ASSESSMENT OF THE SIMULTANEOUS CARBON, NITROGEN, AND

PHOSPHORUS REMOVAL POTENTIAL OF ADVANCED AERATED

SUBMERGED BIOFILM REACTORS (POO-GLOOS)

TREATING MUNICIPAL WASTEWATER

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

Established characteristics of aerated submerged biofilm reactors (ASBRs)

include sustenance of multiple microclimates within the system, high biomass

accumulation, and highly diverse bacterial population. Besides presenting

important advantages over the traditional use of suspended growth activated sludge

systems, these properties also make ASBRs a more suitable environment for the

achievement of simultaneous carbon, nitrogen and phosphorus removal from

sewage. By incorporating air cycling into their operation, simultaneous carbon and

nutrient removal employing ASBRs has been well established and documented. Air-

on and air-off intervals promote the coexistence of aerobic, anoxic and anaerobic

zones within the system, allowing the concurrent biological metabolization of

carbon, nitrogen, and phosphorus compounds.

This research assessed the simultaneous carbon and nutrient removal

potential of specially designed structures treating primary clarified municipal

wastewater effluent at low temperatures. For this, two pilot-scale bioreactors were

constructed and operated during 115. One bioreactor held a series of six dome

shaped aerated submerged biofilm devices, called Poo-Gloos, while the second

bioreactor held a series of six aeration bases, intended to emulate a controlling

suspended growth process. With both bioreactors receiving the exact same influent

wastewater constitution and flow rate, and with operational variables adjusted equally to both reactors on a weekly basis, a quantitative, qualitative and comparative analysis of the nutrient removal capacity of the two systems was performed.

In terms of COD removal, average weekly percentage removals of up to 77±5% and as low as 50±5% were achieved by the Poo-Gloo system under air cycling conditions. In contrast, the control system exhibited an average weekly removal percentage range between 8±8% and 39±6%. In terms of total nitrogen (TN) removal, a consistent increase in average weekly removal percentages from 42±6% to 47±3%, and to 49±4% was observed in the case of the Poo-Gloo system conforming air-off periods were increased from 2 hours to 3 hours, and to 4 hours, respectively. In contrast, the control system exhibited an erratic behavior under air cycling conditions achieving weekly percentage removals in the range between -7±13% and 14±5%. Finally, in terms of total phosphorus (TP) removal, an optimum air cycling composition of 21 hours on/3 hours off was observed, allowing for the largest average weekly TP percentage removal achieved, 22±4%. Meanwhile, the control system accomplished an average weekly removal percentage of only 0±6% under the same air cycling conditions.

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CHAPTER I

INTRODUCTION

Nutrients in Wastewater

Since its introduction in the early 20th century, biological treatment has evolved into the most reliable method for secondary and advanced treatment of sewage. Standard wastewater operations have proven microorganisms to be extremely useful and effective for treating sewage, while intensive research has identified the exact metabolic pathways through which microorganisms consume different wastewater constituents. As more protective regulations and higher requirements for water quality have been introduced, biological treatment has evolved accordingly. Many configurations have emerged in order to provide microorganisms with optimum conditions for the metabolism, or biodegradation, of specific contaminants found in the waste stream. However, rapid population growth, intensified water consumption, reduced freshwater availability, and increased contamination of water bodies continue to challenge the technology and demand for more efficient and economical ways to remediate wastewater.

Among contaminants found in municipal sewage, nitrogen and phosphorus compounds, generally referred to as nutrients, are of great concern. When

discharged in high concentrations, nutrients pose a well-documented threat to surface waters known as eutrophication (Bouwman, Van Vuuren, Derwent & Posch, 2002; de Jonge, Elliot & Orive, 2002; Smith, 2003), which is the increase in productivity of a water body due to intensified input of inorganic nutrients. Eutrophication can lead to exhaustion of oxygen levels in the aquatic environment as intrusive algae and phytoplankton consume dissolved oxygen (DO) through respiration. Along with oxygen depletion, other symptoms of eutrophication include marked shifts in food-web structure including possible extirpations of some fish species and other organisms, taste and odor problems, obstruction of sunlight to light-dependent submerged aquatic vegetation, changes in the natural composition of the aquatic biota, escalated oxygen demand as bacteria growth becomes fostered by the abundance of decaying organisms, as well as many other problems (Smol, 2008; USEPA, 2009). Massive nutrient discharge persists from sources such as urban stormwater runoff, food processing and dairy wastes, leaching of agricultural and landscape fertilizers, and treated or untreated sewage discharge. Despite the fact that control of eutrophication has extensively been targeted by research institutions and government agencies for the last 5 decades, it continuous to rank at the top of the water quality hardships in the United States.

Other concerns, apart from eutrophication, can emerge from the presence of nutrients in wastewater effluents. Nitrogen in the form of ammonia is known to be toxic, even in fairly low to moderate concentrations, to a variety of aquatic fauna which includes fish, amphibians, and invertebrates such as unionid mussels, whose alarming decline in population has recently been related to the ammonia content of

inland waters (Metcalf & Eddy, 2003; Halling- Sørensen & Jørgensen, 1993; USEPA, 1993; USEPA, 2009). Biological oxidation of ammonia to nitrite and nitrate imposes an oxygen demand load of up to 4.57 g O_2/g NH_4-N which contributes to the reduction of dissolved oxygen levels in rivers and estuaries (Rittmann & McCarty, 2001; Sawyer, McCarty & Parkin, 2003). Nitrate concentrations greater than 10 mg NO_3-N/L can be fatal to infants under 6 months of age as methemoglobinemia, commonly known as "blue baby syndrome," is induced. This condition restricts the oxygen-carrying capacity of hemoglobin and can finally lead to infant suffocation (Halling- Sørensen & Jørgensen, 1993; Kapoor & Viraghavan, 1997). Finally, different types of cancer in humans and animals have been associated with the excessive ingestion of N-nitroso compounds. Epidemiological evidence shows an important connection between high nitrate ingestion and gastric cancer (USEPA, 1993; Walsh & Wright, 1995).

The Utah Department of Environmental Quality, Division of Water Quality, recently performed a "Statewide Nutrient Removal Cost Impact Study" (2010). In this analysis, the technical and economic requirements for upgrading 30 mechanical and 22 lagoon-based public owned treatment works (POTWs) to achieve a range of increasingly stringent discharge standards for the nutrients nitrogen and phosphorus were determined. Effluent nutrient discharge standards considered in the study include Tier 1N, with limits of 0.1 mg/L for total phosphorus (TP), and 10 mg/L for total nitrogen (TN); Tier 1, 0.1 mg/L for TP, no limit for TN; Tier 2N, 1.0 mg/L for TP, 20 mg/L for TN; and Tier 2, 1.0 mg/L for TP, no limit for TN. Economic requirements were reported in terms of net present value (NPV) as a function of

estimated capital, operation and maintenance cost. This way, NPVs of $114, $233, $1,089 and $1,352 million were reported in the case of all mechanical POTWs for tiers 2, 2N, 1 and 1N, respectively. In the case of discharging lagoon systems, NPVs of $31.1, $242, $161 and $387 million for the respective tiers 2, 2N, 1 and 1N, were reported.

At the national level, in compliance with the Clean Water Act section 516(b)(1)(B), the United States Environmental Protection Agency (USEPA) has to prepare and present to the congress a Clean Watershed Needs Survey Report every 4 years. In its 2008 version, USEPA estimates the country's overall water quality needs at $298.1 billion, with a fraction of $105.2 billion required by wastewater treatment systems alone (USEPA, 2008). The report highlights New Jersey, California, Massachusetts, New York, Pennsylvania, Nevada, Iowa and Utah as the states with the largest increases in overall needs since 2004. It also exposes Utah as one of the top two states with the largest percentage increase in wastewater treatment needs for the same period, as one of the top three states with the largest per capita needs for wastewater treatment at $833 per person, and as one of the top four states in the case of overall needs for small communities. Overall needs of the nation's small communities accounted for $22.7 billion, about 8% of the total documented needs. The report cites rehabilitation of aging infrastructure, facility improvements to meet more protective water quality standards, and expanding capacity to accommodate population growth, as the main three factors affecting the increase in national sewage treatment needs.

Biological Nitrogen Removal

Conventional biological nitrogen removal from wastewater is achieved through the processes of nitrification, which requires an aerobic environment, and subsequent denitrification, which requires an anoxic environment. Biological nitrogen removal has been extensively studied and well established in both suspended growth systems and attached growth systems.

The anaerobic ammonium oxidation (ANAMMOX) process was first developed at Delft University of Technology in the 1990s and has been investigated by numerous researchers, most recently Feng, Tsenga, Hsiab, Hob and Chou (2007), Fernández, Vázquez-Padín, Mosquera-Corral, Campos and Méndez (2008), Kindaichi et al. (2007), Park, Rosenthal, Jezek, Ramalingam, Fillos and Chandran (2010), Ping (2009), Tsushima, Kindaichi and Okabe (2007a), Tsushima, Ogasawara, Kindaichi, Satoh and Okabe (2007b), van der Star et al. (2007). ANAMMOX gains its attention due to its great efficiency and significant reduction of costs for aeration and exogenous electron donor as compared with conventional nitrogen removal. However, due to the ANAMMOX slow growth rate, with doubling times of around 2 weeks reported in the literature, time consuming startup including needs for special sludge seeding, and a myriad of inhibitory conditions and substances that include high ammonia and nitrite concentration themselves (Dapena-Mora, Fernández, Campos, Mosquera-Corral, Méndez & Jetten, 2007; Fernández, Dosta, Fajardo, Campos, Mosquera-Corral & Méndez, 2010; Kuenen, 2008), conventional biological removal is still the reliable and manageable process widely used in wastewater treatment applications.

Nitrification

Nitrification is a two-stage aerobic biological process in which ammonia is initially oxidized into nitrite, and nitrite then oxidized into nitrate, for cellular energy production. The first oxidation step is commonly facilitated by the bacterial genus *Nitrosomonas*, although *Nitrosococcus*, *Nitrospira*, *Nitrosovibrio*, and *Nitrosolobus* have also been identified as ammonia oxidizing bacteria (AOB) (Rittmann & McCarty, 2001; USEPA, 2009). In the case of the second oxidation step, genera *Nitrobacter*, *Nitrococcus*, *Nitrocysts* and *Nitrospira* are identified as nitrite oxidizing bacteria (NOB). *Nitrobacter* is the dominant nitrite oxidizer in most wastewater-treatment processes. Racz, Datta and Goel (2010) recently studied the effect of organic carbon on the ecology of nitrifying bacteria in a mixed culture. The investigation concludes that a more complex organic carbon source produces a more diverse overall bacterial community than a community fed with a simpler organic carbon source.

Both ammonia and nitrite oxidizing bacteria are classified as autotrophs, chemolithotrophs, and obligate aerobes. Being obligate aerobes, nitrifiers consume a significant amount of oxygen to complete the reactions, produce a small amount of biomass, and cause destruction of alkalinity through the consumption of carbon dioxide and production of hydrogen ions (Metcalf & Eddy, 2003). As chemolithotrophs, nitrifiers rely on inorganic nitrogen compounds as electron donors, and as autotrophs, nitrifiers must fix and reduce inorganic carbon. These conditions constitute the two main reasons for the slower growth of nitrifiers with respect to aerobic heterotrophs, as nitrogen electron donors release less energy per

electron equivalent than do organic electron donors, and as fixation and reduction of inorganic carbon is an energy-expensive process (Rittmann & McCarty, 2001).

Multiple factors can have an impact on nitrification rates; these include presence of toxic chemicals, pH, alkalinity, DO levels, and temperature. Nitrifiers are reputed to be highly sensitive to chemical inhibition (Rittmann & McCarty, 2001). Benzene, carbamates, ethers, cyanates, alcohols, tannins, phenolic compounds, chlorinated organic compounds, solvents, proteins, and amines are organic chemicals known to be toxic to nitrifying bacteria (Hockenbury & Grady, 1977; Sharma & Ahlert, 1977). Unionized ammonia (NH_3) at high pH conditions, nitrous acid (HNO_2) at low pH conditions, anionic surfactants, and heavy metals are inorganic compounds known to cause inhibition of nitrifiers (Rittmann & McCarty, 2001).

At pH values lower than 6.8 and larger than 8.5 a considerable decline of nitrification rates has been observed (Kholdebarin & Oertli, 1977; USEPA, 1993). Also, because nitrification is an alkalinity consuming process, sufficient alkalinity must be assured in order to reach satisfactory nitrification rates and to avoid self-inhibition by inducing low pH conditions as the buffering capacity is consumed. If alkalinity addition is needed, it is typically added in the form of lime, soda ash, sodium bicarbonate, or magnesium hydroxide (Metcalf & Eddy, 2003). Sufficient dissolved oxygen levels are an absolute requirement for the growth of nitrifiers and a controlling parameter of nitrification rates. A minimum ratio of 3.6 mg O_2 per mg NH_4-N is sufficient to be used for the nitrification process in the biological treatment of wastewater treatment (Halling- Sørensen & Jørgensen, 1993). Regarding

temperature, although it is considered to have little effect on nitrification in the range of 15°C to 35°C, values bellow 10°C perturb nitrification rates to the point at which nitrification is sometimes considered impossible for low-water temperatures (Rittmann & McCarty, 2001; Wild, Sawyer & McMahon, 1971). However, nitrification at temperatures of 5°C or lower is feasible if enough DO and solids retention time (SRT) are considered.

Denitrification

Biological denitrification refers to the use of microorganisms for the reduction of nitrate or nitrite to nitric oxide, to nitrous oxide, and ultimately to nitrogen gas (N_2). Because N_2 is volatile and minimally soluble in water, it transfers to the gas-phase, where it is harmless. Denitrification is the result of the ability of bacteria to utilize nitrogen oxides as terminal electron acceptors in the absence of oxygen.

Denitrifying bacteria are widespread in nature. Both, heterotrophic and autotrophic microorganisms are able to carry out denitrification reactions in soils, sediments, surface waters, ground waters, and wastewater treatment plants (Rittmann & McCarty, 2001). Some of the best studied species of autotrophic bacteria associated with denitrification include *Thiobacillus denitrificans*, *Thiomicrospira denitrificans*, *Thiobacillus versutus*, *Thiobacillus thyasiris*, *Thiosphaera pantotropha*, and *Paracoccus denitrificans* (Cervantes, 2009). However, the microbial diversity of autotrophic denitrifiers is still not fully known and current knowledge is largely based on laboratory-scale denitrification reactors. The

heterotrophic organisms include the genera *Achromobacter, Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter, Bacillus, Chromobacterium, Corynebacterium, Flavobacterium, Hypomicrobium, Moraxella, Neisseria, Paracoccus, Propionibacterium, Pseudomonas, Rhizobium, Rhodopseudomonas, Spirillum, Vibrio, Halobacterium*, and *Methanomonas* (Metcalf & Eddy, 2003).

Most denitrifying bacteria are facultative aerobes. Dissolved oxygen concentration is the controlling parameter for whether microorganisms employ nitrogen oxides or oxygen as the electron acceptor (Rittmann & McCarty, 2001). In biological wastewater treatment, denitrification is carried out in the absence of DO or under low DO concentration conditions in order to employ nitrate and/or nitrite as electron acceptors and, thus, induce their reduction. Such conditions of zero or near zero oxygen, along with the presence of nitrate and/or nitrite, are technically known as anoxic conditions. In spite of the low dissolved oxygen imposition, denitrification has been observed in activated sludge and fixed film systems in which the bulk liquid DO concentration is well above zero. This is due to the establishment of an anoxic zone within the floc or biofilm depth, so that denitrification occurs in the floc or biofilm interior, while nitrification occurs at the exterior. Hence, a single system can carry out simultaneous nitrification and denitrification (USEPA, 2009).

Considering $C_{10}H_{19}O_3N$ as the common composition of biodegradable organic matter in domestic wastewater, the balanced stoichiometric formulations for heterotrophic denitrification when wastewater and methanol act as carbon source are, respectively, as follow (Metcalf & Eddy, 2003):

$$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \tag{1}$$

$$5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \tag{2}$$
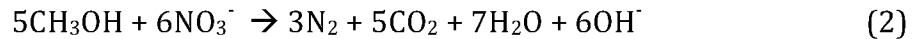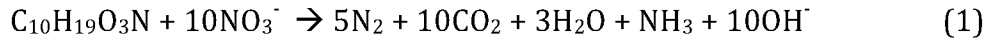
Inspection of the above equations shows that for every equivalent of nitrate as nitrogen reduced during denitrification, one equivalent of alkalinity is produced. This corresponds to the production of 3.57 g of alkalinity as $CaCO_3$ for every gram of nitrate (as nitrogen) reduced. For this reason, it is beneficial to couple denitrification to nitrification as the former would supply approximately one half of the alkalinity requirement for the nitrification reaction.

One concern during the biological denitrification process is the permanent demand of organic compounds to serve as electron donor. Enough biodegradable organic matter must be ensured in the interest of achieving a desired level of denitrification. As a general rule, 4 g of BOD is needed for every 1 g of nitrate reduced (Metcalf & Eddy, 2003), although the precise ratio will depend on the system operation conditions and the exact type of electron donor used for the reduction reaction.

<center>Biological Phosphorus Removal</center>

Phosphorus removal by biological means is regarded as one of the most economical and efficient methods for preventing eutrophication of surface waters. Commonly known as Enhanced Biological Phosphorus Removal (EBPR), it has been demonstrated in many laboratory-scale, pilot-scale and full-scale installations. Consequently, EBPR has been increasingly implemented in many wastewater

treatment plants worldwide. Biological phosphorus removal presents two principal advantages over the more traditional removal by chemical precipitation, reduced chemical costs and less sludge production (Metcalf & Eddy, 2003).

