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Temperature impact on nitrification and bacterial growth kinetics in acclimating recirculating aquaculture systems biofilters

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TEMPERATURE IMPACT ON NITRIFICATION AND BACTERIAL GROWTH KINETICS IN ACCLIMATING RECIRCULATING AQUACULTURE SYSTEMS BIOFILTERS

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Abstract

This project assessed short-term temperature effects on total ammonia nitrogen (TAN) utilization rates in a batch laboratory-scale recirculating system. The tank system was designed for experiments on short term steady state and diurnal temperatures. A set of numerical models was developed to simulate observed results. The performance of the biofilters was determined with three tank replicates at fixed temperatures of 13, 20 and 30^0C; and at diurnal transient (sinusoidal) temperature regimes of (20 ± 3^0C; 30 ± 3^0C). Ammonia utilization rates and biofilter performance for beads acclimated at different temperatures regimes separated and mixed were also determined.

Total ammonia utilization rates increased with increased temperatures. The ammonia removal rates (Pseudo Zero Order) with slope (K) did not significantly differ ($P > 0.05$) for 13^0C ($K = -0.02$) and 20^0 C ($K = -0.04$); but differed ($P < 0.05$) for 13 and 30^0C ($K = -0.12$) and also differed ($P<0.05$) for 20^0C and 30^0C. Diurnal temperatures values differed ($P = 0.001$) for 20^0C $\pm 3^0$C ($K = -0.08$) and 30^0C $\pm 3^0$C ($K = -0.19$). Ammonia utilization rate values for beads that were acclimated and mixed at temperatures of 13, 20, 30^0C and subjected to diurnal temperatures differed ($P = 0.024$) at 20^0C $\pm 3^0$C ($K = -0.12$); and 30^0C $\pm 3^0$C ($K = -0.36$). Biofilter performance increased with temperature linearly with increased performance occurring at higher temperatures and high bacterial mass.

Ammonia utilization rate simulated models matched the observed data and assisted in determination of bacterial mass. Future designs and acclimation of the bead filters may be further enhanced by decreasing biofilter acclimation periods using higher temperatures in recirculating systems.

Keywords: ammonia nitrogen, temperature regime, bacteria, recirculating systems, aquaculture, biofilters, process control and nitrification.

Chapter 1: Overview

Information on biofiltration and bacterial effect on biofilter performance is important to the contributions of growth in aquaculture production. Recent growth in US and world aquaculture is attributed to: (1) human population increase; (2) limited expansion or losses of traditional fisheries; (3) increased demand for high protein; and (4) emphasis on healthy low fat foods (Wortman and Wheaton, 1991). Consequently per capita fishery consumption has increased in the US nearly 30% since 1990. The increased consumption has outpaced beef and pork with aquaculture production accounting for 11% of sea food consumed in the last decade (Wortman and Wheaton, 1991). In 2005, consumption increased such that more than 38% of all seafood consumed around the world was produced by aquaculture (Timmons and Ebeling, 2007; USDA, 2008). Although aquaculture is still in its emergent phase in the US, one aspect of aquaculture that is contributing to production is closed-cycle (recirculating) systems. Closed-cycle systems essentially utilized water in a recycled manner which helps to conserve water, a global natural resource.

Global concern about water resources becoming limited, including the rising cost of pre-treatment and post-treatment of water, have favored applications of water reuse and reconditioning technologies (Watten et. al, 2004; Lyssenko and Wheaton, 2006, Timmons and Ebeling, 2007). Water reuse has led to emerging technologies for improving water quality in closed recirculation systems. Improved water quality also minimizes discharge of toxic effluent from recirculation systems. Improving water quality in recirculating systems conserves water resources. Aquaculture has contributed to increased demand for water (Wortman and Wheaton, 1991) in the United States to meet high protein demand from consumers of fish. Recirculation systems treat water that deteriorates in quality over time from feeding the cultured species. The water treatment involves multiple processes including recirculation of water through the biofilter

to remove toxic nitrogenous waste and eventually some small discharge of waste water. The effluents from these systems usually contain waste including nitrogenous waste (NH_3, NO_2 and NO_3), total suspended solids (TSS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The effects of these effluents after treatment remain a concern for productivity of the systems and the environment in general.

The key focus of treating water in recirculation systems is a two-step ammonia removal microbial filtration process called nitrification (Kim, et al., 2006; Sousa and Foresti, 1996 and Carvantes et al., 2001). The process of ammonia removal is usually quantified in terms of nitrogen content of the nutrient removed from the system. The first step in the ammonia nitrogen removal process is the aerobic oxidation of ammonia to nitrite, and then to nitrate. The process is performed by ammonia oxidizing bacteria (AOB) called nitrifying bacteria, which get their energy from the oxidation of nitrogen compounds. The bacteria convert ammonia to nitrite (Wiesmann, 1994; Tanwar et al., 2007). Another group of bacteria in the system use nitrite as an electron acceptor to convert nitrite to nitrate (Zeng et al., 2003; Lucas et al., 2005). The process thus converts the nitrogen compound ammonia to nitrate a less toxic form in a recirculation system and eventually to N_2 gas as in a natural ecosystem (Figure 1.1). The less toxic nitrate nitrogen water in the recirculation system could then be diluted or discharged to surface waters.

Discharged nitrogen is of great concern in natural ecosystems (Jorgensen and Halling-Sorensen, 1993) considering the fact that nitrogen (a) contributes to eutrophication (Loehr, 1984); (b) increases oxygen demand due to oxidation of organic and reduced forms of inorganic nitrogen; and thirdly (c) has a toxic potential in certain forms which is harmful to aquatic species including brown blood disease in fish (Lawson, 1995). This is a major concern of this study in reducing the concentrations to levels that will be less toxic to fish raised in recirculating

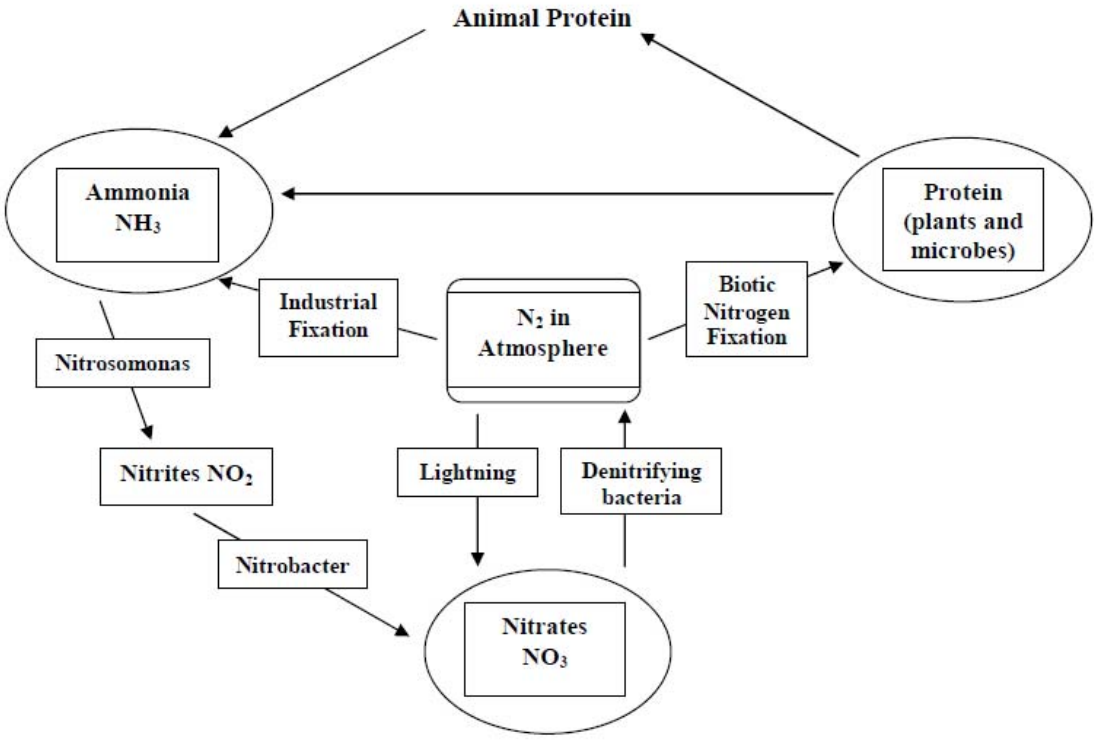

Figure 1.1: The nitrogen cycle in a natural ecosystem: This shows ammonia conversion to nitrite and nitrate.

aquaculture systems. In recirculating aquaculture systems nitrogen is produced by the fish through their metabolic process using protein and the accumulation of the nitrogen is controlled to prevent toxicity. A key nitrogen management practice in recirculation systems is to pass the water through a biofiltration system (Greiner and Timmons, 1998) to reduce the concentration to acceptable levels. The process is normally influenced by factors such as biochemical oxygen demand (BOD), pH, oxygen and temperature (Manthe et al., 1988; Michaud et al., 2006).

The influence of temperature in the nitrification process in a recirculating aquaculture system could help or harm production. Control of the production environment, leads to advantages such as uniform quality products, limited water use and the ability to grow fresh products (Wortman and Wheaton, 1991; Timmons and Ebeling, 2007). In addition to the advantages of control for production of fresh products, temperature control could also enhance

the removal of ammonia and other nitrogen compounds. Controlling the nitrification process could increase production volume, reduce discharged water and enhanced profitability for system managers.

The advantages mentioned above could be achieved if effects of temperature control on the nitrification process could be further explored. The current goal was to contribute to ongoing work in the area of temperature and nitrification interaction predictions for recirculating aquaculture systems. The research reported here utilized comparative studies of ammonia removal rates at different temperature regimes using bench scale biofilters in laboratory scale tank systems. This research also develops a frame work for commercially adopting temperature impacts on biofiltration to improve production.

The objectives were to: 1) determine ammonia removal rates at steady state temperature regimes; 2) determine ammonia removal rates during diurnal temperature regimes; 3) determine ammonia removal rate at diurnal temperatures, with mixed acclimated media (where mixed acclimated media refers to media that was acclimated at different temperature regimes prior to mixing); 4) determine ammonia removal rates at step-up (raising temperature from a lower to a higher value) regime and step-down (dropping temperature from a higher to a lower value) and 5) model the process to predict biofilter performance and to enhance future design.

The results presented in this dissertation call attention to the requirement for researchers to standardize ammonia removal in recirculation systems including acclimation and temperature variations. Currently, recirculating systems and wastewater treatment plants are the leading areas of biofiltration application with considerations for temperature. The most important steps in determining biofiltration performance in a recirculation system are sizing, designing & construction of the system components and understanding temperature effects.

The research in this dissertation involved constructing a laboratory scale self-contained system of tanks that were use to perform the experiment. One of the earliest efforts in the study was to standardize the sampling analysis methods using a spectrophotometer to determine the ammonia concentrations in sample. The procedure described in Appendix F focused on the steps to sampling and analyzing the samples from the tanks. In chapter 3 the results of ammonia concentrations and removal rates at different temperature regimes was reported. Chapter 4 contains simulation model results reported to determine validity of the experimental results and also predict what would happen over time in the bifiltration process. In chapter 5 summary, discussions and conclusions are elaborated on the results of the experiments while possible future commercial applications are also integrated.

Findings in this study support the importance of temperature effects on ammonia nitrogen nitrification process. In addition developing a model that predicts the rate of ammonia nitrogen removal for a closed recirculation system is an approach for potential future commercial application. This considers the fact that most commercial systems operate biofilters on an assumption of steady state temperature all year round, even though the biofilters are exposed to temperature fluctuations at different regions, weather seasons and time of the day. Work of this kind presents several challenges including system design and construction, temperature control water quality sampling and growing the bacteria biomass which takes time due to inoculation and growth phase of the bacteria. All of these are challenges similarly faced by commercial systems and thus the technological and pragmatic problem solving approach here could very well apply to future commercial systems.

This work was supported in part by Louisiana State University Agricultural Center; Biological and Agricultural Engineering Department and the Louisiana Sea Grant College

program. The results of this project have been presented at several scientific meetings (Table 1.1). In addition, two short articles related to this project, have been published. Chapters 3 and 4 are intended for submission for publication in peer-reviewed journals (Table 1.2). For consistency, all chapters of this dissertation have been presented in the format of the *Journal of Aquacultural Engineering* with specific formatting required to meet LSU dissertation format and style.

Table 1.1: Conference presentation and abstracts based on research presented in the document.

Date	Title	Conference	Location
July 2005.	Use of Temperature Control to improve Sustainability via Studies of Biological Effects in Aquatic Species.	American Society of Agricultural & Biological Engineers (ASAE), Paper # 054148.	Tampa, FL.
February 2006.	Design and Testing of Improved Process Control System for Time - Temperature Studies with Eastern Oysters - *Crassotrea Virginica.*	American Fisheries Society (AFS), Louisiana Chapter Meeting.	Natchez, MS.
February 2006.	Temperature and Environmental Control Effect in Recirculation Aquaculture Systems.	World Aquaculture Society (WAS).	Las Vegas, NV.
February 2007.	Controlled Temperature Effects on Biofiltration of Recirculation Systems for Oyster Studies.	World Aquaculture Society (WAS).	San Antonio TX.
March 2007.	Process Control Transient Temperature Effects on Biofiltration of Recirculation Systems.	Institute of Biological Engineers (IBE).	Saint Louis, Missouri.
February 2008.	Effects of Environmental Temperature Fluctuations on Biofilter Performance.	World Aquaculture Society (WAS).	Orlando FL.

Table 1.2 Published articles and manuscripts in preparation based on the research presented in this dissertation.

Number	Title	Journal/Magazine	Status
1.	Automated Temperature-Controlled Recirculation Systems.	Global Aquaculture Advocate, 2006. 9 (3): 40.	Published.
2.	Temperature Fluctuations Affect Biofilter Performance In Preliminary Study.	Global Aquaculture Advocate, 2008. 11 (6): 54.	Published.

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Chapter 2: Introduction

2.1 Biofilters

Nitrogen as a nutrient is essential for all living organisms (Timmons et al., 2002, Timmons and Ebeling, 2007). In recirculating aquaculture systems fish express nitrogenous wastes through urine and feces. Decomposition of these nitrogenous compounds is of primary concern because of the toxicity of ammonia, nitrite and to some extent nitrate (Timmons et al., 2002). Ammonia in recirculation systems is often removed by a biological (biofilter) filter through the process called nitrification. Selection of a biofilter influences capital and operating costs of recirculating aquaculture systems, water quality, and even the consistency of water treatment (Gutierrez-Wing and Malone, 2006). An ideal biofilter should remove all of the ammonia entering the unit (Davidson et al., 2007), produce no nitrite, and support dense microbial growth (biofilm) that require little or no water pressure and maintenance (Summerfelt, 2006). Such demand on the biofilm is unfortunately not feasible in any biofilter, although there are advantages and limitations to each filter design.

Biofilters are a key component in any aquatic recirculation system, responsible for nitrification in a biological filtration process. Biofilters are classified into two general groups: (a) emerged- water cascades over the media to maximize the transfer of oxygen, and (b) submerged- which consists of a biofilm in which the microbial body responsible for the bio-filtration process is completely in the bulk water nutrient solution. The submerged processes of a fixed-film consist of three phases: a packing, biofilm, and liquid (Tchobanoglous et al., 2003). In the fixed film only the active biofilm (nitrifying bacteria) on the filter surface containing the microbial body is responsible for the bio-oxidation of the substrates, irrespective of the total biomass present in the biofilm (Malone and Pfeiffer, 2006; Tseng and Wu, 2004).

Biofilms enable nitrification which is accomplished by autotrophic nitrifiers under aerobic conditions (Zhu and Cheng, 2001). The nitrification rate of a biofilter is normally inhibited by the existence of carbonaceous organic matter in water due to presence of fast growing heterotrophic bacteria. The biodegradable organic matter in recirculating systems enhances the growth of heterotrophic bacteria (Chen et al., 2006) which compete with the autotrophic bacteria for oxygen and surface area. This is especially prevalent in fixed film biofilters which are generally applied in recirculating fish culture systems.

In recirculating systems, the toxic total ammonia nitrogen waste from the fish is converted to nitrite which can be maintained in water at relatively high concentrations (Nijhof and Klapwijk, 1995). Nitrite is then converted to nitrate (Timmons et al., 2006) a less toxic form of the nitrogen compound and eventually may be returned to the atmosphere (See Figure 1). The efficiency of the conversion process is to a large extent dependent on the filter design and the characteristics of the biofilm and its environment.

2.2 Biofilm

A biofilm is a layer-like aggregation of microorganisms often found in aerobic suspended and attached growth treatment processes (Tchobanoglous et al., 2003). Biofilms are normally found in or on streambeds, ground water aquifers, lake-benthos, water pipes, ship hulls, piers, and aquatic plants and animals (Rittman and McCarty, 1980). Engineered processes that utilize biofilms include trickling filters, rotating biological contactors (RBC), biological activated carbon beds, submerged filters, land treatment systems and biofilters such as described here. The biofilm structure plays a major role in the biodegradation bacterial kinetics. Structure of the biofilm plays a vital role in the description of how materials are transported into the biofilm (Bishop, 1997). Biofilms consist of living cells, dead cells and cell debris attached to a surface.

Accumulation of microorganisms at these interfaces has been described for every aqueous life supporting system. Microbial structure of these communities ranges from a monolayer of scattered single cells to thick, mucous structures of macroscopic dimensions consisting of microbial mats and algal-microbial associations (Wimpenny et al., 2000). Factors that influence the spatial structure of the biofilms include microcolonies, extracellular polymeric substances (EPS), and channels.

Conclusive explanations for the structures observed in biofilms, requires the cooperation of fields of investigation, mathematical modeling and experimental research (Wimpenny et al., 2000). The use of current models applied to such complex cell structures in the biofilm assumes that substrate nutrients diffuse from the bulk phase, through a liquid boundary layer at the surface of the film to be utilized by the cells in the bacteria growth (Bishop and Kinner, 1986). The expansion of molecular field techniques not only allows more and more detailed documentation of the spatial distribution of species, but also of functional activities of single cells in their environment of the biofilm.

Microbial cells growing on the filter media surfaces include nitrifying bacteria and heterotrophs which are also sensitive to temperature and other water quality parameters. The functional capability of the biofilter is dependent on the layer of biofilm which is near the surface overlaid by nitrifying populations. However the outer layer of the biofilm is usually dominated by heterotrophs in the presence of organic carbon which increases biofilm thickness (Malone and Pfeiffer, 2006). Substrate bulk water concentration penetrates the biofilm layer dependent on the water boundary layer prior to the biofilm (Tchobanoglous et al., 2003; Zhu and Chen, 2001) and the thickness of the biofilm. The development of the biofilm layer which is based on the reproduction rate of the bacteria that make up the layer is also temperature and substrate

dependent. The temperature influence on biofilm growth process has influence on the ammonia removal rates which are also related to the filter characteristics (Lyssenko and Wheaton, 2006) in both suspended and attached growth systems.

2.3 Characteristics of Filters

Numerous variations in biofilter configurations have been used in semi-closed recirculation systems. This is partly due to the fact that many individuals construct their own biofilters, perhaps because of the same novelty which led people to the industry. In the characterization of any nitrification system, the two most important physical characteristics of biofilter media are a high surface area to volume ratio or specific surface area, and low clogging (biofouling) properties (Wheaton et al., 1991). The latter is often strongly influenced by the hydraulic characteristics of the particular biofilter as what may clog in one system may thrive in another system. Characteristics of filters also include durability, availability, cost, and the often related characteristics of specific gravity, weight, and buoyancy (Lawson, 1995).

However, the most common configurations designed include submerged, trickling, rotating biological contactor (RBC), and fluidized bed biofilters (Lawson, 1995; Wheaton et al., 1991; Timmons and Ebeling, 2007). Fluidized bed filters have a proven reputation for good performance in aquaculture systems (Summerfelt, 2006; Timmons, et al., 2006), wastewater (Tang et al., 1987), and solid waste land fill leachate systems (Martienssen et al., 1995; Kim et al., 2006). Although fluidized bed sand filters have advantages which include high surface area, non-clogging, high nitrification rates, high biomass concentrations, high hydraulic loading rates, and close contact between the liquid and solid phase (Tang et al., 1987), fluidized bed sand filters have their disadvantages too. Some of these include the difficulties in controlling of bed expansion below wash-out or bed carry-over (Chang et al., 1991) and the requirement for high

flow rates over the entire cross-section for fluidization (Wheaton et al., 1991). Therefore good design is needed for effective biofiltration.

2.4 Fluidized Bed Flow

A number of different designs have been developed based on fluidized beds. One type of flow distribution mechanism possibly developed specifically for applications in recirculating aquaculture systems incorporated a pipe-manifold, originating at the top of the vessel, and then distributing the flow into vertical pipes that extended down to the base of a sand bed (Weaver, 2006). The injection pipes were equally spaced across the filter with orifices in each tube for uniform distribution of flow directly into the sand bed. However the emergence of different filter designs including upflow and fluidized bed filters (Tchobanoglous et al., 2003) have revolutionalized biofiltration processes in recirculation systems. Fluidized beds are an efficient, relatively compact and cost-competitive technology for removing dissolved wastes from recirculating aquaculture systems, especially in applications that require maintaining consistent low levels of ammonia and nitrite (Summerfelt, 2006; Weaver, 2006).

Fluidized biofilters have been widely adopted in North America, especially in recirculating systems that require excellent water quality to produce species such as salmon, smolt, arctic char, rainbow trout, endangered fish and Ornamental or tropical fish (Weaver, 2006; Summerfelt et al., 2004; Pfeiffer and Malone, 2006). Uniform flow distribution of water at the base of the media bed (Figure 2) in a fluidized bed is critical for effective and reliable operation (Summerfelt, 2006). There are various flow distribution mechanisms with at least five different flow distribution mechanisms which have been used to uniformly inject water at the base of large filters in recirculating aquaculture systems. A downward flow into the filter avoids penetration of the vessel's walls with distribution pipes which also prevents the hydraulic head of water in the

vessel from back flowing through the distribution piping when there is a check valve malfunction (Summerfelt, 2006; Summerfelt et al., 2004). Single flow distribution filter systems consist of either a media covered pipe-manifold or false-floor distribution chambers that sometimes are used in relatively small recirculating systems (Summerfelt, 2006). In addition to the design complexity, the media used in fluidized bed filters demands high flow rates needed for the filter operation. The high flow rates required by fluidized bed filters may be the reduced in bead filters.

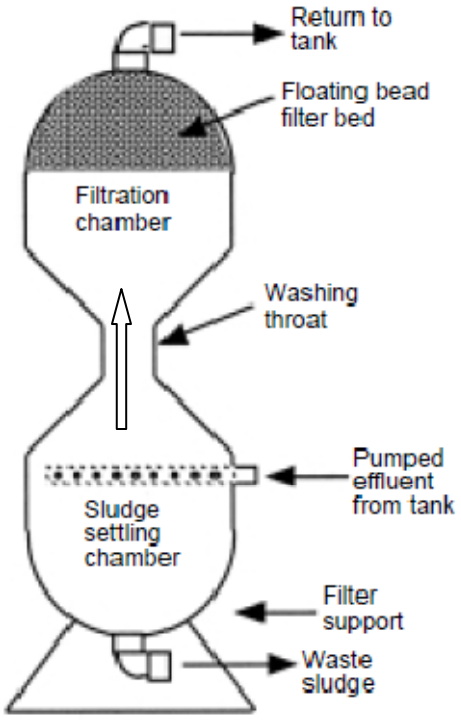

Figure 2.1: Uniform flow distribution of water from base of media (U.S. Patent No. 5,232,586 by Ronald Malone, Department of Civil Engineering, Louisiana State University).

2.5 Bead Filters

Bead filters generally work in pressured vessels and use a media that is only slightly buoyant (Timmons et al., 2006). Bead filters use polyethylene beads (Malone and Beecher, 2000) that are 2-3 mm in diameter. Specific surface area of beads typically used for the filtration beds is 1150 – 1475 m^2 m^{-3}. The surface area per unit volume can be calculated based on the

surface area of a sphere ($4\pi r^2$) (Timmons et al., 2006; Greiner and Timmons, 1998). Surface area and media porosity also enhance water flow characteristics of such filters. The bead filters are mostly operated in the filtration mode.

In the operation mode water passes through the packed bead bed capturing solids (Pfeiffer et al., 2008) while enabling an active biofiltration process simultaneously. The volume of water flowing through the media and the direction of flow is related to ammonia removal rates of the filters (Zhu and Chen, 2001). Although increased flow could increase substrate utilization rates, it also enhances solids capture and growth of heterotrophic bacteria on media which requires cleaning (Backwash). Cleaning of the media bed is periodically required to release excess biofloc and solids from the beads. The bioclarification and cleaning process is accomplished by using a mechanical (Malone, 1992; Malone et al., 1993; DeLosReyes, 1997b) pneumatic or hydraulic method (Malone, 1993; Pfeiffer et al., 2008). Although the filter serves as a bioclarifier the biofilm that feed on carbon-nitrogen nutrient in the bulk water are cells that exist in two forms in their biomass growth process: (a) suspended in solution as particulate material and (b) fixed or attached to media (Tchobanoglous et al., 2003). The attached media is the most popular form used in recirculating systems.

2.6 Attached Growth

Nitrification reactors based on bacterial films attached to fixed beds such as in trickling filters and submerged filters, are generally attractive for treatment of water in intensive recirculating aquaculture systems (Bovendeur et al., 1990). This is because high cell residence times are maintained even when the hydraulic loading rates are high. The attached growth biofilms are generally used for nitrogen removal because they allow longer biomass retention time for reliable nitrification (Okabe et al., 1996). Microorganisms that make the film adhere to

surfaces are of a broad population density while development of the biofilms that attach to the surface is a net result of several physical, chemical and microbial processes. The processes include the following: 1) transport of dissolved particulate matter from the bulk liquid to the biofilm surface; 2) firm microbial cell attachment to the surface; 3) microbial transformations (growth, reproduction, death etc.) within the biofilm and 4) partial detachment of biofilm due to fluid shear stress (Characklis, 1981). A combination of the above describes biofilm development as a growth process. Biofilm growth is also dependent on the configuration of the reactors.

Fluidized biofilter configurations are the most common utilized by aquaculture system managers (Wheaton et al., 1991). Fluidized bed filters have large surface area per unit volume compared to other fixed film bioreactors. They therefore allow the capability of operation as a plug flow at the liquid phase and mixed flow on the biological phase. As the concentration of pollutant nutrient decreases in the aquatic systems, removal rate per surface area decreases. In conventional bioreactors, the biological and liquid phases are mixed as in moving bed or plug flow systems. In the moving bed system the filters operate at a minimum substrate concentration (S_x), below this minimum concentration the bacteria cannot grow (Weaver, 2006; Zhu and Chen, 1999; Summerfelt, 2006). Although the fluidzed bed has the advantage of surface area, there are disadvantages, which include control of bed expansion below wash-out or bed carryover (Chang et al., 1991) stage. High flow rates required over the total surface area for the expansion may wash some of the bacteria away which may affect the nitrification rate of the filter.

2.7 Nitrification

Nitrification was initially documented in waste water in 1887 (Peters and Foley, 1983). Although nitrification is referred to as a two step biological process of converting ammonia

(NH_3) to nitrite (NO_2) and nitrate (NO_3^-) through the action of autotrophic nitrifying bacteria. The nitrification process is of great concern to commercial recirculating aquaculture systems (Lawson, 1995; Boyd, 1990). This is due to the fact that efficiency of the nitrification process in the biofilter influences the profit or loss in operation. Nitrification has been accomplished using many technologies including sand and bead filters of different designs. The first step is undertaken by bacterial populations which involve the oxidation of ammonia to nitrite by ammonia-oxidizing bacteria (AOB) and the second step is subsequent oxidation of nitrite to nitrate by nitrite oxidizing bacteria (NOB) (Kim et al., 2006).

The biological reaction processes involved are shown in equations (1) and (2) below after (Chen et al., 2006; Timmons et al., 2002):

Ammonia oxidation:

$$55\ NH_4^+ + 5\ CO_2 + 76\ O_2 \rightarrow C_5H_7NO_2 + 54\ NO_2^- + 52\ H_2O + 109\ H^+ \text{---------- (1)}$$

Nitrite oxidation:

$$400\ NO_2^- + 5\ CO_2 + 55\ NH_4^+ + 195\ O_2 + 2\ H_2O \rightarrow C_5H_7NO_2 + 400\ NO_3^- + H^+ \text{ -------- (2)}$$

When proteins and nucleic acids undergo catabolism in fish, ammonia-nitrogen is given out as waste by the fish (Lee, 2000). Accumulation of ammonia concentration in recirculation systems can be toxic to fish in the system. Consequently the ammonia in the system is removed through the nitrification process above. The process also reduces the volume of waste water discharged directly to surface waters. Water discharged from such systems pollutes surface waters and is toxic to fish in surface waters (Tilley et al., 2002). Subsequently the process of removing ammonia in recirculation systems generally incorporates biofiltration units in system designs preventing accumulation of ammonia to levels that are unsafe within the recirculation system (Summerfelt et al., 2004). Performance of a biofilter is generally estimated in terms of ammonia removal rates in mass per unit volume per day.

2.8. Estimating Ammonia Removal Rates

Ammonia removal rates in biofiltration are determined from the kinetics of the processes of converting ammonia to the intermediate product NO_2-N and the final product NO_3-N (Watten et al., 2004). The rates of reactions are also influenced by the hydraulic characteristics of the filter in terms flow (Watten et al., 2004; Malone et al., 2006). However the core theory of reaction rate is rooted in Michaelis-Menton enzyme kinetics which is simply represented after (Malone et al., 2006):

$$Rate = V_{\max} \frac{S}{K_m + S} \text{ ---------- (3)}$$

Where V_{max} is the maximum specific rate of substrate utilization (g day $^{-1}$)

S is the substrate concentration (g m $^{-3}$)

K_m is the half saturation constant (g m $^{-3}$)

However the equation is only applicable in conditions of constant enzyme availability (Malone et al., 2006). When bioreactors exhibit dynamic bacterial growth, the reaction rates is expressed as:

$$Rate = K_{\max} \frac{XS}{K + S} V_b \text{ ---------- (4)}$$

Where K_{max} is the biomass normalized maximum rate constant in g of S converted per gram of biomass per day.

X is the bacterial biomass (g m^{-3})

V_b is the reactor volume (m^3)

The actual reaction rates could also be affected by other factors including diffusion of substrate into the biofilm, velocity of substrate flow over the surface of biofilm (Harremoes, 1982; Malone and Beecher, 2000) and the kinetics of bacteria responsible for the nitrification process.

The Monod-type expression (Zhu and Chen, 2002, Zhu and Chen, 2001) for substrate removal

rate further explains the bacterial kinetics in a biofiltration process expressed as:

$$R = \mu_{\max} \frac{XS}{Y_s(K_s + S)} \text{ ---------- (5)}$$

Where R = Substrate removal rate ($gm^{-3}\ d^{-1}$)

μ_{max} = Specific growth rate (d^{-1})

X = Bacterial mass concentration (g cell m^{-3})

Ys = Yield of bacterial mass per unit of substrate used (g cell g^{-1} substrate)

S = Limiting-substrate concentration (g m^{-3})

Ks = Half saturation constant (g m^{-3})

This expression is substantiated by numerous studies and is considered a basic concept of microbial kinetics. Although the Monod equation is empirical, it is basically identical to the mechanistic model of the Michaelis-Menten equation, derived from the rates of chemical reactions catalyzed by enzymes (Jorgensen and Halling-Sorensen, 1993; Malone et al., 2006). It differs from the Michaelis-Menten equation by incorporating the bacterial biomass per substrate concentration and yield of bacterial mass per unit of substrate used. Typical Michaelis-Menten reaction kinetics is represented by the graph below (Figure 3). The models apply to typical nitrification processes that occur in recirculating aquaculture systems (Zhu and Chen, 2002). Biofilm kinetics is considered complex (Harresmoes, 1982) as the substrate supply within the aggregate layer of bacterial film is affected by diffusion as well as the water boundary layer. In addition there are also several other factors that influence nitrification rates.

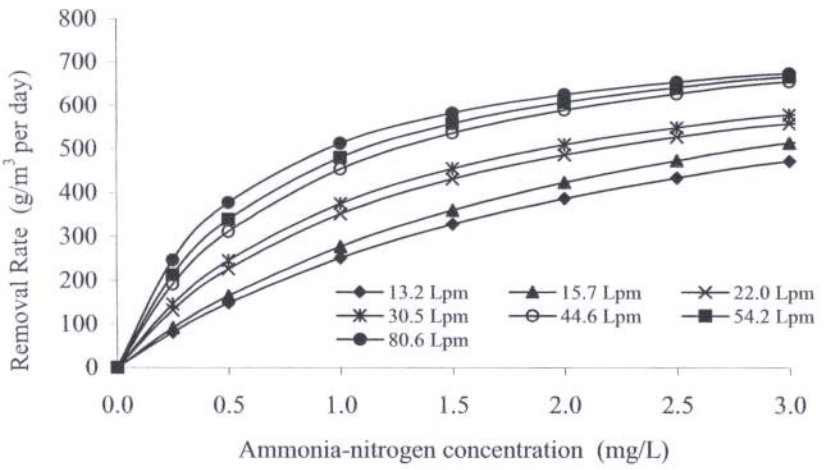

Figure 2.2- A Michaelis Menten graph of reaction kinetics (Recirculating Aquaculture Systems; Timmons et al., 2002; Timmons and Ebeling, 2007).

2.9 Factors Affecting Nitrification Rates

Removal of nitrogen from recirculating aquaculture systems considers the combination of two competitive biological processes: autotrophic bacteria feeding on ammonia and heterotrophic bacteria feeding on organic carbon (Ciudad et al., 2007). This is however affected by many factors extraneous to the process, which include but are not limited to dissolved oxygen (DO), pH, temperature, alkalinity, substrate concentration and biochemical oxygen demand (BOD) (Zhu et al., 1999 and Ling and Chen, 2005). However the supply of oxygen in a recirculating system is critical to maintain the nitrification process by bacteria.

2.9.1 Dissolved Oxygen

The supply of oxygen for the bacteria in the nitrification process is observed to be the principal factor for the upper limit carrying capacity of filters (Manthe et al., 1988; Ling and Chen, 2005). Oxygen concentrations must not fall below a limiting bulk water concentration such as 2 mg liter^{-1} for submerged rock filters if complete nitrification is to occur (Manthe et al., 1988). One of the major constraints of submerged biofilters is the limited supply of oxygen to

support the nitrification process (Manthe et al., 1988; Manthe et al., 1984). The filter in a nitrification process is normally assumed to have no internal source of oxygen. The oxygen flow to the filter is therefore a function of the water flow rate to the media surface area. This relationship can be expressed in terms of oxygen consumed by the filter:

$$OCF = Q*(C_i - C_o) \text{ ---------- (6)}$$

Where OCF = Oxygen consumed in the filtration process (mg of O_2 day^{-1}; Q = flow rate through filter (in liters day^{-1}); C_i = filter influent oxygen concentration (in mg-O_2 $liter^{-1}$); C_o = filter effluent oxygen concentration (in mg-O_2 $liter^{-1}$). The OCF also reflects the amount of oxygen consumed in the filter due to instantaneous impact of biochemical oxygen demand and the respiration of the bacteria (Manthe et al., 1988).

Assuming a minimum oxygen concentration of 2.0 mg $liter^{-1}$ is required to avert inhibition of bacteria in the filter. The oxygen carrying capacity of the filter is expressed as:

$$OCC = Q*(Ci - 2.0) \text{ ---------- (7)}$$

Where OCC = oxygen carrying capacity of filter (in mg-O_2 $liter^{-1}$). It is therefore necessary to have the oxygen flow in the system above the minimum required concentration for removal of ammonia.

2.9.2 Total Ammonia Nitrogen (TAN)

The concentration of total ammonia nitrogen (TAN) substrate in the nitrification process is also an important factor to consider in the design and operation of biofilters (Chen et al., 2006). Nitrification reaction which occurs in the biofilm by utilizing ammonia substrate depends on the local substrate concentration in the biofilm (Zhu and Chen, 2002). The nitrifying populations are therefore substrate limited in the nitrification process by the concentration of substrate available to the bacteria population deep within and at the surface of the biofilm (Horn,

1994; Rasmussen and Lewandowski, 1998; Zhu and Chen, 1999). Ammonia concentrations falling below the limiting substrate concentrations may impair the nitrification process while excessive substrate concentration affects the ratio of food to microorganism (F/M). Subsequently the nitrification process is independent of the concentration when there is high F/M ratio (Tchobanoglous et al., 2003; Tchobanoglous et al., 1991). Although the nitrification process occurs above limiting substrate concentration, it is also influenced by pH of the system.

2.9.3 Effects of pH on Nitrification

The reported pH range for optimum nitrification is 7.0 to 8.8 (Antoniou et al., 1990; Chen et al., 2006). The optimal range is determined by three effects the pH exerts on the nitrifying bacteria, a) Activation – deactivation of nitrifying bacteria, b) Nutritional effect associated with alkalinity and c) Inhibition through free ammonia and free nitrous acid (Villaverde et al., 1997). The nitrification process consumes alkalinity (HCO_3^-) and produces carbonic acid (H_2CO_3). Changes in alkalinity due to HCO_3^- consumption lowers water resistance while the carbonic acid lowers the pH. The pH drop in aquaculture applications is normally due to CO_2 accumulation (Loyless and Malone, 1997). A possible cause of such change is due to poor or inadequate aeration and degassing. Free ammonia concentration depends heavily on pH based on the following equation:

$$[NH_3 - N]_{free} = \frac{[NH_{4+} - N] * 10^{pH}}{K_a / K_w + 10^{pH}} \text{ ---------- (8)}$$

The hydrolysis of ammonia reaction constant is dependent on temperature:

$$K_a/K_w = \exp[6334/(273 + T)] \text{ ---------- (9)}$$

The variation of pH is to a large extent associated with poor aeration of the system, which implies aeration is considered an integral part of the system dynamics in the entire nitrification

process. Proper pH management is required for obtaining optimum performance for recirculating systems (Loyless and Malone, 1997), for CO_2 control and alkalinity adjustments.

