a single sampling period (Table 4-27).

Table 4-27: Overall mass transfer coefficient table for the ammonia volatilization experiment. Each value represents the kLa for a sampling period (10AM – 12PM for example).

	kLa, d^{-1} @ pH 8.0			kLa, d^{-1} @ pH 8.4			kLa, d^{-1} @ pH 9.4		
	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3
	3 mg/L	10 mg/L	17 mg/L	3 mg/L	10 mg/L	17 mg/L	3 mg/L	10 mg/L	17 mg/L
10:00 AM	0.04	0.37	0.14	0.54	0.67	0.19	0.22	1.45	0.56
12:00 PM	0.41	0.33	0.56	0.26	0.63	0.83	1.23	0.88	1.25
2:00 PM	0.59	0.42	0.27	0.77	0.00	0.67	2.34	1.67	1.58
4:00 PM	0.35	0.14	0.33	0.62	1.26	0.58	0.63	1.44	2.10
6:00 PM	0.09	0.09	0.11	--	--	--	0.54	0.45	0.75
9:30 AM									

The results of the ammonia experiment can be used to determine a rough estimate of ammonia volatilization throughout experimentation. Since the experiment was run in the field under natural conditions, the findings would not be valid for use in high-rate ponds that do not have similar geometry or operational procedures. Further, volatilization may be further affected by other constituents in the pond, such as total dissolved solids, other nitrogen species, and so on. Since the experiment was run with potable water, there was negligible biological activity, and it was assumed that all other water quality constituents were very low. During carbon dioxide supplementation in Experiments I and IV, the mechanical injection of gas may have functioned as a periodic stripping action. This would have made ammonia volatilization slightly higher than would be predicted in this experiment, as no gas injection occurred during Experiment V. If a similar experiment

were performed in the future, simulating CO₂ supplementation would be necessary to determine its impact on volatilization.

Since the data were rather sporadic and pond temperatures varied between test runs (mainly morning temperatures during the pH 8.0 run), rough estimates were developed to compare against the volatilization found in previous nitrogen balances (Table 4-15 through Table 4-18). For these estimates, kLa values from the ammonia volatilization study were combined with pH, temperature and total ammonia nitrogen data from the July 21, 2010 diel study. The estimated ammonia volatilization range developed using this rough method was then compared to the nitrogen losses (assumed to be volatilization) found in the nitrogen balance developed from data on samples collected July 19, 2010.

Determining kLa values for each sampling point of the July 21, 2010 diel study was difficult because of the lack of consistent data from the ammonia volatilization experiment. As expected, there was no correlation between TAN concentration and kLa value, so the main factors when choosing a kLa appropriate for a sampling point were pH and temperature. If a pH and temperature combination were similar to the conditions of an ammonia volatilization test point, the range of kLa values calculated for that range was used.

To make rough estimates of ammonia volatilization for points that did not have a similar pH to the tested pH levels of 8.0, 8.4 or 9.4, linear relationships were assumed between the three tested pHs. For each pH tested, the calculated kLa values were averaged for each sampling point (Table 4-28). A linear fit was assumed to produce a kLa value for a given pH.

Table 4-28: Average kLa values during the ammonia volatilization experiment. The data points from Pond 2 at 4 PM and 6 PM were omitted because the 0.00 kLa value was deemed to be an experimental error.

	pH 8.0			pH 8.4			pH 9.4		
	Temp, °C	kLa, d ⁻¹	Std Dev	Temp, °C	kLa, d ⁻¹	Std Dev	Temp, °C	kLa, d ⁻¹	Std Dev
10:00 AM	17.3			14.4			14.8		
		0.18	0.17		0.47	0.25		0.75	0.63
12:00 PM	20.9			17.7			18.1		
		0.43	0.12		0.57	0.29		1.12	0.21
2:00 PM	22.0			20.1			20.2		
		0.43	0.16		0.72	0.42		1.86	0.41
4:00 PM	21.6			21.4			20.7		
		0.27	0.12		0.60	0.38		1.39	0.74
6:00 PM	19.9			20.3			19.9		
		0.10	0.01		n/a	n/a		0.58	0.15
9:30 AM	13.9			n/a			14.2		

Using the kLa values for each sample point, the rate of volatilization was back calculated using the pond TAN concentration and length of the sampling period. Ammonia concentrations in the ponds were assumed to decrease or increase to the next sampling point in a linear fashion to produce intermediate results (TAN was only measured every 5 hours). To determine the percentage of ammonia volatilized, the mg/L equivalent of influent ammonia was compared to the sum of the calculated pond volatilization losses. The results are presented below (Table 4-29).