EBPR is carried out by phosphorus accumulative microorganisms (PAOs) that have the ability to accumulate phosphorus over and above what is required for growth. The process requires alternating anaerobic and aerobic conditions to enrich PAOs, which release orthophosphate (PO_4^{3-}) during the anaerobic phase and uptake more PO_4^{3-} than is released during the aerobic phase, therefore removing phosphorus from the system (Zeng, Lemaire, Zhiguo & Keller, 2003a).

Under anaerobic conditions, volatile fatty acids (VFAs) are formed by the fermentation of biodegradable soluble organic matter (bsCOD) and then taken up by PAOs that store them in intracellular granules as polyhydroxyalkanoates (PHAs). The energy required to accumulate PHAs under anaerobic conditions is supplied by the cleavage of energy rich, and previously stored, polyphosphate. This degradation process produces orthophosphates, which are largely released to the wastewater, causing the phosphorus concentration in the bulk liquid to increase. Under aerobic conditions, PAOs utilize stored PHAs to generate energy for growth and for the uptake of orthophosphate from the liquid in order to replenish their intracellular polyphosphate pools (Metcalf & Eddy, 2003; Rittmann & McCarty, 2001). At the end of each anaerobic-aerobic cycle, the PAOs' population increases and phosphates become intracellularly trapped. Thus, phosphorus is significantly removed from the system once biomass is settled and wasted.

Phosphorus accumulation is not limited to aerobic conditions. Phosphate uptake has been found to also take place under anoxic conditions by following the same biochemical mechanism for aerobic conditions, and replacing oxygen for nitrate or nitrite as the oxidant (Oehmen et al., 2008). Two important advantages for denitrifying phosphate removal are efficient utilization of carbon source and savings in aeration costs since phosphorus and nitrogen are removed simultaneously from the wastewater (Kuba, Murnleitner, van Loosdrecht & Heijnen, 1996; Oehmen et al., 2008).

Several authors have evaluated phosphate accumulation under aerobic and anoxic conditions. Kerrn-Jespersen and Henze (1993), and Meinhold, Filipe, Daigger and Isaacs (1999) observed more rapid phosphorus removal under aerobic conditions than under anoxic conditions and concluded that PAOs can be divided into two groups with respect to the process, one group capable of utilizing only oxygen as the electron acceptor, and another group capable of utilizing both oxygen and nitrate as electron acceptors. Wachtmeister, Kuba , van Loosdrecht and Heijnen (1997) performed a series of activated sludge batch tests to find higher phosphorus uptake rates under aerobic conditions than under anoxic conditions and to demonstrate that activated sludge not previously exposed to anoxic conditions also showed small anoxic phosphorus uptake. Although the results could be explained by two different groups of PAOs, the authors tentatively concluded that the biological phosphorus removal population was only one population with different levels of denitrifying activities depending on the environmental conditions. While the pattern of lower uptake rates under anoxic conditions prevailed, results

regarding the microbial characterization of denitrifying phosphorus removal continue to be contrasting.

With the use of molecular tools, efforts to determine the identity of denitrifying PAOs (DPAOs) and establish if a difference exists between these and common PAOs have increased. Ahn, Daidou, Tsuneda and Hirata (2002) demonstrated the presence of three different population structures in three reactors utilizing oxygen, nitrate, and oxygen together with nitrate as electron acceptors. Kong, Nielsen J.L. and Nielsen P.H. (2004), and Zeng, Saunders, Yuan, Blackall and Keller (2003b) recognized high numbers of *Accumulibacter* in reactors performing simultaneous denitrification and phosphorus uptake. More recently, results found by He, Gu and McMahon (2006) suggest the existence of multiple subgroups between the *Accumulibacter* group with different phenotypic characteristics at the same time with different abilities to denitrify while taking up phosphorus. To date, complete characterization of DPAOs and PAOs is still a matter of considerable study.

Several environmental factors such as organic matter content, pH, retention time, and severity of the anaerobic phase can affect the performance of the EBPR process. Satisfactory availability of organic material is crucial for the anaerobic phase since phosphate release will take place only if PAOs find sufficient amounts of VFAs on which to feast. As a rough estimate, to remove phosphorus to an effluent concentration less than 1.0 mg/L, the COD:P ratio typically should be about 40 or more (USEPA, 2009). Phosphorus removal efficiency is greatly reduced at pH values below 6.5, and it has been shown that EBPR is not possible to be established when

the pH is less than 5.5 (Metcalf & Eddy, 2003). Both anaerobic and aerobic

hydraulic retention times (HRT) can affect the amount of phosphorus stored by

PAOs. Sufficient time should be given in the anaerobic stage for the formation of

VFAs and subsequent PHA storage. It is known that phosphorus uptake in the

aerobic phase can be lower than achievable if insufficient PAHs were stored in the

anaerobic zone. It is also known that the best EBPR performance is obtained when

complete absence of oxygen or nitrate is guaranteed in the anaerobic stage. If these

are present in the anaerobic phase, PAOs can be easily outcompeted by other

microorganisms using oxygen or nitrate as electron acceptors (USEPA, 2009).

Nutrient Removal in Submerged Biofilm Processes

Biofilm attached to a support media has proven to be an effective alternative

to the widely used suspended growth activated sludge process (Schlegel & Koeser,

2007). Trickling filters, rotating biological contactors (RBCs), moving bed biofilm

reactors (MBBRs), bio-filters, and submerged biofilm reactors (SBRs) are among the

most accepted fixed-growth biofilm systems applied in the biological treatment of

wastewater. With their application dating as far back as the early 1900s, the major

advantages of biofilm systems over suspension treatment are the high microbial

density that can be achieved, leading to smaller treatment system footprints, and the

inherent development of aerobic, anoxic and anaerobic zones which enable

simultaneous biological nutrient removal (Ehlers & Turner, 2012). Other

advantages of attached growth processes over the activated sludge process include

lower energy requirements, simpler operation, no bulking problems, less

maintenance, and better recovery from shock loads (Metcalf & Eddy, 2003).

Submerged attached growth processes were first introduced in the 1970s.

Submerged biofilm reactors have evolved into one of the most attractive attached

growth systems because they operate as a high-rate biological and mechanical filter

in the same reactor, eliminating the requirement for separate secondary

clarification (Gálvez, Gómez, Hontoria & González-López, 2003). System stability,

long retention time of microorganisms, much higher biomass content in terms of

mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids

(MLVSS) are other important characteristics of submerged biofilm systems (Wang

B., Li Ju, Wang L., Nie & Li Ji, 1998). There is also less surplus sludge because of

longer food chains in biofilms consisting of abundant amounts and various species

of metozoa, protozoa, bacteria and fungi.

Utilization of specially shaped plastics has displaced the early use of stone,

coke, laths and ceramic elements as binding materials (Rusten, 1984). These

plastics commonly consist of molded polyethylene (PE), polypropylene (PP) or

polyvinyl chloride (PVC), but woven polyester fibers or stripes of PVC films are

employed as well; media properties that are of primary interest because of their

effect on performance are durability, specific surface area density (ratio of

geometric surface area to volume), and percent void space (Schlegel & Koeser,

2007). Greater surface density permits a larger biomass per unit volume, while

greater void space allows for a higher oxygen and mass transfer to the biofilm and

reduces the clogging risks of the channels of the support media by excessive biofilm growth.

Biological removal of nitrogen and phosphorus in submerged attached growth systems was accomplished in the early 1990s by enriching microorganisms with suitable environmental conditions in order to trigger the specific metabolic pathways. González-Martinez and Wilderer (1991) operated a fixed film sequencing batch reactor (SBR) with cycle durations of 6, 8 and 12 hours with anaerobic periods of 25, 45 and 63 percent of the total cycle period. Gonçalves and Rogalla (1992) experimented with two laboratory-scale fixed film biofilters in series, in which aeration conditions were alternated so the continuous flow was serially exposed to anaerobic and aerobic conditions. Gonçalves, Le Grand and Rogalla (1994) used five continuously fed pilot-scale bio filters in series with an anaerobic phase of 2.5 hours and an aerobic phase of 12 hours. This was later adjusted to a 12-hour-cycle duration with an anaerobic phase of 1.75 hours and an aerobic phase of 9.6 hours. By alternating anaerobic and aerobic phases, it was possible to attain denitrification activity and phosphorus release under anaerobic conditions, and ammonia oxidation and phosphorus uptake under aerobic conditions.

More recent research efforts have validated the operational procedures presented in the preceding investigations. Using a biofilm SBR under a 36 hours duration cycle divided into 10 hours anaerobic, 20 hours aerobic, 3 hours anoxic and 3 hours aerobic, Garzón-Zuñiga and González-Martinez (1996) accomplished COD, phosphate and ammonia removals of 89%, 75%, and 87%, respectively. Wang et al. (1998) employed phosphorus nuclear magnetic resonance (P-NMR) to verify

biological phosphorus release and uptake in the anaerobic, oxic and anoxic phases of a submerged biofilm SBR (SB-SBR). They concluded that phosphorus-accumulating bacteria contained in the submerged biofilm released phosphorus due to the degradation of polyphosphates into orthophosphate under anaerobic conditions and took up phosphorus and carried out polyphosphorylation under oxic and anoxic conditions. Li, Xing and Wang (2003) subjected a SB-SBR to a cultivation period of 6 months under a sequence of 3 hours anaerobic and 6 hours aerobic for a total HRT of 9 hours. By the end of the cultivation period, the system reached steady state and the biomass concentration in the biofilm reached a high level of 8855 mg/L in terms of MLSS in comparison to the mere 60 mg/L in terms of MLSS of the suspended biomass concentration in the liquid phase. A TP percentage removal of 90% was achieved along with a TN percentage removal of 57%, which was attributed to the presence of anoxic zones in the thick biofilm. A vertical submerged membrane bioreactor (VSMBR) composed of anoxic and oxic zones in one reactor was developed and operated by Chae, Kang, Watanabe and Shin (2006). Under an optimal anoxic zone/oxic zone volume ratio of 0.6, internal recycle ratio of 400%, and HRT of 8 hours, average removal efficiencies of 75% and 71% were achieved for TN and TP, respectively. Coexistence of oxic, anoxic and anaerobic conditions within the bioreactor and biological film has been established as the key parameter for simultaneous organic matter, nitrogen and phosphorus removal in biofilm systems.

Research Objective

As the overall goal of this study, the potential of advanced aerated submerged biofilm reactors for the simultaneous removal of carbon, nitrogen and phosphorus from municipal wastewater was evaluated. The main objective of this investigation was to show that by incorporating air cycling into the operation of a pilot-scale system composed of dome shaped submerged biofilm structures treating municipal sewage, organic matter and nutrient removal can occur simultaneously.

CHAPTER II

MATERIALS AND METHODS

Investigation of simultaneous organic matter and nutrient removal in

aerated submerged biofilm reactors (ASBRs) was conducted in a pilot-scale set-up

at Central Valley Water Reclamation Facility (CVWRF), in Salt Lake City, between

October 25th, 2010, and February 17th, 2011. Three factors influenced the selection

of CVWRF as the location to conduct this research: first, its proximity to the

University of Utah campus; second, the high organic and nutrient content of the

municipal wastewater treated at the plant; third, CVWRF counts in its premises with

an EPA-certified water analysis laboratory which allowed sample analyses to be

crosschecked as a quality control measure.

Pilot-scale Reactor Configuration

A partitioned pilot-scale reactor tank was constructed to hold an aerated

submerged biofilm system and its resembling control system running in parallel.

The pilot test vessel was made from a commercial dumpster and divided lengthwise

into two parallel tanks, each with a capacity of 1650 gal (6246 L). In the first

partition, the control system contained a linear arrangement of six concrete bases

with each base crowned by approximately 300" of porous bubble-emitting tubes. Analogously, the biofilm system in the second partition contained six scaled dome shaped aerated submerged biofilm devices arrayed in series, as can be seen in Figure 1. Each of these devices, called Poo-Gloos, consisted of three concentrically nested domes with high surface-to-volume LANPAC packing material (Lantec Products, Inc., Agoura Hills, California) placed in between the dome layers, mounted on concrete bases, with the same length of bubble tubes as in the control bases. Sizing of the concentric domes was 38", 32" and 26" in diameter, and 24 ½", 19" and 14" in height, for the outer, middle and inner domes, respectively. In its entirety, the internal structure of the six Poo-Gloos provided about 3000 ft^2 (279 m^2) of available surface for biofilm colonization.

The pilot plant was located beside an aeration ditch between the primary sedimentation tanks and the trickling filters at CVWRF. Wastewater from the transfer ditch was pumped to feed the bioreactors at one end. A flow splitter was used to equally divide the influent into each tank. Concentration of the pumped wastewater varied moderately, but this disparity was canceled by comparing the effluents of the two side-by-side tanks. A small compressor fitted with regulators, oil/water traps, and a knockout tank supplied air to both systems.

<center>Pilot-scale Reactor Operation</center>

The 17 week experimental run had three primary operational variables: a) wastewater flow rate and its associate constituent loading into the tanks, b) periods of air-on and air-off, and c) temperature. The influent pump was operated to control

the hydraulic and nutrient loading, with corresponding HRTs that varied from 3 days to 9 days. Air-on and air-off intervals were introduced to impose the necessary environmental conditions for biological removal of nitrogen and phosphorus. During the time the air was on, air-flow rate was held constant at about 3 L/min per scaled Poo-Gloo (18 L/min total per side). Air cycling varied from 22 hours on/2 hours off to 19 hours on/5 hours off. To promote the release of stored phosphorus, air was shut off for an entire week at two intervals. Finally, water in both tanks was allowed to follow the weather-induced temperatures, which varied from 12.6°C to 0.2°C. Table 1 summarizes the adjustment of operational parameters considered during the experimental run. Figure 2 shows the bioreactor in operation.

Figure 1- Overview of the pilot-scale reactor configuration. Control tank is on the left, Poo-Gloo tank is on the right (Nearest base and dome are out of the picture).

Table 1- Weekly variations in operational parameters.

Week	Dates	Inflow Rate [L/d]	HRT [d]	Air Cycling On/Off [h/h]	Goal
1	10/25/10 - 10/29/10	1938	3	24/0	Establish biofilm
2-4	11/1/10 - 11/19/10	795	7-8	24/0	Allow nitrifying and other bacteria to reach steady state
5-6	11/22/10 - 12/6/10	696	9	19/5	Air cycling to promote denitrification. Possible P uptake
7-8	12/6/10 - 12/17/10	1181	5-6	19/5	Increase loading to improve denitrification and possible P uptake
9	12/20/10 - 12/24/10	0	∞	0/24	Possible P release
10	12/27/10 - 12/31/10	0	∞	24/0	Possibe P uptake
11	1/3/11 - 1/7/11	863	7	24/0	Return system to steady state
12-13	1/10/11 - 1/21/11	863	7	22/2	Show significant BOD and ammonia removal. Possible P uptake
14	1/24/11 - 1/28/11	863	7	21/3	Show significant BOD and ammonia removal. Possible P uptake
15	1/31/11 - 2/4/11	863	7	20/4	Show significant BOD and ammonia removal. Possible P uptake
16	2/7/11 - 2/11/11	0	∞	0/24	Possible P release
17	2/14/11 - 2/17/11	0	∞	24/0	Possibe P uptake

Figure 2- Overview of the pilot-scale reactor in operation. Control tank is on the left, Poo-Gloo tank is on the right.

Sample Collection and Analytical Methods

Sampling of the tank influent along with effluent from the Poo-Gloo and control partitions was carried out on a daily basis. Influent to the tank was sampled once per day and its values compared to the lab results obtained by CVWRF for quality control. Poo-Gloo and control effluents were sampled 1 - 3 times per day, depending on the week. Along the experimental run, lab triplicates were executed during randomly selected weeks for quality control and statistical analysis. Calculated standard deviations allowed the assessment of the reliability of the data and are shown as error bars on the associated figures in the following chapter.

Laboratory analysis of all samples was performed in the Department of Civil and Environmental Engineering at The University of Utah. Water measurements included influent and effluent concentrations of chemical oxygen demand (COD), total suspended solids (TSS), ionized ammonia (NH_4^+), total oxidized nitrogen (TOXN), total nitrogen (TN), total kjeldahl nitrogen (TKN), alkalinity (ALK), orthophosphate (PO_4^{3-}), and total phosphorus (TP). Methods selected and employed for the laboratory analysis of all parameters are listed in Table 2.

Additionally, field measurements were taken with a Horiba W-2010 Water Quality Checker. Parameters including turbidity, pH, temperature, conductivity, oxidation/reduction potential (ORP), and dissolved oxygen (DO), were measured daily. This monitoring produced a wealth of data, with over 5500 field measurement values, and over 4000 lab measurement values.

Table 2- Selected analytical methods for water quality analysis.

Parameter	Method
COD	HACH TNT 82206 (20 - 1500 mg/L)
TSS	Standard Methods APHA, AWWA, & WPCF, with VWR Filters
Ammonia	HACH TNT (0.4 - 50 mg/L)
Total Oxidized Nitrogen	HACH s-TKN TNT 880 (0 - 16 mg/L)
Total Nitrogen	HACH s-TKN TNT 880 (0 - 16 mg/L)
TKN	HACH s-TKN TNT 880 (0 - 16 mg/L)
Alkalinity	HACH TNT 870 (25 - 400 mg/L)
Reactive Orthophospate	HACH TNT (0 - 5 mg/L)
Total Phosphorus	HACH TNT 844 (0.5 - 5 mg/L) - HACH TNT 845 (2 - 20 mg/L)

CHAPTER III

RESULTS AND DISCUSSION

Overview

Operation of the pilot plant was maintained on a continuous basis for 17

weeks. Meanwhile, operational parameters were altered weekly in hope of

assessing the removal of different wastewater constituents. Week-to-week

performances of the Poo-Gloo and control systems were compared with each other.