2.9.4 Alkalinity

The conversion of ammonia to nitrate consumes alkalinity in the form of bicarbonate supplement. Alkalinity in the form of carbonate and bicarbonate becomes the nutrient element for nitrifying bacteria (Chen et al., 2006). Alkalinity is normally consumed at approximately 7.14 g/g $N_{oxidized}$ during nitrification (Timmons et al., 2002). It should be emphasized that pH dependent equilibrium exists between carbon dioxide (CO_2) and alkalinity to maintain concentrations of CO_2 below the animal stress level of 15 mg/l. The equilibrium prevents potential ion imbalance. It is recommended that a maximum alkalinity concentration of 200 mg/l as $CaCO_3$ for nitrification in fresh warm water (< 10 ppt salinity) recirculating aquaculture systems be used for the pH range 7.5 to 8.0 (Loyless and Malone, 1997). The interdependence between the two can be used to adjust pH physically through the (HCO_3^-) contents of the water or chemically adjusting the alkalinity. A greater alkalinity presents a higher nitrification process (alkalinity consumption) while nitrification increases linearly with increase in alkalinity (Chen et al., 2006). However, maintaining the alkalinity level for optimal nitrification may be undermined by the presence of excessive organic material, which encourages growth of heterotrophic bacteria.

2.9.5 Organic Loading

The ratio of organic carbon and inorganic nitrogen (C/N) in water connects the availability of growth area per media and competition for organic carbon and ammonia (Michaud et al., 2006). Although the impact of organic matter on nitrification has attracted increasing research attention recently, however quantitative information regarding the degree of impact on

biofliters in aquaculture is still being studied (Zhu and Chen, 2001). A decrease in nitrification is generally associated with increased organic loading (Bovendeur et al., 1990) due to the fact that the biodegradable organic matter in recirculating aquaculture systems generally supports growth of heterotrophic bacteria which compete with the autotrophic nitrifiers, especially in fixed film bioreactors (Zhu and Chen, 2001).

The inhibition of nitrification efficiency due to the presence of organic carbon is called allelopathy (Michaud et al., 2006). Inhibition of the nitrification process in the presence of organic carbon is partly attributed to the increase in the growth of the heterotrophs consuming oxygen which subsequently reduces dissolved oxygen concentration in the biofilm for the nitrifying bacteria (Chen et al., 2006; Satoh et al., 2000). This effect is also due to the faster growth rate heterotrophic bacteria which finally outgrow nitrifiers, subsequently reducing substrate penetration of the biofilm. Finally temperature changes warming the system associated with growth favors faster growth of most microorganisms (heterotrophic & other bacteria) (Malone and Pfeiffer, 2006).

2.9.6 Temperature

Recirculating aquaculture systems are controlled by system managers. However in several recirculation systems, water temperature fluctuates based on exposure to environmental or other factors. Temperature change influences the metabolic processes of fish in the system producing ammonia with increasing fish size (Tseng and Wu, 2004). Temperature also influences bacterial kinetics of the nitrification process in the biofilter operation. Bacterial reaction rates tend to increase with rising temperatures for suspended film filtration systems while no significant effect has been acknowledged for fixed film series bioreactors (Zhu and Chen, 2002; Zhu and Chen, 1999). However, nitrification with biodrums and landfill leachate

studies indicated an increased nitrification effect with increase in temperature (Wortman and Wheaton, 1991; Kim et al., 2006). Impact of temperature on fixed film filtration systems is still widely studied (Pedersen et al., 2007) because of the complexity of the bacteria kinetics involved (Harresmoes, 1982) in the nitrification process. Bacterial reaction nitrification kinetics have been described with respect to temperature using the Van't Hoff-Arrhenius equation which provides a general estimate of reaction rates expressed as:

$$\mu_{max} = \mu_{20}\,\theta^{T-20} \quad \text{---------- (10)}$$

Where μ_{max}=*S*pecific growth rate (d^{-1})

μ_{20} = the value of μ at 20^0C (d^{-1})

θ = temperature coefficient (Dimensionless)

T = Temperature in ^{0}C

Expanding the Monod equation (5) for the bacterial reaction rate in the nitrification process is expressed after (Zhu and Chen, 2002) as:

$$R = \mu_{20}\theta^{T-20}\,\frac{X}{Y}\,\frac{S}{(K_s + S)} \quad \text{---------- (11)}$$

Where *X* is mass concentration (g cell m^{-3}), *Ys* is yield of bacterial mass per unit of substrate used (g cell g^{-1} substrate), *S* is limiting-substrate concentration (g m^{-3}), *Ks* is half saturation constant (g m^{-3})

The effect of temperature on the nitrification process is an area of interest in ongoing research with diverse opinions on the temperature–biofilm interactions. In the ever increasing demand for fish protein, the focus of system managers has shifted to optimization of system

productivity while reducing cost of operations (Malone and Beecher, 2000) including improving biofilter performance. These important components of recirculating systems perform complex bacterial biofiltration at varying temperature regimes depending on the location of the system and the temperature of the surroundings. It is therefore noteworthy to further investigate transient (short term) temperature effects on nitrification at steady state and diurnal temperature (daily or possible long term) variation.

Numerous studies of temperature interaction with biofiltration, specifically removal of total ammonia nitrogen (TAN) in recirculation systems have been devoted to optimizing biofilter performance at steady state temperatures. However apart from those factors mentioned above, other factors such as temperature variations for seasonal cold fronts, daily diurnal temperature variations and media acclimation at steady state temperature while exposed to diurnal regimes; could also affect the ammonia removal rate in the biofiltration process. This dissertation also calls attention on the importance of the cumulative effects arising from daily diurnal temperature variations in the nitrification process. This section also points out where and how this research differs from previous studies and the importance of modeling for standardizing biofilter performance for the future.

Earlier studies in the area of biofiltration focused on understanding the kinetics of biofiltration (Harresmoes, 1982). Since then more biofiltration studies have been done in areas of design characteristics (Malone and Beecher, 2000) and enhancing performance (Wortman and Wheaton, 1991; Zhu and Chen, 2002; Malone et al., 2006). In contrast to the extensive studies done in biofiltration, similar work has been limited to enhancing performance of the biofilter at steady state temperatures. Two main groups of researchers have looked at temperature effect on biofiltration. Zhu and Chen (2002) could not find any significant effect of temperature on

biofiltration performance within a range of 14 - 27^{o}C. Wortman and Wheaton (1991) found a linear effect of temperature on biofiltration performance across a broader range 7- 35^{o}C. All other studies have been supportive of a linear or non linear relationship between temperature and biofilter performance.

A limited number of studies are known to have contributed to the temperature performance relationship. However little is known of short term temperature effects on biofiltration especially in the acclimation and seeded stage of operations. One of the major factors in managing recirculation systems is the environmental temperature the system is exposed to during operation. Temperature of the environment is not necessarily steady as presumed in most of these studies. In fact exposure to temperature variations in uncontrolled facilities is subject to daily diurnal temperature variation. Finally media acclimation done indoors at a controlled temperature and utilized in outdoor systems prior to a steady biofilm may be subject to temperature acclimation changes in performance, continued bacterial growth in addition to daily diurnal temperature variations. These factors have not been adequately addressed in other studies.

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Chapter 3- Temperature Effect on Biofilter Total Ammonia Nitrogen Removal Rate Performance

3.1 Introduction

Biofilter performance is one of the most important aspects of recirculating aquaculture systems in controlled fishery production. Fishery product consumption per capita increased in the US by nearly 30% over the last two decades, with aquaculture accounting for 11% of the seafood consumed (Wortman and Wheaton, 1991; USDA, 2008). Although seafood supply from captured fisheries grew at annual average rate of 1.2%, aquaculture supply grew at a rate of 9.1% (Gutierrez-Wing and Malone, 2006). The increase in supply is due to growing consumer demand for sea food which has further encouraged import of sea food to the United States to supplement production from traditional fishing and aquaculture systems (USDA, 2008).

However systems are challenged with constraints including water shortages, discharge regulations, demand for high quality product, coastal land use, and decreasing water quality in natural waters (Zhu and Chen, 2002). Recirculating aquaculture systems are a possible solution to the demand and challenges of traditional aquaculture (Wortman and Wheaton, 1991; Malone and Beecher, 2000; Ayer and Tyedmers, 2009). It is therefore no surprise that recirculating systems are gaining popularity for use in effectively managing removal of waste and pollutants where water quality and disease issues dominate (Davidson et al., 2009). Typical applications of recirculating systems are for conditioning of marine brood stock, production of larvae, fry and fingerlings for grow-out systems (Malone et al., 2006, Malone 2002).

Recirculating aquaculture systems are designed to accommodate the cultured organism and to maximize production. Although the design and increased adoption of recirculating systems appear to have stemmed from the need for precise control of temperature and light,

utilization of recirculating systems is now mostly driven by the desire to control water quality, disease and nutritional conditions in the systems (Malone et al., 2006).

Recirculating aquaculture systems are well established in aquaculture, with capability of performing essential mechanical, chemical and biological processes to ensure quality aquaculture products (Watson and Hill, 2006). The biological process is a key aspect of recirculating systems criteria in addition to aeration, clarification, solids capture and circulation, impacted by system management and control (Malone and Beecher, 2000). A biological filtration process consumes toxic soluble waste (ammonia) produced in recirculation systems (Nijhof and Klapwijk, 1995; Timmons et al., 2006). This process is critical to improving the cost effectiveness and productivity of recirculating aquaculture systems if they are to compete directly with other production methods including net pens and open pond systems (Malone and Beecher, 2000; Wortman and Wheaton, 1991; Lyssenko and Wheaton, 2006).

The biofiltration process is influenced by several factors such as pH, alkalinity, ammonia concentration, oxygen, salinity, organic loading and temperature (Tseng and Wu, 2004; Hao and Huang, 1996; Groeneweg et al., 1994). In a recirculating system the control of temperature for organisms raised also impacts the bacteria reaction rates in the biofiltration process. The temperature impact related to the removal of toxic ammonia in the system is a major parameter in biofilter performance (Wortman and Wheaton, 1991; Watson and Hill, 2006).

An approach to examine short-term temperature impact on biofiltration performance while understanding the ramifications on acclimation of biofilters will contribute to possible best management practices to commercial recirculating aquaculture producers.

3.2 Background

Biofilters are designed based on their capacity to utilize ammonia (in grams) per cubic meter of

media volume per day ($g/m^3/d$) or milligrams per square meter of media surface area per day ($mg/m^2/day$) in a biofiltration process (Silapakul et al., 2005; Malone et al., 2006). The rate of utilizing ammonia in the biofilm is an equilibrium process between the demand for substrate from bacterial biomass growth and the rate of substrate supply. The substrate supply is influenced by diffusion transport limitations (Rasmussen and Lewandowski, 1998; Chen et at., 2006). However factors that affect substrate diffusion and bacterial growth also influence the nitrification process and bacterial reaction rate (Chen et al., 2006).

Previous studies and theoretical principles have been core to determining influence of temperature in the design and operation of biological reaction filters (Zhu and Chen, 2002; Wortman and Wheaton, 1991). General predictions of the relationship between temperature and suspended bacteria reaction rates, follows a Van't Hoff Arrhenius curve (Zhu and Chen, 2002; Tchobanoglous et al., 2003). It is also indicated in previous studies that increasing temperature for bacterial reactions above an optimal value can disable enzymes on denature protein (Sawyer et al., 1994; Zhu and Chen, 2002; Tchobanoglous et al., 2003). A representation of the general estimate of temperature effect on biological reaction rates is indicated by the expression (Tchobanoglous and Burton, 1991) below:

$$\mu = \mu_{20}\theta^{T-20} \text{ ---------- (12)}$$

Where μ is the rate coefficient (d^{-1}), μ_{20} is the value of μ at 20^oC (d^{-1}), θ is temperature coefficient dimensionless and T is temperature (^oC). However, short term temperature impact on biofilter performance has not been examined in detail for fixed film and dynamic growth biofilter systems (Wheaton et al., 1994).

Temperature variation of biofilters in recirculating aquaculture systems during each 24 hr cycle may also vary, inducing a 24 hr cyclic diurnal temperature pattern in biofilters exposed to

outdoor or intermediate environmental fluctuating temperature regimes. Determining the effect of such temperature variations on biofilters in relation to total ammonia nitrogen (TAN) utilization may be essential for design and performance of biofilters in recirculating systems.

Ammonia utilization in biofiltration systems can be divided into three stages (Zhang and Bishop, 1994; Harremoes, 1982; Hagopian and Riley, 1998), with the first involving the substrate diffusion through the fluid boundary layer leading to the biofilm. The second stage involves substrate diffusion through the biofilm to the bacteria. The final stage involves nutrient availability to the bacteria within the biofilm. These stages are generally incorporated into reaction kinetic models to adequately describe the biofiltration process. Monod models that are used to represent fixed film nitrification kinetics in biofilters indicate the complexity of transport and enzyme reactions (Malone et al., 2006; Bishop, 1997; Golz et al., 1999). The stages of the reaction and enzyme kinetics that occur are simply represented by the Michaelis-Menton expression which is used to represent fixed film nitrification kinetics (Knowles et al., 1965; Zhu and Chen, 2002):

$$R = \frac{V_{\max} S}{K_m + S} \text{ ---------- (13)}$$

Where R is the substrate removal rate (g m^3 day^{-1}), V_{max} is the maximum specific rate of substrate utilization (g day^{-1}); S is the substrate concentration (g m^{-3}); and K_m is the half saturation constant (g m^{-3}). Equation (13) above is applicable to systems with controlled biomass. However when the equation is applied to more dynamic bacterial growth systems, it is expressed by the Monod formulation:

$$R = \mu_{\max} \frac{X}{Y_m} \frac{S}{K_m + S} \text{ ---------- (14)}$$

Where μ_{max} is maximum specific growth rate (day^{-1}), X is the bacterial biomass concentration (g cell m^{-3}), R is the substrate removal rate (g m^{-3} day^{-1}) Y_m is the yield of bacterial mass per unit of substrate used (g cell g^{-1} of substrate) and K_m is the half saturation constant.

The volumetric TAN removal rate is similarly modeled as:

$$R / V_b = Vtr = \frac{Vtr_{\max} S}{K_m + S} \text{ ---------- (15)}$$

Where Vtr (g m^{-3} day^{-1}) is the TAN conversion rate, Vtr_{max} (g m^{-3} day^{-1}) is the maximum Vtr that can be attained and V_b is the filter volume. An in-depth understanding of the biofilter TAN removal rate performance in relation to temperature interaction with nitrification process could contribute to the much needed improvement in biofilter performance, management practices and cost effective production of recirculation systems. The objectives of this study were to examine short term temperature impact on biofilter performance at steady state, diurnal and step temperature regimes. Setting up the experiments required development of custom designed laboratory temperature controlled tank systems while using laboratory sampling procedures that are applicable universally.

3.3 Materials and Methods

A batch bioreactor system of tanks with intake airlift pumps attached for continuous water flow and aeration of the reactors was designed and developed for this study. The system consisted of 36 tanks, 9 were used to perform the 3 X 3 experiments. Enhanced nitrification polyethylene media (EN-Media manufactured by Aquaculture Systems Technologies, LLC-New Orleans, LA) beads with a diameter of ≈ 3 mm (Figure 3.1) were used for bacterial growth for reactors in the tanks. The total biofilm surface area was assumed to include the media surface area and the inner walls of the bead contact area (Appendix E) in the reactor, while the

calculated volume was based on the dimensions of the media water volume of biofilter.

All tanks were 40 L in volume. Each reactor had approximately 24 recirculating passes per tank volume per day. Synthetic substrate consisting of ammonium chloride (NH_4Cl) and other trace nutrients (Table 3.1) suitable for ammonia oxidizing bacteria (AOB) growth was mixed in a separate tank (Figure 3.2), and pumped into the individual tanks containing the reactors. The reactor volume (4×10^{-4} m^{-3}) was calculated (Appendix E) at the construction stage. Bacteria growth on reactor media was induced by inoculating with seed bacteria from an existing aquarium. The inoculated tank was loaded with synthetic substrate from the general mixing tank at 5 g m^{-3} ammonia nitrogen concentration for bacteria growth and consistency in concentration. Inoculation and bacteria growth lasted from 3-8 weeks. The acrylic bioreactors were centered 0.15 m (6″) from one end of the tank (Figure 3.3).

The biofilters were designed using an acrylic tube with the following dimensions.

1. The height of filter is 19.1 cm (7.5″)
2. Internal diameter is 5.1 cm (2″)
3. The base/stand dimension is 5.1 x 5.1 cm (4″ x 4″)
4. Inflow screen porosity is 2 mm x 3 mm = 6×10^6 μm^2
5. Outflow screen porosity is 2 mm x 3 mm = 6×10^6 μm^2
6. Bead loading depth in filter 6.4 cm (2.5″)

The bacterial growth stage was considered complete when a steady removal rate was achieved for samples, but later analyses of the data indicated that the bacterial mass was still in the growth phase. The temperature of the acclimation growth stage was set at 25°C which was considered to be optimal for growth of nitrifying bacteria (Lyssenko and Wheaton, 2006) and pH was maintained at 7.0 - 8.6 (Hagopian and Riley, 1998, Wheaton et al., 1994).

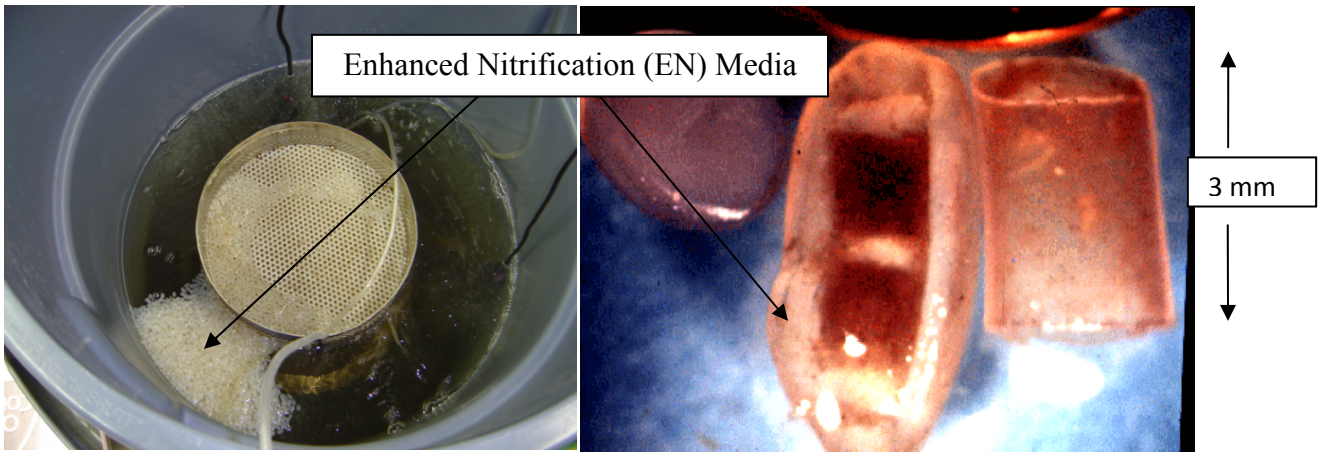

Figure 3.1: Enhanced nitrification media in the bacteria culture tank.

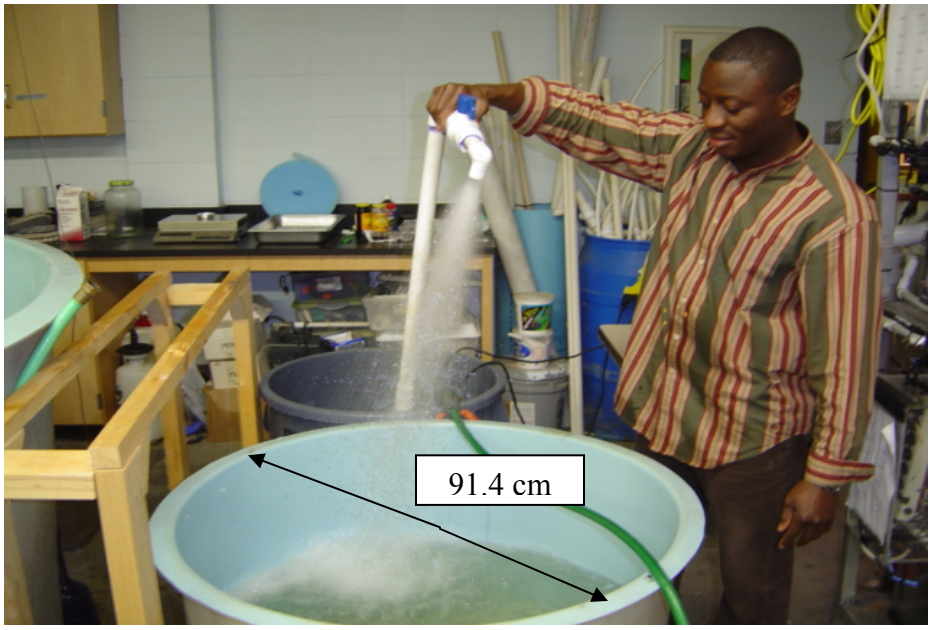

Figure 3.2: Fiberglass nutrient mixing tank (0.91 m in diameter- Total volume ≈ 500 L).

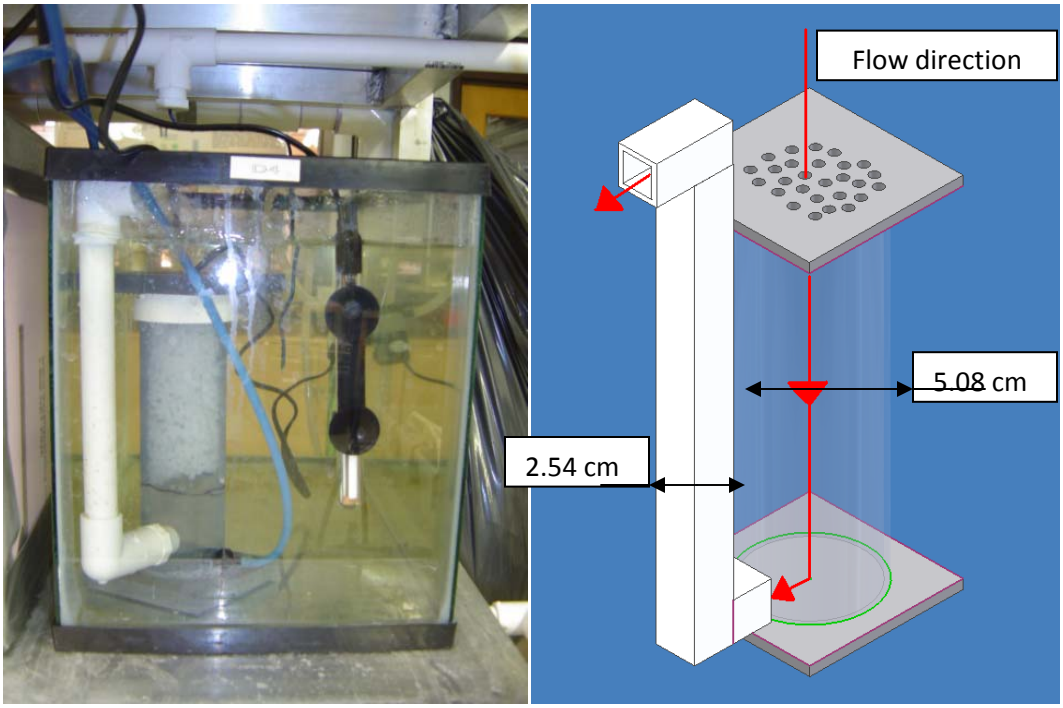

Figure 3.3: Biofilter at one end of tank with airlift for water circulation.

Table 3.1: Chemical composition of substrate nutrients: (Wortman and Wheaton, 1991).

Compound	Source	Concentration (mg/liter)
$NaHCO_3$	Louisiana State University Store/VWR.com	100
K_2HPO_4	″	15
$CaCl_2$	″	15
NH_4Cl	″	5 (as N)
$MgSO_47H_2O$	″	100
$FeCl_36H_2O$	″	5

Dissolved oxygen was maintained above 6 g m^{-3} considered to be above limiting concentration levels (Zhu and Chen, 2002; Zhu and Chen, 1999). Beads in the biofilters were acclimated prior to experimental sampling.

3.3.1 Biofilter Bead Acclimation

EN-Media used in the bacterial inoculation and growth stage were used for loading the acrylic

biofilter columns with attached PVC airlifts. This ensured that the beads were from the same pool (inoculation tank) under same growth conditions. Media beads were acclimated at three steady state temperatures (13, 20 & 30^{o}C), and mixed after the 3 d steady state experiments in a common container. The mixed beads were loaded into separate biofilters and subjected to the diurnal temperature (20 ± 3^{0}C and 30 ± 3^{0}C) regimes.

All cylindrical filter columns were loaded with beads to a depth of 2.5 in (6.4 cm). The unit biofilters were submerged into nine independent 40 L tanks loaded with nutrient solution. The independent tanks (Figure 3.4) were randomized for triplicates at each temperature regimes using a computer automated control system. The tanks were acclimated at the temperature regimes settings for 3 consecutive days before starting experimental sampling. The tanks containing loaded bead reactors were sampled after acclimation, and each reactor started at a fixed concentration (5 g m^{-3}) after acclimation.

3.3.2 System Sanitization

The acrylic reactor cylinders were sanitized between runs to minimize in-situ-nitrification which had proven to be problematic in previous studies (Malone and Beecher, 2006; Malone et al., 2006). After each set of experiments the beads were taken out of the reactor and set aside in individual 20 L containers for 2 h to maintain consistency of the biofilms during the sanitization stage. The reactors were then washed with diluted 1250 parts per million (ppm) bleach (chlorine) water, with all the pieces taken apart, scrubbed out and dropped into the tanks for bleach water circulation in the tank. Tanks were each loaded with 50 ml of concentrated bleach (Clorox purchase from Walmart) per 40 L tank achieving a concentration of 1250 parts per million (ppm) of bleach per liter of water in each tank. The chilling pumps were activated to circulate the diluted bleach within the tank and through the heat exchangers which had the potential of

bacterial growth on the inside. The system was allowed to run for 2 hr, after which the tanks were drained using electric motor operated suction pumps.

The reactors were rinsed with deionized distilled water. The tanks were flushed with tap water (chlorine < 0.2 ppm) using the same chilling pump activation setup. This ensured that the residual bleach water in the heat exchangers was also flushed out. The system was drained for a second time to be reloaded with synthetic substrate for the next experimental setup. The reactors were taken out of the drained tanks and further rinsed out with deionized distilled water before loading the beads into the reactor. The loaded reactors were repositioned in the tanks and the tanks were filled with the synthetic substrate for acclimation. Total time for cleaning was approximately 2½ hr and all tanks were loaded within 15 min of each other.

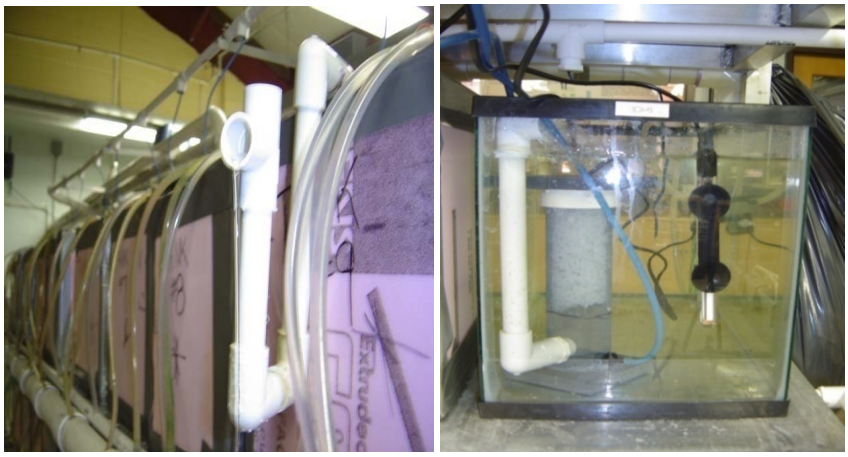

Figure 3.4: Individual tanks with all components (pump, biofilter, thermocouple and heater).

3.3.3 Temperature Control and Data Acquisition

The hardware for controlling the system was custom designed using a 32-channel CIO EXP-32 multiplexer board (Appendix M), one 16-channel PCIM-DAS 1602/16 analog-to-digital converter board, one CIO-DAS24 analog-to-digital converter (ADC) (Measurement Computing Inc., Middleboro, MA.), T-type thermocouples (Omega Engineering Inc., Stamford, CT) and

eight sets of 15A, 4-28 VDC solid-state relays (P/N 611489, Eastman Kodak Co). The hardware and software of the system were customized to process analog and digital data detected by the thermocouples. Junction ends of the thermocouples were soldered with lead-free electric solder (Radio Shack Inc.) and were made water resistant using plumber's "Goop" sealant.

The measuring ends were connected to each of the first 16 analog input channels of a CIO-EXP 32 multiplexer board. Temperatures were read as differential voltages and the multiplexer amplified the voltage signals by a factor of 100. A personal computer (PC-Pentium III), with embedded ADC processed the signals. The multiplexer was connected to the ADC via an EXP2DAS16-10 special 37-conductor cable (Measurement Computing Inc., Middleboro, MA).

Software for the control system was developed in micro-soft Visual studio using C++ (Version 6.0, Microsoft Corp, WA) executed on a Windows XP-professional based platform. The feedback control loop program was executed every 5 min while the program measured the temperature approximately every 3 sec. Average reads of the data were calculated over 5 min intervals for each tank computed and displayed on the monitor. In the feedback computed average temperatures were compared with the desired temperature. Each temperature cycle executed determined "on" and "off" logic for each heater or pump per tank.

3.3.4 Experimental Design and Sample Analysis

The system consisted of 36 tanks and nine tanks consisting three treatments in batches of three tanks per treatment were setup within the system (Table 3). The tanks were arranged in a randomized order for sampling. Each tank was independent of all others (Figure 6) with a unique individual temperature control unit consisting of a thermocouple, heater, pump, heat exchanger bioreactor with an attached airlift for internal (in-take) water recirculation through the filter.

Table 3.2: Thirty-six tank layout shows nine tanks in the top row which were randomized for the experiments. A T,x,y configuration was used such that T is temperature (13, 20 and 30^{o}C; 20 ± 3^{o}C and 20 ± 3^{o}C) x is the row and y is the tank number in that column.

$T_{1,1}$	$T_{1,2}$	$T_{1,3}$	$T_{1,4}$	$T_{1,5}$	$T_{1,6}$	$T_{1,7}$	$T_{1,8}$	$T_{1,9}$
$T_{2,1}$	$T_{2,2}$	$T_{2,3}$	$T_{2,4}$	$T_{2,5}$	$T_{2,6}$	$T_{2,7}$	$T_{2,8}$	$T_{2,9}$
$T_{3,1}$	$T_{3,2}$	$T_{3,3}$	$T_{3,4}$	$T_{3,5}$	$T_{3,6}$	$T_{3,7}$	$T_{3,8}$	$T_{3,9}$
$T_{4,1}$	$T_{4,2}$	$T_{4,3}$	$T_{4,4}$	$T_{4,5}$	$T_{4,6}$	$T_{4,7}$	$T_{4,8}$	$T_{4,9}$

Determining ammonia removal from the tanks is a method of evaluating the performance of a biofilter in the recirculation system. The important factors that influence ammonia removal including pH, flow rate, oxygen and ammonia concentration were relatively constant for the applied temperature regimes. The tanks were sampled at 6 h intervals over the 72-h period. Samples were analyzed using a spectrophotometer (Thermospectronic "Genesys-20", Thermo Electron Corporation product, Krackeler Scientific, Inc., Albany, NY) to determine removal rate when bacteria consumed the ammonia substrate. A calibration curve (Appendix I) was plotted, against which samples were tested. All analyses were performed within 2 hr of collecting the samples to reduce reaction potential from suspended bacteria flocculants. Samples were stored in disposable 50-ml centrifuge tubes before sampling. The difference between the initial loading and the final concentration was the substrate concentration removed by the bacteria in the process. In an assumption of "zero order" reaction kinetics, the equation that fits the linear regression (Malone et al., 2006; Malone and Pfeiffer, 2006) of the *Vtr* is expressed as:

$$Vtr = t\,(S) + C \quad \text{---------- (16)}$$

Where $\boldsymbol{t}$ (d^{-1}) is the slope of the line relating *Vtr* and S, while C is the y-intercept of the equation.

The slope of the lines that fit the linear regression equation (16) for ammonia removal rates at different temperature regimes was used to determine the biofilter performance. The slopes were interpreted for the data at each temperature regime. A T-test was used to compare the ammonia removal rates for the temperature regimes. The linear regression lines of volumetric TAN removal rates determined from equation (16) were normalized using intercepts forced to zero. The new slopes were calculated using sum of squared errors (SSE) to determine the value of parameter ***t*** for the entire analysis of results. It was assumed that $Vtr = 0$ when C= 0 for the linear regression lines to determine the value of ***t***. Although this may neglect the theory of minimum substrate concentration needed to maintain a steady state biofilm (Malone et al, 2006; Rittmann and McCarty, 1980), however minimum substrate concentrations can be maintained over short durations (Furamai and Rittmann, 1994) as applied to this experiment. The direct linear relation (DLR) normalized equation was fitted to the data as in previous studies (Malone et al., 2006; DeLosReyes and Lawson, 1996). The analyzed data was examined for short term temperature biofilter performance at a) steady state temperature regimes 13, 20 & 30^{o}C b) diurnal temperature regimes of 20 ± 3^{0}C and 30 ± 3^{0}C c) diurnal temperature regimes of 20 ± 3^{0}C and 30 ± 3^{0}C using mixed acclimated beads d) step temperature regimes at 30^{o}C stepped-down to 13^{o}C and 13^{o}C stepped-up to 30^{o}C. To further understand the effects of temperature on biofilter performance kinetics, biofilter ammonia conversion rates were compared under different regimes: steady states versus step temperatures, steady state versus diurnal transient regimes, and diurnal transient regimes versus mixed beads at diurnal transient regimes. The data collected for each experimental run was analyzed and presented in a graphical representation as indicated in the results and discussion.

3.4 Results and Discussion

The automated temperature control part of the system was tested at steady state and diurnal (sinusoidal) regimes prior to experiments. Steady state and diurnal temperatures were maintained in the tanks for a 3 d period. The system monitored the water temperatures every 5 min, each day with 288 temperature readings over a 3 d period resulting in a large 864 (288 x 3) number of observations per tank. Actual daily average temperatures in the tanks were within ± 0.5°C from the expected temperature control. The system showed that the lowest chilling average temperature achieved was 11.87^{o}C. The chilling process temperature profiles are as shown in the section to follow. Specifications, design considerations and testing of the system are discussed in Appendix M with details that were applicable to the experiments in the following sections.

3.4.1 Steady State Temperature (13, 20 & 30^{o}C) Ammonia Removal Rates

Temperature profile data was collected for tanks arranged in triplicates and held at 13, 20 and 30^{o}C post acclimation for 72 h for the duration of the experiment. Temperature profiles were plotted showing the steady state temperatures at 13, 20 & 30^{o}C (Figure 3.5) with minimal temperature deviations ≈ ± 0.1^{o}C. Experimental applied temperature regimes were the core in determining the ammonia conversion rates as indicated in the following section.