Table 4-29: Summary of ammonia volatilization analysis for the July 21, 2010 diel study. The average TAN and maximum pH values correspond to the data collected during the diel study. The estimated volatilization was determined using data from the ammonia volatilization experiment. A TKN analysis was conducted on July 19, 2010, along with other nitrogen-based tests, which enabled the calculation of a volatilization estimate for that sample day. The two values were compared since the diel study and TKN sample date were conducted in close proximity.

	Pond 1 (w/ CO ₂)	Pond 2 (control)	Pond 3 (w/ CO ₂)	Pond 4 (control)
Average TAN, mg/L	5.8	5.8	4.1	4.7
Max pH	8.50	8.69	8.54	8.84
Volatilization Range (estimate), %	13-23	16-26	10-17	16-25
Volatilization (N-balance), %	16	20	18	18

The estimated volatilization rate ranges bracketed the rates determined with the nitrogen balance in three of the four ponds. These results indicate that the ammonia volatilization percentages from the nitrogen mass balances are reasonable. However, the variability in kLa values during the ammonia volatilization experiment does not lend much confidence to the precision of these results.

Chapter 5: Conclusions

The main goals of this thesis project were to observe the effects of carbon dioxide supplementation and return activated sludge addition on the treatment efficiencies of algal high-rate ponds and the settleability of the algal-bacterial biomass produced. Approximately six months of data were taken for each of these pond operation strategies.

For the treatment of SLOWRF primary effluent, carbon dioxide supplementation provided no indications of increased algal productivity compared to the control ponds. Carbon limitation would still be expected when HRPs were used to achieve complete nutrient removal from already partially-treated effluent, for example, from an upstream HRP or conventional treatment process.

The addition of return activated sludge appeared to result in higher settling efficiency and increased ammonia removal efficiency, although there was considerable variability between both ponds receiving RAS.

Carbon dioxide supplementation may not have affected algal productivity if inorganic carbon levels were not a limiting factor. If the limiting growth factor was light, organic load, or nutrients (nitrogen or phosphorus), adding carbon dioxide would have no effect. In that case, carbon dioxide supplementation would actually become disadvantageous because pH would be capped at the CO₂ setpoint (in this case 8.4). Higher pH levels lead to more efficient pathogen inactivation, phosphorus precipitation, and odor control (Shilton 2005). Future research should include determination of the limiting growth factor of a given setup. Factors to consider include local climate, municipal wastewater water quality properties, and pond operational strategy.

Even though carbon dioxide supplementation did not increase algal growth, important questions were raised concerning ammonia volatilization due to both pond pH and CO₂ sparging. Assuming that total nitrogen losses were entirely attributable to volatilization to the atmosphere, both CO₂-supplemented and control ponds lost very similar amounts of nitrogen. The ammonia experiment conducted at the end of the project confirmed that ammonia volatilization increases with temperature and pH in HRP. The control ponds experienced pH levels between 8.9-9.8, while the experimental ponds maintained a pH between 8.4-8.5 because of carbon dioxide addition. Based on daily pH differences and the volatilization experiment results, the control ponds should have exhibited higher volatilization. Both the CO₂-supplemented and control ponds exhibited similar nitrogen loss, however. Gas sparging was not taken into account in terms of promoting volatilization. However, sparging could have made a large contribution to ammonia volatilization. The same ammonia experiment could be redesigned to include ponds with various flows of continuous gas sparging to determine the effect on ammonia volatilization.

Suppressed pH in the CO₂-supplemented ponds may have provided favorable conditions for nitrifying bacterial activity. During Experiment IV, the CO₂-supplemented ponds had average nitrate concentrations of 5.8 mg/L, compared to 3.8 mg/L in the control ponds.

The results of Experiment III indicated that return activated sludge can aid in bioflocculation and ammonia removal. The average 2-hour and 24-hour settling efficiencies for the RAS-inoculated ponds were 25% and 21% higher than the control ponds. On average, 24-hour settled effluent was less than 30 mg/L in the ponds receiving RAS. Heightened nitrate levels and reduced TAN concentrations in the ponds receiving

RAS indicated that nitrifier populations in the RAS were active once added to the pond. The total ammonia removal efficiencies in the ponds receiving RAS were 6-29% higher than the control ponds during Experiment III.

Water quality data for the influent wastewater was produced from samples taken between 10 and 11 AM on sampling days. Considering the daily variability in domestic wastewater quality, composite sampling would produce influent water quality data that better represents nutrient loading to the ponds.

In consideration of locations that might not use chemical disinfection of effluent, future research should consider evaluating disinfection efficiency between control ponds and CO₂-supplemented ponds. Disinfection is boosted by the high pH and oxygen content of ponds during sunny hours. The removal of bacterial pathogens has been shown to improve at pH levels above 9, which will occur in ponds without pH regulation (Shilton 2005). Carbon dioxide supplementation reduces pond pH and may decrease the destruction of pathogens.