Overall, the Poo-Gloo system exhibited a much higher efficacy under all different

operational conditions than the control system. Figures 3 to 26 illustrate the

performance of the Poo-Gloo and control systems for each of the constituents of

interest for this investigation over the 17-weeks period. Discussion on the

performance of the two systems in terms of each wastewater constituent is

presented in the following sections.

Organic Removal Measured as Chemical Oxygen Demand (COD)

Due to the ease of measurement with the Hach Colorimetric System, COD was

used for the bulk of the dissolved organic oxygen demands. Values collected in the

laboratory were crosschecked with those obtained by CVWRF staff with no

discrepancies being found. Figure 3 depicts a comparative evaluation on the removal efficiencies accomplished by the Poo-Gloo and control systems. Weekly profiles of the COD values obtained for influent, Poo Gloo effluent, and control effluent are shown in Figures 4 and 5.

From the beginning, the Poo-Gloo tank performance surpassed the control tank at removing dissolved organics. From week 1 to 4, with continuous aeration and both systems largely dominated by heterotrophs, Poo-Gloo removal efficiency progressively escalated from 47±11% in the first week to 68±9%, 71±6%, and 80±6% in the 2nd, 3rd, and 4th weeks, respectively. On the contrary, removal percentages in the control side oscillated between 38±5%, 51±8%, and 44±7% for the first 3 weeks, and a final value of only 53±19% in the 4th week. In terms of concentrations, average COD levels for this initial experimental period were 222.8±53.4 mg/L for influent, 69.9±26.8 mg/L for Poo-Gloo effluent, and 118.4±25.3 mg/L for control effluent.

From week 5 to week 8, removal efficiencies in both tanks gradually reduced. Values in the control side dwindled from 59±13% to 53±18%, to 41±17%, and lastly to 39±6% in the 8th week. Meanwhile, values in the Poo-Gloo side decreased from 77±7% in week 5, to 68±9% in week 6, to 55±13% in week 7, and to 50±5% in week 8. This observation coincides with the system being heavily loaded during weeks 7 and 8, and air-off periods being included into the airing cycle, which emphasizes the importance of aeration in the biological removal of organics.

The stability of the biofilm system was manifested in the months of January and February. With water temperatures getting lower and air-off periods remaining

as part of the airing cycle, control performance continued to deteriorate and between weeks 12 and 15 the highest weekly removal percentage achieved by the control system was only 27±7% in week 15. Meanwhile, Poo-Gloo performance bounced back into the same efficiency levels shown in weeks 4 and 5 and by the 13th week the biggest gap in performance was observed when Poo-Gloo efficiency reached 70±3% and control efficiency reached only 8±8%.

Afterwards, high mass and oxygen transfer appears to be characteristic of the Poo-Gloo device as this system outperformed the control system throughout the experiment, especially on those weeks in which increasing air-off periods were introduced, achieving consistent high COD reduction.
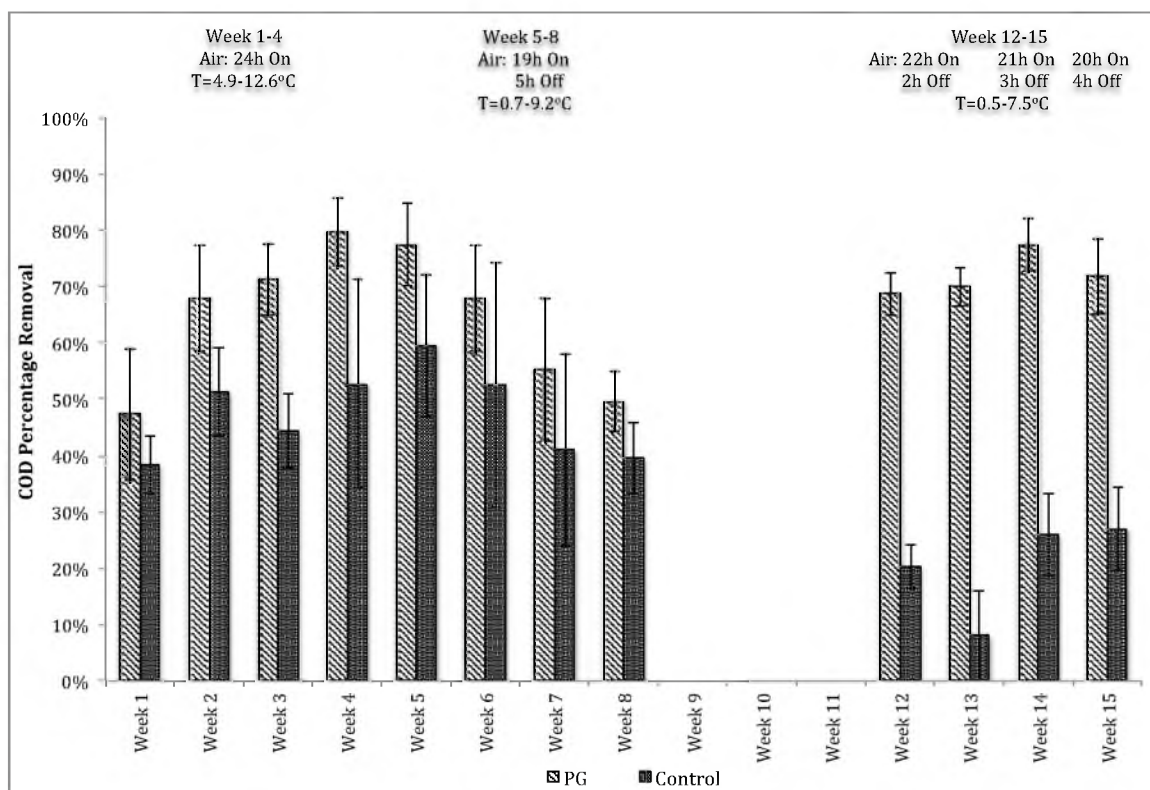

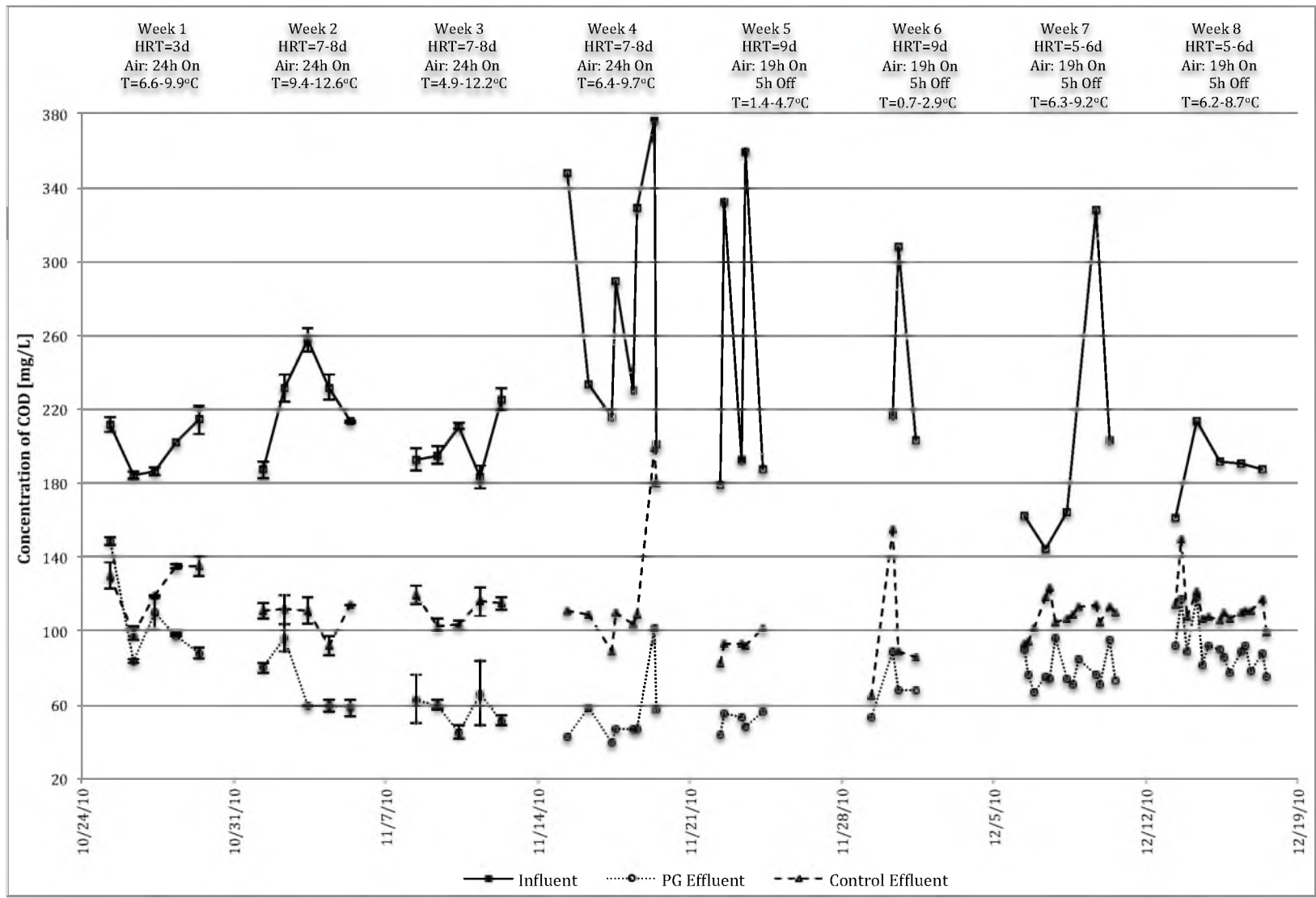Figure 3- Comparative graph of COD percentage removal for Poo-Gloo and control systems.

Figure 4- Graph of COD levels for influent, Poo-Gloo effluent and control effluent (Weeks 1-8).
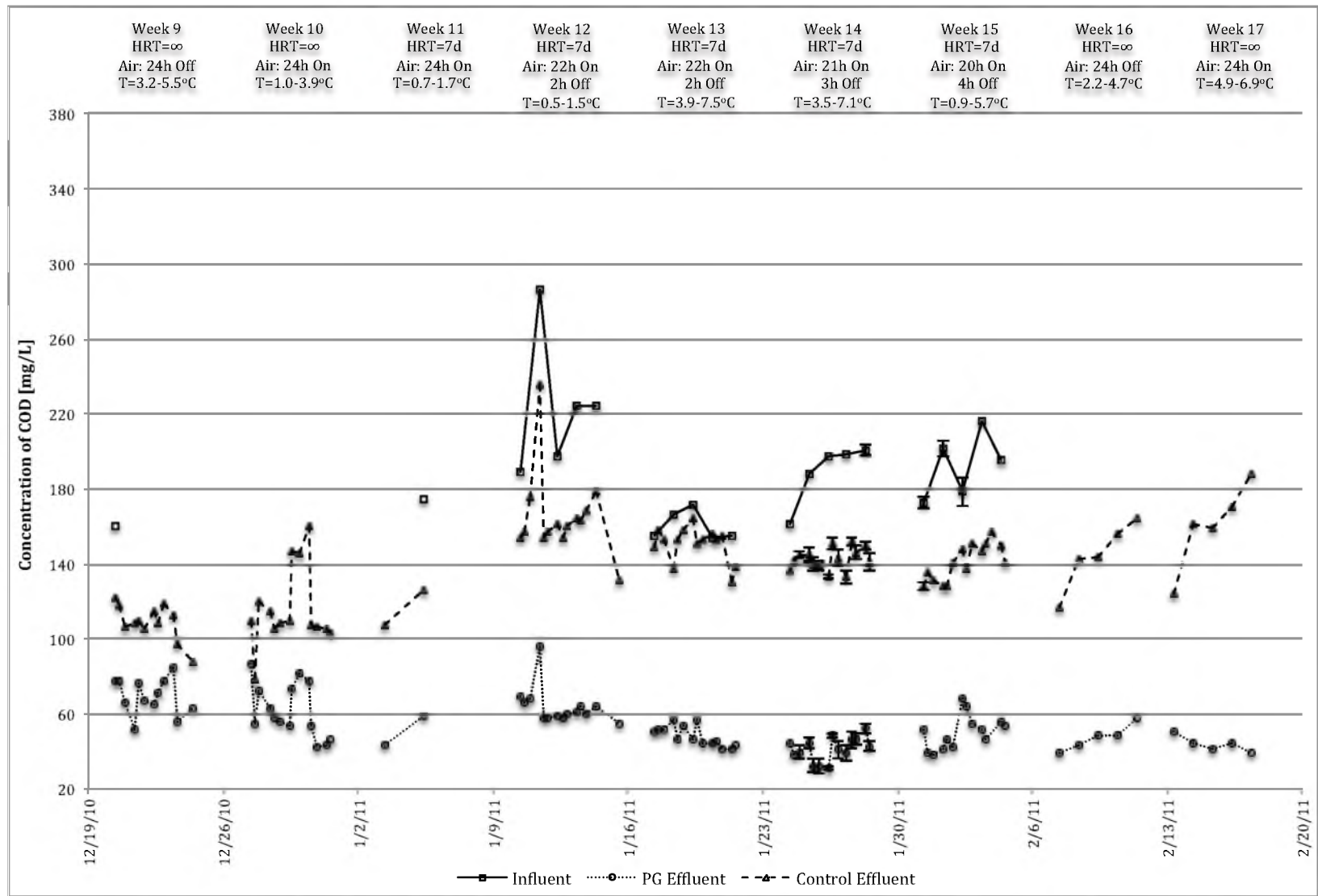
Figure 5- Graph of COD levels for influent, Poo-Gloo effluent and control effluent (Weeks 9-17).

<u>Removal of Total Suspended Solids (TSS)</u>

Solids reduction performance of the Poo-Gloo and control set-up is illustrated in Figures 6, 7 and 8. Figure 6 presents a comparison between the Poo-Gloo and control setups in terms of percentage removal, while Figures 7 and 8 present weekly TSS level profiles for influent, Poo-Gloo effluent, and control effluent.

Trough the experiment, influent, Poo-Gloo effluent and control effluent TSS concentrations averaged 56.6±14.1 mg/L, 8.6±5.6 mg/L and 37.6±16.2 mg/L, respectively. Visually, effluent of the Poo-Gloo tank was consistently more transparent than that of the control tank, with both tanks showing a behavior that
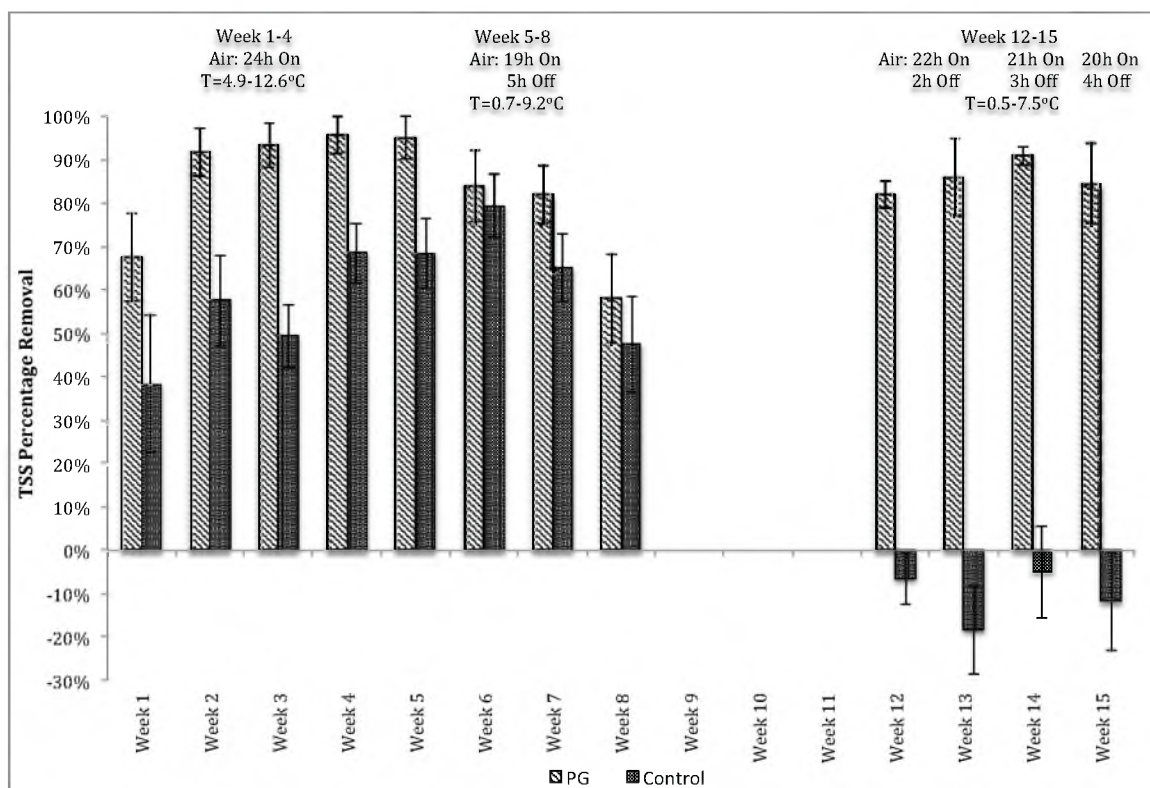