Sample concentrations for ammonia over 72 h (3 d) were plotted (Figures 3.6) for 13, 20 and 30^{o}C steady state regimes. Change in mean concentrations from the experimental data fitted a linear regression plot used to determine specific TAN removal rates per day. The specific ammonia removal rates V_{max} were calculated using the expression:

$$V_{\max} = \frac{dS}{dt} \approx \frac{\Delta S}{\Delta t} = \frac{FinalConcentration - InitialConcentration}{ChangeinTime} \text{ ---------- (17)}$$

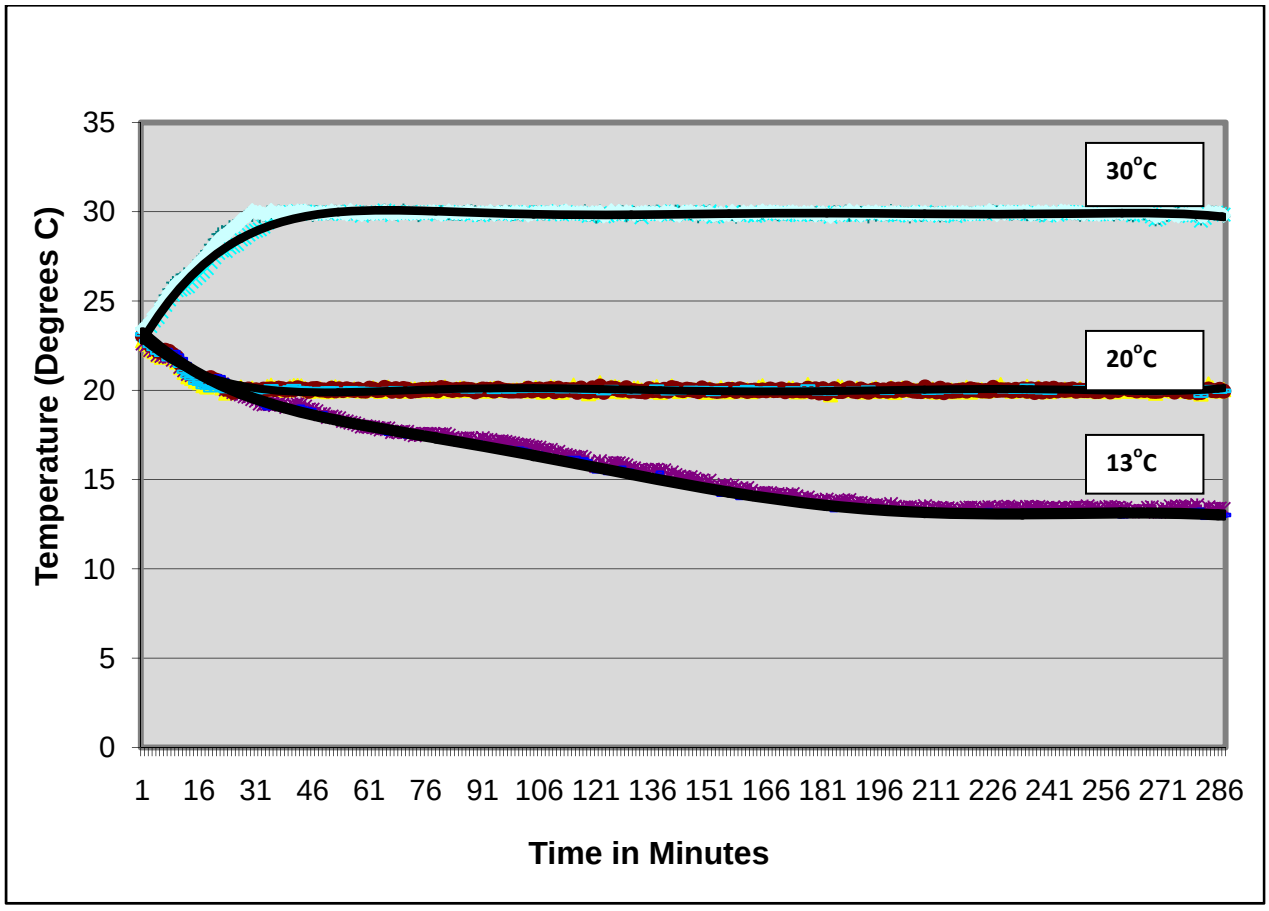

Figure 3.5: Typical approaches to steady state temperature profiles for 13, 20 and 30°C.

Volumetric TAN removal rates were also calculated and the linear regression plot fitting the data were normalized to zero intercepts (Zhu and Chen, 2002; Malone et al., 2006) at different temperature regimes as discussed above.

Ammonia removal rate at 13°C steady state (Figure 9) had a mean TAN slope -m = 0.107 with a correlation coefficient of R^2 = 0.930. The removal rate at 20°C steady state also had a mean TAN slope -m = 0.152 with a correlation coefficient of R^2 =0.963 and, the mean TAN for 30°C steady state had a slope of -m = 0.349 with a correlation coefficient R^2 =0.986. Volumetric

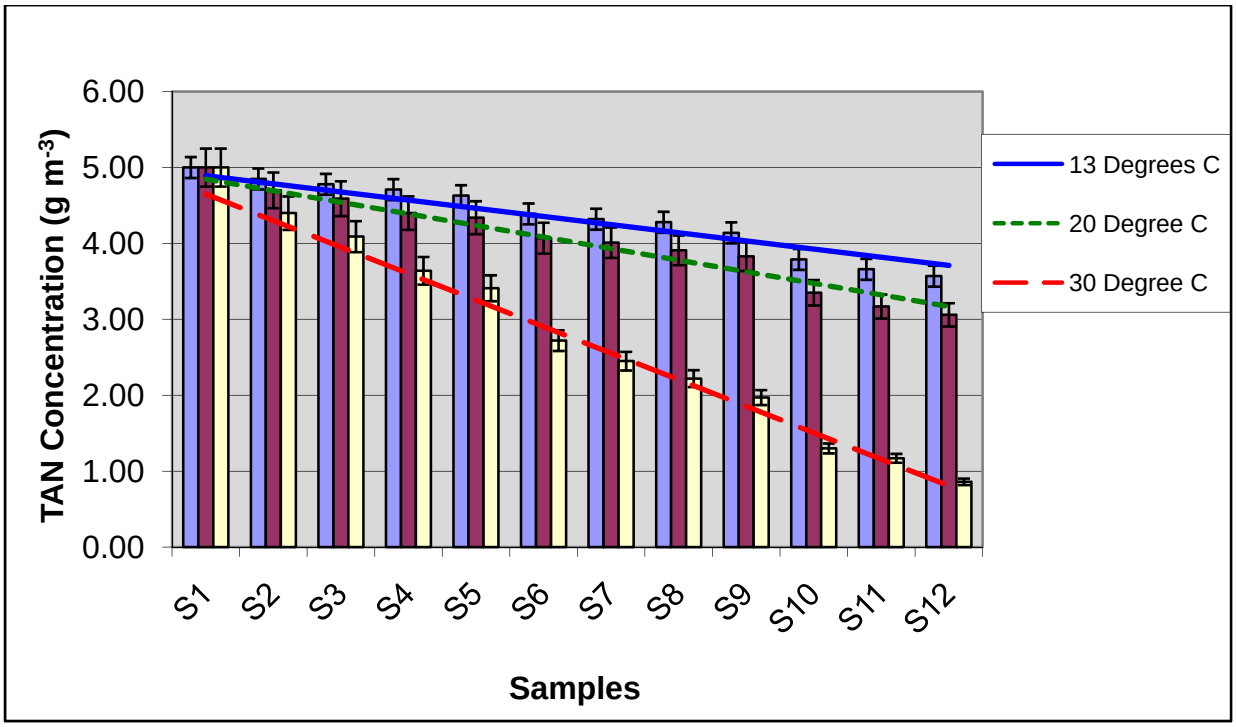

Figure 3.6: Steady state 13, 20 and 30°C mean ammonia (TAN) concentrations of the biofilters and S1 is the initial solution concentration normalized to 5 gm^{-3}.

ammonia removal rates were calculated from the observed mean concentration data. A linear regression from the plot of volumetric ammonia concentration against concentration (Figure 3.7) was used to determine the performance (**t**) of the biofilter.

An equation relating biofilter performance to steady state temperature regimes (Figure 3.8) was also developed as a prediction tool for filter performance at different temperature regimes. The specific differences in ammonia removal rates for temperature regimes were determined using student T-test for observed data. Ammonia removal rates were significantly ($P < 0.05$) faster for 30 than for 20, confirming the numerical variation. However the variations between 13 and 20°C were not significantly different. In previous studies (Zhu and Chen, 2002) concluded numerical variations in the range of 14 -27°C but no statistically significant difference in substrate removal rates. This may be partially supportive of our data as indicated.

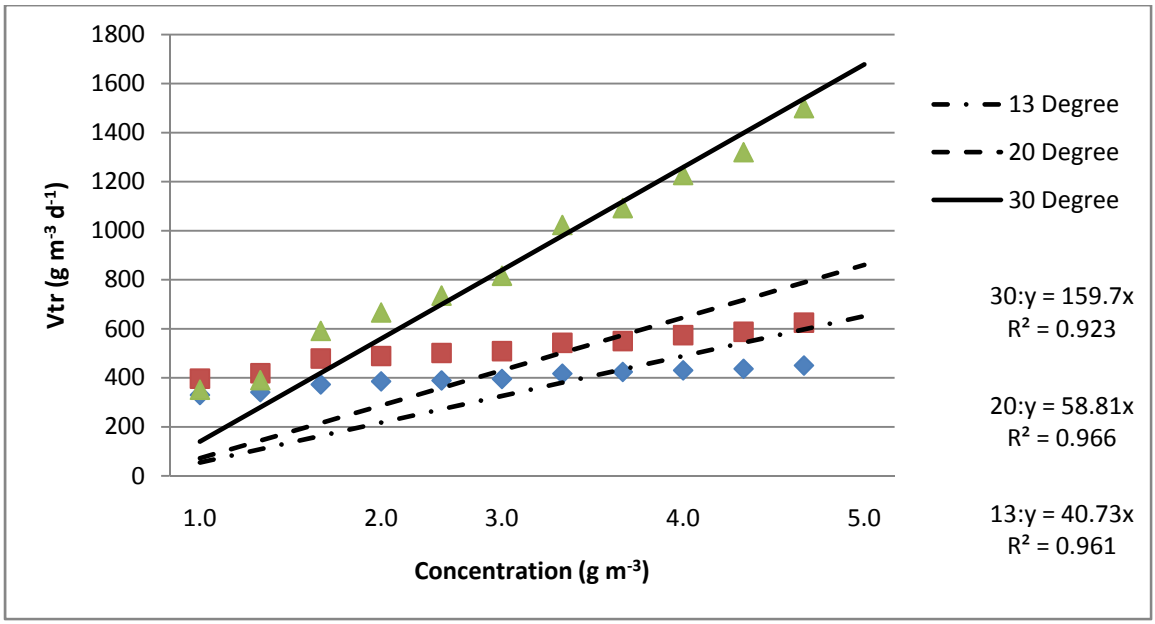

Figure 3.7: Graph of volumetric total ammonia nitrogen removal rates at steady state (13, 20 and 30°C) regimes.

This means that the nitrification rate does not defer significantly between 13 and 20°C. Suggesting that could be biofilters operating within this range may be relatively consistent in TAN removal rates. The removal rates were in agreement with previous biofilter studies reviewed (Malone et al., 2006; Wortman and Wheaton, 1991; Malone and Beecher, 2000). Ammonia removal rates at 30°C were reasonable within the range of values for previous studies (Wortman and Wheaton, 1991, Malone et al., 2006; Zhu and Chen) which may be attributed to optimum growth temperatures for nitrifying bacteria. Temperature effect on ammonia removal rates were further observed from the results of diurnal temperature regimes in the second experimental batch of tanks.

3.4.2 Diurnal Temperature (20 ± 3°C and 30 ± 3°C) Ammonia Removal Rates

The maximum specific TAN removal rates for biofilters at diurnal temperature regimes 20 ± 3°C and 30 ± 3°C (Figure 3.9) were also determined in this experiment. Biofilter

performance was calculated from mean ammonia concentrations (Figure 3.10) and volumetric TAN removal rates (Figure 3.11).

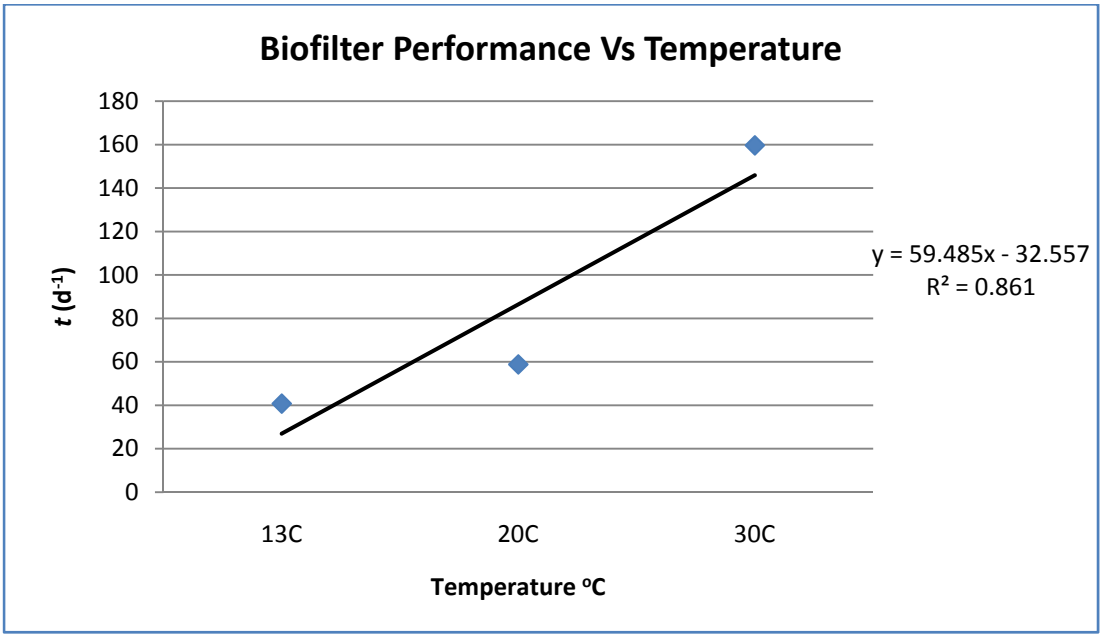

Figure 3.8: Graph shows biofilter performance ***t*** with increase in steady state temperature profiles 13, 20 and 30°C.

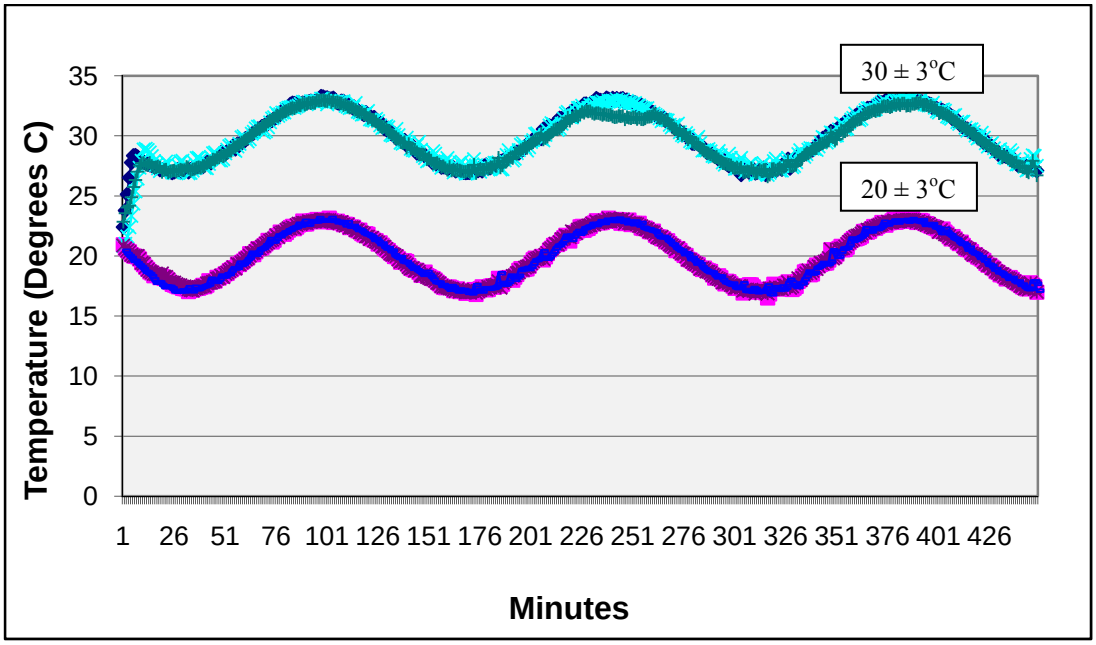

Figure 3.9: Typical diurnal temperature profiles at 20 ± 3°C and 30 ± 3°C.

The slopes of the mean TAN concentration (Figure 3.11) indicated that the 30 ± 3°C temperature regime had a higher removal rate with a slope –m = 0.424 and 20 ± 3°C had a slope –m = 0.285. Mean ammonia concentrations were significantly different ($P < 0.05$) for 20 ± 3°C and 30 ± 3°C temperature regimes. This implies that performance of the biofilter volumetric removal rate at 30 ± 3°C was higher than removal rate at 20 ± 3°C. The maximum specific ammonia removal rates are summarized in Table (3.3). Diurnal results however indicated higher volumetric removal rates compared to the corresponding steady state temperature regimes in the previous experiments. This may be a result of the increase in temperature effects above average dominating the bacterial reaction rates in the experiment.

3.4.3 Diurnal Temperature (20 ± 3°C and 30 ± 3°C) Ammonia Conversions with Mixed Beads

Maximum specific ammonia removal rates (Table 3.3) were determined from mean ammonia concentrations. Volumetric removal rates were calculated from mean ammonia

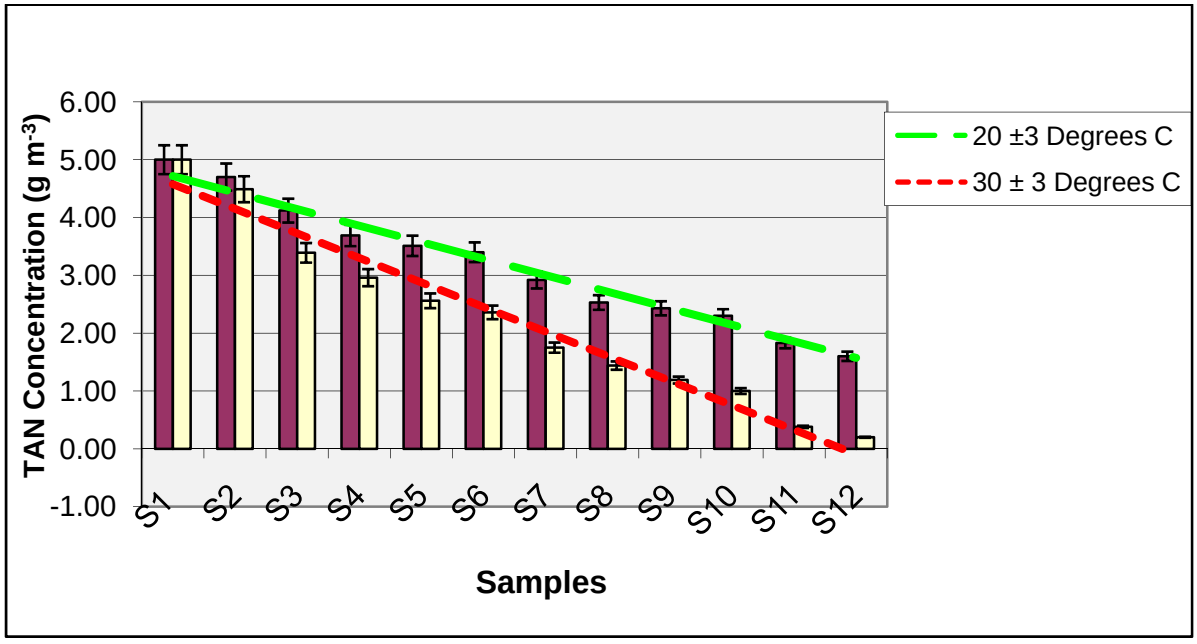

Figure 3.10: TAN mean ammonia concentrations at diurnal (20 ± 3 and 30 ± 3) regimes.

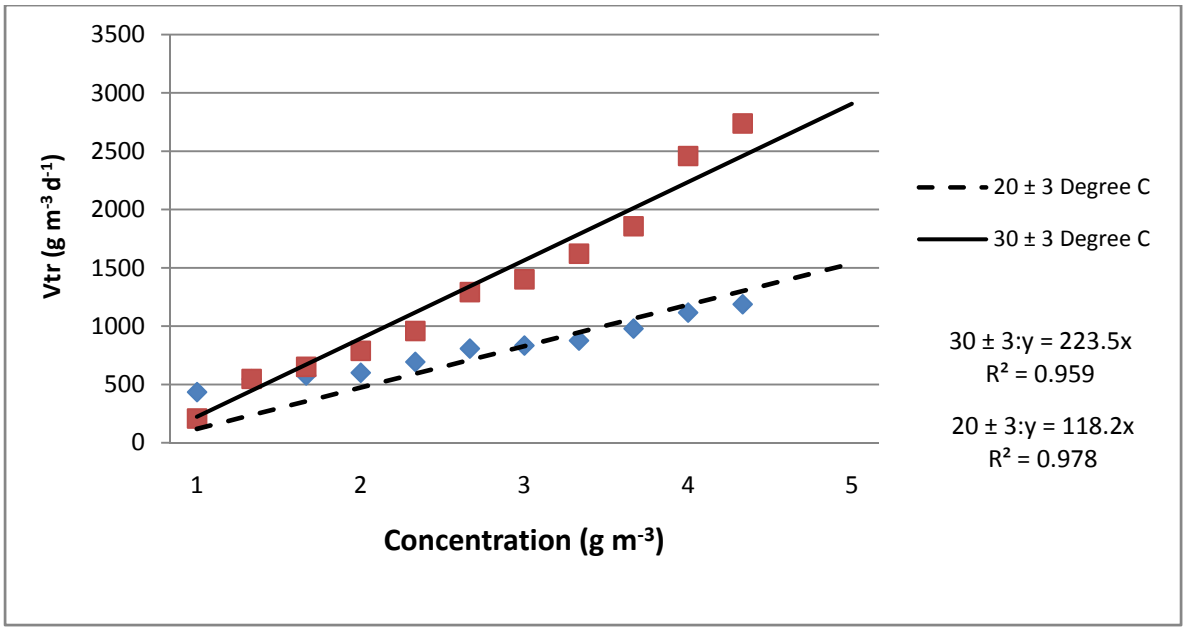

Figure 3.11: Volumetric TAN removal rates at diurnal (20 ± 3 and 30 ± 3) temperature regimes.

concentrations (Figure 3.12) at diurnal temperature regimes for the mixed beads. Biofilter performance was also determined from the calculated volumetric ammonia removal rates with mixed acclimated beads. Performance of the biofilter TAN removal rates with the mixed beads was significantly different ($P < 0.05$) at 20 ± 3 from removal rates at 30 ± 3. This may be due to an increased biomass concentration from the mixed media compared to biomass at 20 ± 3.

3.4.4 Ammonia Removal Rate at Step-down Temperature (30 Step down -13°C)

Tanks in the step-down temperature regime were acclimated at 30°C , stepped down to 13°C and held over a duration 72 hr. Mean ammonia concentration was determined for the triplicate tanks for experimental regime 30 -13°C (Figure 3.13). Mean ammonia concentration slopes for step-down regime was –m = 0.1033 at $R^2 = 0.968$. Performance of the biofilter from volumetric removal rates (Figure 3.14) was also calculated (Table 3.3). Result of step-up experiment was as indicated in next section.

Table 3.3: V_{max} (Specific Removal Rate), R^2 and t values at the steady state, diurnal and step regimes.

Temperature Regime	V_{max}(g d^{-1})	t(day^{-1})	Performance Equations	R^2
13^0C	0.460	40.73	Y= 40.73x	0.938
20^0C	0.640	58.81	Y= 58.81x	0.932
30^0C	1.380	159.7	Y=159.7x	0.827
20 ± 3^0C	1.130	118.2	Y= 118.2x	0.975
30 ± 3^0C	1.600	223.5	Y= 223.5x	0.928
20 ± 3^0C (Mixed Beads)	1.590	189.2	Y= 189.2x	0.959
30 ± 3^0C (Mixed Beads)	1.660	431.5	Y= 431.5x	0.826
Step-Up 13- 30°C	1.438	183.4	Y=183.4x	0.952
Step-down 30- 13°C	0.386	46.90	Y= 46.90x	0.968

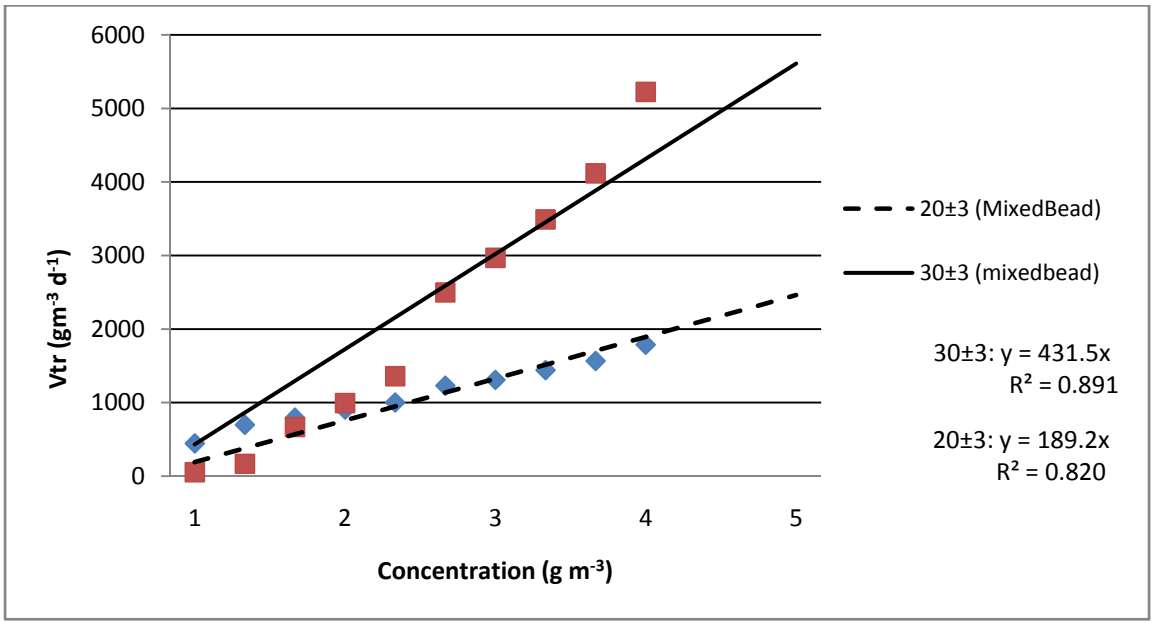

Figure 3.12: TAN volumetric removal rates for diurnal temperatures (20 ± 3 and 30 ± 3) with mixed beads.

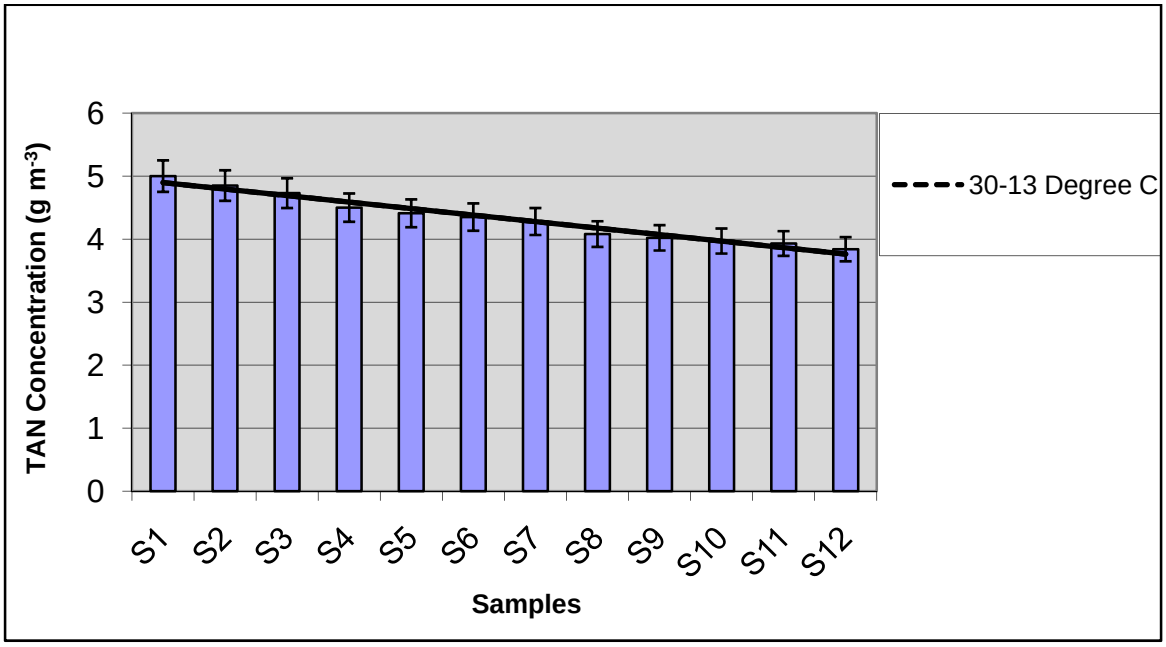

Figure 3.13: Step temperature TAN mean concentration at 30-13 degree step-down regime.

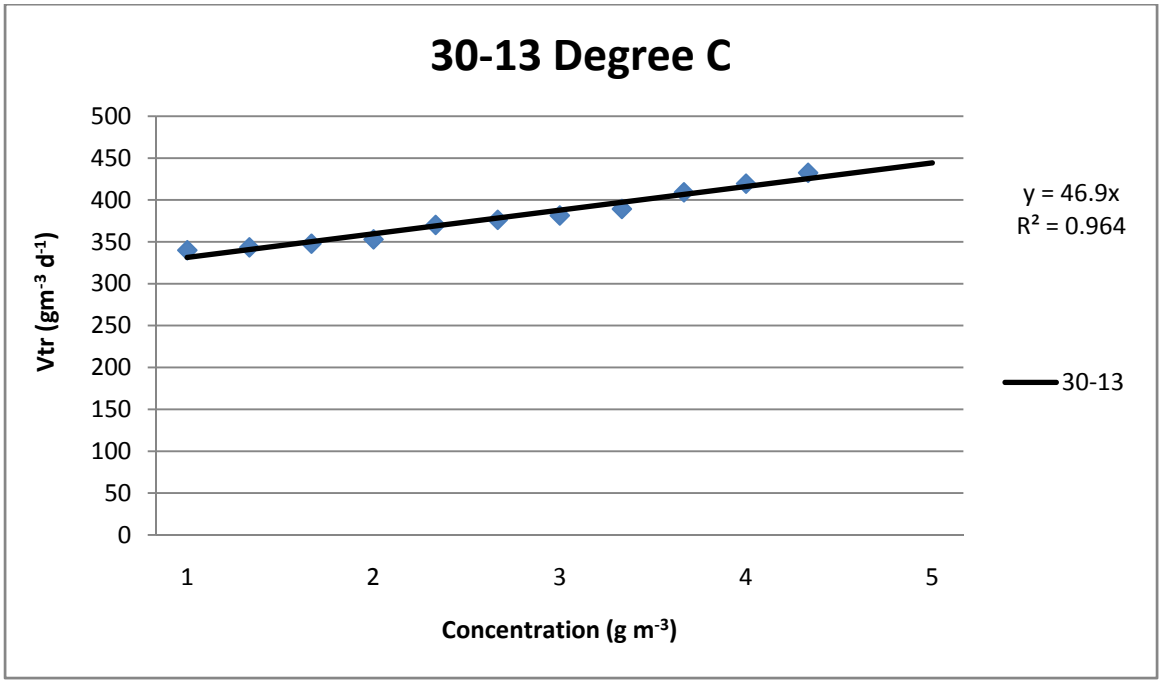

Figure 3.14: Volumetric TAN removal rate at step-down (30-13) regime.

3.4.5 Ammonia Removal Rate at Step-up Temperature (13 Step up-30°C)

Step-up tanks were acclimated at 13°C and stepped up to 30°C and held over the duration of 72 h. Mean ammonia concentration (Figure 3.15) slope for the step up was –m = 0.363 and R^2 = 0.973. Volumetric ammonia removal rates (Figure 3.16) were also calculated for the mean ammonia concentrations.

Mean ammonia concentrations for step temperature regimes 13-30°C were significantly different ($P < 0.05$) from 30-13°C. Although mean concentrations rates at 13-30°C were not significantly different from 30°C, step-down 30-13°C mean values were also not significantly different from 13°C. The differences in TAN removal rates were further analyzed by comparing mean ammonia concentrations at different temperature regimes and the results were as indicated in the following section.

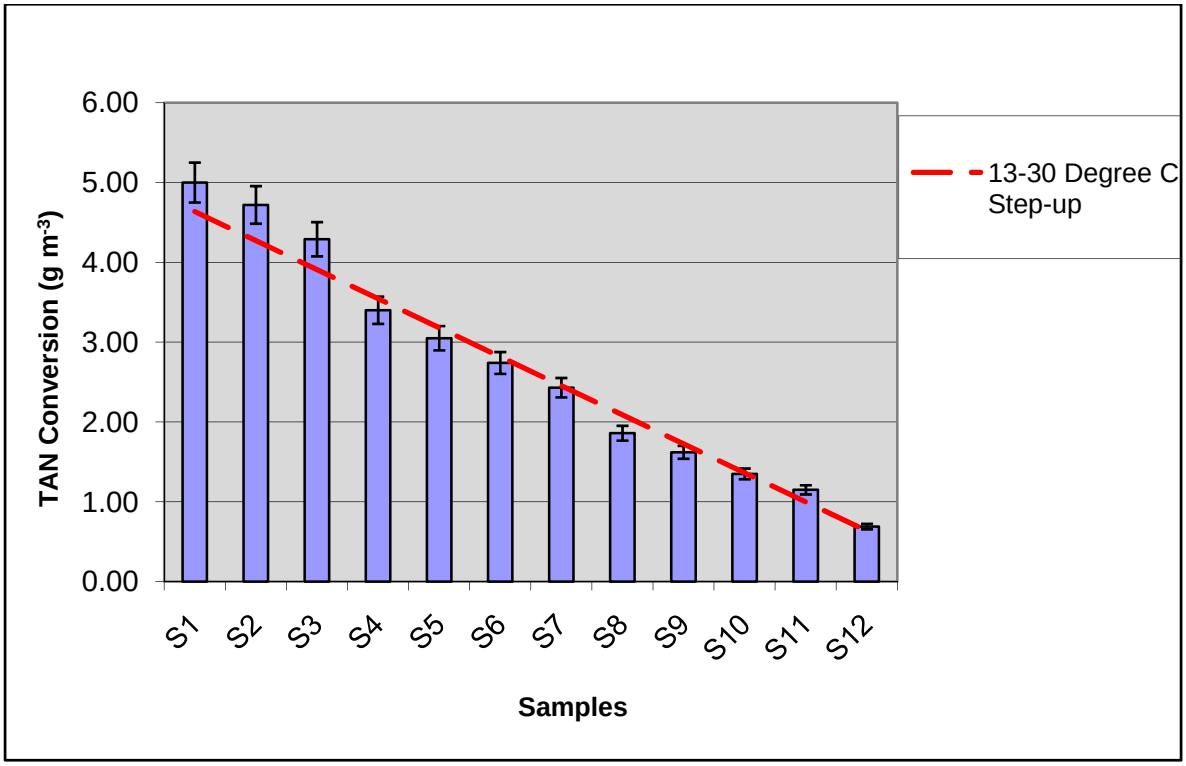

Figure 3.15: Step temperature TAN mean concentration at 13-30°C degree step-up regime.

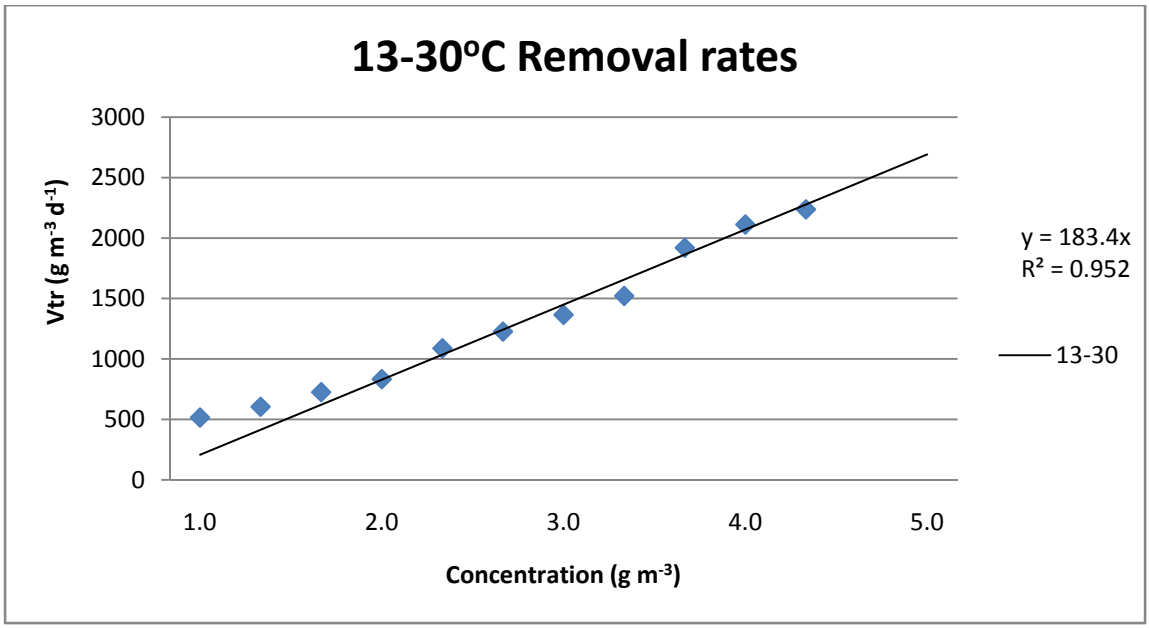

Figure 3.16: Volumetric TAN removal rates at step temperature regimes (13-30°C).

3.4.6 Comparison of Ammonia Removal Rates

Mean ammonia concentrations for the controlled tanks held at constant temperatures 13 & 30°C were compared with the step-up temperature 13-30°C, step-down temperature 30-13°C, diurnal temperatures 20 ± 3°C & 30 ± 3°C were also compared to diurnal temperatures 20 ± 3°C & 30 ± 3°C with mixed beads.

- **Ammonia Removal Rate at Steady State versus Step Temperatures**

Mean ammonia concentrations for triplicate tanks held at step temperature regimes 13-30°C were compared with mean value from triplicate tanks subjected to steady state 30°C (Figure 3.17) regime. Mean concentrations for 30-13°C and 13°C steady state (Figure 3.18) were also compared. The mean concentration slope at step-up 13-30°C was –m = 0.363 at R^2 = 0.973. Slope of mean concentration the steady state 30°C was –m = 0.349 at R^2 = 0.986. There was no significant difference (P > 0.05) in the mean concentrations for 13-30°C versus 30°C.