Since zooplankton blooms can cause over-predation of algae in high-rate ponds, periodic characterization of pond biology is recommended. During the experiments, ostracods were routinely observed in the ponds, while *Daphnia* appeared once after a culture crash. Previous research found that ostracod populations did not substantially reduce algal productivity, while *Daphnia* greatly reduced algal populations (Diner 1986). Thus, it can be concluded that while ostracod populations should be monitored, the presence of *Daphnia* should prompt attempts to eliminate their presence from ponds.

Periodic crashes were also an issue throughout experimentation, as the start and stop dates for each experiment were driven by decreases in biomass productivity. The ponds

also had to be stopped and remixed after a crash during Experiment III. Possible factors contributing to pond crashes include zooplankton blooms, infection by fungi, and toxins in the incoming wastewater. Although one crash did coincide with the presence of *Daphnia*, more in-depth analysis is needed in characterizing and monitoring zooplankton in ponds. The relative small size of the ponds used in this experiment may have made them more susceptible to crashing. Larger ponds may be able to withstand certain levels of contaminants better than small ponds. These considerations should be made in developing future research plans.

Pond performance was highly variable in the two control and two experimental ponds throughout the research project. Biological systems that are open to the atmosphere are harder to analyze than closed laboratory systems due to the uncontrollable nature of wastewater feed and the surrounding environment. These uncontrollable variables result in the need for greater numbers of replicate ponds, which would also support statistical hypothesis testing.

The following are a summary of the main conclusions drawn from this research effort:

- Carbon dioxide supplementation did not consistently increase productivity or nutrient removal, presumably because there was sufficient inorganic carbon in the primary clarifier effluent that was the pond influent. Future research should focus on implementing pond effluent clarification, followed by additional algae growth. The second crop of algae would be expected to require CO₂ supplementation in order to decrease nutrients concentrations to low levels.

- The daily addition of return activated sludge produced pond effluent that settled more efficiently than effluents from control ponds. However, settling efficiencies were variable throughout experimentation. Ammonia removal efficiency was similar in two control ponds and one pond receiving RAS. The remaining pond receiving RAS had much higher ammonia removal efficiencies. Further research is needed to determine an optimal RAS-inoculation system that provides reproducible results and consistent effluent settling.
- A better understanding behind the causes of pond crashes and development of potential remedies should be a point of emphasis in future research. Monitoring zooplankton more closely and devoting resources to a more thorough investigation of pond microbiology may provide some insights into crash mechanisms.

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Appendix A: Supplemental Data

Zooplankton count data for the entire project. During Experiment III, Ponds 1 & 2 received RAS, and during Experiment IV, Ponds 1 & 3 received CO₂. *Daphnia* were observed in the ponds but did not show up on sampling days with zooplankton counts.

Date	Ostracods, organisms/L				Rotifers, organisms/L				Stalked Ciliates, organisms/L				Experiment Number
	Pond 1	Pond 2	Pond 3	Pond 4	Pond 1	Pond 2	Pond 3	Pond 4	Pond 1	Pond 2	Pond 3	Pond 4	
2009-10-23	750	490	930	100	1340	1430	2650	720	0	0	0	0	III - Seg 1
2009-10-29	0	100	0	0	760	0	200	0	0	0	0	0	III - Seg 1
2009-11-04	150	0	0	0	1650	0	250	200	0	0	0	0	III - Seg 1
2009-11-10	100	50	0	0	2600	990	200	770	0	0	0	0	III - Seg 1
2009-11-17	400	0	0	0	3490	250	100	200	0	0	0	0	III - Seg 1
2009-11-19	200	0	0	0	3290	400	50	350	0	0	0	0	III - Seg 1
2009-12-03	850	0	0	0	2770	390	0	0	0	0	0	0	III - Seg 1
2009-12-16	980	50	0	150	1180	400	300	100	0	0	0	0	III - Seg 1
2010-01-14	450	300	0	0	100	1000	50	2680	0	0	0	0	III - Seg 2
2010-01-28	100	250	400	300	0	150	300	400	0	0	0	0	III - Seg 2
2010-02-04	50	350	580	0	0	600	250	100	0	0	0	0	III - Seg 2
2010-03-04	200	0	0	0	0	0	0	0	200	350	0	0	III - Seg 2
2010-04-16	2500	2300	900	400	500	0	200	0	500	1800	0	0	III - Seg 3
2010-04-23	600	300	200	300	100	0	100	0	0	370	200	0	III - Seg 3
2010-04-26	200	300	400	300	300	200	100	0	1800	290	0	0	III - Seg 3
2010-05-03	50	530	780	500	0	500	150	0	0	900	0	0	III - Seg 3
2010-05-10	50	100	350	540	0	0	50	100	0	250	0	0	III - Seg 3
2010-05-24	150	620	420	250	300	0	430	400	0	0	0	0	IV - 4d HRT
2010-06-14	900	600	1300	350	250	0	600	150	0	0	0	0	IV - 4d HRT
2010-07-28	1570	1690	750	1970	0	0	150	50	0	0	0	0	IV - 4d HRT
2010-09-08	50	200	730	150	580	4210	800	250	0	0	0	0	IV - 4d HRT