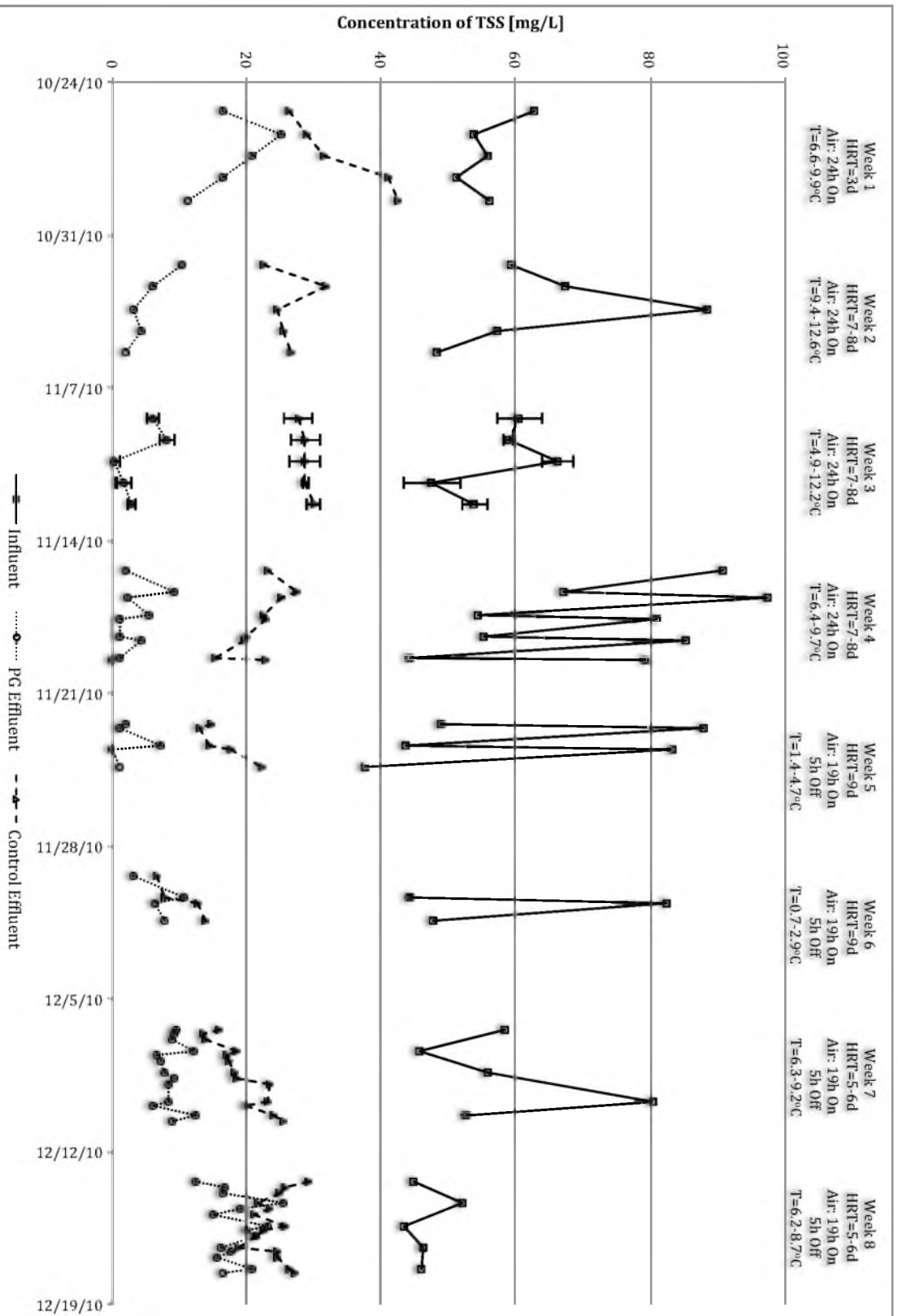Figure 6- Comparative graph of TSS percentage removal for Poo-Gloo and control systems.

Figure 7 - Graph of TSS levels for influent, Poo-Gloo effluent and control effluent (Weeks 1-8).
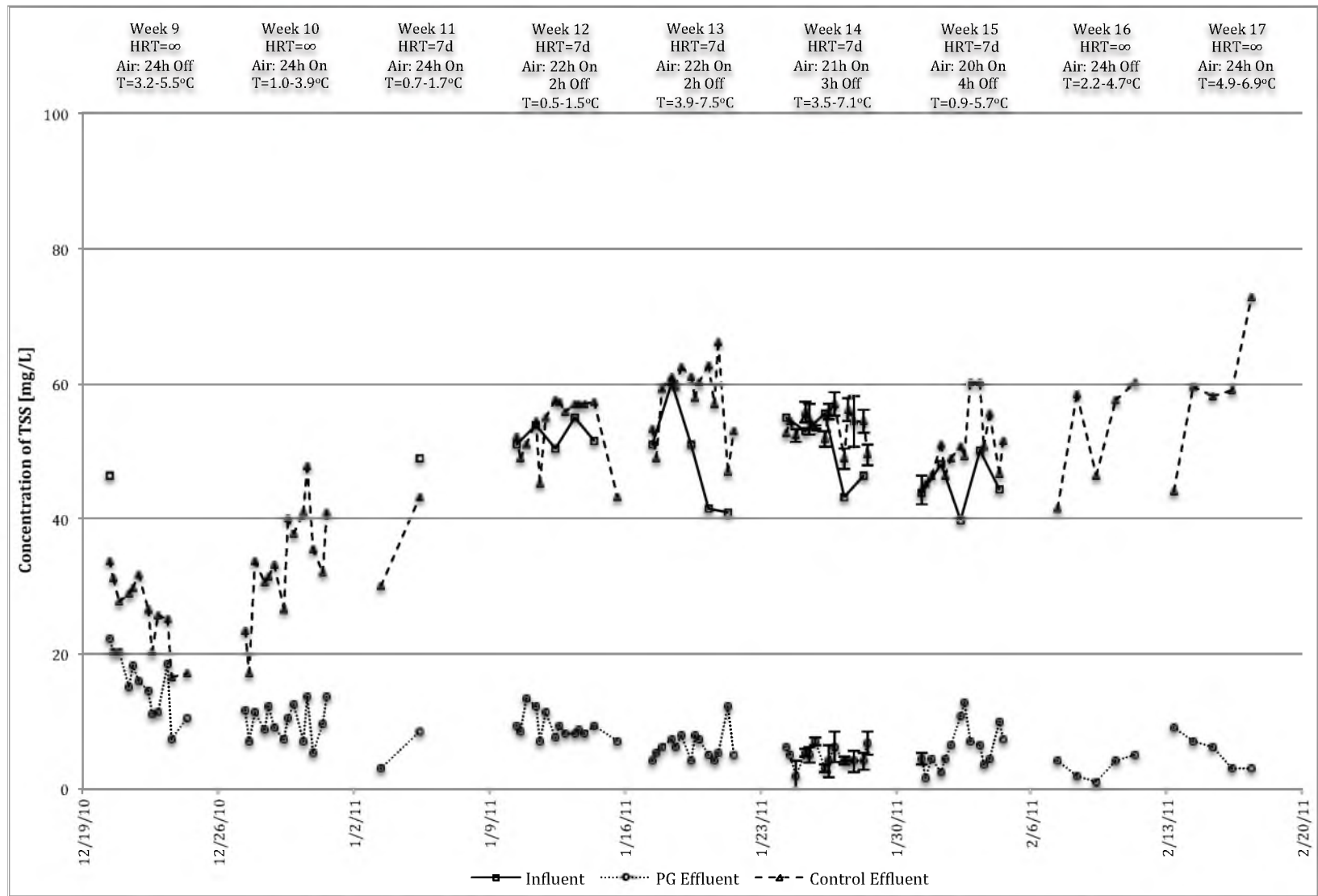
Figure 8- Graph of TSS levels for influent, Poo-Gloo effluent and control effluent (Weeks 9-17).

resembled dissolved organics removal. TSS removal capacity of the Poo-Gloo system was greater than the removal capacity of the control system through the entire experiment. Removal efficiencies of both tanks peaked in week 4 at 96±4% in the case of the Poo-Gloos, and 68±7% in the case of the control. During weeks 5 - 8, efficiencies gradually dropped until reaching, during the 8th week, 58±10% for Poo-Gloos, and 47±11% for control. Finally, on the period between weeks 12 and 15, control efficiency levels continued to deteriorate and were characterized by negative removal values. Meanwhile, Poo-Gloo efficiency levels recuperated from weeks 5 to 8 and got to afford weekly removal percentages in the ranges of 82±3% and 91±2%.

Finally, one needs to consider that biomass in the control tank was suspended and being continuously washed out with the effluent, and in this way, contributing to the high TSS concentration at the outlet. Meanwhile, biofilm in the Poo-Gloo system acted as a very efficient biofilter regardless of the variations in solids loading, trapping suspended matter and increasing the retention time of microorganisms.

<u>Nitrogen Removal</u>

Assessing Poo-Gloo's potential for simultaneous removal of dissolved organics, nitrogen, and phosphorus on a pilot-scale was set as the main objective of this investigation. Previous studies (Choi, Johnson, Hayes & Xu, 2008; Choi, Johnson, Hayes, Sung & Xu, 2010) had validated the outstanding capacity of the Poo-Gloo

device to perform simultaneous COD removal and nitrification, however, viability of the application to remove nutrients from sewage remained unknown.

The most common and important forms of nitrogen in wastewater include: ammonia nitrogen, nitrogen gas, nitrite nitrogen, nitrate nitrogen, and organic nitrogen (Metcalf & Eddy, 2003). Tests for ammonia, total oxidized nitrogen (TOXN), which is defined as the sum of the concentrations of nitrite and nitrate nitrogen, alkalinity, and total nitrogen (TN), which is the sum of the concentrations of organic nitrogen, ammonia nitrogen and TOXN, were performed on pilot plant samples to determine the extent of nitrification, denitrification and overall nitrogen removal in the Poo-Gloo and control systems.

Ammonia Nitrogen

The pilot plant ran for 2 weeks before nitrifiers proliferated in the biofilm. With Poo-Gloo and control removal percentages presented in Figure 9, establishment of a functioning nitrifying biofilm is shown by the drastic increase in Poo-Gloo's efficiency between the 2nd (32±4%) and 3rd week (79±11%). This upward tendency continued in week 4 when Poo-Gloos achieved almost complete nitrification in all tested samples as denoted by a percentage removal of 97±3%. In the case of the control system, plots of the detected ammonia-nitrogen levels shown in Figures 10 and 11 revealed a much inferior performance when compared with the Poo-Gloo side during the initial 4 weeks. Furthermore, the control tank suffered a dramatic drop in nitrification in week 3, achieving a negative percentage removal of -1±4%. With water temperature reaching in week 3 its lowest reading (4.9°C)
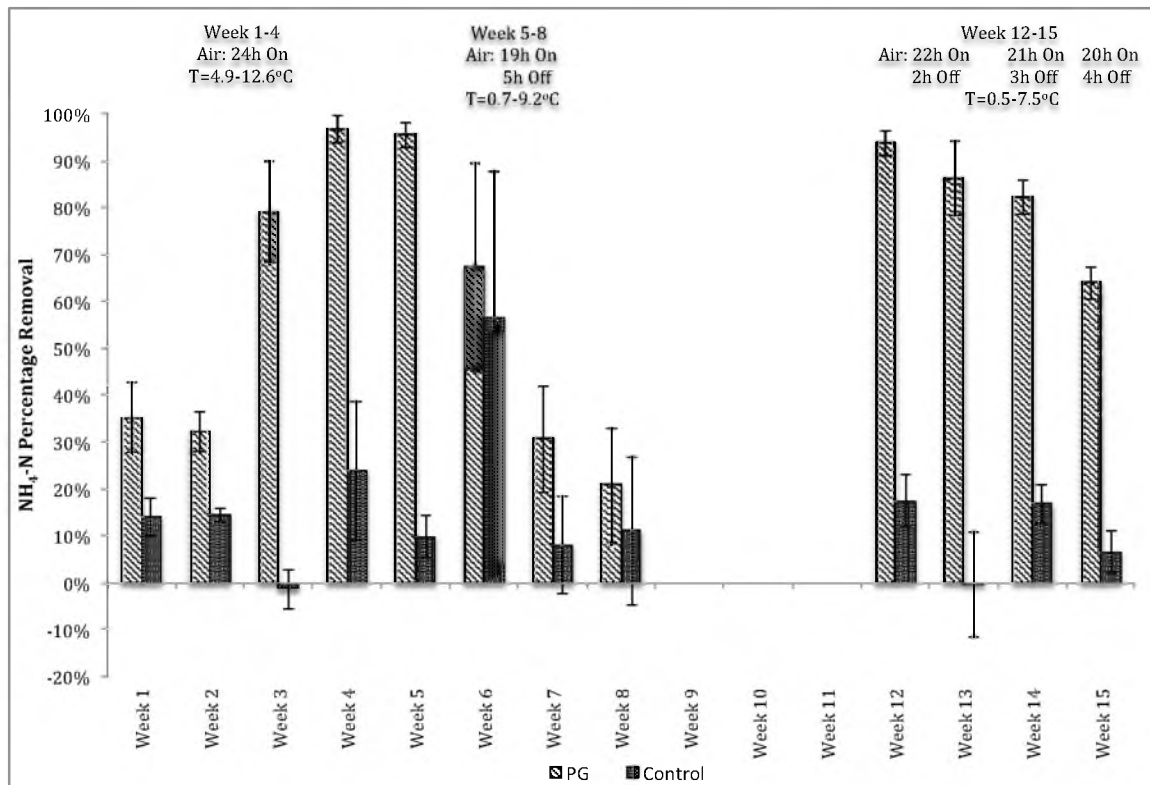
Figure 9- Comparative graph of NH$_4$-N percentage removal for Poo-Gloo and control systems.

since the beginning of the experiment, and with low temperatures recognized as a well-known inhibitor of nitrifiers in suspended growth systems, poor performance of the control side within this week could be attributed to temperature inhibition.