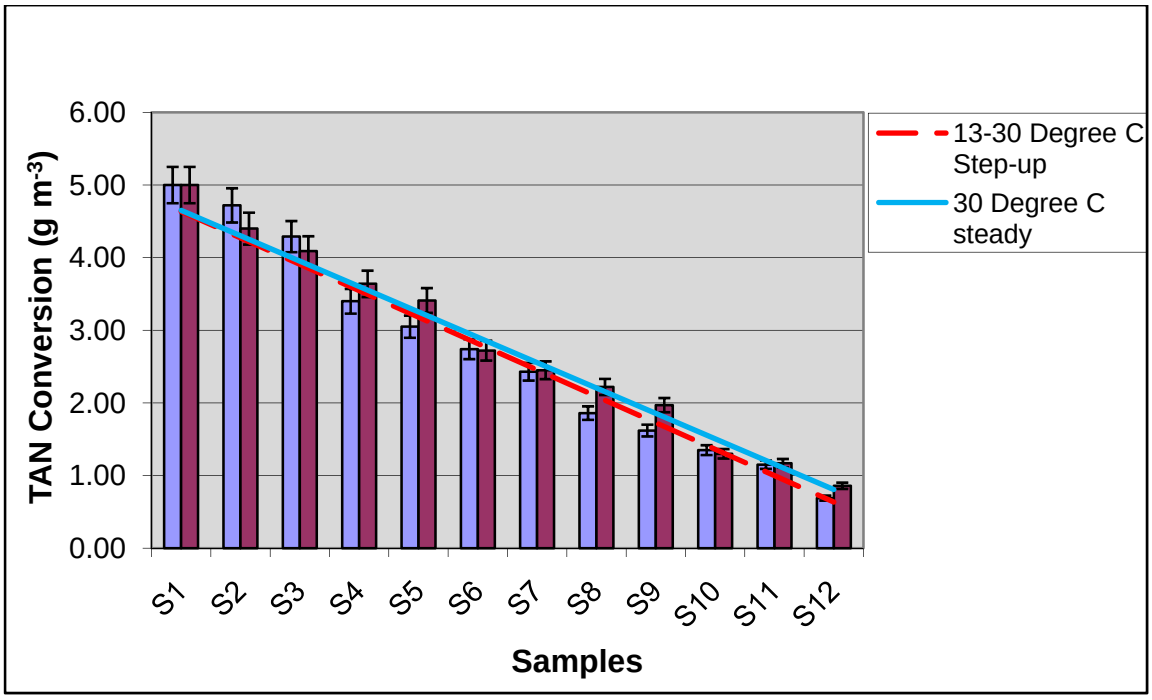

Figure 3.17: Mean TAN concentration removal rates at 13-30 Vs 30^{o}C.

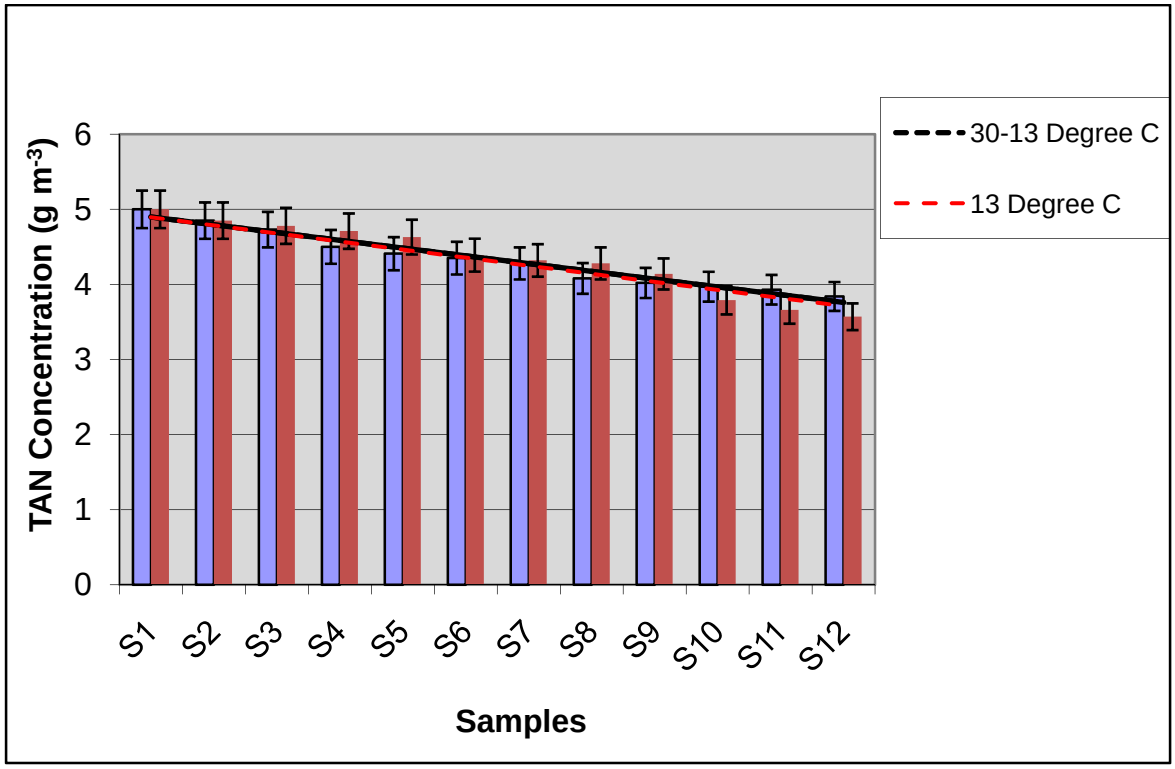

Figure 3.18: TAN mean concentration removal rates at 30-13 Vs 13^{o}C.

The step down 30-13^{o}C regime had a slope –m = 0.103 and R^2 = 0.968, while 13^{o}C had slope –m = 0.107 and R^2 = 0.930. There was no significant difference (P > 0.05) between the removal rates of 30-13^{0}C versus 13^{0}C. This may also suggest that the biofilter and the nitrifying bacteria in their entirety adapt to temperature changes. The result may be explained also by "Brownian kinetics" effect on bacteria metabolic process. It may explain the decrease in reaction with temperature decrease within the shortest possible time to make up for the conversion rates. Considering the fact that the corresponding steady state temperature regimes do not differ in the removal rates from the step temperature regimes, it may be plausible to suggest that the bacteria do rebound and adapt their performance at a certain temperature regime at least during these 3-days. Comparison of results to determine the biofilter performance was done for steady state to diurnal regimes in the following section.

- **Ammonia Removal Rate at 20 versus 20 ± 3^{o}C Diurnal Regimes**

Mean ammonia concentrations from the removal rate slope at 20^{o}C steady state was compared with mean concentration at 20 ± 3^{o}C (Figure 3.19) temperature regimes. The ammonia conversion rates for both treatments were plotted together to determine any difference in the conversion of ammonia. The mean ammonia concentration slope for diurnal 20 ± 3^{o}C was -m = 0.285 and R^2 = 0.979. The slope of conversion at steady state 20^{o}C was -m = 0.152 and R^2 = 0.963. The mean ammonia concentrations were significantly different (P < 0.05) at 20^{o}C from ammonia removal at 20 ± 3^{o}C. This may also suggest that biofilter beads acclimated at a steady state and subjected to diurnal temperature profiles may perform better than those at a steady state. . A possible cause may be an average higher temperature at the diurnal regime accounting for increased ammonia removal rate at the crest of the diurnal temperature profile. This may also be a result of the bacteria still in the growth phase of the biofilm consequently

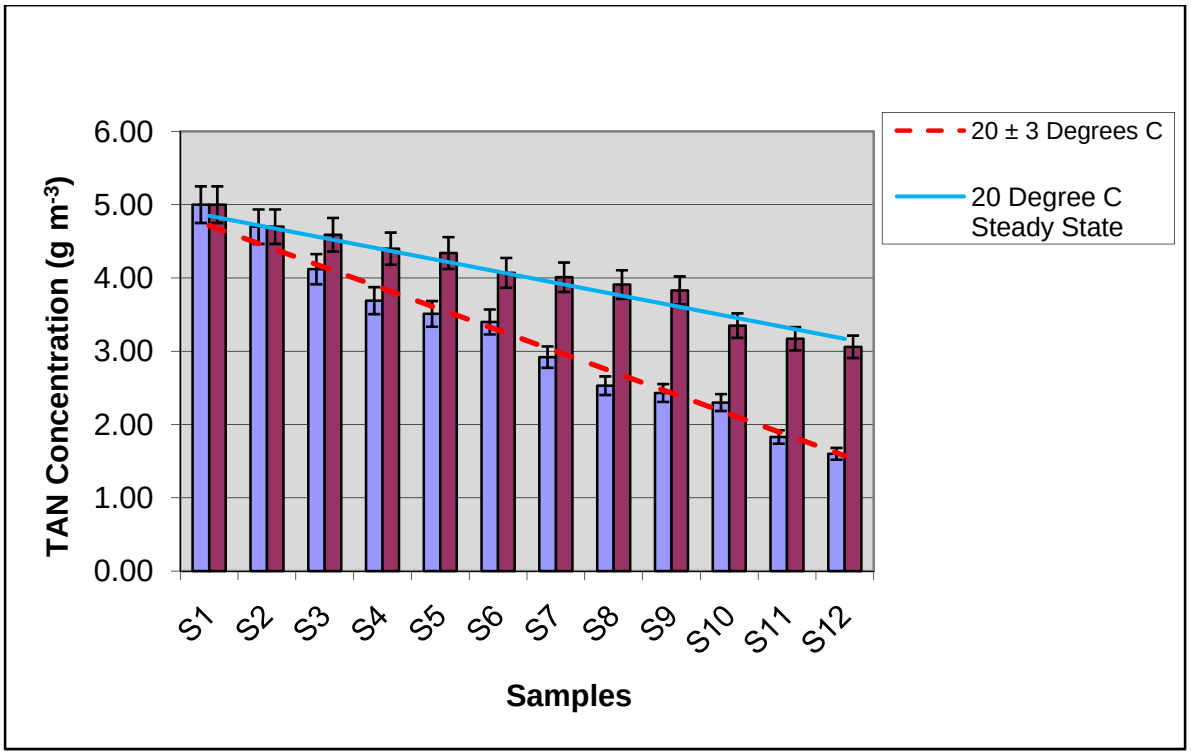

Figure 3.19: TAN mean concentrations at 20 Vs 20 ± 3°C diurnal temperature regimes.

responding to the temperature increase, which may have led to a resulting higher performance than the steady state regime.

- **Ammonia Conversion at 30 Steady State Vs 30 ± 3°C Diurnal Regime**

Mean ammonia concentration using triplicate tanks at steady state temperature 30°C was compared to mean concentrations at 30 ± 3°C (Figure 3.20) diurnal regimes. Mean ammonia concentration removal rate for 30 ± 3°C had a slope -m = 0.424 with $R^2 = 0.965$. The mean concentration removal rate for steady state 30°C had a slope –m = 0.349 with $R^2 = 0.986$. The mean concentration removal rates were significantly different ($P < 0.05$) for diurnal and steady state regime. This may again suggest that biofilter beads acclimated at a steady state and subjected to diurnal temperature profiles may perform better than those at a steady state.

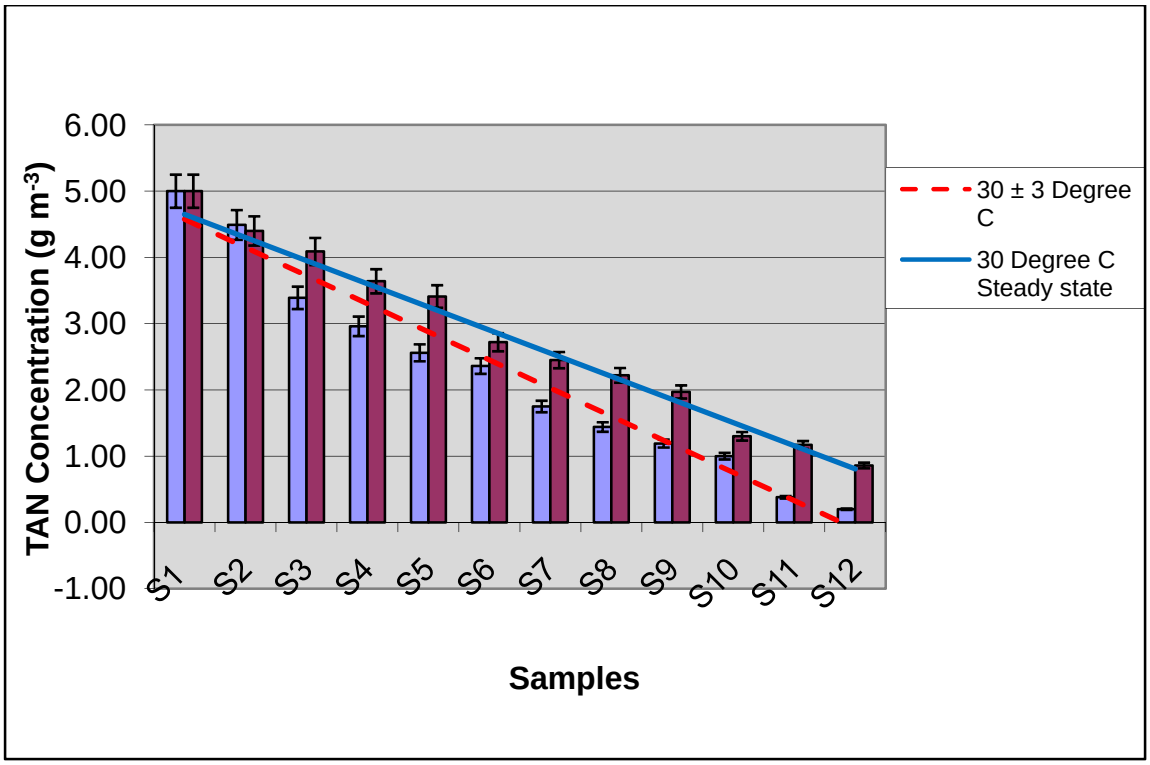

Figure 3.20: TAN mean concentrations at 30 Vs 30 ± 3°C diurnal temperature regimes.

- **Ammonia Removal Rate at 20 Vs 20 ± 3°C Diurnal Regimes (Mixed Beads)**

The result of mean ammonia concentration at 20°C steady state was compared with 20 ± 3°C mixed beads diurnal removal rate (Figure 3.21). Mean concentration for mixed beads at 20 ± 3°C had slope –m = 0.36 at R^2 = 0.977. Mean concentration at 20°C had slop –m = 0.152 at R^2 = 0.963. There was a difference ($P < 0.05$) between the removal rates of the 20°C steady state versus the 20 ± 3°C mixed bead diurnal temperature regimes. This may suggest again that the average higher temperature amplitudes on the temperature profile curves accounts for the increase in mean removal rates by the biofilters.

- **Ammonia Removal Rate at 30 Vs 30 ± 3°C Diurnal Regime (Mixed Beads)**

Mean concentration for 30°C steady state was compared with values at 30 ± 3°C diurnal regimes with mixed beads (Figure 3.22).

The mean removal concentration for 30 ± 3°C had –m = 0.517 at R^2 = 0.950, while mean values at 30°C had slope –m = 0.349 at R^2 =0.986.

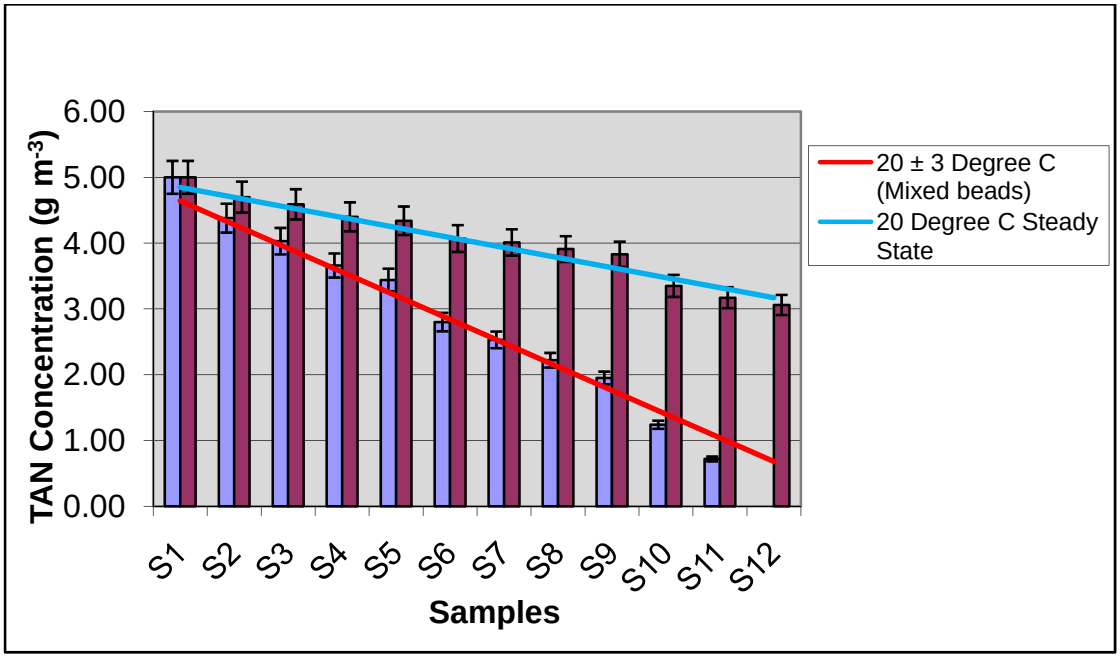

Figure 3.21: TAN mean concentrations at 20 Vs 20 ± 3°C diurnal temperature regimes using mixed beads.

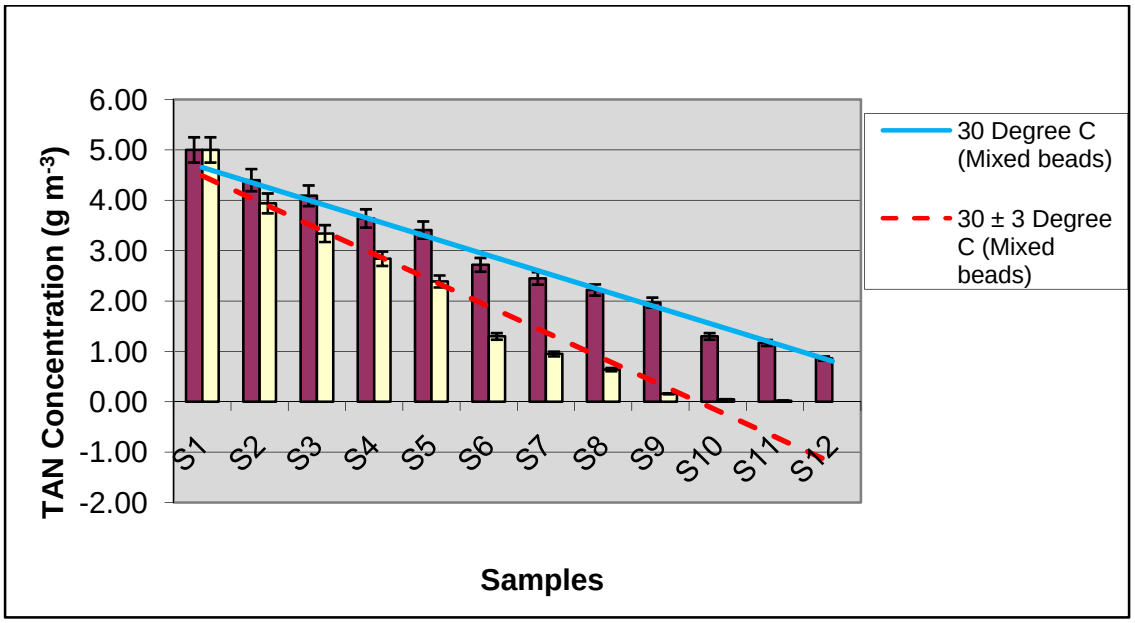

Figure 3.22: TAN mean concentrations at 30 Vs 30 ± 3°C diurnal temperature regimes using mixed beads.

The comparison between the ammonia conversions at 30 versus 30 ± 3°C (Mixed Beads) were also significantly ($P < 0.05$) different. This also suggests that temperature increase may affect the rate at which the substrate ammonia is being converted in a biofiltration system. Further experiments were also performed to test the conversion rates between beads at diurnal temperature regime with mixed beads subjected to diurnal temperature regime in as shown in the next section.

- **Ammonia Removal Rate at 20 ± 3°C Vs 20 ± 3°C Diurnal Regimes (Mixed Beads)**

Ammonia conversion data for beads exposed to diurnal temperature regimes were compared to beads acclimated at three different temperature regimes 13, 20 and 30°C, mixed and loaded in the biofilters. The mixed beads were exposed to diurnal temperature regimes 20 ± 3 and 30 ± 3°C. Mean concentration removal for 20 ± 3°C diurnal regimes were compared to 20 ± 3°C diurnal mixed beads mean concentration removal rates (Figure 3.23). Although the data bars in the graph appear to have numeric differences, comparing for the diurnal 20 ± 3°C to 20 ± 3°C (mixed beads) diurnal regimes, there was also no significant difference ($P > 0.05$) in mean concentration removal rates. This may suggest that even though the beads were mixed, temperature is the probably the major factor contributing to the non difference in the conversion rates. The mixed and non-mixed were both at the same temperature fluctuation amplitudes. Further experiments were performed with larger temperature fluctuation amplitudes as shown below.

- **Ammonia Removal Rates at 30 ± 3°C Vs 30 ± 3°C Diurnal Regimes (Mixed Beads)**

The mean concentration removal rate for 30 ± 3°C diurnal was also compared to 30 ± 3°C diurnal mixed beads removal rates (Figure 3.24). The mean concentration removal rate for 30 ± 3°C diurnal was $-m = 0.424$ with an $R^2 = 0.965$.

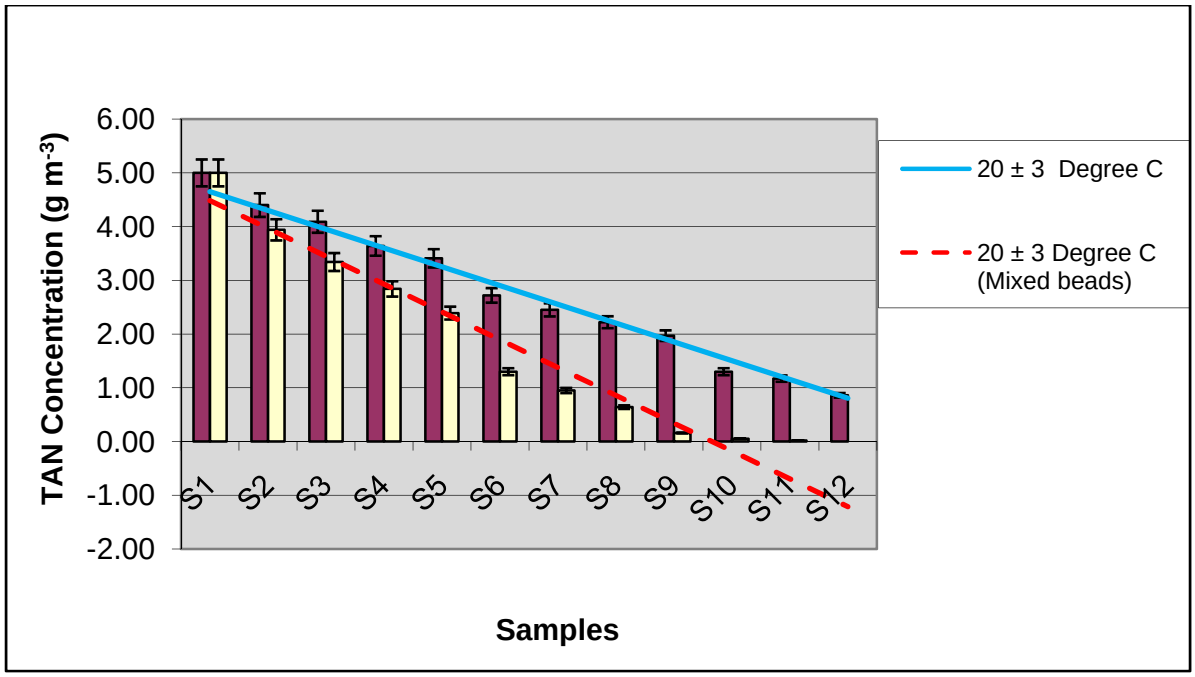

Figure 3.23: TAN mean concentrations removal rate at 20 ± 3°C transient Vs 20 ± 3°C diurnal mixed beads.

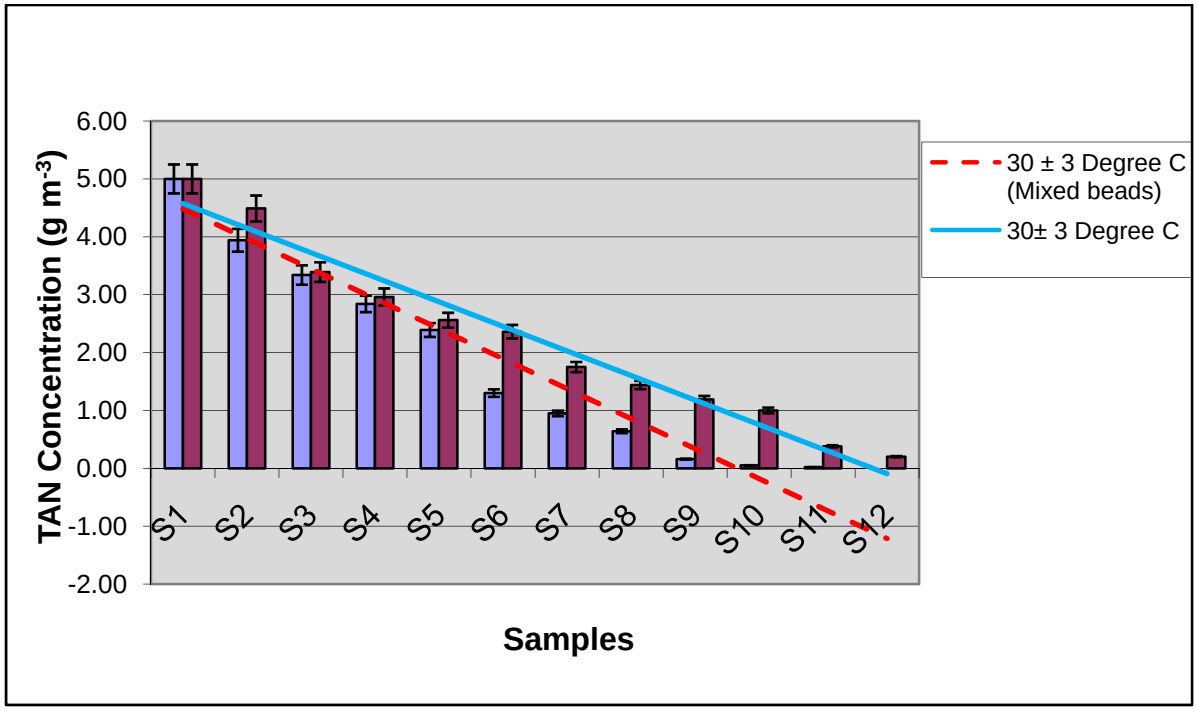

Figure 3.24: Ammonia conversion at 30 ± 3°C diurnal Vs 30 ± 3°C mixed bead diurnal regime.

The mean concentration removal rates for the mixed bead at 30 ± 3°C was -m = 0.517 and correlation coefficient R^2 =0.950. Although the data bars in the graph appear to have numeric differences, however conversion rates compared for the diurnal 30 ± 3°C to 30 ± 3°C mixed beads diurnal regimes were not significantly different (P > 0.05). The diurnal regime and the mixed beads at diurnal regime were exposed to the same temperature fluctuation amplitudes. This may suggest that even though the beads were mixed, temperature may be the affecting the removal rates based on the reaction of the bacteria to temperature change.

- **Discussion**

Data from the mean concentrations were observed to fit a linear regression for all temperature regimes (13, 20 & 30°C; 20 ± 3°C and 30 ± 3°C) indicating a pseudo zero order. The change in mean concentrations used to calculate ammonia removal rates were significantly different for 20 and 30°C steady state temperature regimes. This may have been a result of the impact of temperature on bacterial reaction rates (Hanus and Richard, 1968; Tchobanoglous et al., 2003). However there was no significant change in removal rates between 13 and 20°C steady state. It may be possible that the bacterial density and growth rates (Modeled later in the next chapter) could have had an effect on the removal rates. It is also noted that the optimum growth range for a particular microorganism occurs within a fairly narrow range of temperature (Tchobanoglous et al., 2003) although most microorganisms can survive within a wider range. In fact it was been suggested in literature that bacterial growth changes for every 10°C in temperature change (Madigan et al., 2000) until they reach an optimal temperature. This may have had an effect on the nitrification rate considering the range in temperature from 13 to 20°C and 20 to 30°C. This may explain why removal rates at 13 and 20°C had no significant difference. In this study the experiments were short term (3 d experimental sampling) after a

prior 3 d acclimation of the media, it is therefore plausible to acknowledge that bacterial biomass density on the media may have been in the lag phase at which the bacteria grow in size but not in counts. This phase is required for the organisms to acclimate to their new environment before and during significant biomass growth (Tchobanoglous et al., 2003; Strathmann et al., 2000).

Results of the diurnal temperatures (20 ± 3 and 30 ± 3^{o}C) were observed to be significantly higher than the corresponding steady state temperatures (20 and 30^{o}C). Since average temperatures at the diurnal regimes were higher than the steady state regimes; this may have positively influenced bacterial growth and acclimation response to temperature. This may explain the increased TAN removal rates at diurnal regimes. It may also be the case for the mixed beads that were acclimated at steady state temperatures and subjected to diurnal regimes. However in the mixed beads, biomass density may have increased from the combination of the constituent media at three different temperature regimes. Biomass density from the higher temperature (30^{o}C) media may have accounted for the increased performance in the biofilter over the lower temperature (13^{o}C) media after the mixing procedure.

Volumetric removal rates for results were analyzed using the direct linear method for the data in this study. The direct linear regression method used for analyzing the performance of the biofilter in this study was proposed in previous study (Malone et al., 2006; Malone and Pfeiffer, 2006) and literature (Tchobanoglous et al., 2003) as a suitable method of evaluating systems with lower TAN concentrations. It was also proposed to be a possible method of evaluating biofilters and this method was also presented to have a lower coefficient of variation in predictions when used compared to a Lineweaver-Burke linearization method (Malone et al., 2006; Malone and Pfeiffer, 2006). The use of this method in this study may suggest that it is also suitable for determining the performance of seeded biofilters and filters in the lag phase where the bacteria

are acclimating and growing in size but not reproducing. It is speculated that the pseudo zero order reaction rates in a batch system may suggest that the biofilter was in the seeded stage with biomass growth in progress. In such instance it may well be possible that there was a high food to microorganism (F/M) ratio which may possibly explain the linear trends seen in the data. The equation for the relationship between biofilter performance (***t = y***) and temperature (^{o}C) was expressed from Figure (10) as: **y = 59.485x - 32.557**, where x is temperature (^{o}C). The results of the biofilter performance presented do not include the effect of heterotrophic bacteria in an organic carbon loaded system, nor does it specifically account for first or second order reaction rates. Subsequently the performance may differ in higher reaction orders. It therefore speculated that the specific removal rates and biofilter performance may not reflect predictions for carbon loaded system with different bacterial growth kinetics. Complexities of bacterial growth in a biofilm should not be understated. A modeling study is suggested to better understand the performance of biofilters.

3.5 Conclusions

(1) Results of TAN removal rates at 13, 20 and 30^{o}C temperature regimes showed that the biofilter performance and temperature relationship had the most accurate fit with a linear regression, although ammonia removal rates, at 13^{o}C is not significantly different from the removal rate at 20^{o}C. However results also indicate that there is a difference in removal rates at 20^{o}C compared to 30^{o}C.

(2) Diurnal TAN removal rates were higher than the corresponding steady state regimes. TAN removal rate at 20 ± 3 was 20% high than the steady state 20^{o}C while removal rates at 30 ± 3^{o}C were 9 % higher than the corresponding 30^{o}C. (3) Diurnal TAN removal rates with mixed beads were higher than the corresponding steady state regimes. TAN removal rates at 20 ± 3^{o}C (Mixed

beads) was 30% higher than removal rate at 20°C, while removal rates at 30 ± 3°C were 32% higher than 30°C steady state regime. Future recirculating systems may be expected to be more efficient in biofiltration and increased production using the results of this study for system management.

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Chapter 4 - Simulation Modeling of Temperature Effect on Bacterial Growth Phase Biofiltration Processes

4.1 Introduction

Modeling biofiltration processes in recirculating systems is a cost effect method of determining biofilter performance and system productivity. Previous studies in determining biofilter performance have been modeled with the assumption of steady state biofilm (Torsten et al, 2009; Zhu and Chen, 2002; Wheaton et al., 1991). However models used with this fundamental assumption do not consider the growth process of the bacteria between the seeded stage of a biofilter and the required acclimation period prior to achieving a steady state biofilm. Determining the performance of biofilters in acclimation and bacterial growth phase will contribute to understanding ammonia utilization in a biofiltration process. Biofilter performance in recirculating systems may be also affected by other parameters such as temperature and diffusion (Wortman and Wheaton, 1991; Harremoes, 1982). However theoretical (Zhu and Chen, 2002) and empirical (Lyssenko and Wheaton, 2005) evidence also indicates that temperature impacts bacterial kinetics which affects biofilter performance.

Bacterial growth kinetics is influenced by the rate of substrate utilization which improves with increase in substrate concentration. This effect also tends to increase the bacterial biomass in bacterial growth kinetics (Tchobanoglous et al., 2003). However a reduced biomass may be also attributed to limited energy gained from low substrate concentration in the metabolic process of bacteria involved. In fact at sufficiently low substrate concentration, the rate at which energy is captured may be less than energy expended to maintain viability of bacteria (Rittman and McCarty, 1980). This simulation modeling study therefore determines the interaction between temperature, ammonia substrate utilization and bacterial biomass in a nitrification process. The model presented seeks to demonstrate the influence of temperature on substrate

utilization and biomass growth in seeded and acclimating biofilters before a steady state biofilm is developed. Specific objectives focused on in this study were: 1) temperature impact on biofilter removal rate performance during an acclimation period; and 2) temperature impacts on biomass accumulation in the biofilter over time.

4.2 Background

Ammonia utilizing bacteria (nitrifiers) in recirculation systems are generally retained inside biofilters by attaching to a media surface. Although the bacteria have the ability to use a whip like flagella to move as suspended flocs in water and process reactors, biofilters in recirculating systems however use attached growth biofilters. In an attached growth biofilter, the bacterial growth starts at the lag phase, through the log phase before it get to the steady state biofilm where there is neither a net growth nor decay over time (Figure 4.1). At steady state reactions bacterial growth may be assumed to have achieved maturity. However in a dynamic process the growth phase could have significant impact on the acclimation and subsequent biofilter performance.

Figure 4.1: Generalized bacterial growth curve showing the phases in the growth of bacterial colonies from inoculation in medium to steady state biofilm.

Bacterial reaction response to temperature impact in a growth process is generally depicted by a Van't Hoff-Arrhenius plot which plays a significant role in ammonia utilization in a biofiltration process (Hanus and Morita, 1968; Tchobanoglous et al., 2003). Ammonia utilization reaction rates are believed to increase with rising temperatures for suspended floc biofiltration systems although some studies have shown no significant effect in nitrification between 14 - 27^{o}C for fixed film bioreactors (Zhu and Chen, 2002; Zhu and Chen, 1999). However nitrification with fixed film biodrum studies has indicated a linear relationship with increased nitrification effect per degree rise in temperature (Wortman and Wheaton, 1991; Kim et al., 2006).

Previous studies have used the following expression(s) to determine substrate utilization in a bacterial reaction process (Antoniou et al., 1990; Willke and Vorlop, 1996; Zhu and Chen, 2002) as in equation (18).

$$R = \mu(X_{mass} / Y)\frac{S}{K_s + S} \quad \text{---------- (18)}$$

Where R is the TAN removal rate (g m^{-3} d^{-1}), S is TAN concentration in tank (g m^{-3}) K_s is half saturation constant (g m^{-3}), X_{mass} is bacterial biomass concentration (g m^{-3}), Y is bacteria yield g/g. μ is the bacterial reaction rate.

The expression is widely used in modeling a dynamic bacterial reaction with an explanation for change in biomass with time in the reactor. The model is presented in other studies to account for bacterial growth process (Wortman and Wheaton, 1991; Zhu and Chen, 2002; Malone et al., 2006; Rittmann and McCarty, 1980; Chen et al., 2006).

In a dynamic bacterial reaction process, the biomass is considered to be changing until a steady state is achieved. However dynamic growth may influence the substrate diffusion process since conventional diffusion-based concepts considers biofilms to consist of cells attached to the surface through which the soluble substrate diffuses (Bishop, 1997; Rittmann and McCarty,

1980; Harremoes, 1982). In fact Harremoes (1982) presented that the removal of ammonia per unit volume of biofilm was proportional to the concentration of nitrifiers at the surface of the biofilm and increased exponentially with the thickness of the biofilm and growth rate. However in the model (equation 18) presented by previous studies acknowledging temperature effect on substrate utilization and bacterial growth rate, do not have a temperature correction factor for bacterial dynamic growth. This modeling study therefore proposes that a temperature correction factor be included in bacterial growth phase of equation (18) considering the fact that a logarithmic plot of growth rate against reciprocal of time is a linear model (Hanus and Morita, 1968).

4.3 Materials and Methods

Mathematical models which use algorithms to translate input variables into output variables are generally used in modeling experiments (Beuning et al., 2008). Mechanistic models have been developed by a number of investigators to describe biological substrate utilization kinetics in biofilm (Malone et al., 2006; Rittman and McCarty, 1980; Rittman and McCarty, 2001). Models of this type are derived from mass balances of the biofilter while defining the underlying phenomena in such a way that performance of the biofilter may be predicted outside the range of tested conditions (Grady, 1983).