Volatile suspended solids data for the entire project. Experiment I: control Ponds 2 & 4, experimental Ponds 1 & 3 received CO₂ ; Experiment III: control Ponds 3 & 4, experimental Ponds 1 & 2 received RAS ; Experiment IV: control Ponds 2 & 4, experimental Ponds 1 & 3 received CO₂. Insolation is reported in units of W/m².

Appendix B: Experiment V Raw Data

Ammonia Volatilization Experiment Data at pH 8.0

Pond 1	T, °C	% NH ₃	NH _x -N, mg/L	Total % Drop	Rate, mg/L-d	kLa, d ⁻¹
10:00 AM	17.3	3.1%	3.02	--	--	--
12:00 PM	20.9	4.1%	3.01	0.3%	0.12	0.04
2:00 PM	22.0	4.4%	2.91	3.6%	1.20	0.41
4:00 PM	21.6	4.3%	2.77	8.3%	1.68	0.59
6:00 PM	19.9	3.8%	2.69	10.9%	0.96	0.35
9:30 AM	13.9	2.5%	2.53	16.2%	0.25	0.09

Pond 2	T, °C	% NH ₃	NH _x -N, mg/L	Total % Drop	Rate, mg/L-d	kLa, d ⁻¹
10:00 AM	17.3	3.1%	10.50	--	--	--
12:00 PM	20.9	4.1%	10.18	3.0%	3.84	0.37
2:00 PM	22.0	4.4%	9.90	5.7%	3.32	0.33
4:00 PM	21.6	4.3%	9.56	8.9%	4.08	0.42
6:00 PM	19.9	3.8%	9.45	10.0%	1.33	0.14
9:30 AM	13.9	2.5%	8.92	15.1%	0.83	0.09

Pond 3	T, °C	% NH ₃	NH _x -N, mg/L	Total % Drop	Rate, mg/L-d	kLa, d ⁻¹
10:00 AM	17.3	3.1%	16.99	--	--	--
12:00 PM	20.9	4.1%	16.79	1.2%	2.40	0.14
2:00 PM	22.0	4.4%	16.02	5.7%	9.24	0.56
4:00 PM	21.6	4.3%	15.66	7.8%	4.32	0.27
6:00 PM	19.9	3.8%	15.24	10.3%	5.04	0.33
9:30 AM	13.9	2.5%	14.21	16.4%	1.59	0.11

Ammonia Volatilization Experiment Data at pH 8.4

Pond 1	T, °C	% NH ₃	NH _x -N, mg/L	Total % Drop	Rate, mg/L-d	kLa, d ⁻¹
10:00 AM	14.4	6.2%	2.94	--	--	--
12:00 PM	17.7	7.8%	2.81	4.4%	1.56	0.54
2:00 PM	20.1	9.1%	2.75	6.5%	0.72	0.26
4:00 PM	21.4	9.9%	2.58	12.2%	2.04	0.77
6:00 PM	20.3	9.2%	2.45	16.7%	1.56	0.62
9:30 AM	n/a	n/a	n/a	n/a	n/a	n/a

Pond 2	T, °C	% NH ₃	NH _x -N, mg/L	Total % Drop	Rate, mg/L-d	kLa, d ⁻¹
10:00 AM	14.4	6.2%	9.35	--	--	--
12:00 PM	17.7	7.8%	8.84	5.5%	6.12	0.67
2:00 PM	20.1	9.1%	8.39	10.3%	5.40	0.63
4:00 PM	21.4	9.9%	8.39	10.3%	0.00	0.00
6:00 PM	20.3	9.2%	7.55	19.3%	10.08	1.26
9:30 AM	n/a	n/a	n/a	n/a	n/a	n/a