Figure 12 shows the bioreactor in operation between weeks 12 and 15, the coldest period during the experimental run. With the system brought back to steady state after week 11, consistent nitrification was attained in the Poo-Gloo tank from week 12 to week 15; with efficiency again gradually decreasing as air-off periods were increased along the weeks. High removal rates were achieved in weeks 12 and 13 despite incorporating a 2 hours air-off period and water temperatures dropping as low as 0.5°C. Average ammonia levels of 24.0±0.9 mg/L in the influent got
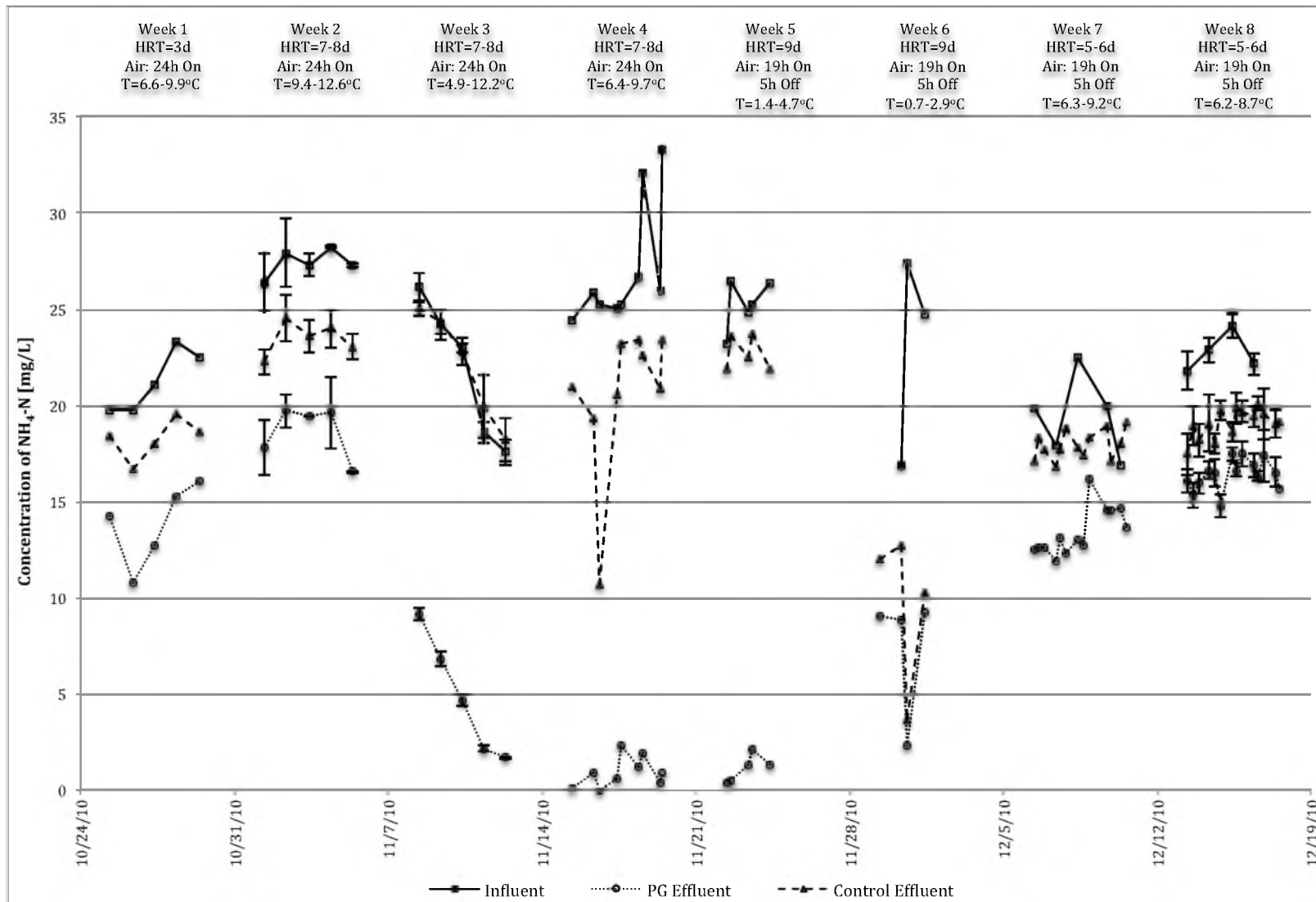
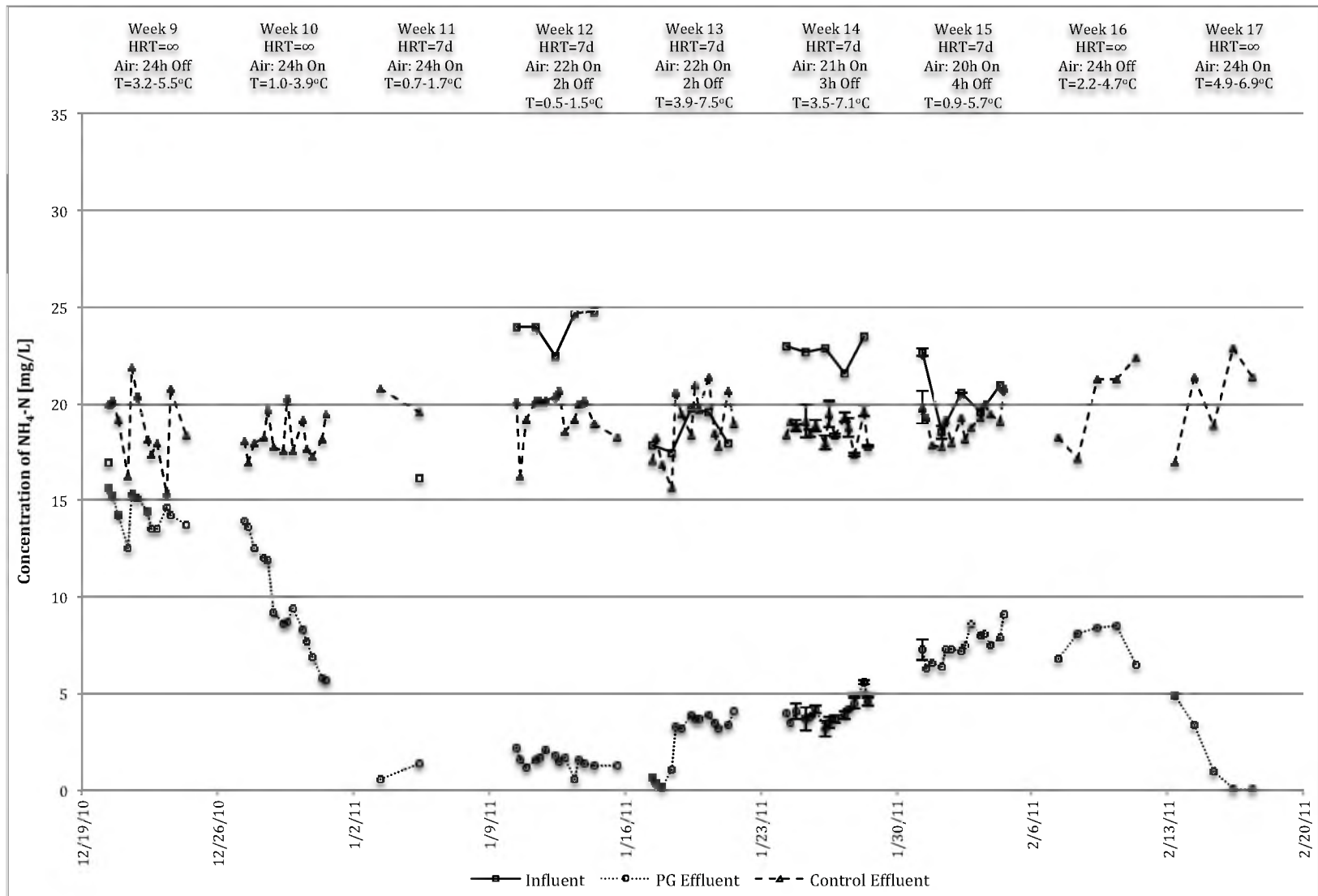Figure 10- Graph of NH$_4$-N levels for influent, Poo-Gloo effluent and control effluent (Weeks 1-8).

Figure 11- Graph of NH₄-N levels for influent, Poo-Gloo effluent and control effluent (Weeks 9-17).

Figure 12- Overview of the pilot-scale reactor in operation during weeks 12-15.

reduced to 1.5±0.4 mg/L in week 12, and from 18.6±1.1 mg/L to 2.7±1.4 mg/L in week 13.

This investigation verifies observations made by many other researchers who have indicated that nitrifying bacteria are inhibited at temperatures below 10°C and subsequently can be washed out of a suspended-growth system. Fixed-film nitrifying bacteria remain in place and continue to nitrify at temperatures approaching 0 °C (Bear & Corapcioglu, 1991; Lewandowski & Defilippi, 1998)

Total Oxidized Nitrogen

Reduction in ammonia levels does not alone prove that nitrification occurs in the submerged biofilm of Poo-Gloo devices. Biological nitrification generates nitrite,

then nitrate in the presence of oxygen. Thus, ammonia removal should result in a measurable increase in the concentration of both the intermediate and the end product.

Samples collected from week 1 until week 15 were analyzed for total oxidized nitrogen (TOXN=NO_3^- + NO_2^-). Results obtained for TOXN and ammonia concentrations in the influent and Poo-Gloo system during weeks 3 and 13 were used to generate Figures 13 and 14, respectively. There is a clear correlation between the rise and decline of ammonia removal rates with the rise and decline of oxidized nitrogen compounds generation, a dependence that was observed to subsist throughout the 15 weeks in which TOXN levels were measured.
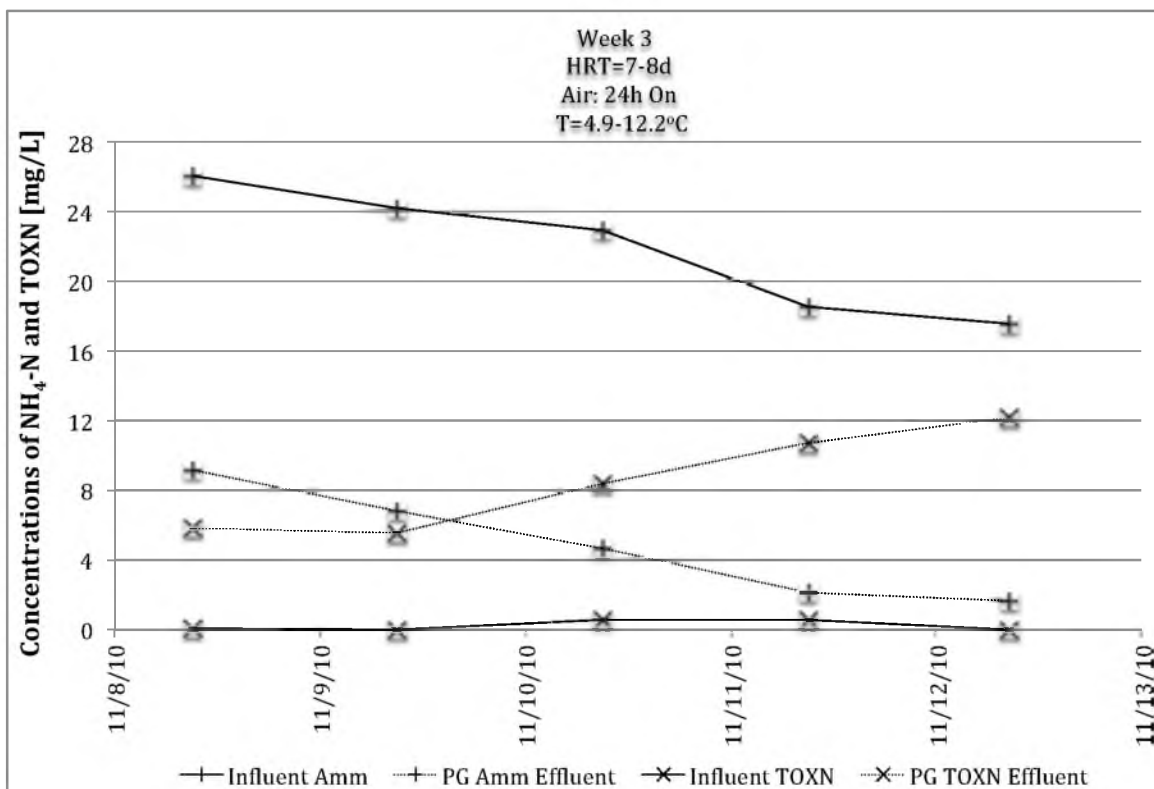

Figure 13- Rise of TOXN concentration while ammonia is being biologically oxidized (Week 3).
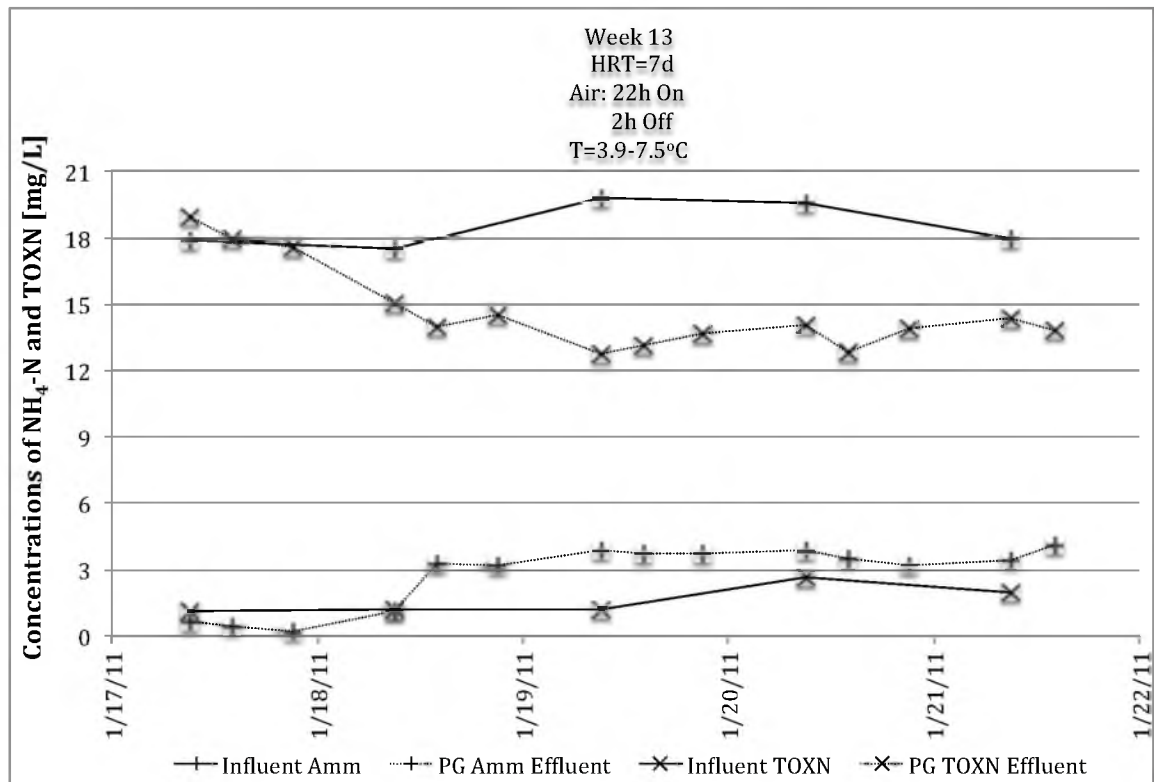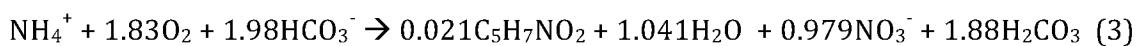
Figure 14- Rise and decline of TOXN concentrations while ammonia is more or less oxidized (Week 13).

Stoichiometry of the complete oxidation of ammonia to nitrate by nitrifying bacteria is shown in the overall reaction (Barnes & Bliss, 1983):

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7NO_2 + 1.041H_2O + 0.979NO_3^- + 1.88H_2CO_3 \quad (3)$$

Mass fluxes of ammonia removed and TOXN generated for weeks 3 and 13 were obtained by subtracting the average effluent concentration from the average influent concentration and multiplying the result by the influent flowrate for that day. The calculated values were then incorporated into a stoichiometric analysis based on Equation #3, assuming the nitrate concentration to be equal to the concentration of TOXN. The analysis shows that during the 3rd week, a total of 93 g of ammonia were removed from the system, an amount that should account for the

formation of 313 g of nitrate. However, using nitrate concentrations detected during this week, only 45 g of nitrate appeared produced. On the 13th week, 95 g of ammonia were removed, which should account for 320 g of nitrate produced. However, detected levels accounted for only 79 g of nitrate in the system. These differences make it worthwhile to discuss the fate of ammonia nitrogen and nitrate nitrogen in the system. It is safe to predict that with ammonia being assimilated by microorganisms in the biofilm to fulfill their anabolic requirements, not all of the ammonia removed from the system can be accused to its biological oxidation. Also, volatilization of ammonia could have taken place to some extent, but at the pH range of the study, between 7.3 and 8.2, this effect should be negligible given that the ammonium/ammonia speciation (NH_3/NH_4^+) is almost entirely in the ionized form, ammonia nitrogen. In the case of nitrate, the gap between the value obtained based on the determined concentrations of each week and the value obtained based on the stoichiometry of the reaction suggest the defined presence of denitrifying bacteria, reducing nitrite and nitrate to nitrogen gas. This scenario is analyzed in more depth in the following section.