The model for the substrate removal rate reaction in this study was developed by incorporating a temperature correction factor into equation (18) to predict nitrification processes in a biofilter under various temperature regimes. This included the use of mass balances for the batch system used in this study. The simple dynamic biofilm model considered used parameter values defined for this modeling study to predict substrate utilization and bacterial growth reaction kinetics under varying temperature regimes.

4.3.1 Modeling Theory

A mechanistic model was developed to simulate ammonia utilization rate in a batch reactor system. Multiple tank replicates of the same biofilter design were used for this study. Simulation tanks were 0.04 m^3 in volume used in the relationship of mass to volume for the model structure development. A theoretical expression in the model for ammonia removal rate was used to determine the process of ammonia utilization (Zhu and Chen, 2002; Harremoes, 1982; Rittman and McCarty, 1980) without a temperature correction factor expressed as:

$$R = K_{\max} X_{mass} \frac{S}{K_s + S} \quad \text{---------- (19)}$$

Where R is the TAN removal rate (g m^{-3} d^{-1}), X_{mass} is the bacterial mass in the biofilm (g m^{-3}), K_{max} is the biomass maximum rate.

Bacterial growth rates tend to respond to temperature changes over time represented by Arrhenius curves (Hanus and Morita, 1968; Tchobanoglous et al., 2003). This may suggest that the temperature correction factor could be a function of biomass (X_{mass}) because of the bacterial growth and temperature relationship. However as the bacterial mass increases on the filter media the biofilm becomes thicker which may have an effect on the half saturation constant (K_s) by increasing the value of this parameter. Since the half saturation (K_s) is a function of bacterial mass and bacterial mass is also a function of temperature, it is therefore proposed in the model that the temperature correction factor be presented as a function of biomass while neglecting the effect of temperature on the half saturation constant (K_s). This is also supported by the Van't Hoff-Arrhenius estimate of temperature effect on biological reaction rates (Tchobanoglous et al., 2003; Zhu and Chen, 1999) expressed as:

$$\mu = \mu_{\max} \theta^{T-20} \text{ ---------- (20)}$$

Equation (19) was then modified to include a temperature correction factor as shown below:

$$R = \frac{\mu_{\max}}{Y} X_{mass} \theta^{T-20} \frac{S}{K_s + S} \text{ ---------- (21)}$$

Where μ_{max} is the maximum bacterial reaction rate, θ is the temperature correction factor.

4.3.2 Modeling Structure and Implementation

The batch tank ammonia model was constructed using STELLA (Systems Thinking Experiential Learning Laboratory with Animation) version 9.0.3 software (High performance systems, Manchester, NH) on a personal computer. The model consisted of stock, state variable, connectors and converters that were inter connected (Figure 4.2). The model used in this study was developed from a combination of mass balance approach and empirical deductions. State variables and coefficients of the model were defined in Table 4.1.

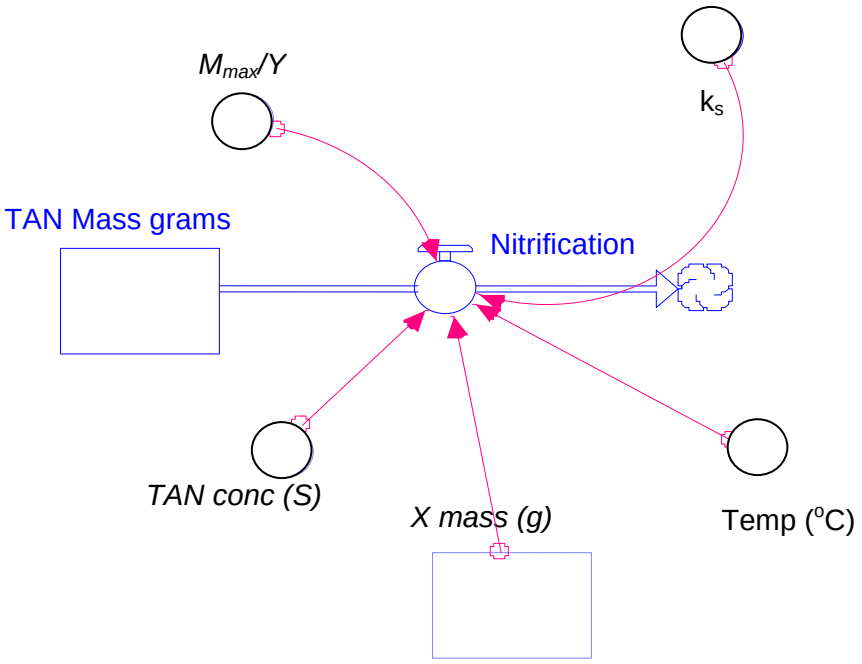

Figure 4.2: Model construct with input parameters in a batch reaction system.

Table 4.1: Parameter values used in the model compared to literature

Parameter Units	Literature Values	References	Values Used
K_s (g m^{-3})	1.3	Knowles et al. (1965),	9.0
	2.0	Zhu and Chen, (1999)	
	3.5	Ulken (1963)	
	10	Hoffman and Lee (1952)	
θ Dimensionless	1.10	Knowles et al. (1965),	1.08
	"	Gujer and Boller(1986)	
	"	Zhu and Chen, (2002)	
X_{mass} (g cell m^{-3})	12700	Rittman and Manem (1992)	6.08×10^{-3} (Initial mass)
		Chen et al. (1989)	
μ_{max} (1/day)	1.48	Gujer and Boller (1986)	1.15
Y (g cell/g TAN)	0.20	Rittman and Manem (1992)	0.10
	0.21	Gujer and Boller (1986)	

To obtain the simulation results for equation (21), the following five parameters were used 1) half saturation constant (K_s) used in the simulation was consistent with literature (Hoffman and Lee, 1952), however low turbulence tends to limit substrate diffusion which may impact the half saturation constant (Zhu and Chen, 2001), 2) temperature correction factor (θ) was also less than literature values and it had a positive effect with temperature increase on the simulations, 3) Initial bacterial mass (X_{mass}) was small compared to values for steady state systems. This indicates this system started with minimal bacterial mass on the media in the biofilters, 4) bacterial reaction rate (μ_{max}) and 5) bacterial yield (Y) were also less than literature values. Bacterial yield for nitrifiers is generally less than heterotrophs. This may also be impacted by starting with a minimal nitrifier population. However the high food to bacterial

mass ratio at the start of a system run may tend to influence bacterial mass increase indicated in the ammonia utilization simulations.

Ammonia utilization was represented by variables, which may in turn be functions of other parameters. Input data requirements included temperature, substrate concentration, bacterial reaction rate, biomass yield, bacterial mass and limiting substrate concentration (Figure 4.2). The Runge-Kutta-4 integration method was used by the simulation package for solving the differential equations with a time step (ΔT) equivalent to 0.0125 d. The simulations were executed for 3 d periods consistent with the actual duration of experiments. Sensitivity analysis of the simulations was also extended to a 7 d period, 10 and 15 g m^{-3} substrate concentrations to determine bacterial mass effects over time on the performance of the biofilter.

4.3.3 Modeling Assumptions and Verification

The ammonia utilization rate equation (21) above was based on a number of assumptions. Bacterial mass in the biofilm was determined by the substrate availability. Flux of substrate into the biofilm was assumed to be influenced by diffusion as the biomass increased. It was also assumed that the presence of heterotrophic bacterial influence in the biofilm was minimized by the elimination of carbon from the feedstock (Bovendeur et al., 1990).

4.4 Modeling Results

The nitrification process in the batch tanks were modeled based on the above assumptions and the results were validated by comparison to observed data. The effect of substrate utilization with temperature was evaluated by comparing substrate utilization for observed data to the model results. However the performance of the biofilter was determined from coefficients of the linear fit on the curve of temperature versus performance (Figure 3.7). The coefficients were compared to data from the results of a study by Malone et al. (2006) in

which a linear modeling method for determining biofilter performance at low to moderate concentrations was proposed.

The model was able to predict 3 day ammonia concentration utilization with change in temperature for bacteria while simultaneously predicting bacterial mass for this dynamic bacteria population. Bacterial mass corresponded closely to changes in temperature in the model, indicating that the dynamic bacterial process in this system accounted for differences in biofilter performance over temperature range in this study. Calibration of model was done for substrate utilization rate by using observed data from previous studies (Zhu and Chen, 2001; Wortman and Wheaton, 1991; Zhu and Chen, 1999).

4.4.1 Model and Observed Data at 13, 20 and 30^{o}C

A comparison of the observed data and the simulated decay concentration at the 13^{o}C temperature regime was expressed in graphical format (Figure 4.3). The substrate utilization curve of observed data corresponded to the simulated curves of the model.

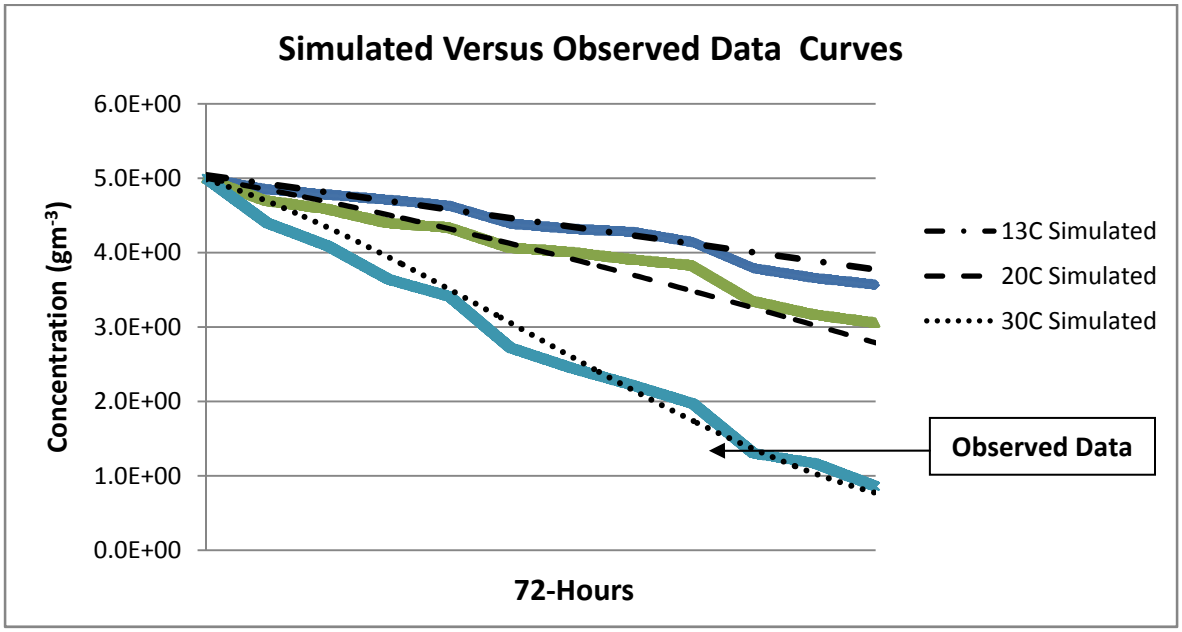

Figure 4.3: Graph of observed data versus simulated data for 13, 20 and 30^{0}C temperature regimes.

The simulated line for 13, 20 and 30°C manifested a linear response that is consistent with zero order growth at which the experiment was performed. This may have been an effect of starting with minimal bacterial mass and the time needed for acclimation of the biofilters. The bacterial mass curves for 13, 20 and 30°C (Figure 4.4) indicated a continuous growth throughout the sampling period. However the curves differed such that 13°C indicating the least growth followed by 20°C and 30°C. Curves for 13 and 20°C manifested shapes that are consistent with the beginning stages of a bacterial growth log phase (Figure 4.1), while the 30°C indicated growth of bacteria consistent with approaching a plateau in growth. However in this system the results confirmed our assumptions of substrate limitation affecting growth. This suggests that the curve at 30°C did not plateau because it was limited by substrate. This curve was also consistent with the Van't Hoff Arrehenius temperature bacterial reaction relationship.

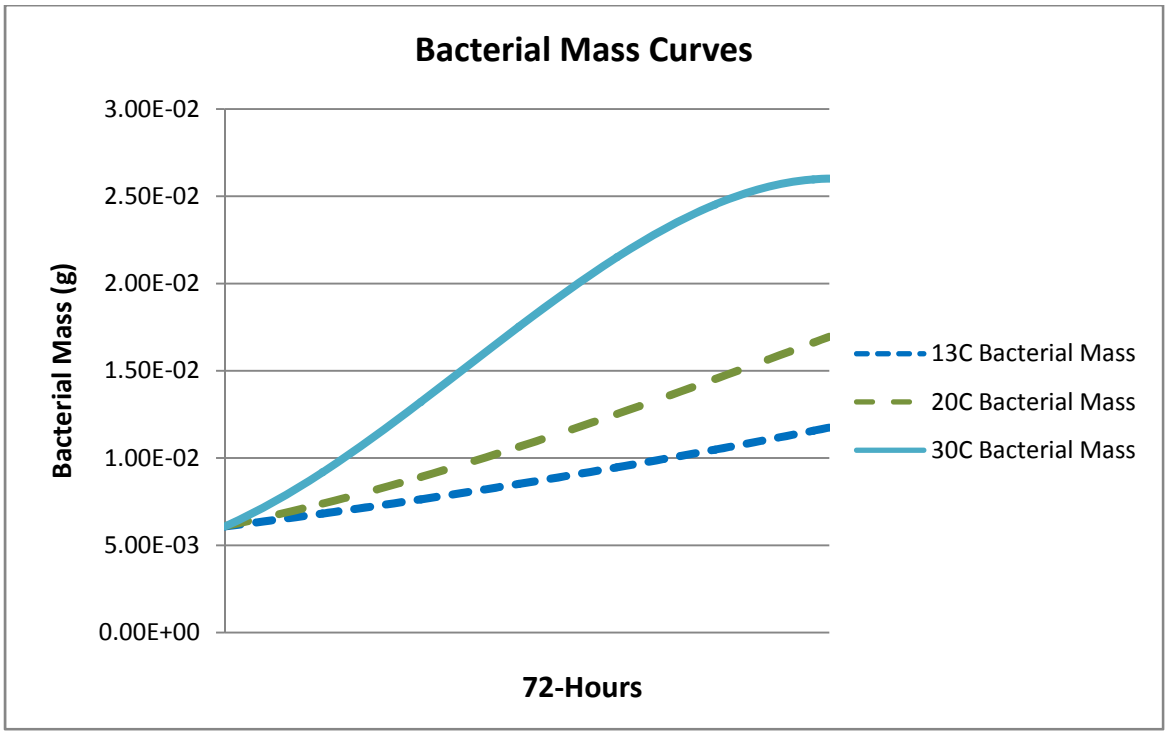

Figure 4.4: Graph of bacterial mass from simulated data 13, 20, and 30°C.

4.4.2 Model and Observed Data at 20 ± 3 and 30 ± 3°C

The simulation curve for 20 ± 3°C (Figure 4.5) diurnal temperature showed deviation of some data sets. However it was consistent with the observed data. Simulation curve at 30 ± 3°C diurnal temperature was also consistent with the observed data. However the curves for the diurnal temperatures appeared to exhibit a non-linear pattern which may suggest that the system was manifesting a transition from zero-order to half to first order reaction. This may have been an effect of the dynamic bacterial mass in the system. Bacterial mass curves (Figure 4.6) exhibited patterns that were consistent with Van't Hoff Arrehenius curve. The bacterial mass curve 20 ± 3°C also manifested bacterial growth at the log phase, while the 30 ± 3°C indicated a pseudo plateau limited by substrate concentration as shown in the ammonia utilization curves. This suggests that the food to micro-organism ratio drops as the substrate in exhausted.

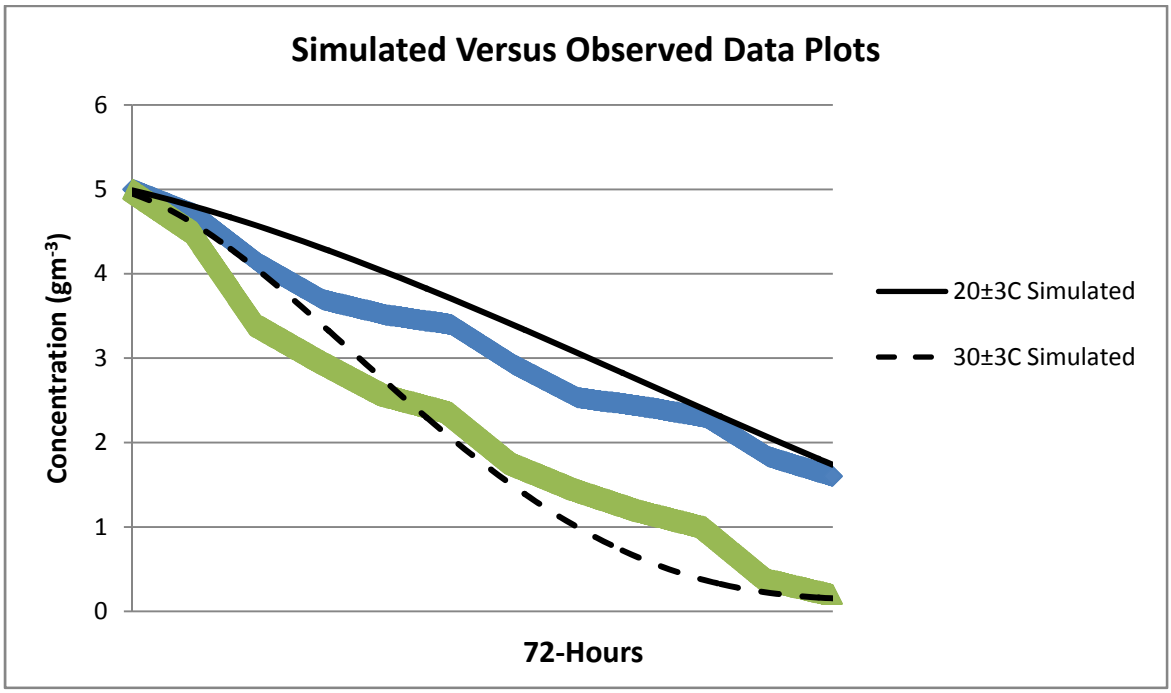

Figure 4.5: Graph of observed data versus simulated curves for 20 ± 3 and 20 ± 3°C.

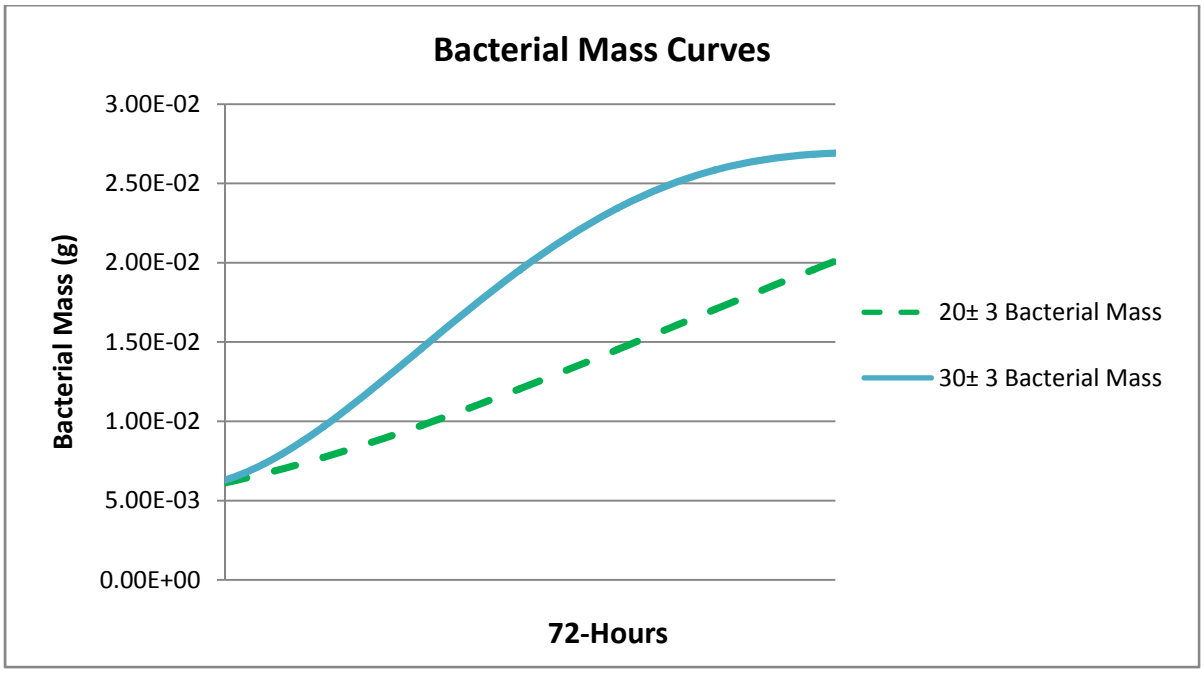

Figure 4.6: Graph of bacterial mass from simulated data 20 ± 3 and 30 ± 3 ^{0}C.

4.4.3 Model and Observed Data at 20 ± 3 and 30 ± 3^{o}C (Mixed Beads)

The simulated curves for 20 ± 3 and 30 ± 3^{o}C (Figure 4.7) were consistent with the observed data. At 20 ± 3^{o}C the curve appeared to exhibit a non linear pattern that suggests there was a transition in reaction order. This was further manifested in the 30 ± 3^{o}C curve indicating a transition in the reaction process from zero to half to first order reactions.

Transitions in the reaction order may suggest that bacterial mass responded to changes in temperature regimes as temperature increased. This is supported by the bacterial mass curves (Figure 4.8) which manifested a step curve at the higher temperature (30 ± 3^{o}C). Temperature impact at 30 ± 3^{o}C was such that the bulk of the curve was consistent with the higher segment of the log phase (Figure 4.1) on a bacteria temperature growth curve. The simulated effect of temperature, substrate loading and time on the bacterial mass in an acclimating and dynamic biofilter was further determined using sensitivity analysis.

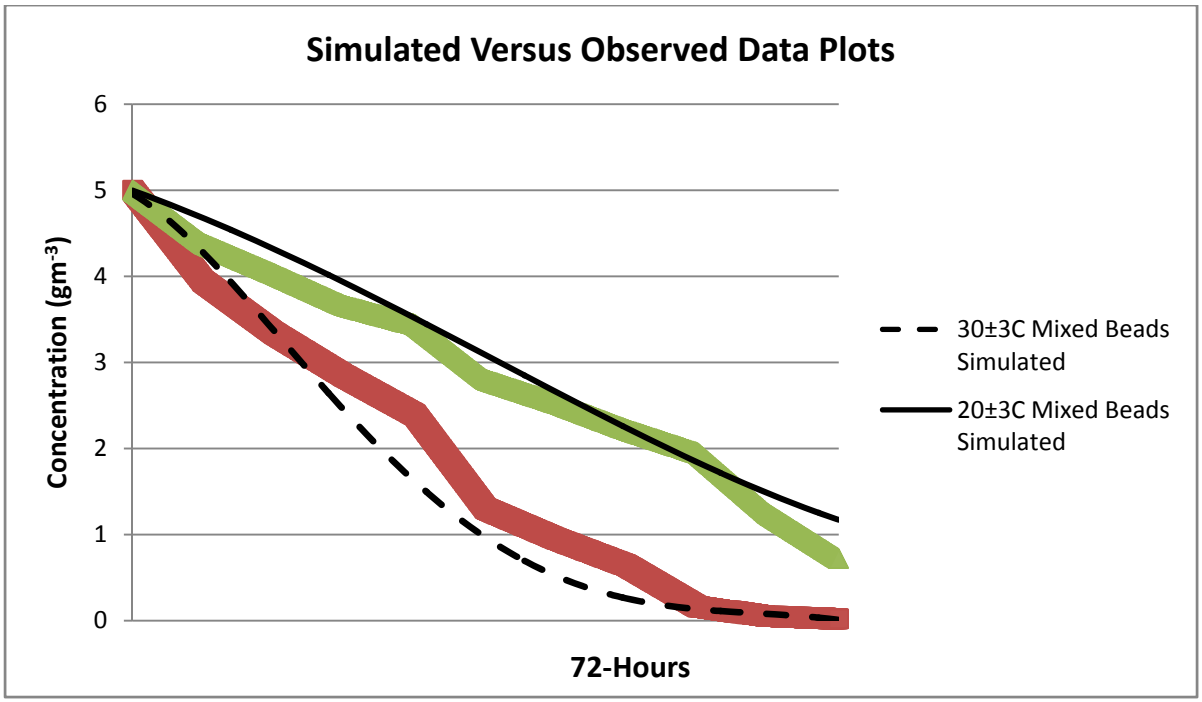

Figure 4.7: Graph of observed data versus simulated curves for 20 ± 3 and 30 ± 3°C (Mixed Beads)

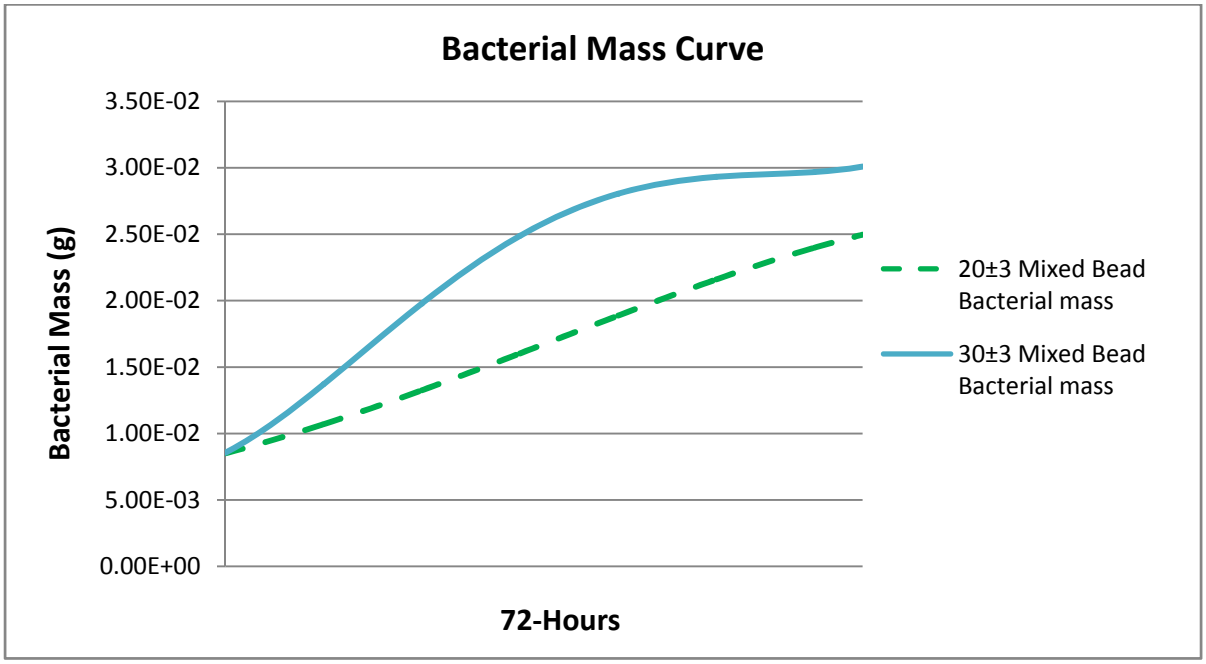

Figure 4.8: Graph of bacterial mass from simulated data 20 ± 3 and 30 ± 3 ^{0}C (Mixed Bead)

4.4.4 Sensitivity Analysis

Analysis of model sensitivity was performed by defining an initial state of the model and changing the value of the parameter of interest in increments across the range defined for the following, 1) Temperature values were varied at 5 degree temperature increment intervals starting at base temperature 13^{o}C, 2) Ammonia concentration was varied at interval of 5 g m^{-3} starting at the base concentration in the simulated experiment 5 - 15 g m^{-3} , 3) The period of run in days was also varied from base 3- 7 days and the effect on bacterial growth mass was determined since the model substrate utilization is a function of bacterial mass. Table 4.2a, 4.2b and 4.2c below shows the percentage effective change that occurred to the bacterial mass when each of the parameters discussed above was varied in the model analysis.

The simulation was started at base bacteria mass and the percentage sensitivity change was calculated using the expression below:

$$\% \text{ change in } X_{mass} = \frac{X_{mass(final)} - X_{mass(base)}}{X_{mass(base)}} X100 \quad \text{---------- (22)}$$

Where $X_{mass(Base),}$ is initial bacterial mass and $X_{mass(final)}$ is the final bacterial mass.

The bacterial growth mass at base concentration 5 g m^{-3} (Table 6a) for 3-day periods changed from 93.3% at 13^{o}C to 347.9% at 33^{o}C. Extending the period to 7-days at same concentration changed bacterial mass from 248.2% at 13^{o}C to 353.6% at 33^{o}C. This clearly suggests that bacterial mass will continue to grow over a given period when substrate is available. However time also had an effect on substrate concentration as the growing bacteria utilized the substrate. It may be suggested that growth of bacteria in a batch system is limited by substrate availability. Increasing the concentration to 10 g m^{-3} (Table 4.2b) increased the bacterial mass in a 3 d period from 172.5 % at 13^{o}C to 706.7 % at 33^{o}C. A period of 7 d at same concentration changed

Table 4.2a, 4.2b & 4.2c: Sensitivity analysis parameter percentage changes

Table 4.2a:

		% Change in Mass at Concentration **5 g m^{-3}**	
Temperature (C)	Initial X (g)	3 day (%)	7 days (%)
13	0.00608	93.3	248.2
18	0.00608	148.9	323.0
23	0.00608	230.1	350.2
28	0.00608	311.5	353.5
33	0.00608	347.9	353.6

Table 4.2b:

		% Change in Mass at Concentration **10 g m^{-3}**	
Temperature (C)	Initial X (g)	3 day (%)	7 days (%)
13	0.00608	172.5	570.3
18	0.00608	306.8	694.9
23	0.00608	524.9	707.0
28	0.00608	684.3	707.2
33	0.00608	706.7	707.2

Table 4.2c:

		% Change in Mass at Concentration **15 g m-3**	
Temperature (C)	Initial X (g)	3 day (%)	7 days (%)
13	0.00608	235.7	918.9
18	0.00608	454.2	1057.2
23	0.00608	843.9	1060.8
28	0.00608	1051.6	1060.9
33	0.00608	1060.8	1060.9

bacterial mass from 570.3 % at 13^{o}C to 707.2 % at 33^{o}C. Although the bacterial mass at 13^{o}C increased in value close 33^{o}C in 7 d, it suggested that bacterial growth had approached substrate limiting conditions. Increasing concentration to 15 g m^{-3} (Table 4.2c) changed the bacterial mass for 3 d period from 235.7 % at 13^{o}C to 1060.8 % at 33^{o}C, while bacterial mass changed from 918.9 % at 13^{o}C to 1060.9 % at 33^{o}C in a period of 7-days at same concentration. However at this concentration (15 gm^{-3}) bacterial mass manifested plateau at temperature 18-33^{o}C. This further confirmed substrate limiting conditions which affected bacterial mass.

4.5 Discussion

The simulation model predicts daily variation of ammonia concentration in laboratory batch systems. The importance of biofiltration is widely appreciated especially with increased use of recirculating systems stretching from hatchery to recreational fish. However the magnitude of the contribution of temperature to ammonia removal in the biofiltration process is less well understood. The model indicates that change in temperature affects bacterial mass and substrate utilization. Since the bacteria mass in this dynamic bacteria system was influenced by temperature, which suggests that performance of a biofilter in the form of ammonia utilization may be impacted by temperature.

The importance of bacterial mass as a regulator for ammonia utilization is also well appreciated especially in relation to slow or rapid growth (Ratkowsky et al., 1982; Tchobanoglous et al., 2003), but the relative importance of ammonia utilization for slow growing bacteria in seeded and acclimation stage of a biofilter is not widely understood. Bacterial mass changed with increase in temperature this suggested bacterial growth. The growth of bacteria with increase in temperature has been previously studied (Hanus and Morita, 1968; Randall et al., 1992; Tchobanoglous et al., 2003). However it was similarly indicated in the model that

bacterial mass increased with temperature while acclimating the biofilter. The model output for bacterial mass indicated that maximum substrate utilization corresponded to higher temperature regimes while bacteria mass increased at higher temperatures. It is possible that diffusion conditions which may be attributed to substrate utilization were more favorable during periods of higher temperature. This may also suggest that seeded biofilters and acclimation periods may be set at higher temperatures from a pragmatic approach for managing a recirculating biofiltration system. The biofilter substrate utilization rate was also increased at higher temperatures (30 ± 3^{o}C) with mixed beads. This may have resulted from a combination of bacterial mass and temperature effect on the reaction. However bacterial mass simulation curves appeared to manifested a plateau at higher temperatures.

Sensitivity analysis indicated that bacterial mass doubled between 13^{o}C and 28^{o}C corresponding to the theoretical predictions that bacterial reaction rates double for every 10^{o}C change in temperature (Tchobanoglous et al., 2003). Bacteria mass was the second sensitive parameter to temperature changes in the reaction system. The curve of bacterial mass manifested an increased mass corresponding to temperature increase until substrate limitation. Changing the sensitivity parameter from temperature to concentration affected the bacterial mass and substrate utilization. Substrate utilization was faster at higher temperatures. Simulating bacterial mass for period(s) longer than 3 d suggested that bacterial continued to grow while bacterial mass exhibited a plateau for longer periods as substrate was depleted. However smaller values of bacterial mass at lower temperatures may suggest reduced ammonia utilization rates. This may suggest that biofilters may perform better in the spring and summer when temperatures are higher. Although substrate utilization rates may not directly respond to temperatures in their immediate environmental, temperature effect may suggest new design opportunities to enhance

biofilter performance in recirculating aquaculture system.

This study is partly consistent with studies conducted by Wortman and Wheaton (1991) and this may suggest that the results may apply to recirculating aquaculture systems. Simulated curves in this study matched observed curves in relation to temperature change and ammonia utilization. However ammonia utilization in this study had no significant change between 13 and 20^{o}C. Although this was consistent with studies of Zhu and Chen (2002) which had no significant difference between 14 and 27^{o}C, the results in this study may have been an artifact of the dynamic bacterial growth response to substrate availability and temperature effect. Since bacterial mass manifested at 13^{o}C by the simulation model was less than bacterial mass at 20^{o}C, it is possible that bacterial growth would have continued under the respective temperature conditions above a minimum threshold to demonstrate significant difference. The bacterial mass curve and sensitivity analysis in the simulation model may also support this explanation.

A detailed integration of the simulation model with respect to other nitrogen removal parameters may be required. Parameters not included in the model, but which may need further explanation, include contributions of bacteria death rate in the biofilm, net substrate utilization rate and organic carbon. Presence of organic carbon loading in the system encourages heterotrophic bacterial growth while competing with nitrifiers and ultimately affecting substrate diffusion. Further studies may be required to validate the model parameters especially temperature effect on bacterial mass in a non limiting substrate system (continuous flow) for biofilter acclimation and performance.

4.6 Conclusions

(1) Substrate utilization is influenced by temperature, bacteria mass, time and substrate concentration.

(2) Bacterial mass increases with temperature increase until a plateau at which substrate is limited.

(3) Bacterial mass increases with increase in substrate concentration.

(4) A combination of time, temperature and substrate concentration could be used to increase bacterial mass in biofilter acclimation.

(5) The proper combination of the above sensitive parameters could be used to improve biofilter performance in cold weather and temperate climates.

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Chapter 5 Summary and Conclusions

This dissertation is part of a larger goal aimed at improving biofilter performance with respect to temperature for application to commercial recirculation systems. The dissertation addressed short term and diurnal temperature effects on dynamic biofiltration nitrogen removal performance. The contributions of this dissertation specifically focused on the following: 1) design, development and testing of a bench-scale research system prior to experiments; 2) demonstration of experimental methodology and use of research system for biofilter studies; 3) determination of short term temperature effects on dynamic growth biofilters in a nitrification process; 4) determining an empirical relationship between short term temperature regimes and biofilter performance and 5) development of a simulation model to verify and validate the experimental data.

Review of previous studies indicates a deficiency in the literature on dynamic growth biofilter performance and short-term temperature effects. Previous studies were also limited based on systems that specifically focused on steady state biofilm. This study was undertaken at the Louisiana State University, Department of Biological and Agricultural Engineering (LSU-BAE) and the approaches taken were oriented to improve commercial application systems. Solutions and problems identified here are applicable to recirculating aquaculture on a commercial scale.

Soluble waste in the form of total ammonia nitrogen (TAN), nitrite or nitrate, have been accepted to have toxic effects on fish (Kuo-Feng, T. and Wu, K., 2004; Atwood et al., 2003; Bovendeur et al., 1990). The process of removing soluble TAN using a biofiltration process (Chapter 3) revealed that degradation kinetics in recirculating aquatic systems were impacted by temperature. This is also confirmed by previous research although the range at which

temperature may impact TAN degradation rates has not been unanimously conclusive as suggested by variety of opinions (Zhu and Chen, 2002; Wortman and Wheaton, 1991). However, it is recognized that previous studies were carried out at steady state temperature regimes (Kuo-Feng, T. and Wu, K., 2004; Zhu and Chen, 2002; Malone and Beecher, 2000). By contrast, most recirculating aquaculture systems and almost all aquaculture systems are exposed to diurnal temperatures from day to night and seasonal effect (Beitinger et al., 2000; Widmer et al., 2006). The impact of such varying temperatures on a typical aquaculture system could directly affect the biofilteration process. This may also suggest considering well known time and temperature interaction effects over a period of time in biological filter acclimation and ammonia utilization in a recirculating aquaculture system.