Pond 3	T, °C	% NH ₃	NH _x -N, mg/L	Total % Drop	Rate, mg/L-d	kLa, d ⁻¹
10:00 AM	14.4	6.2%	17.55	--	--	--
12:00 PM	17.7	7.8%	17.27	1.6%	3.36	0.19
2:00 PM	20.1	9.1%	16.12	8.1%	13.80	0.83
4:00 PM	21.4	9.9%	15.24	13.2%	10.56	0.67
6:00 PM	20.3	9.2%	14.52	17.3%	8.64	0.58
9:30 AM	n/a	n/a	n/a	n/a	n/a	n/a

Ammonia Volatilization Experiment Data at pH 9.4

Pond 1	T, °C	% NH ₃	NH _x -N, mg/L	Total % Drop	Rate, mg/L-d	kLa, d ⁻¹
10:00 AM	14.8	40.4%	3.24	--	--	--
12:00 PM	18.1	46.4%	3.18	1.9%	0.72	0.22
2:00 PM	20.2	50.3%	2.87	11.4%	3.72	1.23
4:00 PM	20.7	51.2%	2.36	27.2%	6.12	2.34
6:00 PM	19.9	49.7%	2.24	30.9%	1.44	0.63
9:30 AM	14.2	39.3%	1.58	51.2%	1.02	0.54

Pond 2	T, °C	% NH ₃	NH _x -N, mg/L	Total % Drop	Rate, mg/L-d	kLa, d ⁻¹
10:00 AM	14.8	40.4%	12.12	--	--	--
12:00 PM	18.1	46.4%	10.74	11.4%	16.56	1.45
2:00 PM	20.2	50.3%	9.98	17.7%	9.12	0.88
4:00 PM	20.7	51.2%	8.68	28.4%	15.60	1.67
6:00 PM	19.9	49.7%	7.70	36.5%	11.76	1.44
9:30 AM	14.2	39.3%	5.73	52.7%	3.05	0.45

Pond 3	T, °C	% NH ₃	NH _x -N, mg/L	Total % Drop	Rate, mg/L-d	kLa, d ⁻¹
10:00 AM	14.8	40.4%	19.01	--	--	--
12:00 PM	18.1	46.4%	18.14	4.6%	10.44	0.56
2:00 PM	20.2	50.3%	16.34	14.0%	21.60	1.25
4:00 PM	20.7	51.2%	14.32	24.7%	24.24	1.58
6:00 PM	19.9	49.7%	12.02	36.8%	27.60	2.10
9:30 AM	14.2	39.3%	7.35	61.3%	7.23	0.75

Appendix C: Zooplankton Counting Procedure

Zooplankton Counting Procedure

Equipment:

- Pond sample
- 10-mL graduated cylinder
- 4 chambered petri dish
- Dissecting scope
- Glycerol/protoslo
- Transfer pipettes
- Clicker-counter
- Rose Bengal (optional)

Procedure:

- Pour 10-mL of pond sample into filter apparatus with 100- μ m screen.
 - Do this at an angle as to concentrate the zooplankton in one area.
- Invert the apparatus so that the screen is facing towards you and place the angled part into a petri dish with four chambers.
- Pour 10-mL of DI/RO water onto the backside of the screen to release organisms.
- Repeat procedure for a duplicate sample.
 - Pour second sample into chamber directly across from previous sample.
- Place petri dish underneath dissecting microscope.
- (Place one drop of Rose Bengal dye into the chamber right before counting.)
- Depending on the vitality of the zooplankton place between 1-3 drops of glycerol into the chamber and mix.
 - Get glycerol/protoslo from solvents cabinet.
 - Using a transfer pipette, place a few drops on a piece of parafilm.
 - Using a separate transfer pipette place the drops of glycerol into the chamber: if 1, place it in the middle, if 2, place them opposite each other, if 3, place them in the corners.
 - More glycerol than this tends to terminate the zooplankton vitality.
- Use the clicker-counter to count the number of ostracods in the sample.
 - Method 1: place the chamber in full view and count from corner-to-corner.
 - Method 2: focus in on the chamber and move the plate to count from corner-to-corner.
 - Do your best to not double count individuals.
- Repeat 5 times for accuracy and take the average.
- Repeat procedure for rotifers.
- Repeat for all ponds.

Appendix D: RAS Data

TSS concentration of RAS throughout Experiment III. The 3.78 L daily addition of RAS was converted to an equivalent mg/L TSS of RAS in the ponds.

Nitrite-N, nitrate-N and TAN concentrations of RAS throughout Experiment III. The 3.78 L daily addition of RAS was converted to an equivalent mg/L N in the ponds for each nitrogen species.