The consolidated TOXN concentrations from week 1 to week 15 are illustrated in Figures 15 and 16 for the influent, control effluent and Poo-Gloo effluent. Overall, the TOXN results illustrate the exponential growth of the biomass in the Poo-Gloo system during the first 3 weeks of the study. The results also illustrate that a stationary phase had been accomplished by the month of December. More important, they ratify that oxidation of ammonia occurred biologically.
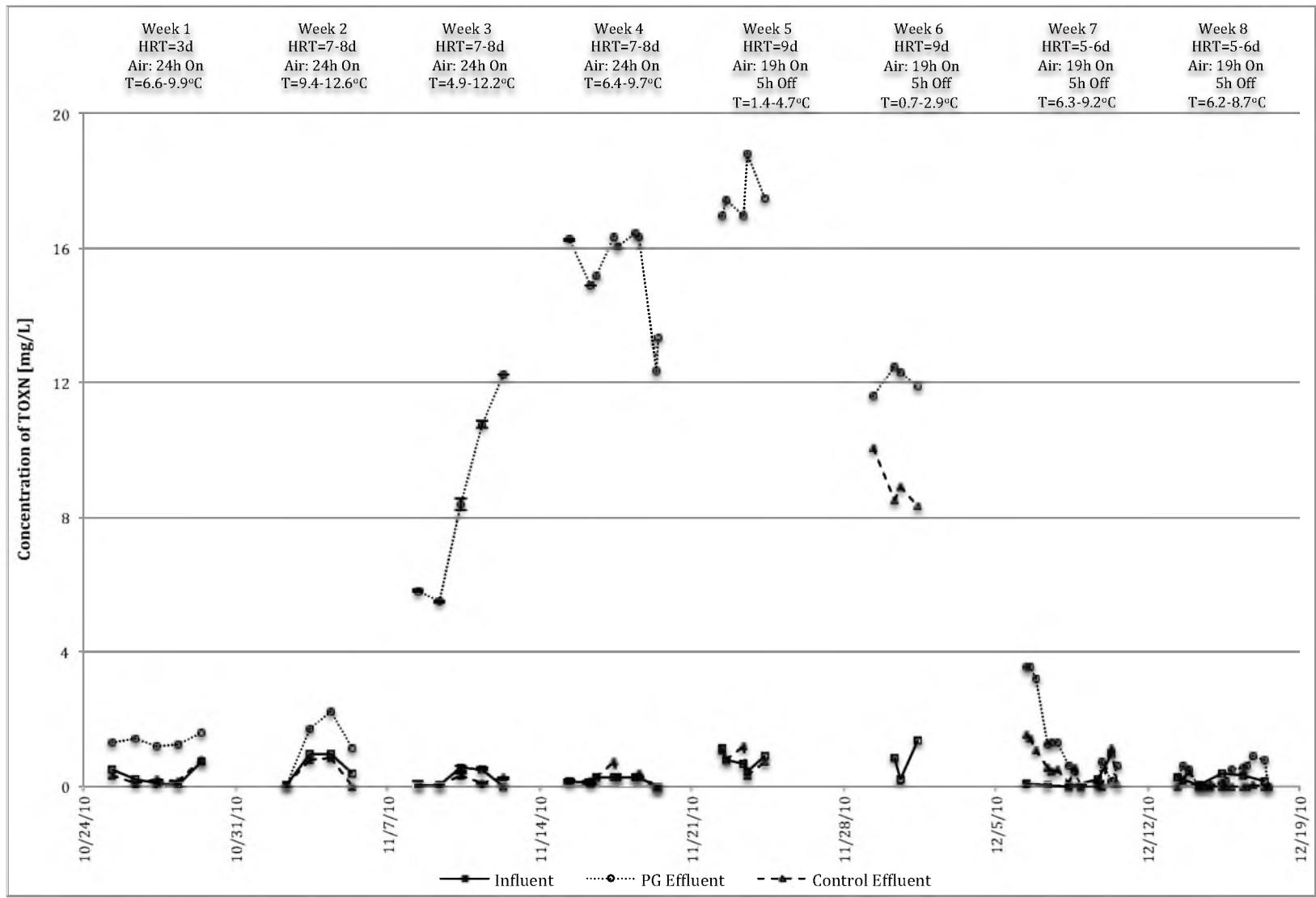
Figure 15- Graph of TOXN levels for influent, Poo-Gloo effluent and control effluent (Weeks 1-8).
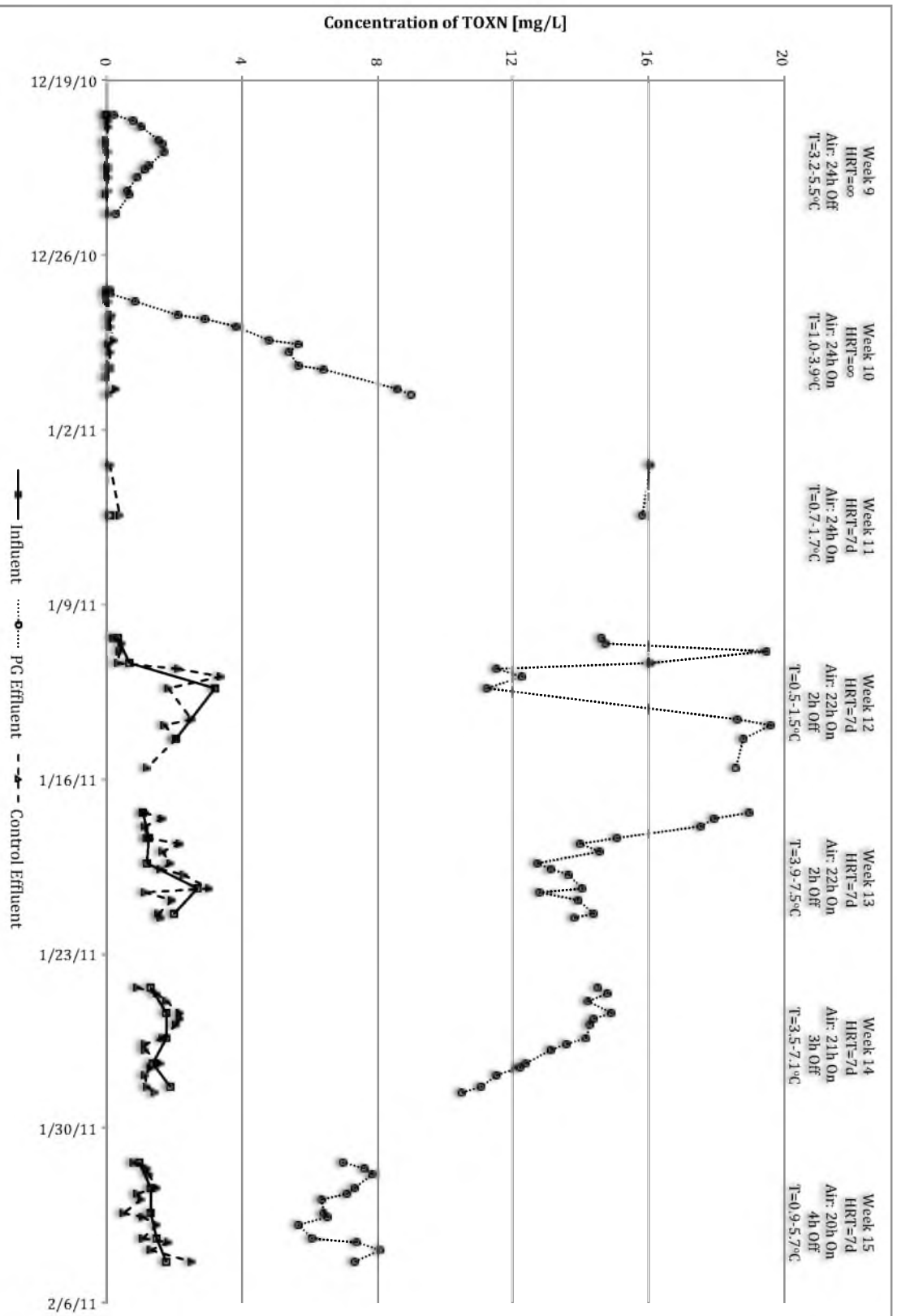
Figure 16- Graph of TOXN levels for influent, Poo-Gloo effluent and control effluent (Weeks 9-15).

Alkalinity

The nitrification reaction shown in Equation #3 describes how autotrophic nitrifiers consume bicarbonate and oxygen to oxidize ammonia to nitrate. At the pH range of the experiment (7.3-8.2), bicarbonate represents the major form of alkalinity; therefore, biological nitrification in the Poo-Gloo tank should cause a decrease in alkalinity.

Alkalinity levels were monitored from week 1 to 10, and the results of each of the sampling points are shown in Figure 17. Between weeks 4 and 5, when ammonia removal percentages reached values over 90%, alkalinity concentrations were reduced from an influent range of 301-358 mg/L as $CaCO_3$ to a Poo-Gloo effluent range of 143-281 mg/L as $CaCO_3$. Reduction in alkalinity was consistent with the extent of nitrification during each week. The largest consumptions occurred during those weeks with the largest ammonia removal percentages, and the smaller consumptions took place in those weeks with limited nitrification. This data depicts the positive correlation between biological removal of ammonia and the consumption of alkalinity by the nitrifying reaction. This observation, along with the consistent production of TOXN, justifies the activity of an effective nitrifying biomass in the Poo-Gloo tank.

Total Nitrogen

The Poo-Gloo system was somewhat efficient in removing nitrogen from the influent, as shown in Figure 18. Between weeks 1 and 4, average TN concentrations were 31±4.9 mg/L for influent, 18.6±3.3 mg/L for Poo-Gloo effluent, and 25.6±2.8
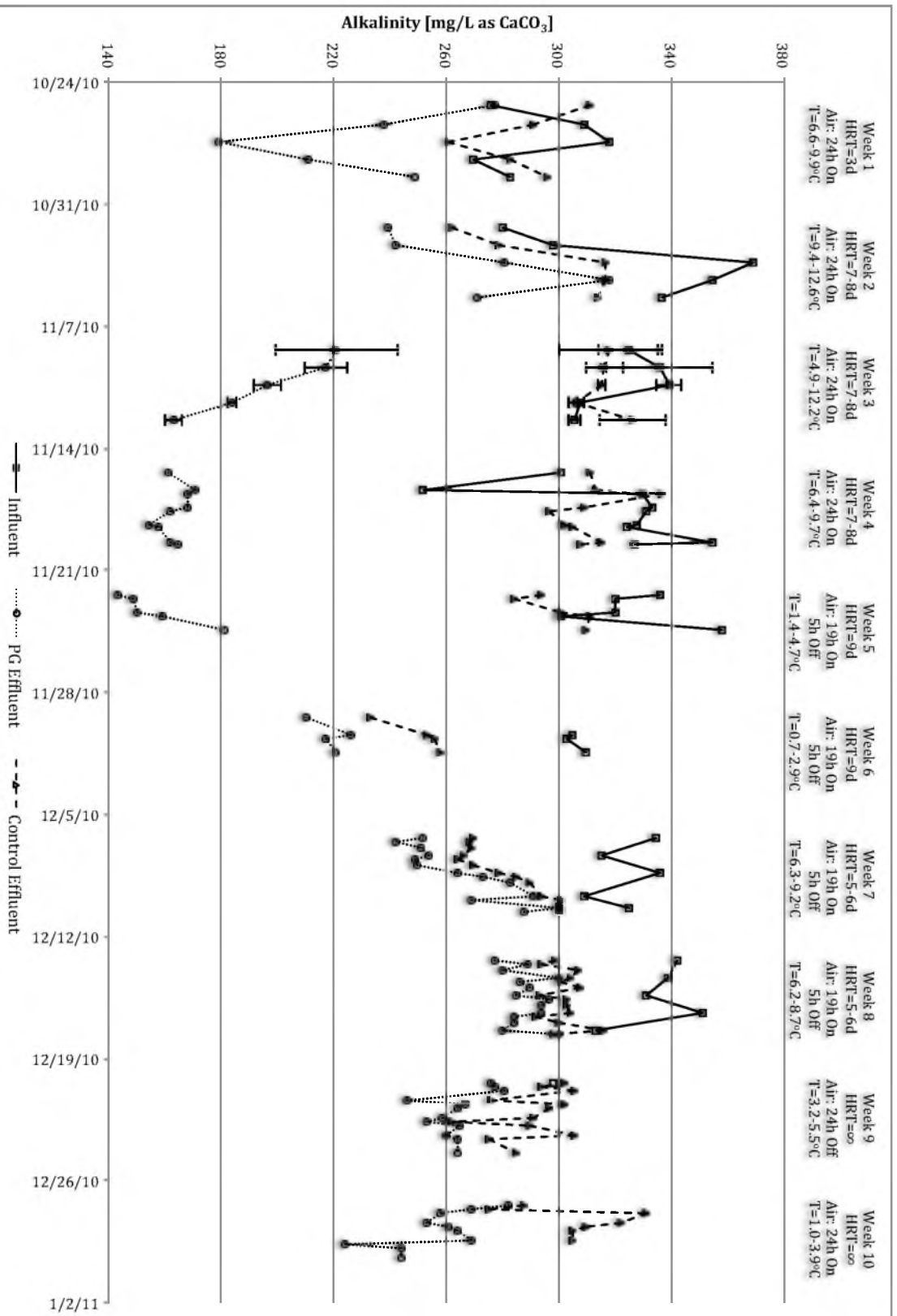
Figure 17- Graph of alkalinity levels for influent, Poo-Gloo effluent and control effluent (Weeks 1-10).

mg/L for control effluent. This reveals a much higher biomass density of the Poo-Gloo system with respect to the control system, as bacteria had to incorporate nitrogen from the water to fulfill their anabolic requirements for growth. It also suggests a larger community of heterotrophic denitrifies in the biofilm than in the control, as removal percentages of Poo-Gloos more than doubled those of the control system, removing between 30-50% of influent nitrogen during this period. Reaching efficiencies of 51±7% in the Poo-Gloo tank under continuous aeration in week 4 emphasizes Poo-Gloo's capability to sustain multiple microclimates, and agrees with the 40-50% elimination of TKN loads observed by Schlegel and Teichgraeber (2000) in submerged fixed bed biofilm reactors (SFBBRs).
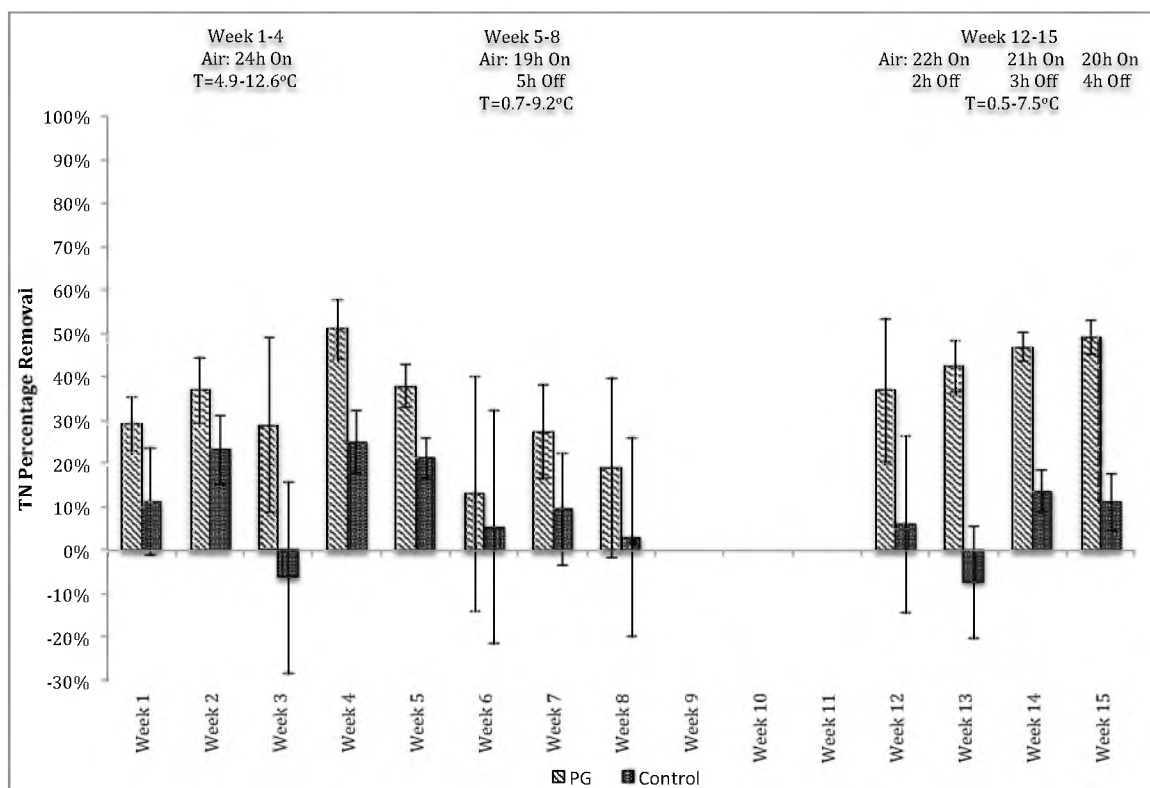

Figure 18- Comparative graph of TN percentage removal for Poo-Gloo and control systems.

Figures 19 and 20 present plots of TN levels detected during each week of the experiment. As air-off periods were incorporated in order to enhance anoxic zones in the reactor, it is noted that removal rates in both tanks did not increase between weeks 5 and 8 despite the fact that air cycling had been set. Denitrification rate is strongly influenced not only by the carbon content but also by its quality (Gómez, González-López, Hontoria-García, 2000). With no external carbon being added to the system, it seems likely that denitrifies were easily outcompeted by other heterotrophs during this period.

Still, air cycling effects on Poo-Gloo denitrification were well manifested in the period of week 12 to 17. Extending the air-off period in 1 hour between weeks 13 and 14, and then between 14 and 15, caused corresponding increments in the removal percentages of the Poo-Gloo system (42±6%, 47±3% and 49±4%). In weeks 16 and 17, influent water flow was shut off and the system was run in batch mode. Aeration was halted in week 16 and then turned back on at the beginning of week 17. During these 2 weeks Poo-Gloo denitrification increased as can be seen in Figure 20. Overall, Poo-Gloo system's performance outweighed the control's performance, coinciding with observations by Schlegel and Koeser (2007), who noted that simultaneous TN elimination without a separate denitrification stage is higher in SFBBRs than in conventional activated sludge plants.