This study therefore used small scale laboratory biofilters to address the short term temperature effects on TAN removal rates at different temperature regimes. The first design was a pilot scale system used for preliminary data. These were 18- 250 liter tanks with in-situ biofilters, heat exchanger chillers and insertion heaters (EARS system). This allowed complete independence between the 18 tanks. The second system was a 36 tank system using 40 liter tanks (BAE system) which was used for data collection for this study. Each system was temperature controlled via PC-computers.

The designed system for BAE was used to determine biofilter performance using steady state temperature regimes (13, 20 & 30^{o}C), diurnal regimes (20 ± 3^{0}C & 30 ± 3^{0}C) and, diurnal regimes (20 ± 3^{0}C & 30 ± 3^{0}C) with mixed beads. Results observed indicated that there was no significant difference in ammonia utilization rates between 13 and 20^{o}C, however there was a significant difference in utilization rate between 20 and 30^{o}C. Results of the diurnal temperature regimes also indicated that there was a difference in the substrate utilization rate between 20 ±

3^0C & 30 ± 3^0C. Similarly there was a difference between 20 ± 3^0C & 30 ± 3^0C using the mixed beads (Chapter 3).

TAN removal rates at increasing temperature regimes in this study under ideal conditions were further illustrated by comparing our observed outcome to results reported in previous studies. Zhu and Chen (2002) presented an observation that temperature within the range of 14-27^0C did not have any significant impact on TAN removal rates. This tends to partially support our findings from our observed data in the range of 13-20^0C, however comparison of the simulation model suggested that there may be a difference in TAN removal rates at 13 and 20^0C had the biofilm achieved its growth steady state biomass. The inconclusiveness may be due to an experimental artifact in the design of system and duration of experiment. Results reported by Wortman and Wheaton, (1991) indicated a linear relationship between TAN removal rate and temperature in the range of 5-35^0C. This seems to support the TAN removal rate differences our results indicated between 13 & 30^0C and 20 & 30^0C. However the relationship for biofilter performance (Chapter 3) with increase in temperature at steady state was linear in this study, although biofilter performance determines the capability of biofilter to remove ammonia, rather than specific or volumetric substrate removal rate. Further analysis of the diurnal temperature regimes indicated that higher temperatures had a higher substrate utilization rate and higher biofilter performance ($P < 0.05$). It is important to note that the higher temperature limit of the 20 ± 3^0C regime was 23^0C and the lower limit was 17^0C, while the higher temperature limit for 30 ± 3^0C was 33^0C and lower limit 27^0C. This may suggest that the increased temperatures at the higher limits could account for increased biofilter performance and substrate utilization differences between the ranges of 17- 33^0C. A final experiment with beads that were acclimated at different temperatures but exposed to diurnal temperature regimes (20 ± 3^0C & 30 ± 3^0C) also

indicated a significant ($P < 0.05$) change in utilization rates. This further confirmed a change in TAN utilization rates within ranges 17- 33^0C for the mixed beads.

It is interesting to note that the substrate utilization rates at the diurnal temperature regimes were significantly different ($P < 0.05$) with a numerical higher utilization rate than their corresponding steady state temperature regimes. This may be an artifact of the nitrifying bacteria in the biofilm adapting to diurnal regimes and specifically to the temperature influence on their growth process in reaction kinetics. Bacteria reactions with change in temperature are known to follow an Arrhenius curve (Zhu and Chen, 2002; Tchobanoglous et al., 2003). It is however important to acknowledge that impact of temperature on bacteria growth process in a seeded and dynamic growth bioilter may significantly affect the biofilter performance. It is a possibility that this may have been a cause for non-significant ammonia utilization rates determined between 13 and 20^{o}C even though there was a numeric difference.

A simulation model developed (Chapter 4) to determine time and temperature effect on the biofilter substrate utilization performance and bacterial growth mass supported speculated temperature effect on observed data. The model indicated that bacteria growth mass was impacted by temperature and increased with increase in temperature and suggested a peak biomass at 33^{o}C. This may explain the difference in substrate utilization rates at higher temperatures between 20 and 33^{o}C. Although simulating a prolonged time period in the system also had a positive effect which increased bacterial growth mass, it is further clarified by the model that over extended period of time the biofilter at the lower temperature will grow more bacteria. However this effect is enhanced by higher temperature starting points since these achieve peak bacteria mass growth faster than the lower temperatures.

Model simulations further clarified results from observed data indicating slightly lower

than expected bifilter performances in commercial systems especially the low temperatures. It was determined that the filters were still in the seeded and growth phase and had not achieved steady state biofilm at lower temperatures of 13 and 20^{o}C. However, at higher temperature regimes of 30 and 33^{o}C, the model indicated that the bacterial growth mass gradually achieved a peak on a curve similar to second order reaction kinetics. This may suggest that biofilter acclimation prior to installation and start up at commercial facilities should be at higher temperatures to encourage faster bacterial mass growth. These findings may even suggest considerations for some future engineering design changes such as incorporating heater systems in the filters to spike temperatures in the filters while maintaining a high flow residence time. This may be initially pragmatic by keeping a balance between high residence time for acclimation and media bed agitation (back-washing) to reduce carbon feeding heterotrophs from outgrowing nitrifying bacteria.

However in a typical recirculation aquaculture system the presence of BOD (Biochemical Oxygen Demand) from feed material in the water contains organic carbon feed source for heterotrophic bacteria. The heterotrophic bacteria compete with nitrifying bacteria for surface area, substrate and oxygen. It is recognized that BOD has a negative impact on biofilter performance (Harremoes, 1982; Zhu and Chen, 1999; Heinsbroek and Kamistra, 1990). The results of this experiment were obtained using tap water and the necessary nitrification substrate nutrients. The results were obtained with the assumption that presence of BOD was not significant in the substrate feed stock. It is therefore noted that the removal rates could be higher than it would have been in a seeded acclimating commercial recirculation system.

The results and observation in this study leads us to conclude the following:

(1) Biofilter performance increased with temperature increase.

(2) There is a significant difference in TAN removal rate for 13 and 30^0C observed and modeled data.

(3) There is a significant difference in TAN removal rate with increase in temperature between 20 and 30^0C.

(4) Diurnal short term temperature regimes 20 ± 3 and 30 ± 3^0C increase TAN utilization rate compared to corresponding steady state temperatures.

(5) Biofilters with beads acclimated at different temperature regimes have a substrate utilization rate significantly different from biofilters at corresponding steady state temperatures.

(6) Bacterial mass in a biofilter is influenced by temperature and substrate concentration.

(7) The overall significance of the temperature effect on biofilter acclimation is the duration of acclimation that is reduced with increase in temperature. This also has a positive effect on the biofilter performance in a recirculating system.

The conclusions drawn above led us to make recommendations that will be necessary to further clarify temperature impact on biofilter performance in future studies as well as pragmatic approaches for recirculation system managers as follows:

(1) Future studies may include BOD in the feed substrate to determine its impact with the different temperature regimes. Heterotrophs which feed on organic carbon present in BOD generally compete with nitrifiers for space. The presence of BOD may increase competition for space which could affect the response of utilization rates in relation to temperature. A possible application could be to incorporate influent from an existing commercial facility, use of artificial carbon loading in the feed stock prepared or use of organic substance such as wood chips, rice hulls versus plastic biomedia.

(2) The duration of the experiment may be extended for a longer period (7day- 2weeks etc) to allow nitrifying bacterial mass to achieve maximum steady state growth.

(3) Biofilters with mixed beads acclimated at different temperature regimes perform significantly better than their counterparts at steady state. This may suggest that acclimating biofilters for different temperature zones or climates and incorporating the filters in series or parallel could enhance the utilization rates of TAN in systems. It is recognized that real life commercial applications are exposed to daily diurnal temperature variations in a 24 hour cycle similar to observations in this study. Interestingly these studies suggest these variations may actually enhance performance because of the higher temperature limit in the fluctuations.

(4) An engineering contribution to system capability could include expanding the range of temperature regimes e.g. 5- 35^0C which could enhance the predictability of TAN removal rate versus temperature relationship.

(5) The system design functional capability may enhance further studies in this direction of engineering applications by incorporating heaters in a biofilter.

This study in its entirety may contribute to developments in recirculation systems for future designs of biofilters and management approaches for commercial facilities. Additionally, the increased clarity and understanding that may result from this study should contribute possible significant economic benefits to the commercial sector that operate on profitability.

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Appendix A

Protocol for Loading Tanks with Synthetic Nutrient (Ammonia) Water

Safety First: Shut down the system (Computer Desktop Program) to avoid any risk of electrical shock.

Note: If not sure shut off power from break switch or unplug relay pack main power lines.

1. To shut-down the system go to the program menu in the bottom tool bar (**C\windows\systems**) of the desk top.
2. Right click on tool bar and scroll down to close, click **close** the program will shut down.
3. Go to tool bar Sine-wave at the bottom of desk top and left click.
4. **Go to** "**Debug**" tool bar at the top of the screen and click
5. **Go to** " Start with out Debugging" (Ctrl + F5) and click
   - Note: leave the blank Black screen as it appears. This ensures that all **heaters** and **chillers** are off.
6. Proceed to drain tanks if there is any water in the tanks
7. Rinse the tanks out with distilled water and check for chlorine and any leaks or cracks.
8. Also check bio-filters for proper air tube connections to air-line.
9. Refill tanks using submersible pump customized for this purpose only (Blue cast iron Pump)
10. Check tank levels to ensure water level is at the 9-1/2 "depth level from base of tank to water surface in tank.
11. Inspect all chilling pump tubes and heater lines to ensure proper location before starting the system program.

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Appendix B

Synthetic Nutrient (Ammonia –Nitrogen) Mixture for the Tanks

The following chemical compositions (adopted from Wortman and Wheaton, 1991) were used to make the ammonia nutrient mixture enriched with trace elements. This composition was scaled down for desired concentration and prepared to support growth of both AOB (ammonia oxidizing bacteria) and NOB (nitrite oxidizing bacteria).

Compound	Source	Concentration (mg/liter)
$NaHCO_3$	Louisiana State University Store	100
K_2HPO_4	″	15
$CaCl_2$	″	15
NH_4Cl	″	5 (as N)
$MgSO_47H_2O$	″	100
$FeCl_36H_2O$	″	5

The mixing procedure started with sanitizing the holding containers (44gallons plastic can) with diluted bleach to minimize the potential residual bacteria which may cause insitu-nitrification.

The individual chemicals in the list above were weighed for an equivalent concentration of 5mg/l based on the container volume of water. The constituent chemicals were added to the distilled water in the can. The water was thoroughly mixed at room temperature of 22^0C and delivered to the individual tanks using a submersible pump. The tank served as both a reservoir and sump for nutrient circulation through the filter.

Appendix C

Steps to Change Temperature of Controlled Tanks

1. Minimize the console (**Dark/Black background**) window with name "C:\windows\system32\cmd.exe"
2. **Right click** the console tool bar at the bottom of the screen.
3. **Click "Close"** from the window that pop-up.
4. **Click** the program window tool bar at the bottom of the screen (Named: Sine of 18-Microsoft to make it active on the screen.
5. **Go to** "**Debug**" tool bar at the top of the screen and click
6. **Go to** " Start with out Debugging" (Ctrl + F5) and click
7. The program temperature console will pop-up with question "**Enter Average temperature for Tank** 1"
8. Key in the desired number and press enter. The program will again pop another question "**Enter the temperature variation for Tank1**"
9. Enter "**0**" if holding tank at steady state temperature. If a daily transient (sine-wave/cyclic) temperature is required key in the desired variation.
10. Repeat **Step 9** for all eighteen tanks and press enter.
11. Wait 5-Minutes to see the console display "Required & Average temperature"

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Appendix D

Steps to Change Transient Temperature (Sine-Wave) Deviations

1. Minimize the console (**Dark/Black background**) window with name "C:\windows\system32\cmd.exe"

2. **Right click** the console tool bar at the bottom of the screen.

3. **Click "Close"** from the window that pop-up.

4. **Click** the program window tool bar at the bottom of the screen (Named: Sine of 18-Microsoft to make it active on the screen.

5. **Go to** "**Debug**" tool bar at the top of the screen and click

6. **Go to** " Start with out Debugging" (Ctrl + F5) and click

7. The program temperature console will pop-up with question "**Enter Average temperature for Tank** 1"

8. Key in the desired number and press enter. The program will again pop another question "**Enter the temperature variation for Tank1**"

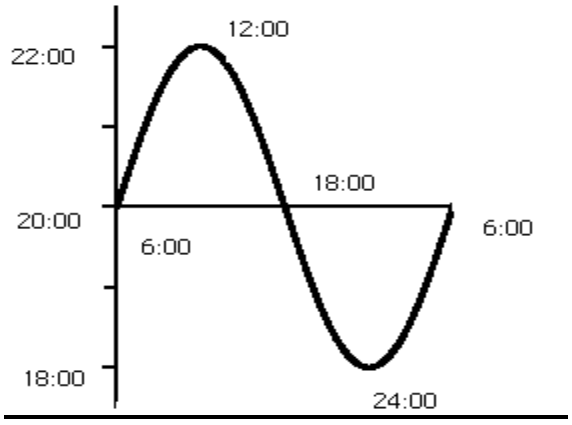
9. Enter "2", "5" or "10" if a sine wave is required for tank temperatures. This applies to any daily transient (sine-wave/cyclic) temperature that is desired.

10. Repeat **Step 9** for all eighteen or thirty-six tanks and press enter.

11. Wait 5-Minutes to see the console display "Required & Average temperature"

Note: The temperature curves for sine-wave simulated as shown below.

Sine code for 18/36 tanks (24 hrs cycle):



Appendix E

Biofilter Sizing and Filter Design Characteristics

(a) Physical design Characteristics

The bio-filters were designed using an acrylic tube with the following dimensions.

7. The height of filter is 7.5″
8. Internal diameter is 2″
9. The base/stand dimension is 4″ X 4″
10. Inflow screen porosity is 2mm x 3mm = 6 X 10^6 microns^2
11. Outflow screen porosity is 2mm x 3mm = 6 X10^6 microns^2

(b) Media Characteristics

1. EN media bead size characteristics can be represented by 1/8 by 1/8 inch cubes
2. Area of cube = 0.015625 inch^2
3. Area of biofilter = $\frac{\pi D^2}{4}$ = 3.142 inch^2
4. Depth of biofilter = 7.5 inches
5. Volume of biofilter = 23.56 in^3

Airlift Design Characteristics

The airlift was incorporated into the bio-filter for the purpose of circulation, nitrification, oxygenation, and Carbon stripping. The following dimensions were used in the design process.

1. Submergence/depth is 8”
2. Tube is 1/2" diameter
3. Lift height is 1.5 “
4. Lift to submergence ratio is 1.5/8 = 0.18
5. Average water flow rate is 1.6 liters/min

Media Porosity

The movement of air, water, and nutrients within media bed generally depends on the media structure, or the geometrical shape of the media, and the porosity of the media. The provision of sufficient media aeration and drainage is characteristic of a good media.

Determining Media Porosity

The determination of sufficient media porosity is essential for calculating total porosity, water-holding porosity and aeration porosity. Porosity can be determined using the following procedure.

- With drainage holes sealed in an empty container, fill the container and record the volume of water required to reach the top of the container. This is the **container volume**.
- Empty and dry the plugged container and fill it with the growing media to the top of the container.
- Irrigate the container medium slowly until it is saturated with water. Several hours may be required to reach the saturation point, which can be recognized by glistening of the medium's surface.
- Record the total volume of water necessary to reach the saturation point as the **total pore volume**.
- Unplug the drainage holes and allow the water to freely drain from the container media into a pan for several hours.
- Measure the volume of water in the pan after all free water has completed draining. Record this as the **aeration pore volume**.
- Calculate total porosity, aeration porosity, and water-holding porosity using the following expression.

Total porosity = total pore volume / container volume

Aeration porosity = aeration pore volume / container volume

Water-holding porosity = total porosity - aeration porosity

This study primarily focused on the total porosity of the EN (enhanced nitrification) media used for the filtration process.

Total porosity calculated for the media was as follows:

Total pore volume = 250 ml

Total container volume = 500 ml

Hence porosity = 250/500 = **0.5**

Appendix F

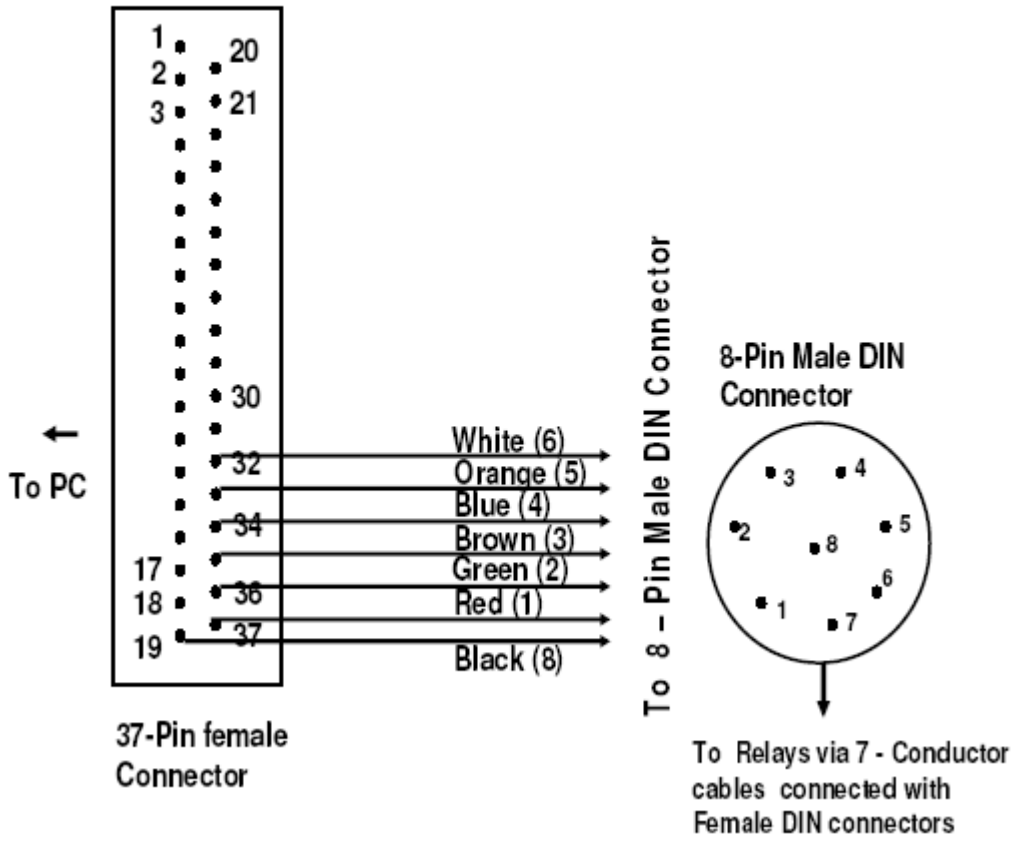
Filter Preparation and Sampling

The filters were loaded with beads to 1/3 the volume of the filter column. The filters were immersed in the tanks and held at the desired temperatures for the acclimation period. The filters were then subjected to the desired temperature regimes steady state, step-down/step-up and cyclical transient temperatures in random order of three tank replicates. The filters were air-lift operated, with the air-lift incorporated into the filter. Water flow through the filter was down ward. Samples were collected at the top of the filter as influent flow. Samples were analyzed for concentration values based on the HACH, spectrophotometer EPA approved Nesslerization method 8038 used to determine the ammonia concentration removed. The transient temperatures were applied to the tanks for three consecutive days for each treatment.

The water DO and pH values were measured once a day in each tank prior to sampling. All measurements were made within the first two hours of drawing samples. Prior to each test, reagents (HACH, Nitrogen-Ammonia reagents) were added to the sample water allowed to react for 1 minute. The samples were then measure in the spectrophotometer for absorbance values. The absorbance values were then matched against a standard curve to deduce the concentration of ammonia in the samples. Tanks were sampled every six hours for the entire 72 hrs. The sampling process required extracting 50ml of water from tank. The volume removed from the tank including evaporated volume was all replenished by dripping a mixture of the nutrient ammonia solution held in an independent reservoir. The replenishing flow to tanks was controlled with a submersible pump (ViaAqua, model no: VA-306, marinedepot.com) located in the mixture reservoir flow valves.

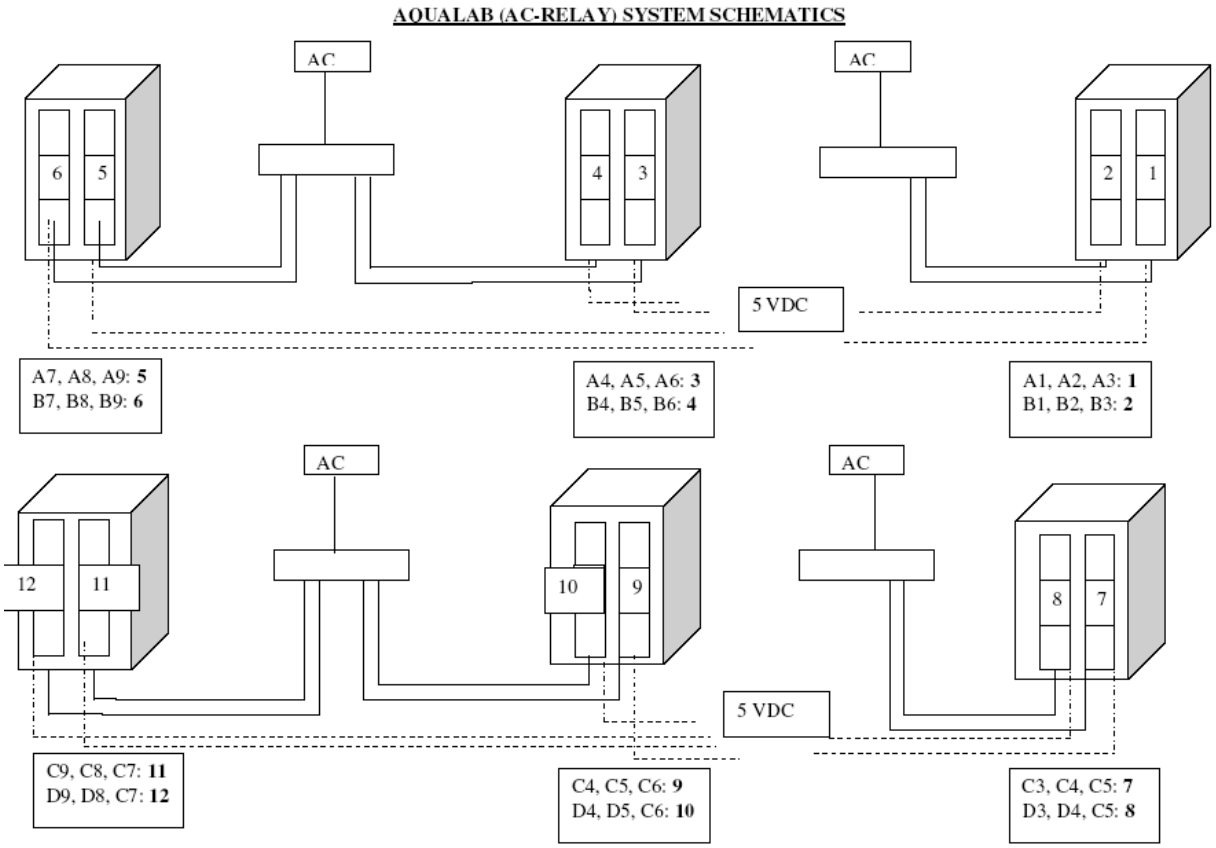
Appendix G

Wiring Color Code For Temperature Control Relay Boxes



Appendix H

BAE Engineering Laboratory- Relay Schematics



Appendix I

Calculations for Standard Dilutions and Standard Curve Plot

The standard curve needed for determining sample concentrations required dilution of standards to make more standard values that were used to plot the curve. The diluted standards were made using the following samples (Table 1) of calculated numbers. Dilutions were made for concentration level of 5 mg/l for the required standard curve in the experiment.

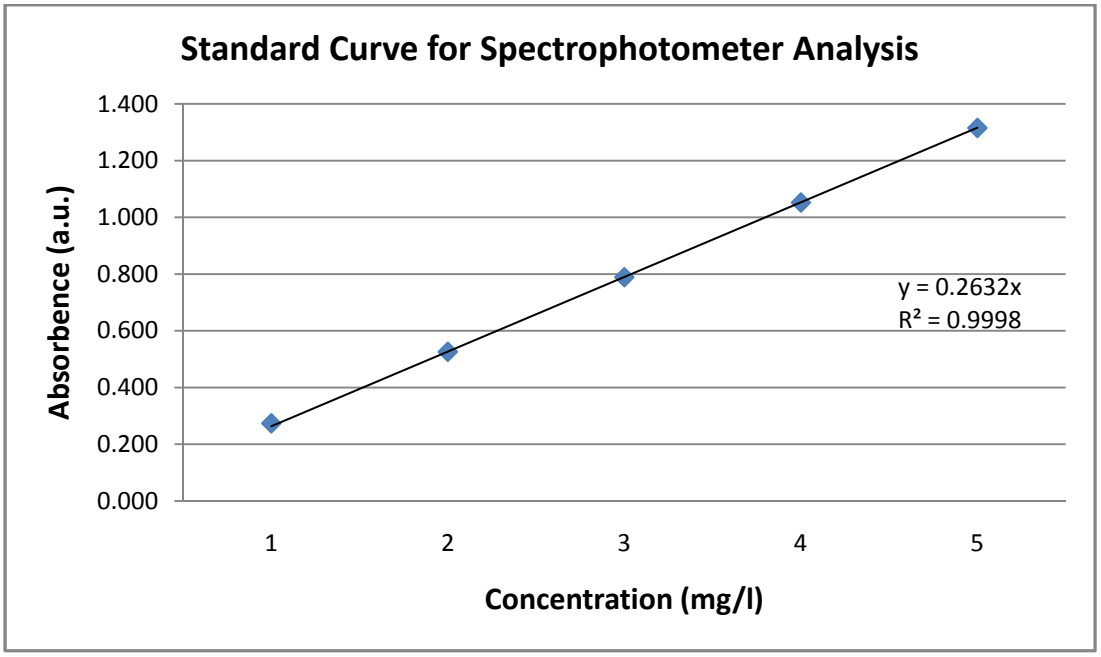
Table 1: Samples of dilution for calibrating standard curve

Dilution Concentration	25 ml	V Stock (ml)	Standard Stock Used	Absorbance
0.2		5	1 mg/l	0.041
0.8		20	1 mg/l	0.177
1.2		3	10 mg/l	0.271
1.8		4.5	10 mg/l	0.411
2.6		6.5	10 mg/l	0.002

Five diluted standards were made by adding calculated volumes to the standard stocks bought off the counter (HACH colorimetric kit, method 8038). The absorbance of the standard samples (Table 2), were recorded from the spectrophotometer at Biological and Agricultural Engineering department. Three successive recordings were taken from the spectrophotometer values and averaged. The averaged (mean) values were then plotted to determine the standard curve.

Table 1: Sample absorbance values for Standards from spectrophotometer

Concentration	Absorbance (a.u.)				
mg/l	1	2	3	Mean	SD
0.2	0.041	0.045	0.034	0.040	0.006
0.8	0.177	0.175	0.163	0.172	0.008
1.2	0.271	0.271	0.264	0.269	0.004
1.8	0.411	0.423	0.405	0.413	0.009
2.6	0.577	0.58	0.578	0.578	0.002



The standard cure was used to calculate ammonia concentrations from the samples collected in the tanks.

Using the equation of the curve y = 0.263X

Where: y = absorbance

X =? Much needed concentration

Therefore: X= y/0.263 to determine any concentration from known absorbance value.

Appendix J

Design and Testing of Laboratory Temperature Control Batch Tank System

This section focuses on the design, development and testing of automated controlled temperature recirculating system, used to study short term temperature impacts on biofilter performance in a biofiltration process. Customized system designs were used to control and mimic temperature regimes example cold & warm water (13^0C, 20^0C & 30^0C); diurnal variation (20 ± 3^0C, 30 ± 3^0C), step temperatures (13-30^0C and 30-13^0C) of natural aquatic systems. The key features of these systems included independent tanks with no mixing of water; controlled temperature and consistent conditions for accurate laboratory testing of samples. Sets of tank systems were constructed at two locations at facilities of the Louisiana State University Ag. Center (LSUAC). A batch of pilot scale tanks were initially constructed at the Engineering laboratory of Aquaculture Research Station (EARS) used for preliminary studies. A batch of laboratory scale tanks were designed at the Department of Biological and Agricultural Engineering (BAE) on the LSU campus after the EARS system. EARS system consisting of 18 pilot scale tanks with 250 l capacity was initially designed and built for oyster biology studies, with drop-in chillers and stainless steel heat exchangers. These were also modified to improve chilling rates and total operational efficiency. The laboratory scale system, constructed at the Biological and Agricultural Engineering Department (BAE) had thirty-six tanks, each with a capacity of 40 liters. Each tank was self contained with a submerged bio-filter.

Heat exchangers used for chilling tank water incorporated polyvinyl chloride tubing (PVC), used in the heat exchanger designs to achieve desired temperatures for each independent tank. The BAE system (vinyl tubing) chilled at the rate of 2.28^0C per hour ($T_{start}\approx20^0$C) for a 40-liter tank while the EARS system chilled at a rate of 1.98^0C per hour ($T_{start}\approx20^0$C) for a 250-liter

tank with stainless steel material and 1.51^{0}C per hour using vinyl tubing. However stainless steel was not used because of the possibility of corrosion and leak in salt water. The lower temperature of 13^{0}C was successfully attained for the experimental regime at BAE although the system design performance was expected to attain a design cooling goal of 10^{0}C which was not achieved due to the chilling limitations. The modifications in the EARS systems achieved the desired 10^{0}C chilling of the EARS system. The upper desired temperatures of 33^{0}C for both systems were attained using glass electric immersion heaters.

The biofilters were also designed to fit the tank dimensions while maintaining active biomass for removal of nitrogen from the tanks. Submerged bioballs were used in the EARS system for pilot studies. The BAE system was custom designed with an airlift for water circulation while using micro-beads to host active biomass responsible for ammonia removal. The system design allowed easy cleaning between experiments and was effective for use in experimental and pilot scale studies to determine ammonia removal rates for different temperature regimes. Experimental and computational results were later used to enhance the design and could be used in future designs.

Introduction

The control of temperature and its impact on water quality, particularly dissolved oxygen and nitrogen (waste) byproducts is very important in the growth and health of cultured species. Temperature affects most biochemical and physiological activities of fishes (Beitinger et al., 2000; Widmer et al., 2006). Water temperature is consequently a vital component of fish habitat. Seasonal changes in natural aquatic system habitats determine variations in temperature over time, which impacts the system dynamics of aquatic systems. These changes in the natural environment are due to multiple factors including released water from reservoirs, water

diversions, loss of vegetation, other land use practices (Poole and Berman, 2001) and daily outdoor temperature variations. Temperature fluctuations due to these changes create diurnal cyclical temperature patterns in water bodies (Sinokrot and Stefan, 1993). However the full implication of the daily and seasonal temperature fluctuations are not fully understood (Zhu and Chen, 2002; Wortman and Wheaton, 1991). The need to study such systems in a laboratory setting encourages incorporating computer control technologies which can simulate temperature (and other water quality factors) regimes of natural aquatic systems. The processes involve controlling the environmental conditions and system constraints (Fang et al., 2004), which include oxygen, temperature, ammonia, feed rate and the density of fish stocked in a system. The controlled output parameter in the process is TAN, which is of significant interest for the benefit of fish and the protection of the environment in general and could be studied and regulated (Lee 2000; Lamoureux et al., 2003; Hall et al., 2002) for improved management practices.

The need to maintain a habitable environment for studies with species such as shellfish and other fish is increasing while resource of water are also becoming limited with the rising cost of pre/post treatment of water. This supports applications of water reuse and reconditioning technologies (Watten et. al, 2004; Lyssenko and Wheaton, 2006). The need for water reuse and reconditioning has led to emerging technologies of closed recirculation systems to improve water quality for aquatic systems (Timmons et al., 2006) but also minimize effluent discharges. It is expected that aquaculture systems which fall under recirculation aquaculture systems (RAS) have a common target which is focused on maintaining the water quality parameters of the cultured species in other to maximize growth and production rates while emphasizing cost effectiveness. Improvements in growth and production are of significant importance to the commercial fishery industry considering the market potential for seafood products in the US is

increasing (Lee, 1995). This is partly due to diet-conscious Americans increasing their consumption of fish (USDA, 1992).

The use of controlled recirculation systems is becoming more of an alternative to meet the demand in the fishery industry. This control also enhances capabilities for research done in indoor facilities. Process control such as temperature control and water quality studies is limited by factors such as environmental concerns, high labor cost, increase in competition for dwindling water and land resources and stringent regulatory measures (Lee, 1995; Lee, 2000). The goal of this section was to develop efficient temperature process control systems to achieve the following objectives: a) modify the existing EARS temperature control system for efficient operations b) Build a small scale temperature process control system at BAE after the EARS system for examining biofilter performance at different temperature regimes.

The temperature control systems used for the studies above were designed and constructed at separate facilities of Louisiana State University. This first system (Figures 1a & 1b) was designed and developed (Kolar, 2005) for studies with shellfish (Eastern Oyster, *Crassostrea virginica*) at EARS. The system was modified during this project for efficiency in chilling and operational cost effectiveness which was used for pilot studies with the desired temperature regimes. The temperature process control system (EARS) was initially designed with four (4) individual drop-in unit chillers located in two central tanks for the entire system as shown (Figure 3a) in the design section to follow.

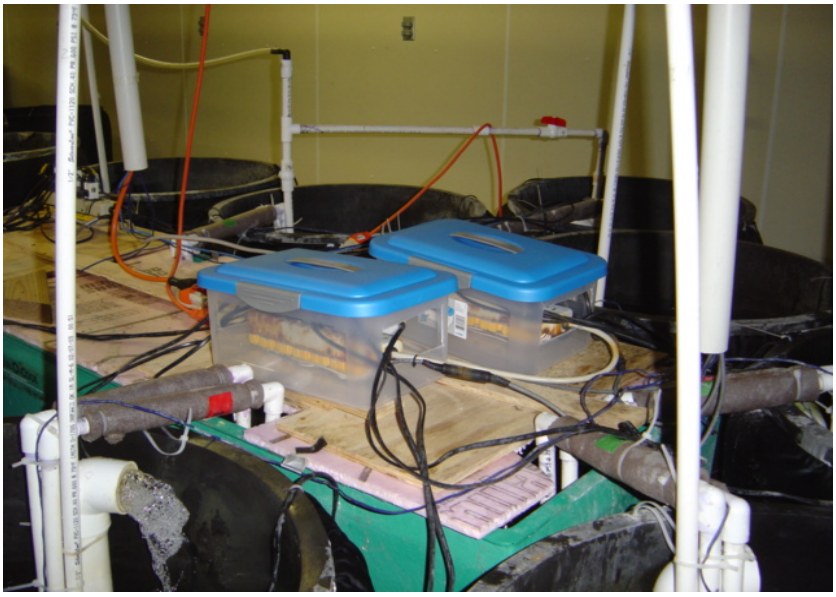


Figure 1a: Engineering lab Aqua Research Station System - This figure shows tanks and control relay boxes of the designed system at the Engineering Aquaculture Research Station EARS (System), of the Louisiana State University AgCenter. Ben Hur Dr. Baton Rouge, LA.

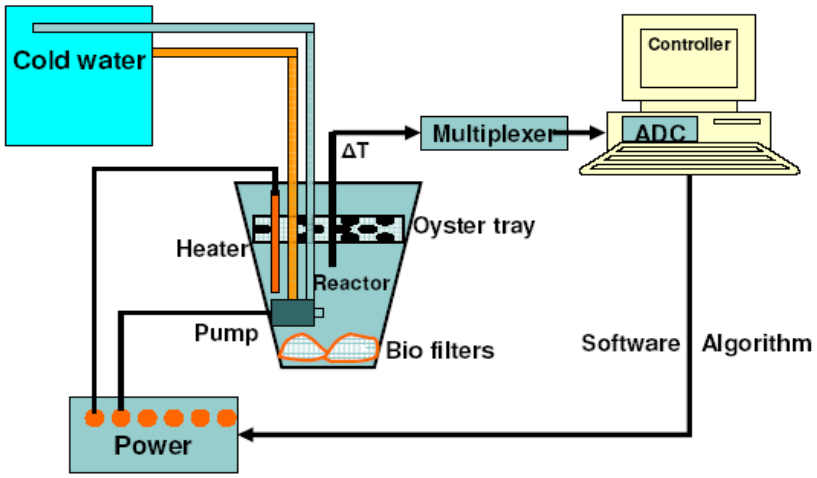


Figure 1b: A Schematic of the system at Aqua Research Station. – Shows closed loop control provided desired temperature regimes.

The system was initially used for holding and conditioning eastern oysters. Although it had functional chilling capacity, its efficiency and effectiveness was limited due to heat transfer to the room from the previous chillers which were located inside the building. Therefore a new chiller system was installed at the EARS to improve the system chilling.

The laboratory scale system (Figure 2a & b) was designed and installed at BAE to perform high accuracy water quality studies water quality studies at a small laboratory scale, simulating lightly to medium loaded nitrogen recirculating aquatic systems. The earlier EARS system at had average chilling rates for the system of tanks that were mostly not low enough to attain desired set temperature below 15^0C for each tank. This was a limitation to undertake any studies that required holding aquatic species such as oysters at a lower temperature. Redesigning and improving the chilling system with a central chiller minimized the limitation(s) and enhanced chilling capacity because of the following:

(1) Replicating experiments in a single process control system consisting of separate tanks, with each carrying its individual heat exchanger and biofilter making a statistically sound system. It was necessary to have a central chilling system (which kept water separated in tanks) that ensured the water accessed by all eighteen heat exchangers was at the same temperature. This minimized the variations in the chilling rates in the individual tanks.