Appendix E: Daily pH and DO Log

Pond pH and DO Log

Date	Time	pH				DO (mg/L)				EXP	KEY
		P1	P2	P3	P4	P1	P2	P3	P4		
2009-08-19	11:15 AM	8.68	9.05	8.53	8.68					I	CO2
2009-08-20	1:00 PM	9.03	9.45	8.58	9.03					I	Control
2009-08-21	12:45 PM	8.80	9.22	8.60	8.80					I	RAS
2009-08-22	2:20 PM	9.18	9.54	8.40	9.18					I	
2009-08-24	11:00 AM	8.95	8.98	8.50	8.95					I	
2009-08-26	3:00 PM	9.08	9.55	8.49	9.08					I	
2009-08-31	2:20 PM	9.87	9.63	8.27	9.87					I	
2009-09-01	10:30 AM	9.38	9.16	8.39	9.38					I	
2009-09-07	1:50 PM	9.47	9.62	8.21	9.47					I	
2009-09-09	11:30 AM	9.20	9.05	8.31	9.20					I	
2009-09-10	11:30 AM	9.47	9.14	8.27	9.47					I	
2009-09-14	10:00 AM	8.91	8.69	7.60	8.91					I	
2009-09-19	1:00 PM	9.23	9.08	8.06	9.23					I	
2009-09-21	10:30 AM	8.58	8.42	7.94	8.58					I	
2009-09-23	10:30 AM	8.75	8.65	8.09	8.75					I	
2009-09-24	11:20 AM	9.05	8.91	8.39	9.05					I	
2009-09-25	12:30 PM	9.24	9.09	8.58	9.24					I	
2009-09-28	11:15 AM	8.34	8.35	8.28	8.34					I	
2009-10-01	10:00 AM	8.15	8.32	8.15	8.15					I	
2009-10-05	12:30 PM	8.55	8.75	8.65	8.55					I	
2009-10-06	12:00 PM	8.64	8.98	8.66	8.64					II	
2009-10-07	12:10 PM	8.63	9.01	8.66	8.63					II	
2009-10-10	12:10 PM	9.26	8.73	8.65	9.26					II	
2009-10-12	11:20 AM	8.45	8.88	8.18	8.45					II	
2009-10-14	12:20 PM	8.72	9.14	8.73	8.72					II	
2009-10-19	11:25 AM	8.25	8.11	8.67	8.25					II	
2009-10-26	8:25 AM	7.64	7.62	7.63	7.64					II	
2009-10-28	12:00 PM	8.14	8.11	8.15	8.14					III	
2009-10-29	10:00 AM	8.03	8.03	8.05	8.03					III	
2009-10-31	12:00 PM	8.26	8.25	8.25	8.26					III	
2009-11-02	8:20 AM	8.00	8.00	7.99	8.00					III	
2009-11-04	12:00 PM	8.24	8.22	8.20	8.24					III	
2009-11-09	9:15 AM	8.08	7.98	8.09	8.08					III	
2009-11-11	11:50 AM	8.46	8.42	8.46	8.46					III	
2009-11-12	10:00 AM	8.11	8.12	8.11	8.11					III	
2009-11-14	12:30 PM	8.55	8.50	8.45	8.55					III	
2009-11-16	11:58 AM	8.49	8.43	8.36	8.49					III	
2009-11-18	11:41 AM	8.15	8.51	8.30	8.45					III	
2009-11-19	9:00 AM	7.66	7.97	8.01	8.05					III	
2009-11-21	12:15 PM	8.31	8.56	8.38	8.43					III	