Phosphorus Removal

Phosphorus exists in inorganic and organic forms, the latter usually being a minor consideration in most domestic wastes (Metcalf & Eddy, 2003; Sawyer et al.
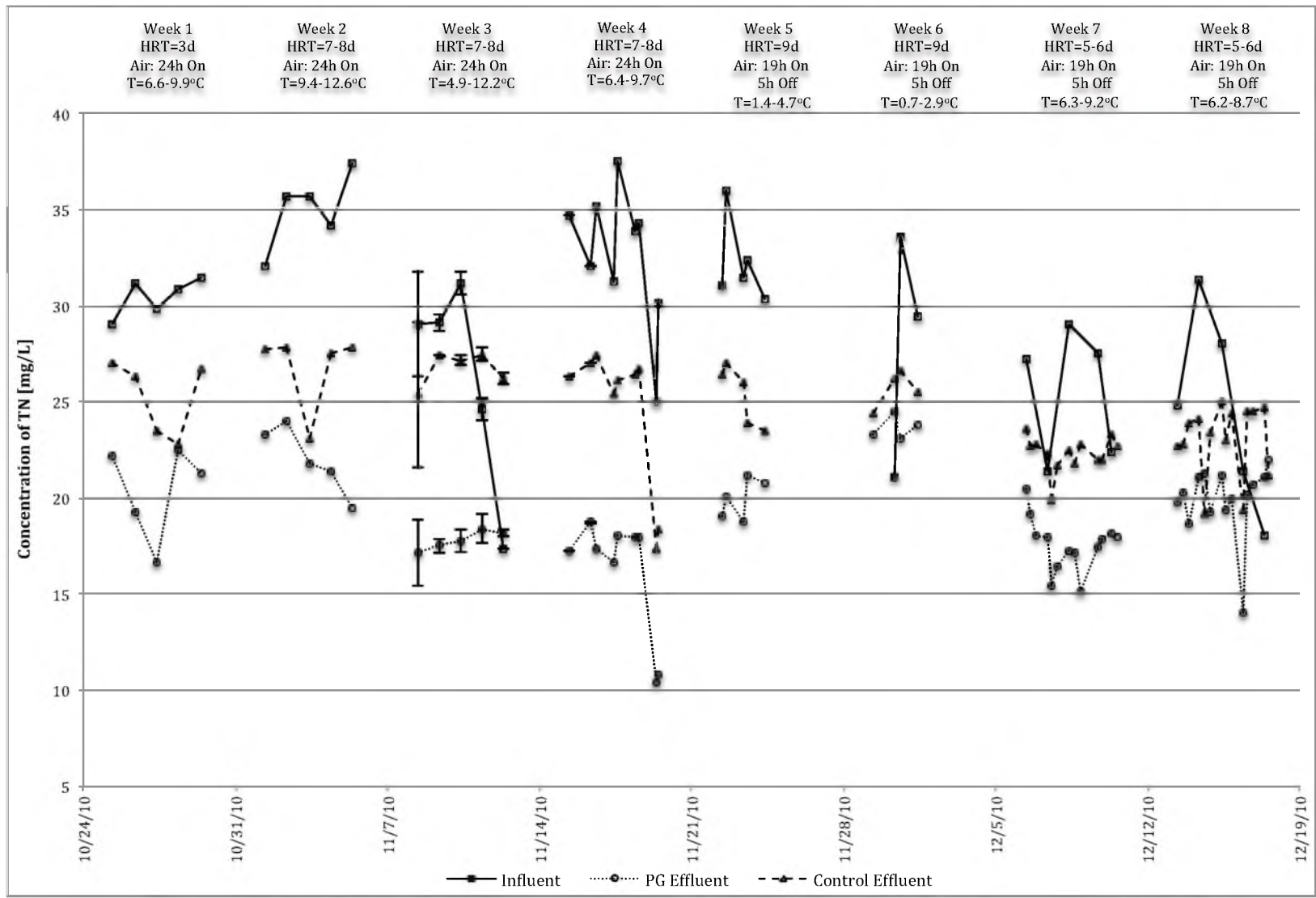
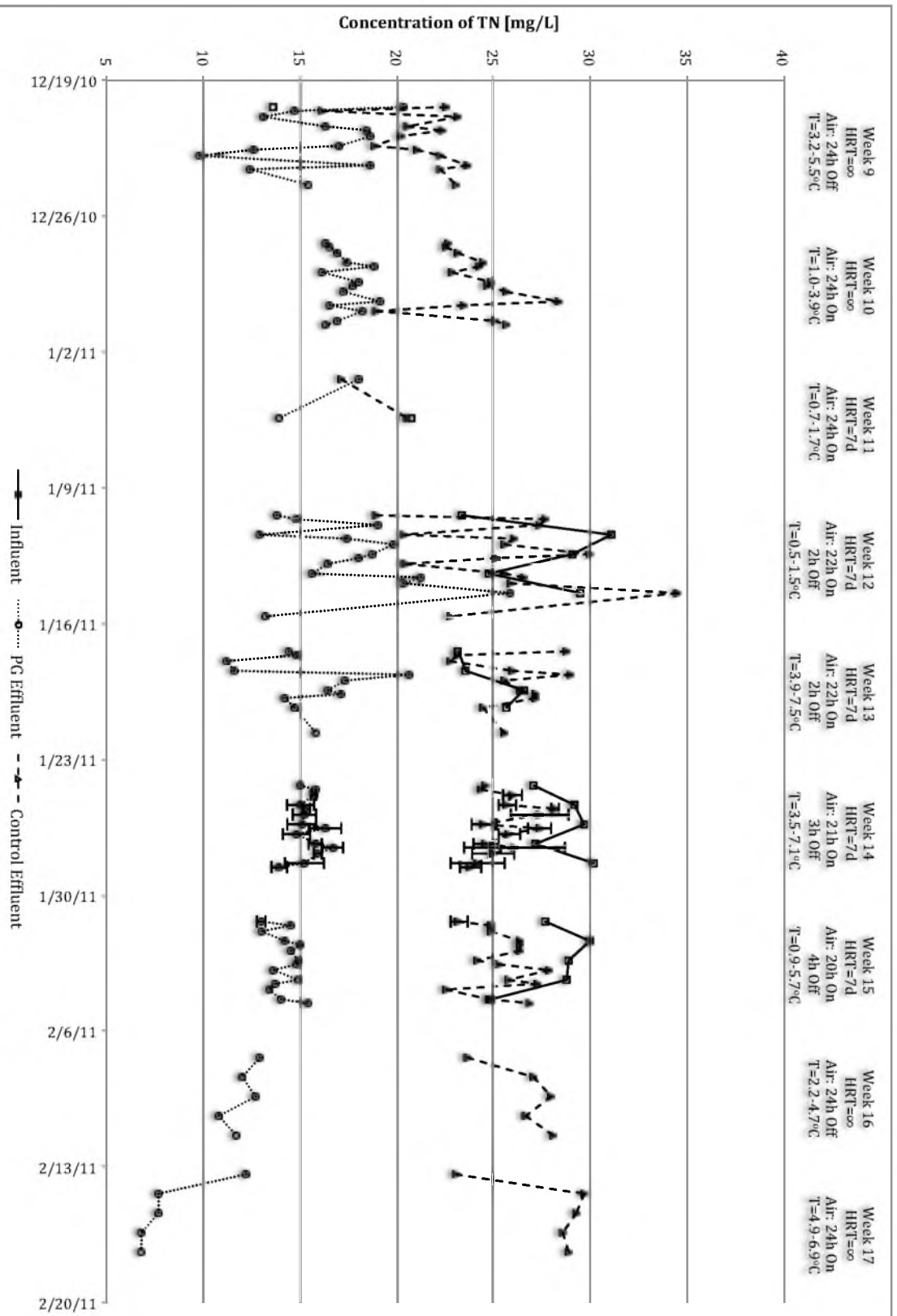Figure 19- Graph of TN levels for influent, Poo-Gloo effluent and control effluent (Weeks 1-8).

Figure 20- Graph of TN levels for influent, Poo-Gloo effluent and control effluent (Weeks 9-17).

2003). The usual forms of inorganic phosphorus are orthophosphate and

polyphosphate. Total phosphorus accounts for all forms of phosphorus including

organic phosphorus. Laboratory measurements of orthophosphate and total

phosphorus concentrations were recorded throughout the experiment to evaluate

the extent of enhanced biological phosphorus removal in the pilot-scale plant.

Removal efficiencies achieved by each of the systems are compared in Figure 21,

while week-to-week plots of detected orthophosphate and total phosphorus

concentrations are displayed in Figures 22, 23, 24, and 25.

Over the 17 weeks study period, the average total phosphorus reduction

rates were 23±10% for the Poo-Gloo tank and 7±11% for the control tank, as can be
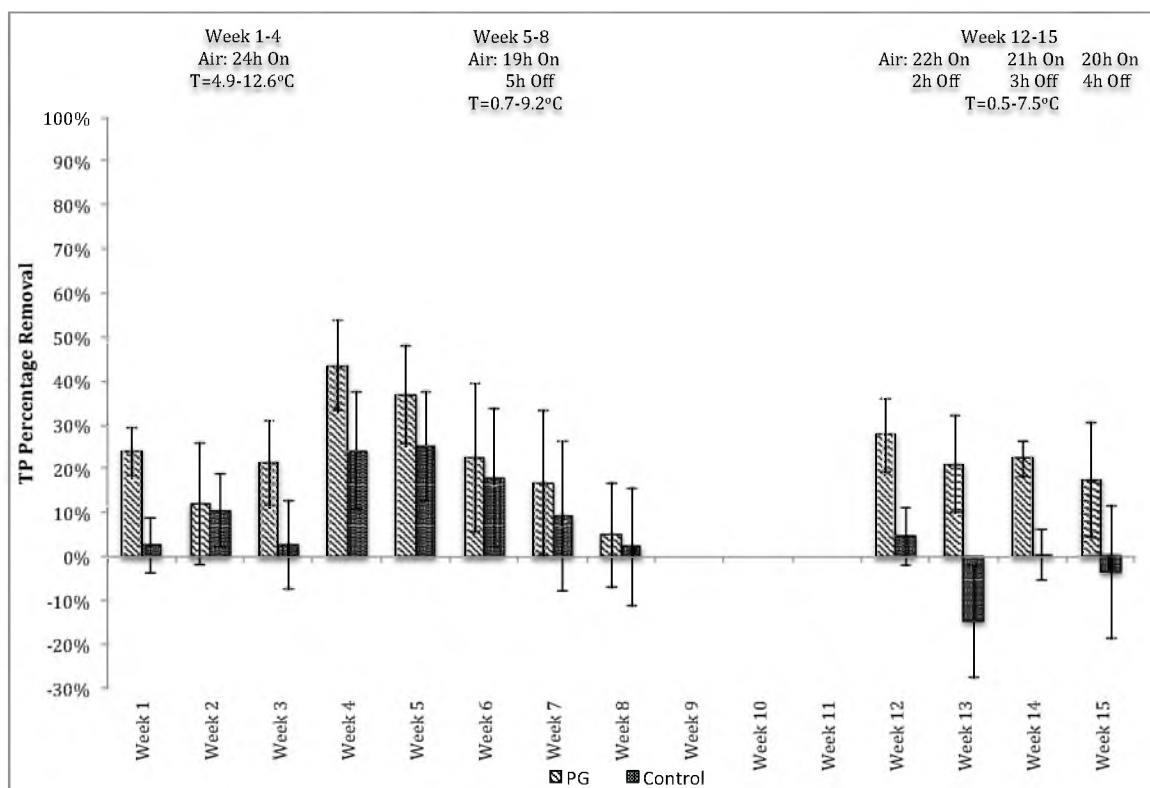

Figure 21- Comparative graph of TP percentage removal for Poo-Gloo and control
systems.

derived from Figure 21. Although the existence of PAOs in the whole bioreactor was not verified, the fact that EBPR activity was found in the final weeks of the experiment infers the presence of these microorganisms in the microbial biota of the two systems. Changes in the reactor operation impacted the removal percentages achieved in both tanks week after week; however, from inspection of Figure 21 it is discernible that PAOs in the biofilm exhibited much better adaptability than PAOs in the suspended growth of the control side.

During the first 4 weeks of operation, the average total phosphorus concentrations for influent, Poo-Gloo effluent and control effluent were 11.7±1.9 mg/L, 8.1±1.1 mg/L and 10±0.5 mg/L, respectively. Removal efficiencies of the Poo-Gloo system ranged between 12±14% and 43±10%, while those of the control system ranged between 3±10% and 24±13%. Phosphorus accumulation during this initial period is tentatively attributed to the growth requirements of bacteria for colonization of the biofilm and suspended growth systems.

Air cycling consisting of 19 hours on/5 hours off was introduced at the beginning of week 5 and maintained until the end of week 8. Far from improving phosphorus accumulation, this adjustment translated into reduction of TP removal percentages. Poo-Gloos' efficiency dropped from 37±11% in week 5, to 17±16% in week 7, while control's efficiency dropped from 25±12% in week 5, to 9±17% in week 7. Short HRT during the 7th and 8th weeks overloaded the system and a steeper drop of percentage removals was observed in both tanks during week 8.

Influent flow was suspended during weeks 9 and 10. Both systems were run in batch mode while aeration was suspended during the 9th week. Air flow resumed