(2) The deviations of the individual tank temperatures from the input set point in the previous system were minimized if not eliminated because of the consistency in the central cooling system. The temperatures were controlled within ranges of $\pm$ 0.1^0C.

Redesigning the EARS system and constructing the small scale BAE system was done by construction of individual units and assembling the units using materials as indicated in the section to follow.

2a 2b

Figure (2a): Design and construction of tanks with encapsulated fiber glass. (2b): A single tank with components.

Materials and Methods:

Design and Construction of System I (EARS)

Eighteen cylindro-conical food-grade tanks each of 250 L capacity (Aquatic Eco-systems, Inc, Apopka, FL) were used as holding tanks for the EARS systems. All tanks were insulated with encapsulated (Figure 3a & b) fiberglass insulation (~10 cm) (Johns Manville, Denver, CO) to prevent heat loss and further covered with a nylon base cloth material to prevent damage to the fiberglass insulation. Water from the tanks did not mix at any point, making them completely independent from each other. Each tank was computer controlled (Figure 4) and had separate heating and cooling mechanisms (Figure 5) and had the following components:

1. The tanks (Figure 3a & b) containing water and the control system components were insulated by fiberglass insulation.

3a 3b

Figure (3a): Tanks insulated with fiber-glass insulation. (3b) Fiber glass covered with cloth lining for protection – The insulation maintains temperature at its current state until changed by the computer

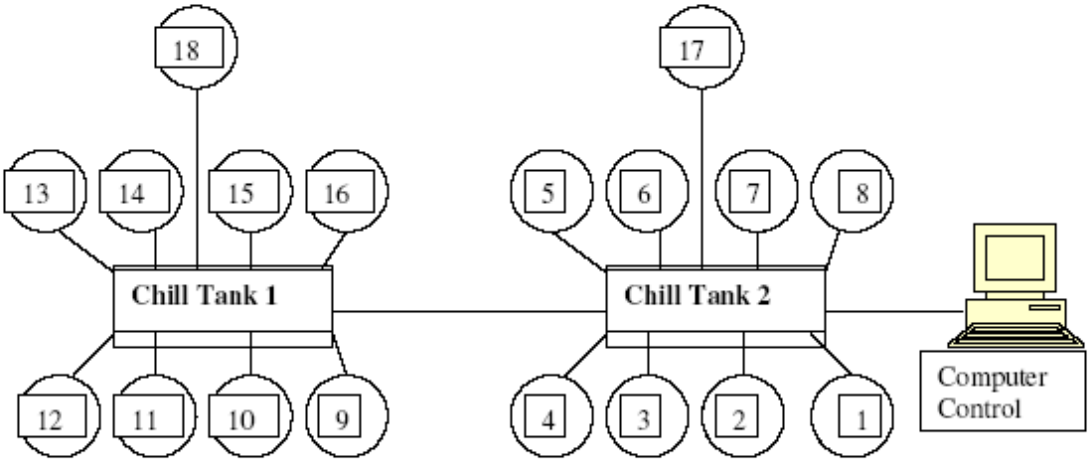


Figure 4: Schematic of tank arrangement at the EARS with computer control.

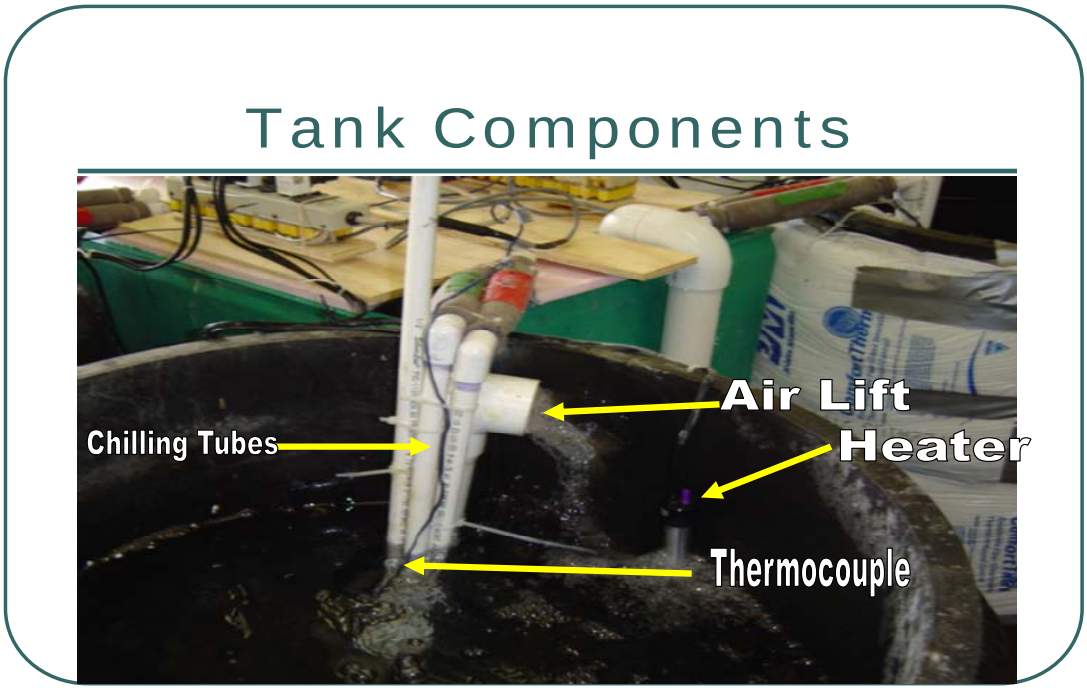
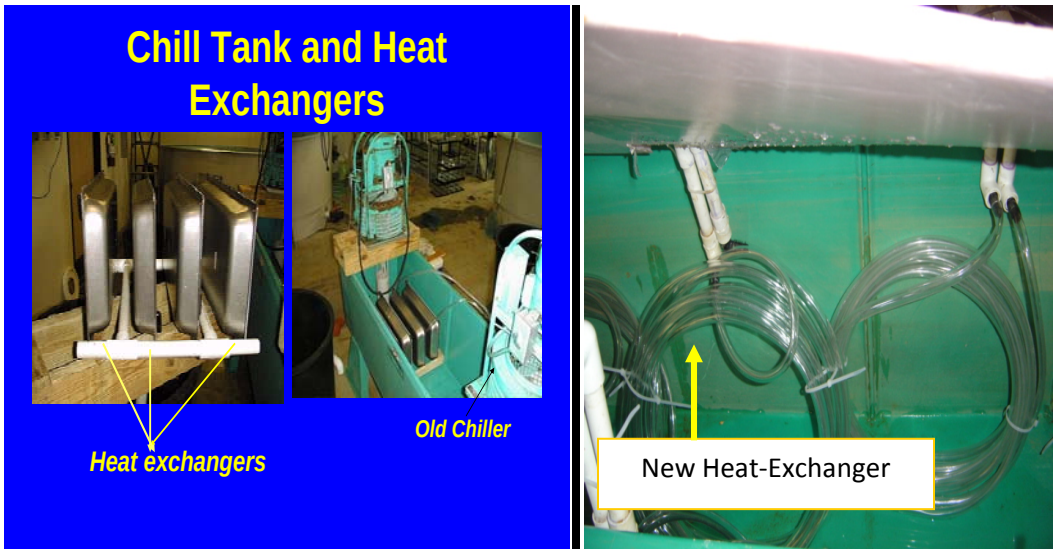


Figure 5: Heating, cooling and temperature sensing components in one tank, maintaining the independent operation of each tank

2. An airlift (Figure 5) consisting of a 2″ (5.08cm) diameter, 31.5″ (80cm) height and gas to liquid ratio of 0.3 PVC tube, with a 90□bend at the top was used for water circulation and aeration. Two 0.8″ (2cm) elbows were glued to the bottom of the airlift pipe to form a U-shaped air injection point. The air was injected via a 0.5″ (1.25cm) polyvinylchloride (PVC) plastic tube connected to the air supply. The airlift performed two functions: aeration of water, and circulation of water in each tank.

3. Heating was provided by 300-W electric glass immersion heaters in each tank (Model # 300, Commodity Axis, San Gabriel, CA).

4. Chilling was provided when needed by circulating water through heat exchangers (Figure 6a & 6b). A submersible water pump (Figure 7) (Model # 305/306, Commodity Axis, San Gabriel, CA; Pump (Via Aqua submersible Pump (VA80) (Dimensions 5 X 4 X 3 cm Rated Max. Flow 1.32 GPM (5 Lpm)), recirculated saline water in the tank through the heat exchanger @ 2 lpm. The heat exchangers were constructed from 8-18 gauge stainless steel (Browne Halco 88002 NSF #1 Korea) trays (21.6″ (54cm) x 13″ (33cm) x 2.4″ (6 cm)) (Figure 6a) and lids sealed with plumbers “Goop” sealant and held in place with stainless steel screws to prevent corrosion. The heat exchangers were later changed to vinyl tubing (5/8″ (1.7cm) OD X 1/2″ (1.27cm) ID X 20′ (6.1m) Length) to avoid the leaks created in the system due to corrosion. Tank temperatures were monitored by T-type thermocouples (Copper-Constantan)



6a 6b

Figure 6a: Heat exchangers and the chillers initially designed and used in system. (6b) The modified heat exchanger (Vinyl) tubing used to replace the stainless steel

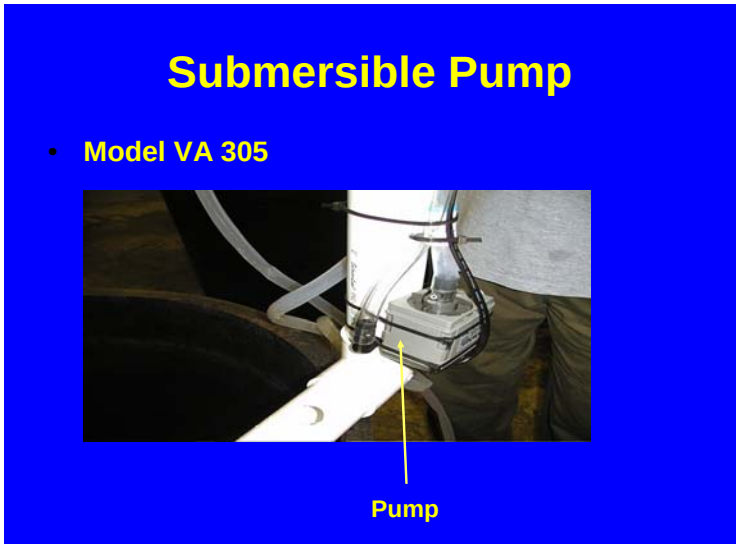
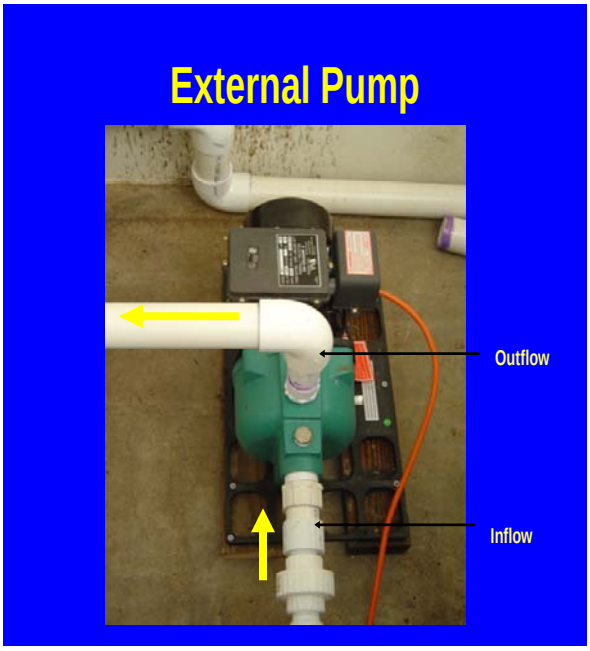


Figure 7: Pump used for water recirculation from tank to heat exchanger and back

The heat exchangers were immersed in fresh water at 4□C in a chill tank of dimensions 2.1 x 0.55 x 0.5 m (Frigid Units, Toledo, OH).

5. Water in each chill tank was initially cooled by a pair of electrically operated chillers (120 V AC and 2000 W approximately). These were later replaced by pumping the water from the tanks and recirculating it through an external chiller (Figure 8a & 8b) using a 1.34 horse power (hp), 1000 W & 120 V pump. Nine tanks were connected to each of the two rectangular chill tanks (0.57 m^3) as shown (Figure 9) in schematics.

6. Two biological filter bags consisting of 25 individual bio- cylinders (4cm in diameter) were acclimated at 28^{0}C in a central tank (Figure 10a & 10b) which were placed at the bottom of each tank for water quality studies. The filter media consisted of plastic bio balls (5 cm diameter) with a void space of approximately 94% and geometric surface area 31ft2/ft3 (Keeton Industries, Inc., Wellington, CO). The filter media was placed in perforated plastic sacks.



8a 8b

Figure 8a: The new external chiller installed to improve chilling capacity at the ARS facility.
8b: Pump used for circulating the chilled water.

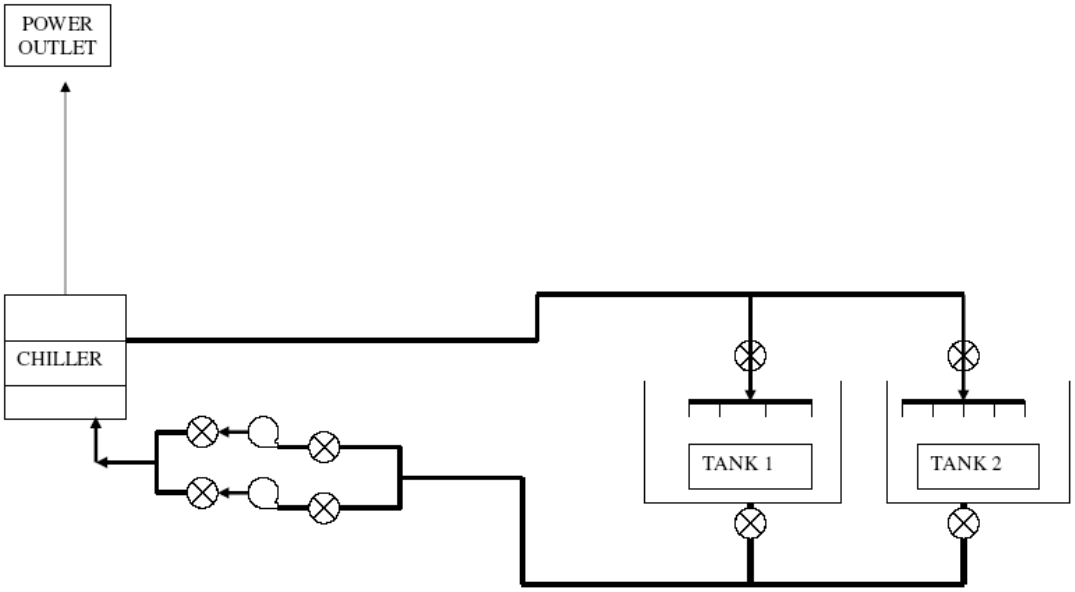
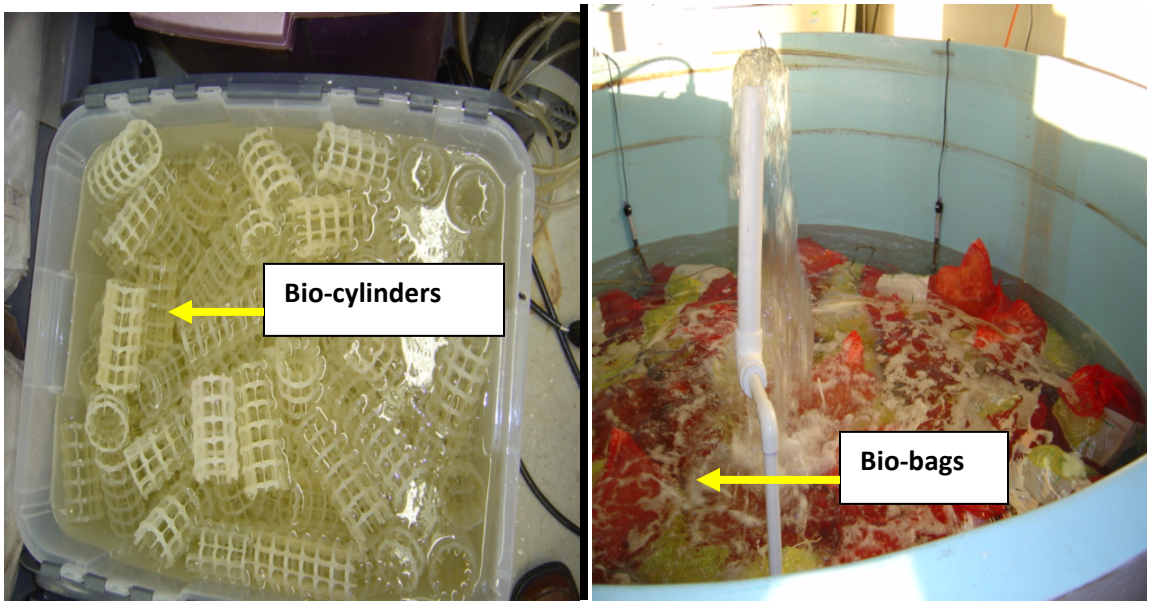


Figure 9: Schematics of improved chilling system with water circulation through an external chiller to reservoirs tanks 1& 2



10a 10b

Figure 10a: The bio-cylinders used for bacterial growth in the biofilters. (10b) Central acclimation tank used for bacterial growth in bio-bags and acclimation at 28^0C

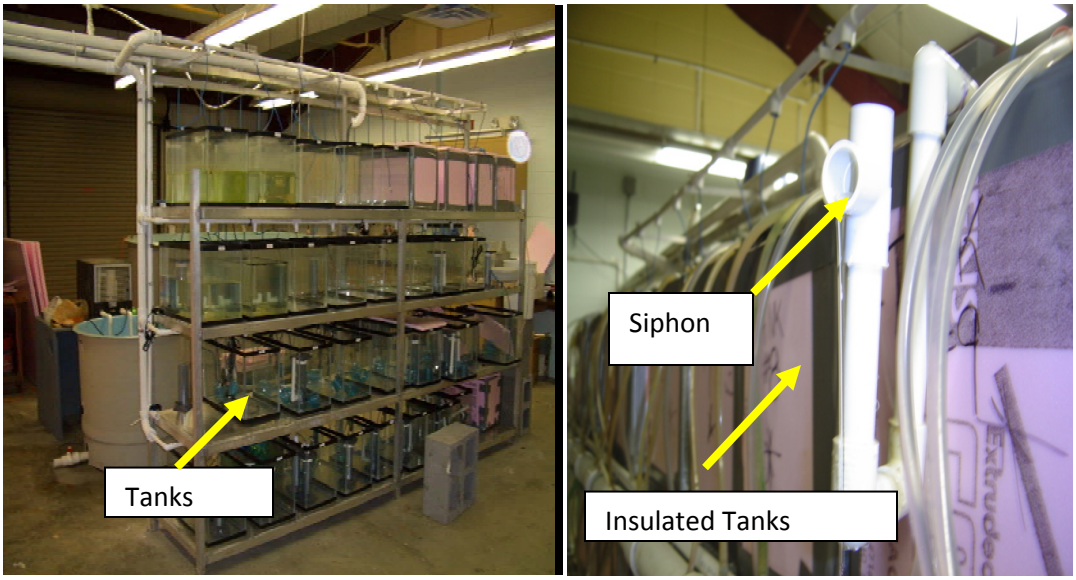
The system of tanks including the biofilters were tested after completion of the redesign and modification phase for efficiency in chilling and operating results are show below. The improved chilling system indicated a marked improvement in chilling capacity from attaining a consistent lower limit chilling temperature of 8^0C in the tanks. This was lower than previous systems lower temperatures limit which was >8^0C with inconsistency in individual tank temperatures. The system was also used for pilot studies of ammonia conversion rates. The pilot studies contributed to sizing and design of the BAE system of tanks as described in the following section.

Design and Construction of System II (BAE)

The BAE system was designed after the EARS system on a laboratory (40 L) scale. The central cooling system design was adopted from the EARS system and replicated for the small scale system in BAE. This system was used for determining total ammonia nitrogen removal rates at different temperature regimes in the objectives above.

The BAE system consisted of the following the design and construction:

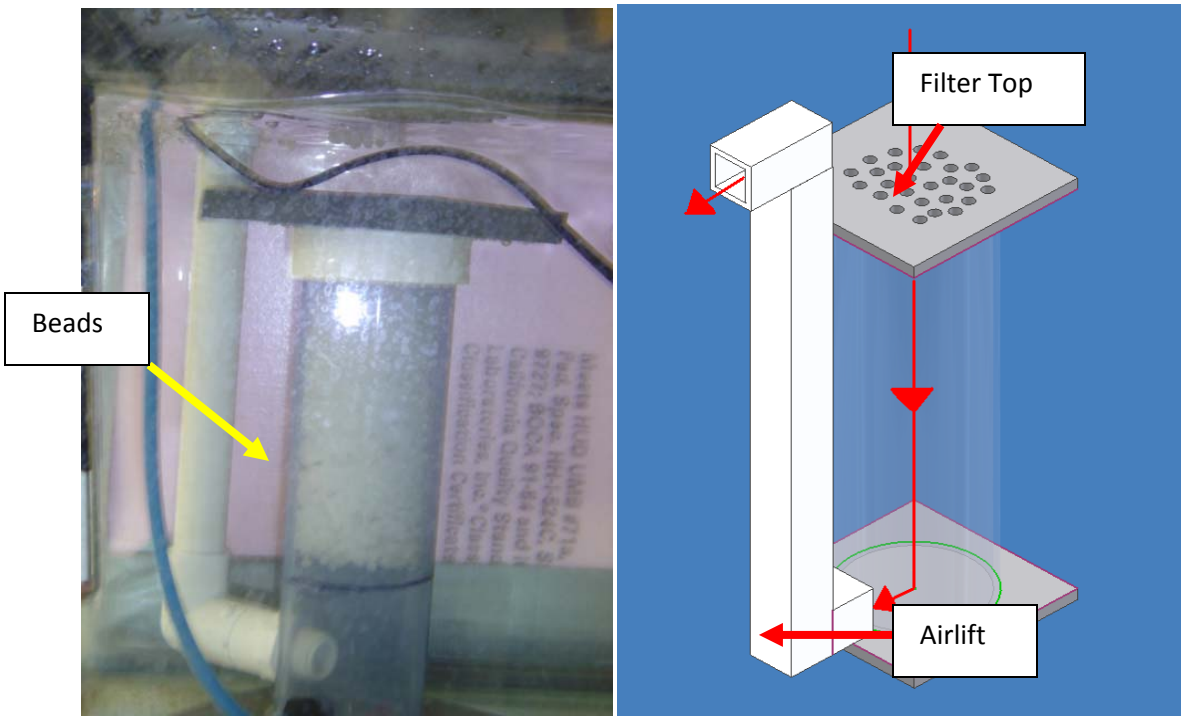
1. Thirty-six rectangular glass tanks (Figure 11a & 11b) each capable of holding approximately 40 liters were setup for holding water. All tanks were insulated (with Foam insulating Sheathing (Owens Cornings) to prevent heat loss. The tanks were completely independent from each other. Each tank had a separate heating and cooling system.



11a 11b

Figure 11a: Rectangular glass tanks setup in the BAE facility. (11b) insulated tanks with plumbed siphon in place for a continuous water flow control.

2. An acrylic tube (7.5″ (19.1cm) high, 2″ (5.1cm) diameter & 4″(10.2 cm) X 4″(10.2 cm) base plates) was designed to be used as bio-filter for water biofiltration with an attached 1/2″(1.27cm) diameter X 10″(25.4 cm) high PVC airlift (Figure 12a & b) for water circulation in the tank. The bio-filter contained enhanced nitrification (EN) beads (5mm X 3mm dimensions). An air compressor (central pneumatic (S-34843) was used for both aeration and airlift purposes.



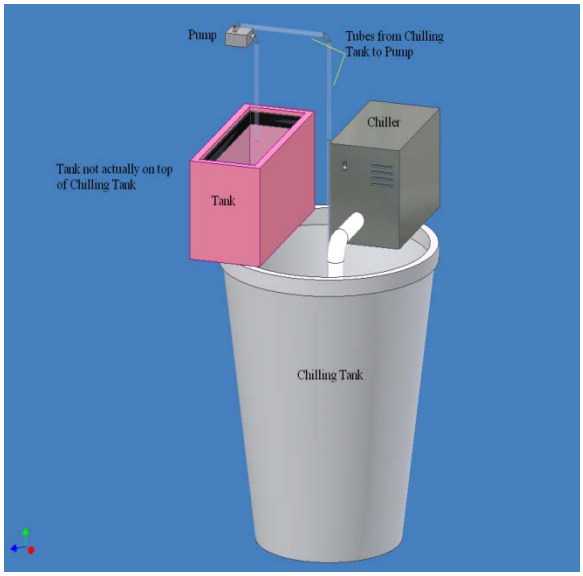
12a 12b

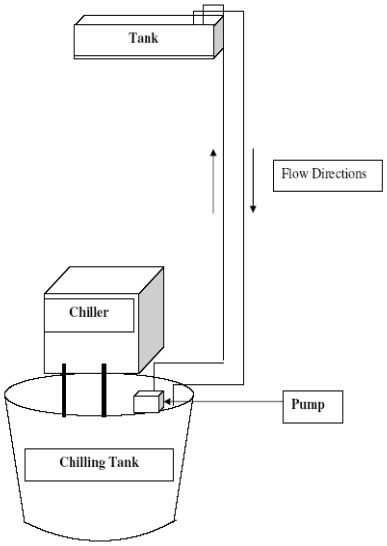
Figure 12a: Airlift and Biofilter as installed in the tank with the bead bed floating at the top of filter. (12b) Schematic with flow direction of water as it passes through the bio-filter and the air lift under normal operation.

3. The materials used for construction of the biofilters included (1/2″ (1.27cm)) polyvinyl chloride tubes (PVC), (2″ (5.08cm)) acrylic tubes, acrylic sheets, (1/2″(1.27cm) 90 degrees PVC elbows, small screen at the top of filter and PVC glue. The tools used included press drill, band saw, drill dice and portable hand drills. The acrylic sheets were cut into squares of size 4” X 4” (10cm X 10 cm) for supporting the base of the filters. The acrylic tubes were then cut into lengths 7.5″ (18.8 cm) and glued to the square bases. Holes 1/2″ (1.27cm) wide, were drilled at the bottom of the acrylic tubes that were glued to the base for an airlift attachment elbow. The airlift PVC tubes were cut to lengths 10″ (25.4 cm) long. Holes were then drilled into the second set of elbows which were mounted on top of the airlifts for air injection tubes to go through to the bottom of the airlift tubes. The airlifts were made to be tight fit but not glued, which

enhances cleaning the tubes to minimize insitu-nitrification from bacteria embedded in those tubes. The top of the filters were covered with circular pieces of mesh screen for down flow of water during circulation and holding of beads in the acrylic tube from float out to the top. The other end of the filter bottom opposite of airlift attachment and close to the base, a hole 1/4″ (0.64 cm) was drilled for attachment of a second air injection hose used for back flushing or vigorously washing the beads. This vigorous washing if needed could have enhanced the nitrification process by knocking off the heterotrophic bacterial which generally outgrow the nitrifying bacteria in the presence of organic material. However due to non presence of biochemical oxygen demand (BOD) loadings in the system the back washing process was not initiated during the entire sampling process. Details of the biofilter design and testing is included in the appendix.

4. Chilling was provided by central chiller(s) (Figure 13a & b) with built-in pump and an external reservoir holding the chilled water which was circulated by a pump (Via Aqua submersible Pump (VA80) (5 X 4 X 3 cm rated max flow 0.53 GPM (2 Lpm)) through the outer jacket of the heat exchangers. Submerged pumps within the individual tanks passed the chilled water through several coils of PVC casing heat exchanger (Figure 14a & b) using vinyl tubing (5/16″ (0.79cm) OD X 3/16″ (0.48cm) ID X 8.5′ (2.59m) Length, Aquatic ecosystems) which circulated water from the tank through the tube. Chilled water flowed over the tube taking heat out of the tank. A digital thermocouple was used to confirm controlled temperatures in tanks. A central Chiller [Figure 14b, (Neslab: Model CFT-25) Neslab instruments Inc, Newington, NH, USA.] was used for the chilling process.





13a 13b

Figure 13a: Layout of chilling system (13b) Schematics of the central chilling system showing a single tank plumbing- This was replicated for all thirty-six tanks.

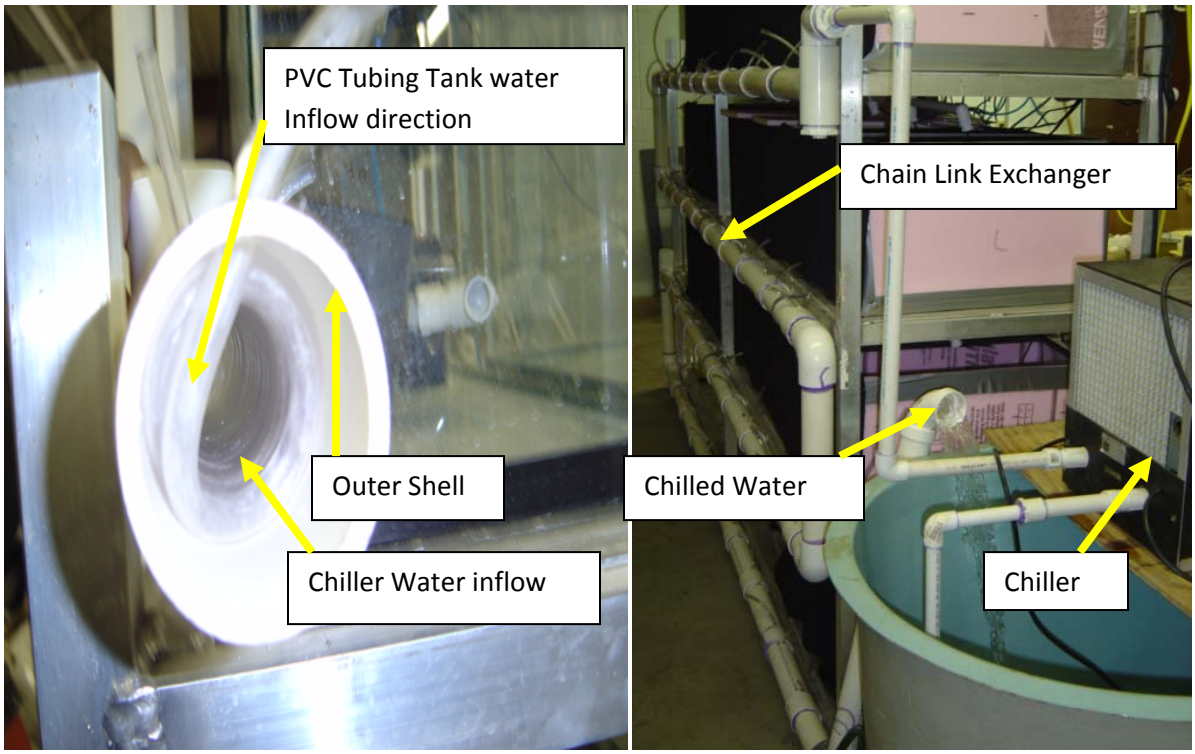


Figure 14a: Heat exchanger coiled vinyl tubing used for an individual tank- This was a section of a series of heat exchangers for the system (14b) A Chain-link system of individual segments glued together forming the heat exchanger for the entire system- Chilled water flowed through from reservoir to the chiller.

Thirty– six units of 2″ X 12″ (5.08cm X 30.48cm) PVC tubes were cut with a band saw. The sections were marked in two places at equidistance from the edges. Holes 1/2″ (1.27cm) diameter were drilled using a press. A round file was used as needed to smooth the edges of the holes. An 8.5' (2.6m) length of clear vinyl tubing was threaded through the hole and coiled in the PVC inner wall. Having the tubing exit at both ends of the pipe section made it easier to attach the tubing to the pump and to place the other end of tubing into the tank that needs to be chilled. The exchanger tubing was sized based on the tank capacity and all installation constraints facing the heat exchangers locations. All thirty-six tanks were stacked after construction in four groups of nine per row from top to bottom with independent units of operation using separate heat exchangers per tank. Although the heat exchangers were independent the individual units were glued together to form one piece (Figure 15) of PVC for proper flow of water. The flow direction of the water from the tanks was counter flow (Figure 16) of water from the chilling tank running through the entire length of heat exchanger (Figure 17) column. This made it easier to vary parameters and control environment in each tank. The electrical hardware, software coding & logic and the operation of control system is described in detail in the section to follow.

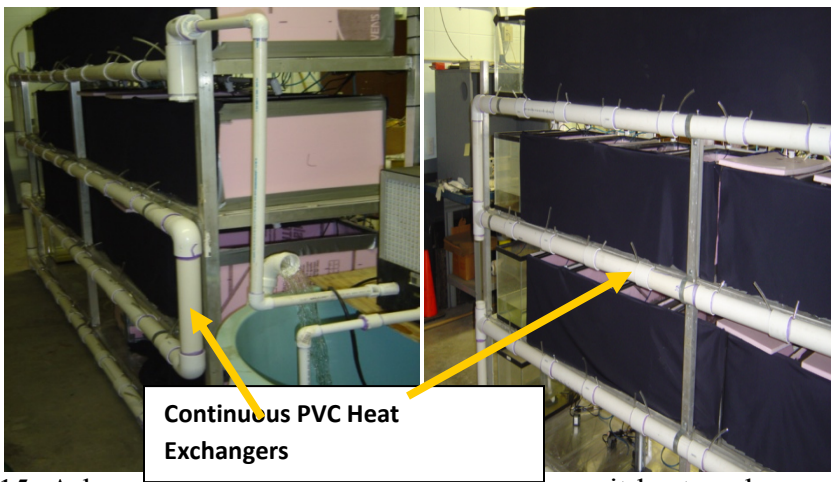


Figure 15: A long heat exchanger made by joining unit heat exchangers to form the continuous loop.

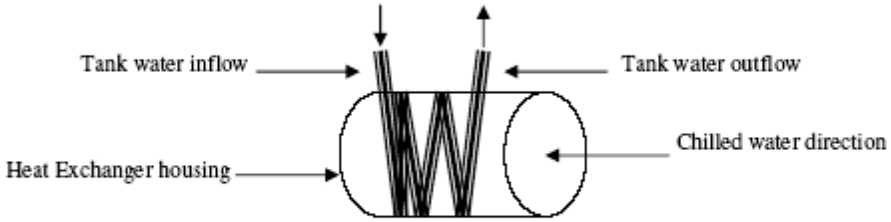


Figure 16: Coiled vinyl tube in the heat exchanger housing design showing flow directions

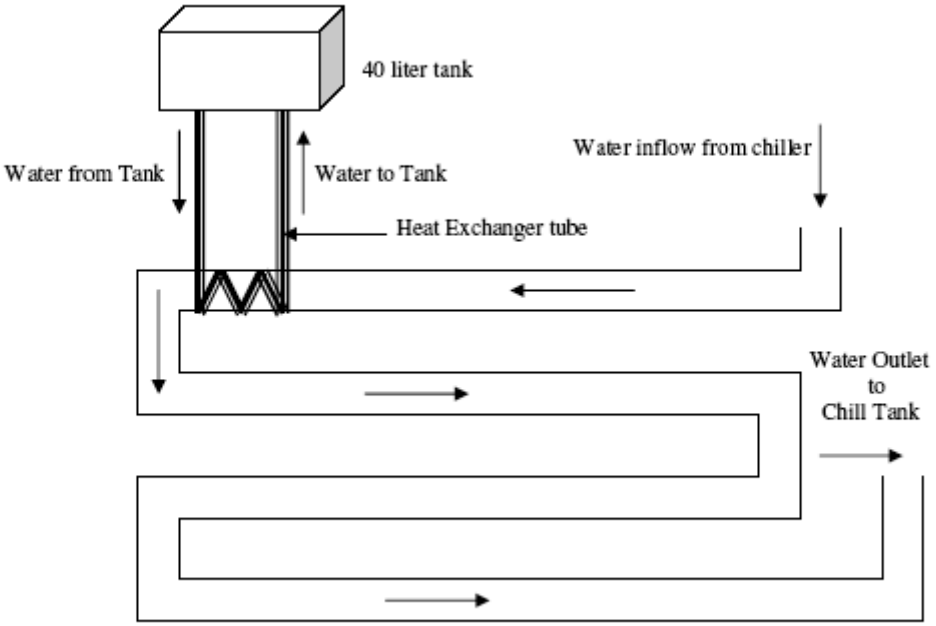


Figure 17: Schematics of complete length of PVC tube forming the heat exchanger housing.

Computer and Electrical Hardware Design Customization

The hardware for controlling the system was similar to that of the ARS system with slight modification to the wire codes and relay layouts which included a 32-channel CIO EXP-32 multiplexer board (Figure 18), one 16-channel PCIM-DAS 1602/16 (Figure 19) analog-to-digital converter board, one CIO-DAS24 analog-to-digital converter (ADC) (Measurement Computing Inc., Middleboro, MA.), T-type thermocouples (Figure 20a & b) (Omega Engineering Inc., Stamford, CT) and eight sets of 15A, 4-28 VDC solid-state relays (P/N 611489, Eastman Kodak Co). The hardware and software of the system were customized to process analog and digital data. The junction ends of the thermocouples were soldered with lead-free electric solder (Radio Shack Inc.) and were made water resistant using plumber's "Goop" sealant. The measuring ends were connected to each of the first 16 analog input channels of a CIO-EXP 32 multiplexer board. The temperatures were read as differential voltages and the multiplexer amplified the voltage signals by a factor of 100. A personal computer (PC-Pentium III), with embedded ADC processed the signals. The multiplexer was connected to the ADC via an EXP2DAS16-10 special 37-conductor cable (Measurement Computing Inc., Middleboro, MA).