Date	Time	pH				DO (mg/L)				EXP	KEY
		P1	P2	P3	P4	P1	P2	P3	P4		
2009-11-22	12:10 PM	8.27	8.50	8.32	8.38					III	CO2
2009-11-23	11:55 PM	8.01	8.24	8.21	8.22					III	Control
2009-11-24	9:10 AM	7.70	7.92	7.90	7.90					III	RAS
2009-11-30	12:20 PM	8.17	8.30	8.17	8.42					III	
2009-12-02	11:35 AM	7.92	8.09	8.29	8.30					III	
2009-12-06	12:15 PM	7.77	8.61	8.13	8.30					III	
2009-12-08	12:15 PM	7.90	7.97	8.08	8.17					III	
2009-12-09	12:15 PM	7.79	7.93	8.05	8.11					III	
2009-12-11	12:15 PM	7.43	7.57	7.76	7.84					III	
2009-12-12	12:15 PM	7.27	7.67	8.24	8.50					III	
2009-12-15	12:00 PM	7.59	7.69	8.11	8.44					III	
2009-12-16	12:00 PM	7.42	7.42	7.64	7.66					III	
2009-12-20	12:20 PM	7.74	7.87	8.00	8.19					III	
2010-01-15	11:00 AM	8.00	8.07	8.15	8.25					III	
2010-01-23	12:50 PM	8.00	8.35	8.40	8.37					III	
2010-01-28	9:30 AM	7.46	7.47	7.50	7.32					III	
2010-02-03	10:45 AM	7.72	7.88	8.15	7.80					III	
2010-02-04	9:00 AM	7.46	7.47	7.68	8.06					III	
2010-02-11	9:05 AM	7.29	7.30	7.43	7.71					III	
2010-02-16	11:00 AM	7.78	7.76	7.84	7.51					III	
2010-02-17	10:00 AM	7.96	7.74	7.79	7.93					III	
2010-02-18	9:00 AM	7.43	7.47	7.54	7.90					III	
2010-02-20	9:30 AM	7.67	7.70	7.78	7.58					III	
2010-02-22	9:30 AM	7.65	7.65	7.73	7.73					III	
2010-02-26	10:30 AM	7.77	7.73	7.76	7.85					III	
2010-02-27	11:00 AM	7.99	7.82	7.83	7.98					III	
2010-03-01	9:00 AM	7.62	7.55	7.62	7.70					III	
2010-03-03	11:00 AM	8.03	7.91	8.09	8.26					III	
2010-03-04	9:15 AM	7.82	7.84	7.96	8.03					III	
2010-03-06	10:00 AM	8.00	7.98	8.30	8.61					III	
2010-03-08	9:00 AM	7.68	7.82	8.00	8.21					III	
2010-03-10	11:00 AM	7.63	7.94	8.27	8.57					III	
2010-03-11	9:00 AM	7.59	7.73	7.98	8.24					III	
2010-03-13	11:00 AM	7.87	8.23	8.49	8.64					III	
2010-03-15	8:30 AM	7.52	7.57	7.85	7.86					III	
2010-04-01	9:40 AM	7.57	7.87	7.61	7.61					III	
2010-04-02	9:00 AM	7.58	7.89	7.63	7.60					III	
2010-04-03	11:30 AM	7.71	8.37	7.95	7.92					III	
2010-04-05	9:00 AM	7.75	8.01	7.85	7.81					III	
2010-04-07	12:40 PM	7.75	8.46	8.28	7.92					III	
2010-04-08	1:30 PM	7.67	8.29	8.31	7.87	6.3	11.7	13.7	6.7	III	
2010-04-09	9:15 AM	7.41	7.46	7.51	7.51					III	

Date	Time	pH				DO (mg/L)				EXP	KEY
		P1	P2	P3	P4	P1	P2	P3	P4		
2010-04-10	1:00 PM	7.44	7.64	7.80	7.66	2.7	3.5	5.8	3.0	III	CO2
2010-04-13	12:00 PM	7.58	7.55	7.63	7.56	7.0	5.1	6.3	4.5	III	Control
2010-04-14	10:00 AM	7.37	7.43	7.48	7.45	3.5	3.5	4.0	3.3	III	RAS
2010-04-16	9:30 AM	7.28	7.35	7.42	7.42	2.8	2.9	3.5	2.8	III	
2010-04-17	9:00 AM	7.32	7.37	7.41	7.37	3.7	3.4	3.8	3.1	III	
2010-04-19	9:30 AM					7.8	3.8	3.8	2.7	III	
2010-04-21	11:30 AM					9.8	6.3	7.1	6.5	III	
2010-04-22	9:45 AM					7.0	5.8	5.6	5.3	III	
2010-04-23	9:45 AM					7.3	5.6	6.0	5.5	III	
2010-04-26	9:30 AM					5.2	4.4	6.7	6.3	III	
2010-04-28	2:30 PM					8.0	5.9	10.6	8.8	III	
2010-04-29	9:45 AM					9.9	9.9	10.7	9.8	III	
2010-05-02	3:30 PM					8.3	7.7	11.8	10.4	III	
2010-05-03	9:30 AM					7.5	7.6	9.9	9.3	III	
2010-05-04	10:00 AM					8.1	8.2	10.4	9.7	III	
2010-05-07	11:00 AM					17.4	12.8	15.4	14.0	III	
2010-05-08	8:45 AM					11.4	9.8	11.3	11.0	III	
2010-05-11	10:15 AM					13.7	10.3	14.8	14.6	III	
2010-05-18	10:00 AM					8.1	6.8	11.2	11.4	IV	
2010-05-22	10:30 AM	8.31	8.33	8.24	8.27					IV	
2010-05-24	10:00 AM	8.18	8.20	8.12	8.20	12.6	11.7	11.8	12.0	IV	
2010-05-25	10:30 AM	8.46	8.43	8.39	8.49	14.2	13.5	13.8	14.3	IV	
2010-05-26	1:00 PM	8.44	8.80	8.44	8.92	20.0	19.4	20.0	20.0	IV	
2010-05-28	9:30 AM	8.32	8.39	8.33	8.41					IV	
2010-05-29	10:30 AM	8.56	8.71	8.50	8.69					IV	
2010-06-01	1:30 PM	9.09	9.09	9.11	9.08	18.2	17.8	17.9	17.1	IV	
2010-06-02	9:30 AM	8.43	8.46	8.42	8.45	14.0	14.1	13.9	13.9	IV	
2010-06-04	10:00 AM	8.59	8.62	8.40	8.64	14.9	14.3	14.9	14.6	IV	
2010-06-22	10:30 AM	8.41	8.63	8.45	8.60	14.5	15.9	15.1	15.4	IV	
2010-07-03	9:45 AM	7.28	8.04	7.92	8.12	9.1	10.8	11.7	11.7	IV	
2010-07-07	10:55 AM	8.11	8.11	8.22	8.13	8.8	8.8	10.2	8.6	IV	
2010-07-13	9:30 AM	8.28	8.10	8.20	8.14	14.0	11.4	12.7	11.7	IV	
2010-07-24	2:30 PM	8.36	8.61	8.52	8.73	18.0	15.6	13.8	17.4	IV	
2010-07-25	9:15 AM	7.09	7.73	7.67	7.80					IV	
2010-07-28	9:30 AM	7.93	7.97	7.99	8.17	10.8	10.2	11.0	11.2	IV	
2010-08-01	9:30 AM	7.79	8.03	8.08	8.24					IV	
2010-08-05	9:30 AM	7.68	7.78	7.78	7.89	7.8	7.6	8.1	7.5	IV	
2010-08-06	9:30 AM	7.74	7.81	7.82	7.94					IV	
2010-08-07	9:30 AM	7.71	7.85	7.90	7.99	7.8	7.4	7.6	7.4	IV	
2010-08-08	2:30 PM	8.50	8.75	8.20	8.83					IV	
2010-08-11	9:30 AM	7.92	8.07	8.03	8.20	10.2	10.6	11.6	11.0	IV	
2010-08-12	10:00 AM	7.94	8.14	8.11	8.29	10.4	11.7	12.9	12.4	IV	