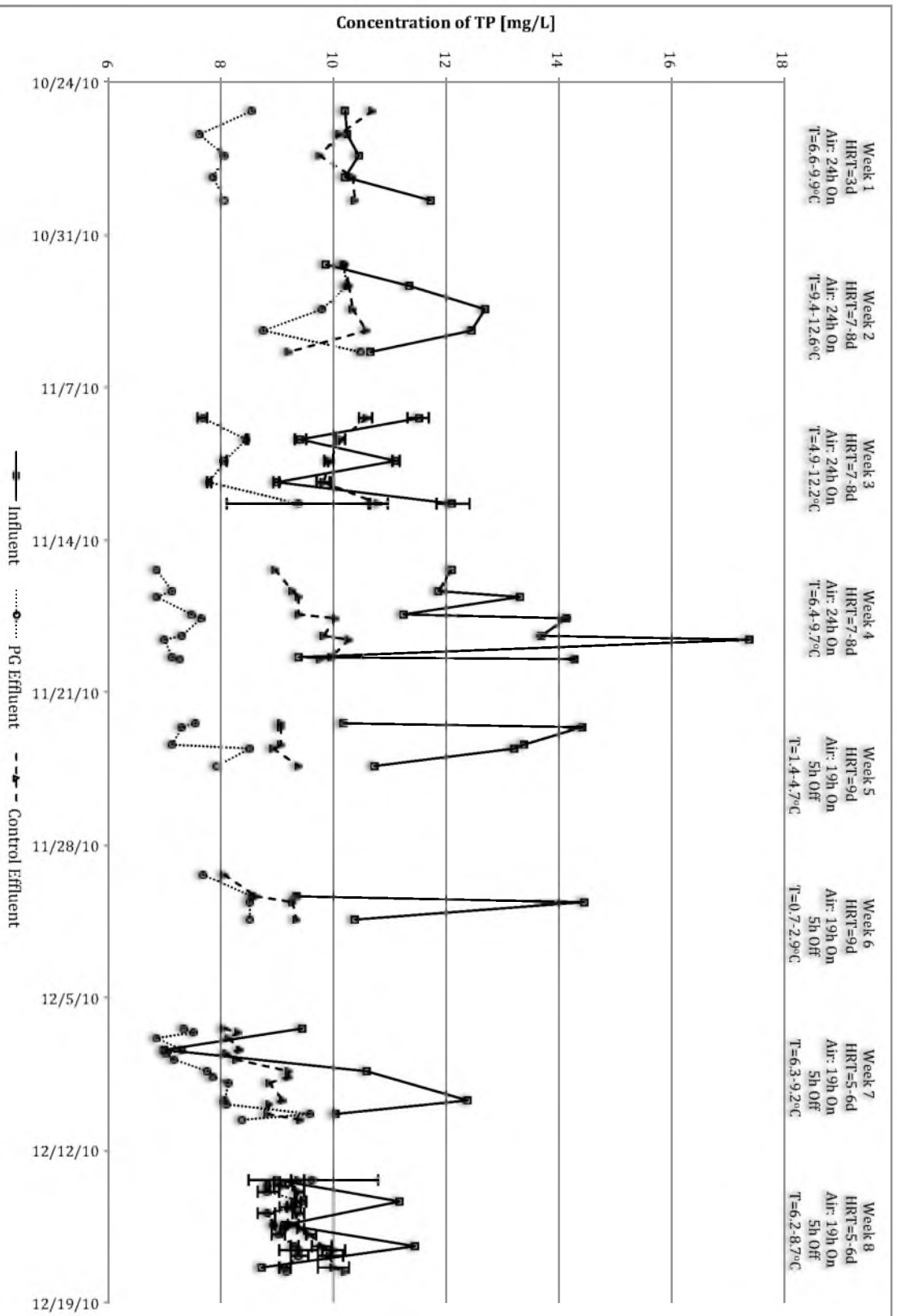Figure 22- Graph of TP levels for influent, Poo-Gloo effluent and control effluent (Weeks 1-8).
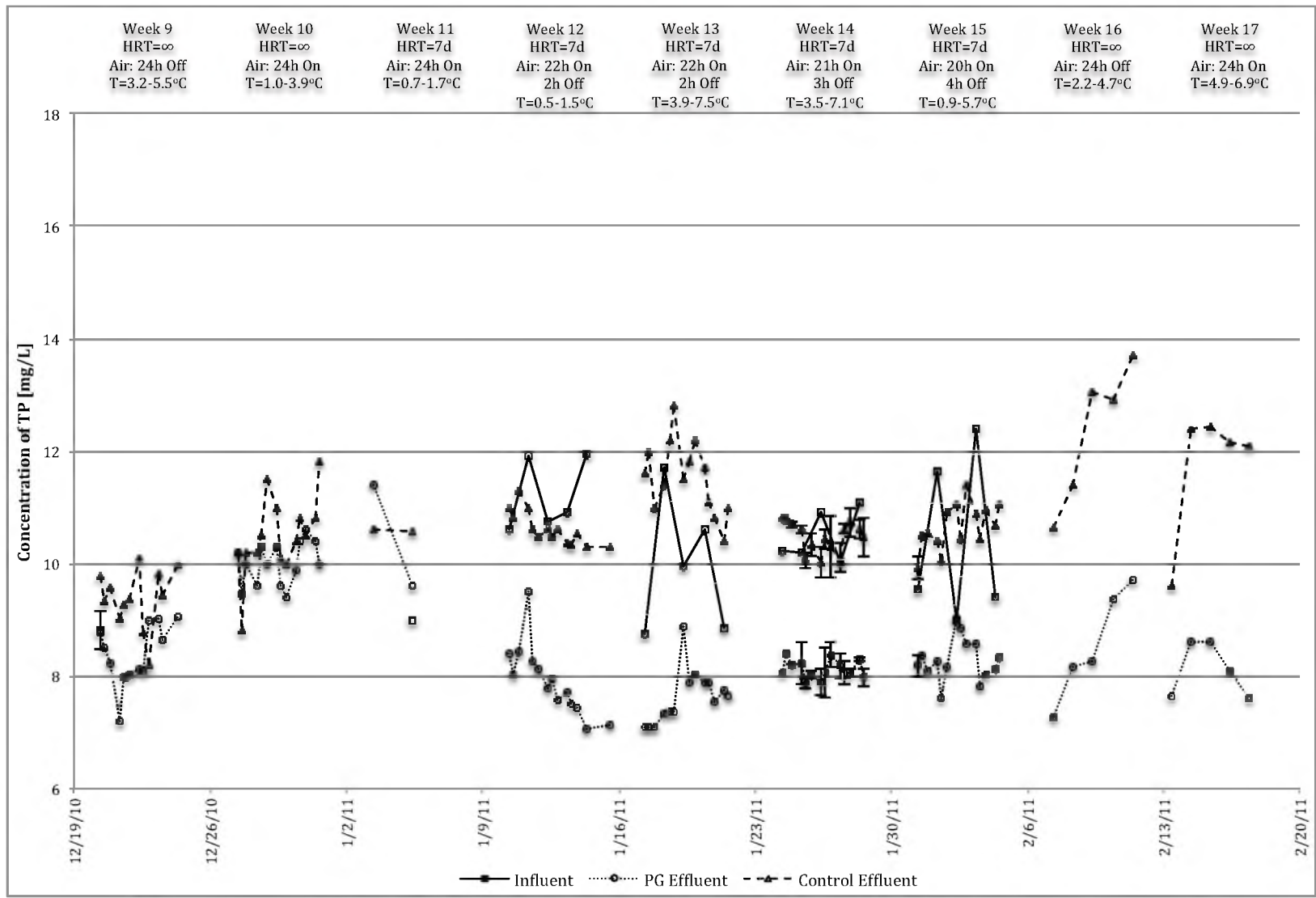
Figure 23- Graph of TP levels for influent, Poo-Gloo effluent and control effluent (Weeks 9-17).
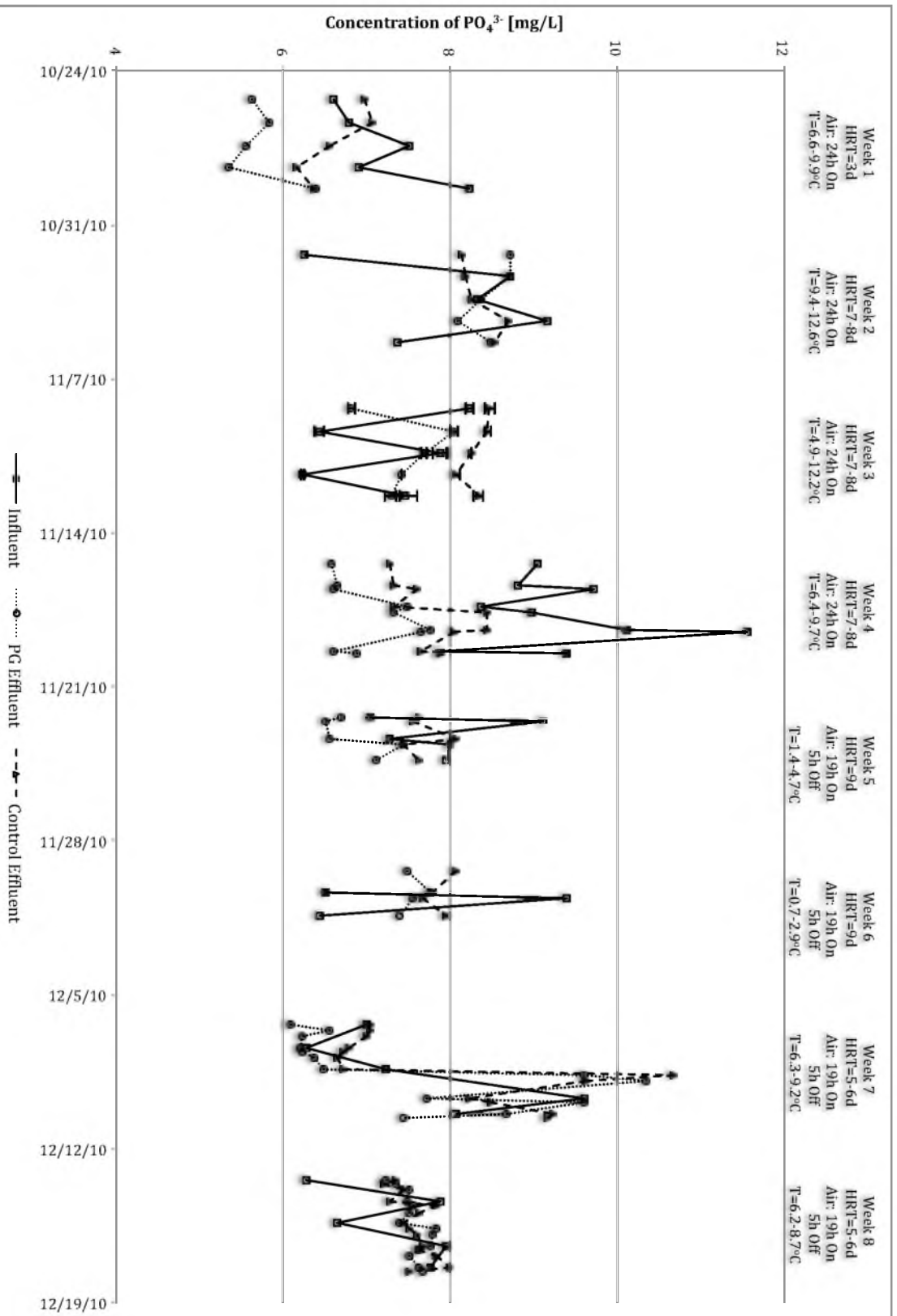
Figure 24- Graph of PO_4^{3-} levels for influent, Poo-Gloo effluent and control effluent (Weeks 1-8).
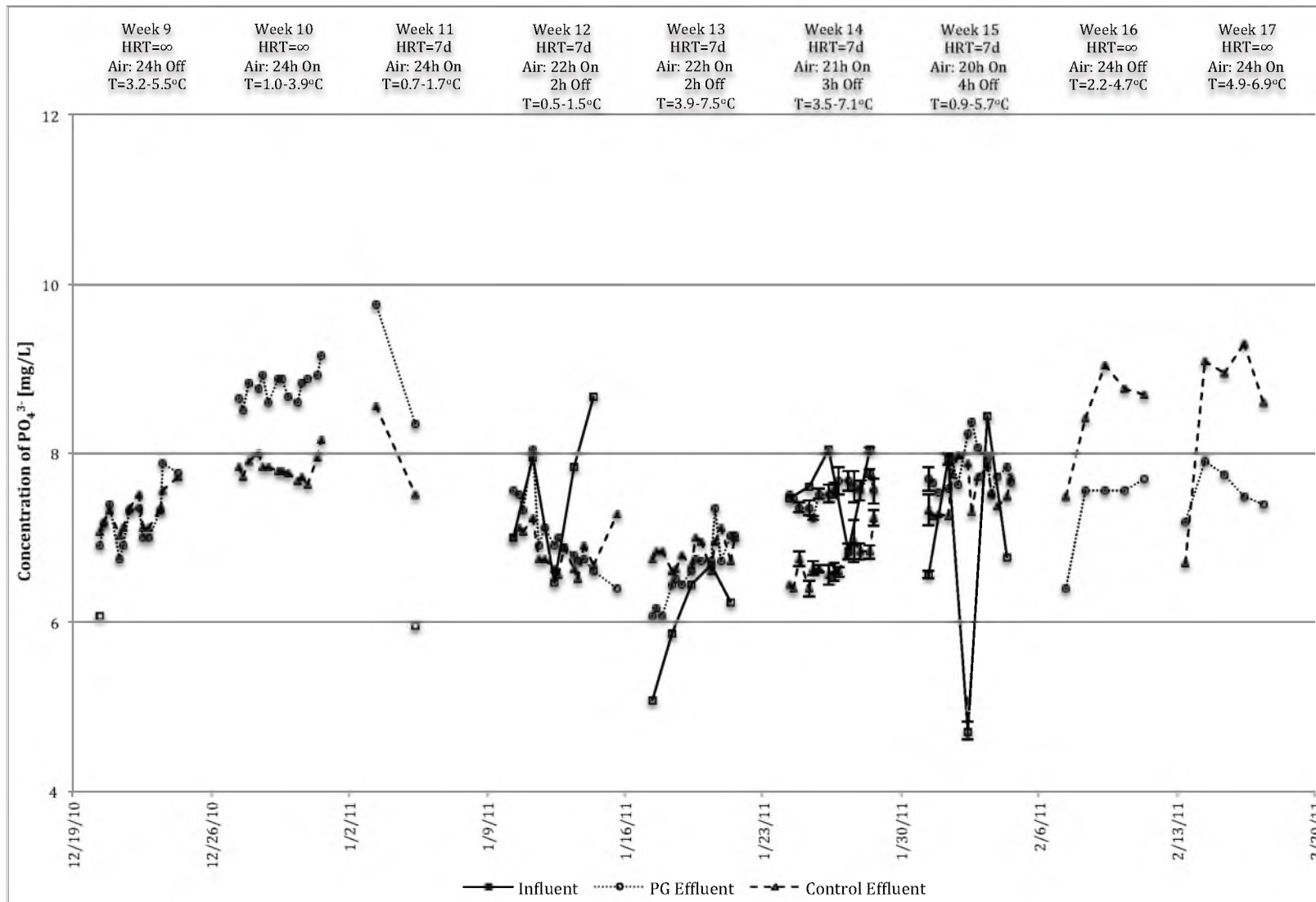
Figure 25- Graph of PO$_4^{3-}$ levels for influent, Poo-Gloo effluent and control effluent (Weeks 9-17).

again during the 10th week. Despite these conditions, there were no clear profiles of orthophosphate release and TP increase during week 9, or orthophosphate uptake and TP decrease during week 10.

In week 11, aeration was continuous and the inflow wastewater rate set at 863 L/day for an HRT of 7 days. This allowed the system to stabilize before air cycling was again incorporated at the beginning of week 12. In weeks 12 and 13, under 22 hours on/2 hours off aeration, reductions of Poo-Gloo influent phosphorus of $28\pm8\%$ and $21\pm11\%$ were attained, respectively. In week 14, under 21 hours on/3 hours off aeration, Poo-Gloo influent phosphorus was reduced by $22\pm4\%$; and in week 15, under 20 hours on/4 hours off aeration, Poo-Gloo influent phosphorus was reduced by $18\pm13\%$. The control tank, on the other hand, did not exhibit consistent phosphorus reduction in this period, even reaching negative efficiencies in weeks 13 and 15.

In the investigation of a lab-scale anaerobic/aerobic sequencing batch biofilter for phosphorus removal, Chiou, Ouyang, Lin and Chuang (2001) suggest an optimum value of 0.5 for the An/Ox time ratio. Meanwhile, using a sequencing batch biofilm reactor for simultaneous P and N removal, Gieseke, Arnz, Amann and Schramm (2002) concluded that simultaneous nitrification and phosphorus removal appears to be only possible with a sufficient long oxic period to ensure oxygen availability for nitrifiers. Although Gonçalves and Rogalla (2000) found the quality of the organic substrate to be a more effective selector of EBPR bacteria in biofilm systems than the length of the anaerobic phase, optimization of the air cycle is still a crucial component of submerged biofilm systems pursuing simultaneous removal of

nutrients and should be the matter of further investigation in the case of the Poo-

Gloo device.

Finally, an additional batch mode run was set in weeks 16 and 17. Influent

wastewater flow was suspended and aeration completely shut down during week

16, and turned back on the first day of week 17. Profiles for orthophosphate levels

in these 2 weeks are shown in Figure 26. Increase and depletion of orthophosphate

was observed in the two tanks at their corresponding week. This would confirm the

presence of PAOs in the system, at least during the final weeks of the experiment.
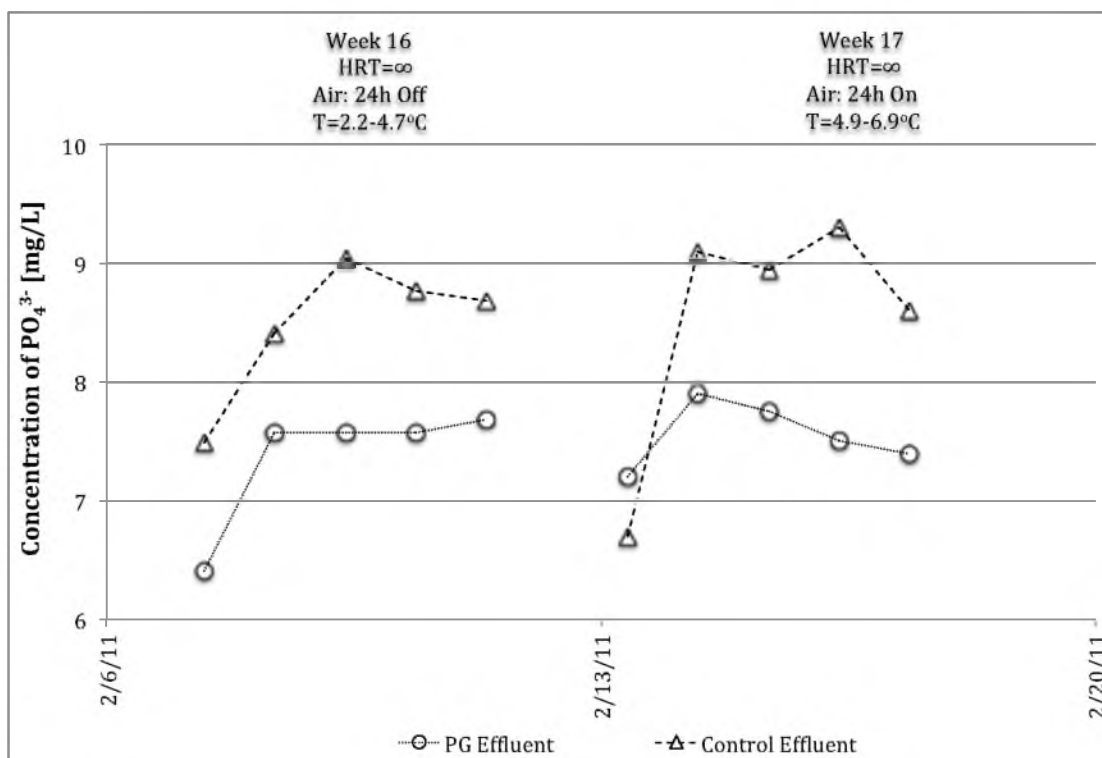

Figure 26- Possible release and uptake of PO_4^{3-} between weeks 16-17 by PAOs
suspected to be part of the heterotrophic biota of both tanks.

CHAPTER IV

CONCLUSIONS AND RECOMMENDATIONS

The nutrient removal potential of advanced aerated submerged biofilms inside domes was assessed in this thesis. Previous research demonstrates successful achievement of simultaneous organic matter, nitrogen and phosphorus removal in lab-scale submerged biofilm installations by subjecting the system to alternative periods of anaerobic and aerobic conditions. It was hypothesized that by following the same operation, Poo-Gloo devices would accomplish simultaneous biological nutrient and organic removal. A pilot-scale installation consisted of two tanks, one holding a system of six Poo-Gloo devices in series and another holding a resembling control system, was built, set in place, and operated with changing operational parameters for the treatment of primary-clarified domestic wastewater during a period of 17 weeks in order to address this hypothesis. According to the results found, the following conclusions can be made:

o Simultaneous organic matter, nitrogen and phosphorus removal is a complex process in which air cycling functions as a control parameter to assure optimum coupling between all processes. Efficient COD removal must guarantee enough organic matter is still available to sustain denitrification.

Air-off periods are intended to enhance denitrification and EBPR while nitrification is affected by the availability of oxygen. Therefore, special attention must be given to the optimization of the air cycle in each installation.

- Poo-Gloos exhibit exceptional COD and TSS removal capabilities, even under conditions of air-on/air-off aeration, attributed to very high mass and oxygen transfer enhanced by a domed design which encloses a dense biofilm that acts as an effective biological filter. Weekly COD removal percentages of up to 76±13% and 77±5% were achieved under conditions of continuous aeration and 21 hours on/3 hours off, respectively. Under the same conditions, corresponding TSS removal efficiencies of 96±4% and 91±2% were achieved.

- Almost complete nitrification was achieved under continuous aeration, while nitrification levels of up to 93.7±3 were achieved under 22 hours on/2 hours off air cycling. The fact that such elevated efficiency levels were accomplished with water temperatures bellow 10°C, sometimes near 0°C, evinces an outstanding capacity of the Poo-Gloo device to retain microorganisms into the biofilm matrix allowing the healthy growth of slow-growing bacteria.

- Denitrifying activity was well observed in the final 6 weeks of the investigation under air cycling conditions varying between 22 hours on/2 hours off, 23 hours on/3 hours off, and 20 hours on/4 hours off. Corresponding weekly total nitrogen removal percentages of 42±6%, 46±3%,

and 49±4% were accomplished. Incorporation of longer air-off periods resulted in enhancement of the anoxic zones in the system, demonstrating Poo-Gloo's capacity to sustain different environments within the biofilm.

o Microorganisms inside the domed structures of the Poo-Gloo devices were capable of taking up phosphorus to the point of immobilizing up to 23% of all phosphorus input into the system. However, weekly profiles of the concentrations of total and reactive phosphorus were consistent with the incorporation of air-off periods only until the penultimate week of the study, making EBPR activity possible to be claimed only during the last 2 experimental weeks. More investigation in the case of biological removal of phosphorus is recommended. Although EBPR appears to be feasible with advanced aerated submerged biofilms, an investigation that shows more consistent results during a period of time larger than just 2 weeks would be recommended.

o Still, under the conditions studied in this investigation, TN removal percentages of weeks 12-15 (42±6%-49±4%) along with TP removal percentages of the same period in the range of 18±13% to 28±8%, coincide with observation of full-scale aerated submerged biofilm applications in which 40-50% of TKN-loads and 20-50% of phosphorus loads have been simultaneously removed from municipal wastewater (Schlegel & Teichgraeber, 2000; Schulz & Menningmann, 2008).

o Finally, the simplicity of its design, the ease of operation, and the outstanding results demonstrated in this study, especially in terms of COD, TSS and

ammonia removal, make the application of the Poo-Gloo device practically

suited for rural communities relying on lagoon systems.

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