Date	Time	pH				DO (mg/L)				EXP	KEY
		P1	P2	P3	P4	P1	P2	P3	P4		
2010-08-13	12:30 PM	8.46	8.79	8.04	8.93	18.2	20.0	20.0	20.0	IV	CO2
2010-08-14	9:15 AM	7.67	7.67	7.81	8.12	8.5	8.5	11.1	10.1	IV	Control
2010-08-15	3:30 PM	8.39	9.05	8.36	9.18					IV	RAS
2010-08-17	9:45 AM	7.92	8.15	8.19	8.37	11.6	12.5	13.5	13.9	IV	
2010-08-18	9:00 AM	7.77	7.98	7.91	8.23	7.6	9.8	10.2	11.0	IV	
2010-08-19	9:40 AM	7.85	8.04	8.01	8.29					IV	
2010-08-20	12:10 PM	8.52	8.67	8.44	8.88					IV	
2010-08-21	10:30 AM	8.20	8.18	8.21	8.43					IV	
2010-08-22	3:45 PM	8.32	8.97	8.43	9.28					IV	
2010-08-24	3:15 PM	9.42	9.05	8.57	9.26					IV	
2010-08-26	8:50 AM	7.90	7.98	7.88	8.13					IV	
2010-08-27	12:45 PM	8.29	8.72	8.19	8.81					IV	
2010-08-28	2:30 PM	8.60	8.95	8.51	8.96					IV	
2010-08-30	8:40 AM	7.84	7.98	7.94	7.99					IV	

Appendix F: Pond Micrographs

Algal-bacterial floc in Pond 1 (receiving RAS) on 12-21-2009, viewed under a microscope at 400x.

Algal-bacterial floc in Pond 1 (receiving RAS) on 12-21-2009, viewed under a microscope at 400x.

Algal-bacterial floc in Pond 2 (receiving RAS) on 12-21-2009, viewed under a microscope at 400x.

Algal-bacterial floc in Pond 2 (receiving RAS) on 12-21-2009, viewed under a microscope at 400x.

Algal-bacterial floc in Pond 3 (not receiving RAS) on 12-21-2009, viewed under a microscope at 400x.

Algal-bacterial floc in Pond 3 (not receiving RAS) on 12-21-2009, viewed under a microscope at 400x.

Algal floc in Pond 4 (not receiving RAS) on 12-21-2009, viewed under a microscope at 400x.

Algal-bacterial floc in Pond 4 (not receiving RAS) on 12-21-2009, viewed under a microscope at 400x.