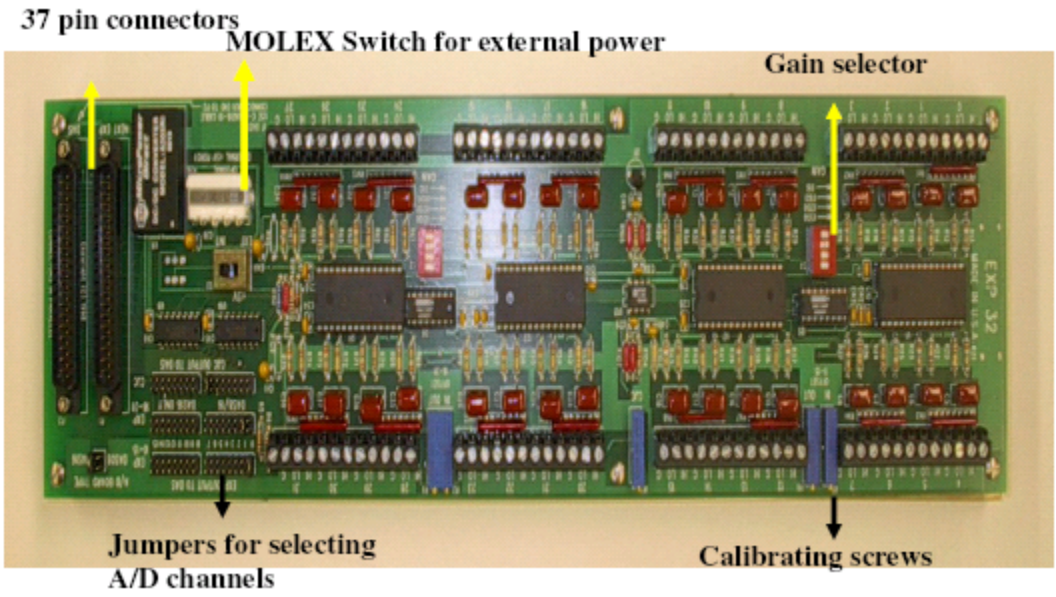


Figure 18: CIO-EXP 32 Multiplexer board was used to measure temperature signals from tanks via thermocouples- Important components are shown using arrows

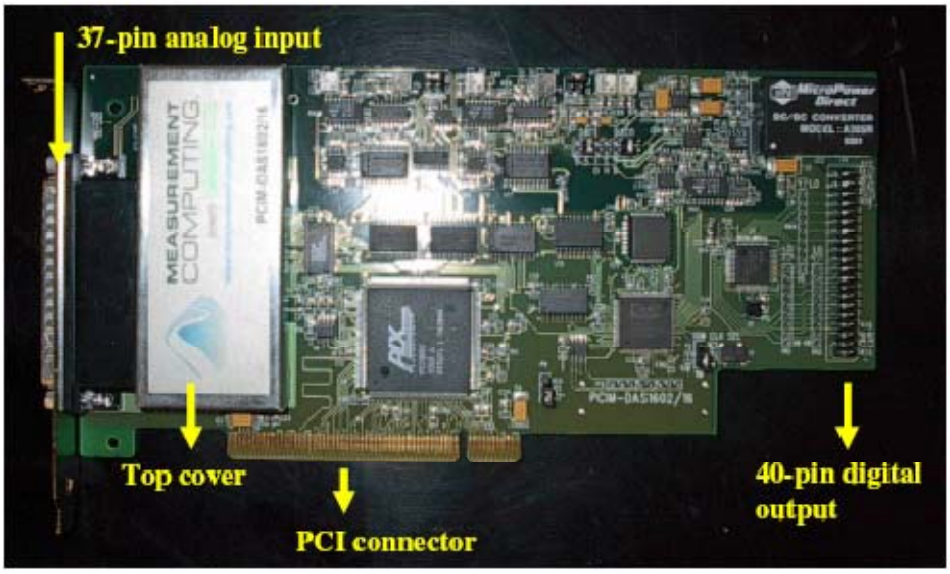
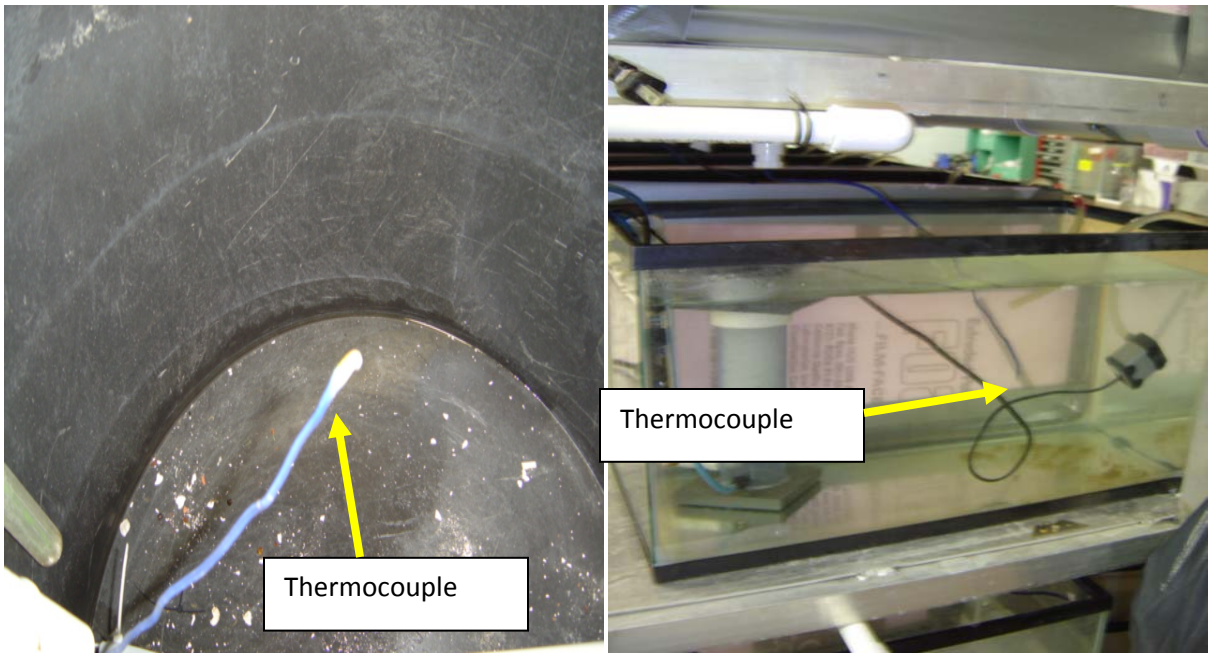


Figure19: Analog-to –Digital Input output board used for signal processing



20a 20b

Figure 20a: Thermocouple in a tank in Engineering Lab Aqua Research Station.
20b: Thermocouple in tank Engineering Lab BAE.

Based on the control software, 5 VDC signals were transmitted by the ADC through a BP 40-37 (Measurement Computing Inc.) ribbon cable that brought 40-pin on-board connections out to 37-pin male connector on computer back plate. Two C37 FF-2 (Measurement Computing Inc.) ribbon cables were used for transmitting output signals. One end of C37 FF-2 cable was connected to the male pin of BP 40-37, and other ends were soldered to an 8-pin standard male DIN connector (Marlin P. Jones & Associates, Inc.,). Seven-conductor cables were used to connect the C37 FF-2 cables. Both the ends of the 7-conductor cables were soldered with 8-pin standard female DIN connectors (Marlin P. Jones & Associates, Inc.). The seven -conductor cable was connected to the relays at one end and connected to the C37 FF-2 cables at the other end. The output signals (5VDC) closed the 110V AC circuitry (Solid state relay) and actuated pumps and heaters in the tanks as customized in the software.

Software, Coding and Logic

The software for the control system was the same as in the previous system, developed in micro-soft Visual studio using C++ (Version 6.0, Microsoft Corp, WA) executed on a Windows XP-professional based platform. The feedback control loop program (Figure 21a) was executed every 5 min. The program measured the temperature every 3 seconds and average calculated over 5 minutes for each tank was computed and displayed on the monitor (Figure 21b). The computed average was compared with the desired temperature. Each temperature cycle executed, determined what unit needed to be turned on or off (Heater or Pump). The software was custom designed and allowed input of variable chilling and heating rates as shown in the selected logic command loop below.

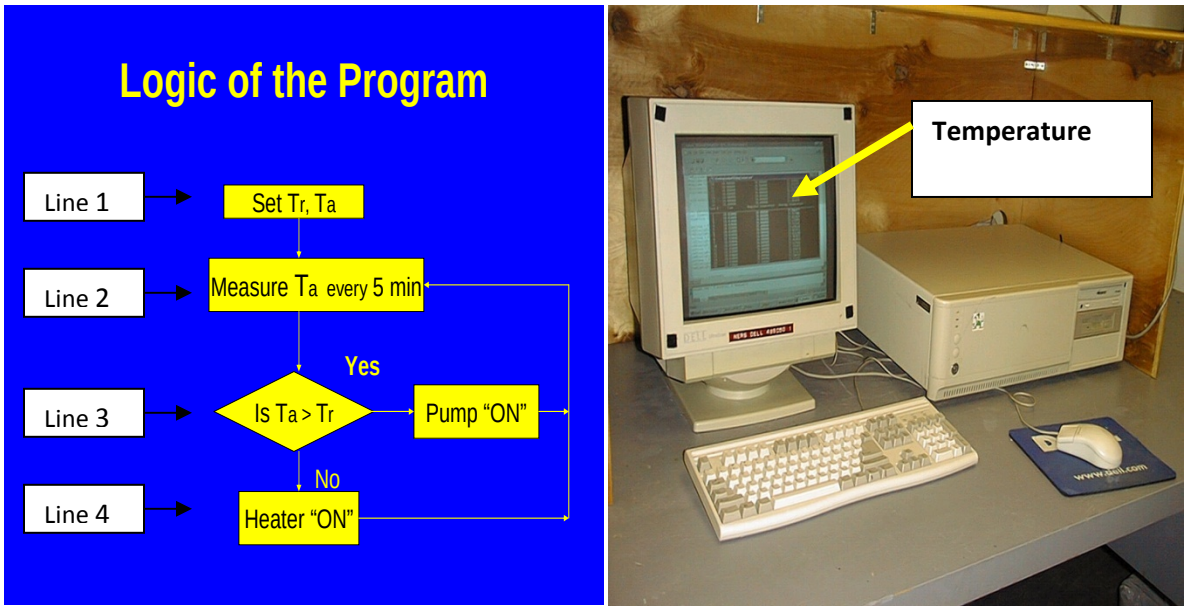


Figure 21a: Feedback control logic. (21b): Temperature display on computer monitor

Line 1: **Printf (“Enter the maximum and minimum temperature for tank %d: “,j);**

Scanf (“%f %f “, &lowtem[j], & hightemp [j];

Printf (“\n”);

The logic software code above asked the system manager to input desired temperature minimum and maximum for a steady state temperature control. In the case of transient cyclical regimes the temperature deviation will be required

Line 2: This line was written in the code to average the temperature data sets every 5 minutes and compare with the reference input temperature in the code [“Temperature [j][x]=tempvalue*slope[j] + intercept[j]; X++; for (i=0;i<300;i++)”].

Line 3: If the current temperature was greater than set temperature computer will send a yes (on) signal to the chilling pumps to start cooling tanks example: [else if (avg[j]>hightemp[j] {high=1; count1=count+1;} else {low = 0; high=0;},case 0: if (low= =1 cmdoutA[0]=1;.

Line 4: If no computer will send yes (on) signal to heaters to continue heating tanks. Detailed software code is in the appendix of this document example [“if (avg[j]<lowtemp[j] {high=1; count1=count1+1;}], else if (high= =1) cmdoutA[1] =1.

The systems were tested for functionality after the code was input to the computer hard drive as discussed below.

Process Control System Testing

Test runs were performed to assess chilling rates, temperature control and chilling capacity in each system. An experiment was conducted to test the EARS system for temperature control and pilot nitrogen removal rates. Preliminary studies were also performed at the BAE system. The bio-filters used in the tanks at the Aquaculture Research Station were also

inoculated with bacteria from filters in previous tanks at a salinity of 20ppt to develop an active bio-film before using for bio-filtration in the tanks. These were then loaded at 5 mg/l of ammonia as substrate for bacteria. Water quality tests were performed to determine volumetric total ammonia removal rates of filters at short term temperature regimes applied to the tanks.

Experimental Designs and Procedures

The experiments and procedures were designed based on the functional efficiency of the system. Results could improve management practices, as well as increase productivity in recirculating aquaculture systems. These fundamental principles of removing ammonia through a three step process (Zhang and Bishop, 1994) were used in performing the biofiltration tests to determine the rate at which ammonia was converted when the bacteria mass was fully established. Experiments were later performed using the customized biofilters to determine the objectives mentioned above.

A batch bioreactor system of tanks with intake airlift pumps attached for continuous water flow and aeration of the reactors (Figure 22) was used in each tank.

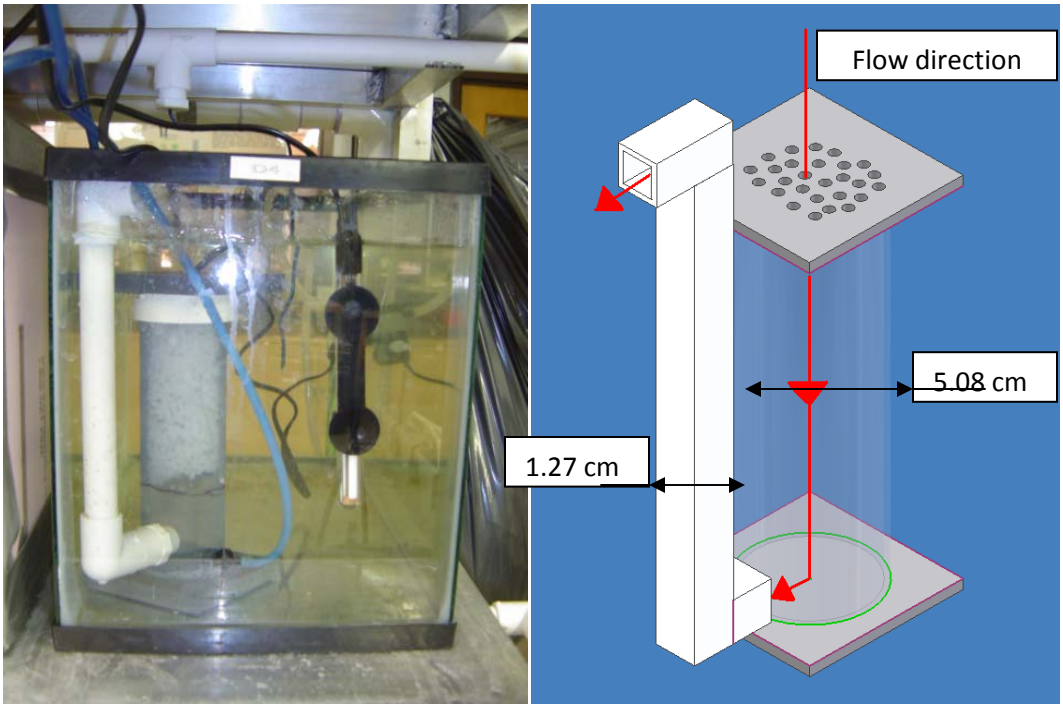


Figure 22: Biofilter at one end of tank with airlift for water circulation

The tanks were loaded with synthetic substrate from the general mixing tank at 5mg/l. The biofilter units were sanitized after each experiment to prevent in-situ nitrification which is problematic (Malone and Beecher, 2000) for precisely determining the ammonia removal rates in bioreactor systems. All components of the systems were tested before performing the actual experiments. The results of the system design testing are indicated below.

System Design Test Results

Three tank replicates were set-up for each temperature regime above. The temperatures were monitored and controlled by a stand-alone process control computer. The data sets from the computer were recorded at regular iterations of 5-minutes and the points were later collated to form one continuous temperature regime data. The data sets were used to show trends of heat

transfer and efficiency of the chilling system under controlled temperature pattern and the total ammonia removal rates at the experimental test temperature regime.

Results (EARS)

The testing process for the system included comparing stainless steel versus (Figure 23) at the EARS before further testing of the vinyl tubing at different temperatures were performed. Chilling was set for the lowest desired values in the system 5^0C. The stainless steel attained its lowest temperature at 6.49 ^{0}C and the vinyl attained its lowest temperature at 8.08 ^{0}C (Figure 26). The stainless steel chilled at a rate of 1.98 ^{0}C/ hour at 25^0C, while the vinyl tubing chilled at the rate of 1.51 ^{0}C/ hour at 25^0C.

Figure 23: Stainless steel heat exchangers initially used in the ARS system- These were replaced with vinyl tubing because of corrosion.

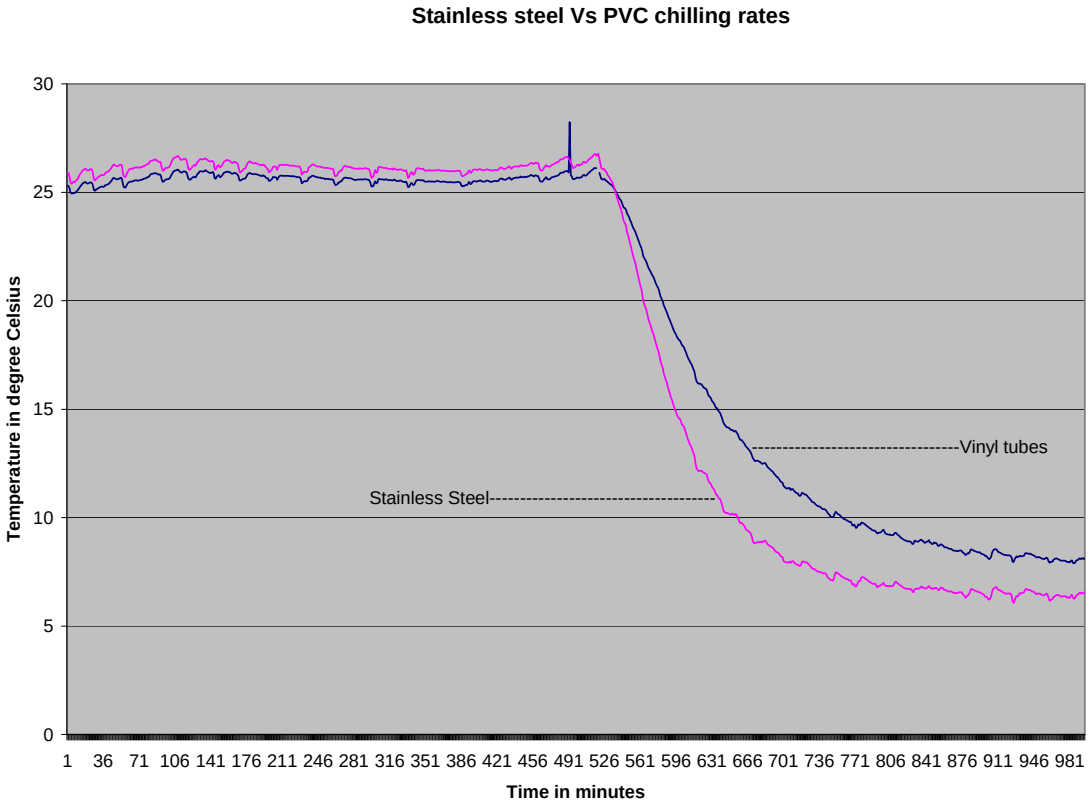


Figure 24: Comparing chilling capabilities of stainless steel and vinyl tubing during the heat exchanger design

Results (BAE)

The BAE system was also tested for chilling capability (Figure 25) and temperature control for steady state (Figure 26) and diurnal transient (Figure 27) regimes, using the vinyl tubing heat exchangers. The lowest temperature achieved by the system was 11.87^0C although the desired set temperature was 10^0C as shown below (Figure 25). The chiller temperature was at 9^0C at its highest performance. It may have been that the limitation of the chiller affected the chilling rate and lower limit temperatures attained in the chilling tanks, due to the fact that 10^0C was not attained in the tanks. The chilling rate for the tank was approximately 2.3^0C per hour for a 40 liter tank.

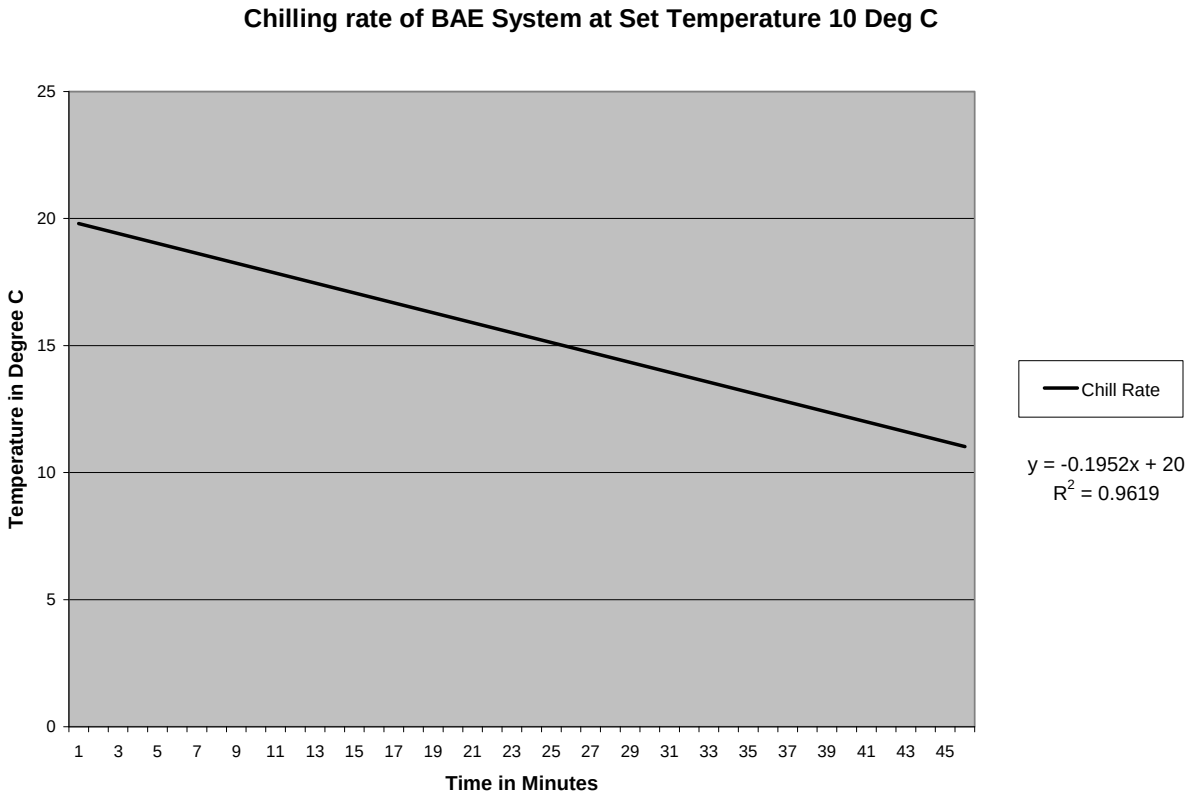


Figure 25: A snapshot of the chilling area from 20^0 C to the lowest holding temperature system attained, with temperature readings at 5minute intervals.

The temperature control output for the systems had the following mean and standard deviations:

1. **13.06** ± 0.08^0C; **20.02** ± 0.09^0C; **30.02** ± 0.01^0C at steady state regime (Figure 26).
2. **12.37** ± 0.8^0C, **20.09** ± 3.1^0C and **30.15** ± 3.1^0C at transient cyclical regime (Figure 27)

The temperatures were accurate to ≈ 0.1^0C which indicates the system had the capability to control tanks effectively. The vertical axis of the graph above (Figure 25) indicates temperature while the horizontal axis indicates the recorded time at intervals of 5 minutes from the computer program. The slope from the graph above indicates that the chilling rate was 0.1952^0C/5minutes data recording interval, this gave 0.039^0C/minute (2.3^0C/hour), with a correlation coefficient (R^2 = of 0.9619). The temperature regimes (Figure 26 & 27) shown below are the temperature profile outputs during the system testing and validation.

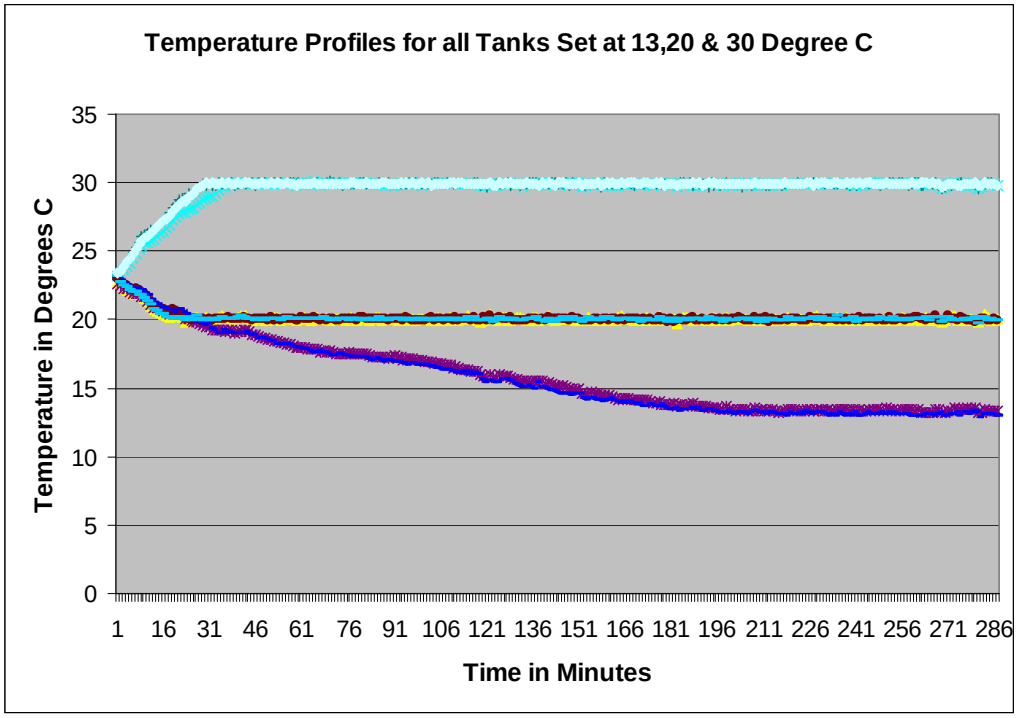


Figure 26: System test temperature control profile for set steady state temperatures 13, 20 and 30^{0}C.

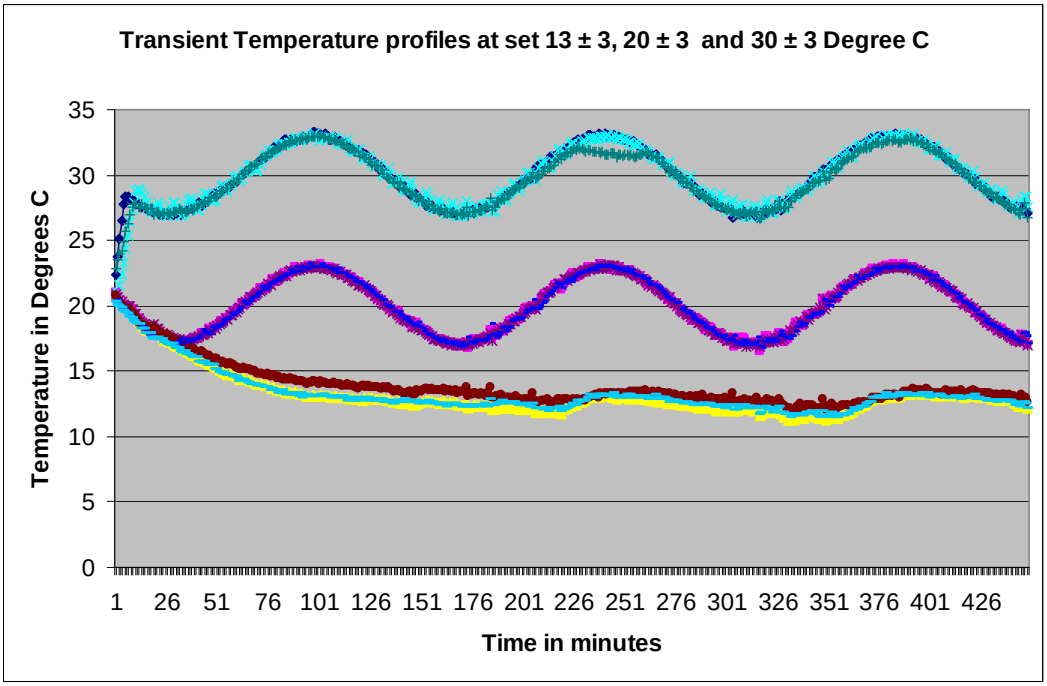


Figure 27: System test temperature control profile for set transient cyclical temperatures 13±3, 20±3 and 30±3^{0}C.

The temperature profiles at steady state (Figure 26) indicate the system of tanks were held constant after the initial chilling and heating range required to attain the different temperatures. However in the diurnal temperature profiles at the 30 ± 3^0C range had one tank with a dip in the profile this can be attributed to a loose electrical connector that prevented the heater from attaining maximum temperature at that point. This was ultimately fixed and resolved. The profile for the 10 ± 3^0C was not achieved although the profile had variation, this was due to limitations of the chiller.

Biofilter Ammonia Removal Test

The biofilters were tested for ammonia removal to determine the functional capability of the filters before starting experiments. Samples were analyzed using a spectrophotometer (Thermospectronic "Genesys-20") to determine change in concentration when bacteria consume the ammonia substrate. A calibration curve was plotted, against which samples were tested. The difference between the initial loading concentration and the determined concentration from the analyses was the decay (Figure 28) concentration that was converted to the nitrite stage of the process. This is the concentration that had been removed by the bacteria in the process over that sampling period. The tanks were monitored in the pre-experimental stage to determine a constant removal rate at which indicated system was ready for running experiments. However some challenges were faced in the pilot phase of the EARS system test that was solved and modifications were made in the BAE system for such limitations as indicated in the next section.

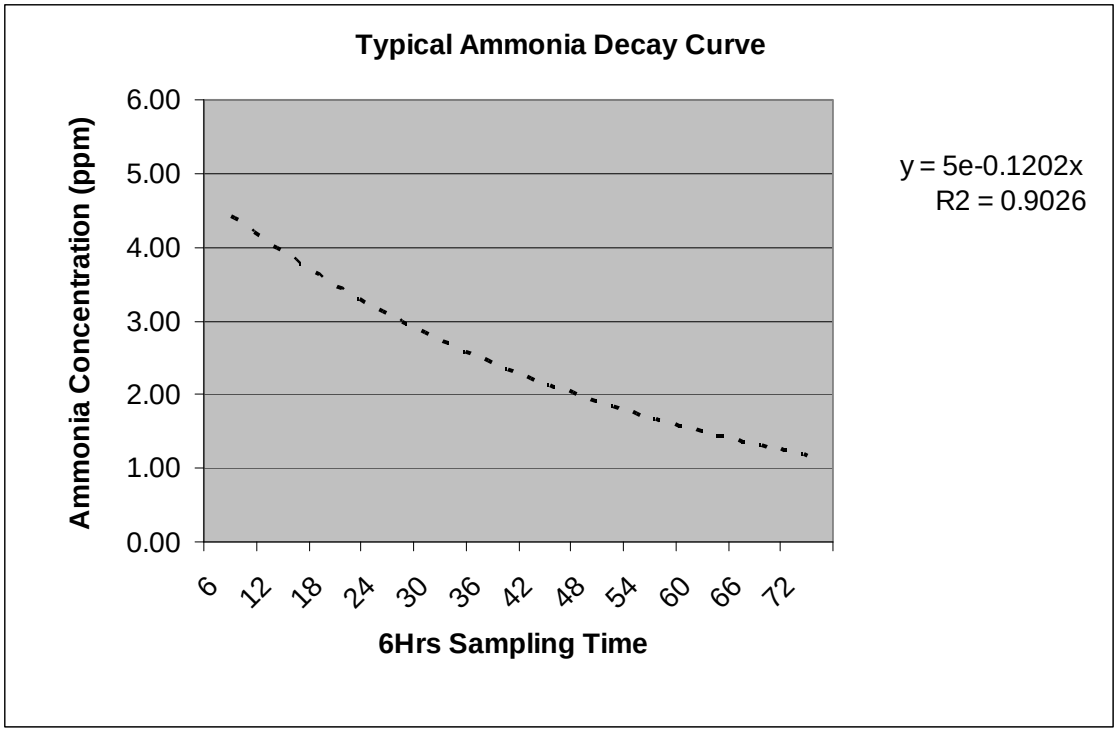


Figure 28: Concentration decay in a typical bacteria ammonia utilization stage

System Test Challenges

The initial test phase was faced with various challenges. Some of these were improved in ongoing pilot stage enabling us continue pilot experiments. A few other components were put off to repair after the initial tests. The following is a brief summary of incidents that occurred during the tests of the EARS system. The BAE system was corrected for these defects during the design process. In continuous test from 03-23-06 to 04-28-06 the following problems were encountered and fixed along side, until the system was considered to be in an efficient running state.

Encounter 1: Tank # 7 was at 22.8^0C instead of desired preset temperature. Wires were crossed and relay pack shut down. Problem was resolved at 9:45am same day. Tank # 17 was at 7.7^0C instead of desired temperature. The same reason as above relay wires were crossed

between the two tanks which happen to be next to each other. A routine check was later done and all tanks were found to be in good order with temperatures maintained as desired.

Encounter 2: The power line circuit breaker tripped, causing computer to shut down. Average temperature in the lab was 17^0C which was not the desired temperature, leaving no cause for alarm for temperature variations. Temporary data file was automatically: Recorded for 3/24/06 (result1 folder)

Encounter 3: On inspection the temperature of tank # 18 was at 23^0C which was not the required temperature not faulty relay was replaced and corrected. All temperatures in all tanks were later inspected as alright although relays in tanks 15, 16, & 18 were under observation. Probable cause could have been irregular signals to relays due to errors in running the program. The program was found not to be compatible with the platform of the old system computer. The system platform was upgraded and software and system functioned as desired.

Encounter 4: The software froze leaving the temperatures varying from set point in some tanks.

Tank# 9: went up to 29^0C

Tank#10: went up to 29^0C

Tank#12: went up to 25^0C

Tank#15: went up to 27^0C

All other tanks were fine. This happened between 2pm and 6pm same afternoon. The system was reset at 7pm. The temperatures at which the computer froze were not the desired temperature. The system performance check showed that the random memory was low due to data storage at

the desk top. Steps were taken to relocate and save data on the hard drive with external backup memory devices.

Encounter 5: Program was shut off leaving pumps in chilling mode. The problem was corrected after detecting the UPS was not on the generator plug to sustain the computer in the event of a power failure. This face of the test had several challenges that were addressed at all levels, especially the hardware, software, heat exchangers and human resources to improve management strategies.

Summary

Two systems were designed, constructed and tested. The first system was designed and constructed at the ARS engineering laboratory for oyster biology studies by a previous master student. It was later modified for improved chilling capability and used as a pilot water quality test system. The process enhanced the design aspect for a second system. The second system was designed and constructed after the ARS system with improvement in design for deficiencies in the heat exchangers in the previous system. The design of the two sets of heat exchangers was challenging, the chilling rates were efficient enough and appropriate for the experimental runs. The lowest average temperature achieved for the BAE system was 11.87^0C. The average chilling rate for the tanks was 2.287^0C per hour for a 40 liter tank.

Chilling water temperature was recorded at 5^0C. The stainless steel attained its lowest temperature at 6.49^0C (over a twelve hour period) and the vinyl attained its lowest temperature at 8.08^0C (12-hour duration) for the ARS system. The stainless steel chilled at a rate of 1.98^0C per hour for a 250 liter tank @ $\approx 20^0$C while the vinyl tubing replacing the stainless steel in the same tanks chilled at the rate of 1.51^0C per hour for a 250 liter tank. The desired temperature set points

were achieved for both EARS and BAE system. The goal designing and constructing a new system was to have a functional and efficient system to enhance the study of short term transient temperature effects on water, for water temperatures ranging from cold water (below 15^0C) and warm water (above 15^0C). The lower limit achieved was still within the justified limits of cold water experimental designs. The automated control of temperature for the range of the chiller, were precise ($\leq 0.5\ ^0$C). In conclusion the system was effective for temperature control. The sizing cost for the vinyl was also more cost effective for the versus the stainless steel heat exchangers per square feet of material. The vinyl heat exchangers lasted longer due to the elimination of rust which affected the stainless steel system in a salt water system during the Oyster biology studies. The assembly was also easier than the stainless steel heat exchangers. The ammonia decay curves were consistent with decay curves in the literature, allowing us to set up the actual experiments.

Future design of the system will focus on making the loops for the vinyl tube permanent by using some form of heat application to shape the tubes. It is intended that future studies will include other parameter (pH, salinity, Nitrite, Nitrate etc) effects in the nitrification process with respect to temperature. Applications of these systems could be extended to wastewater treatment systems and other aquatic species.

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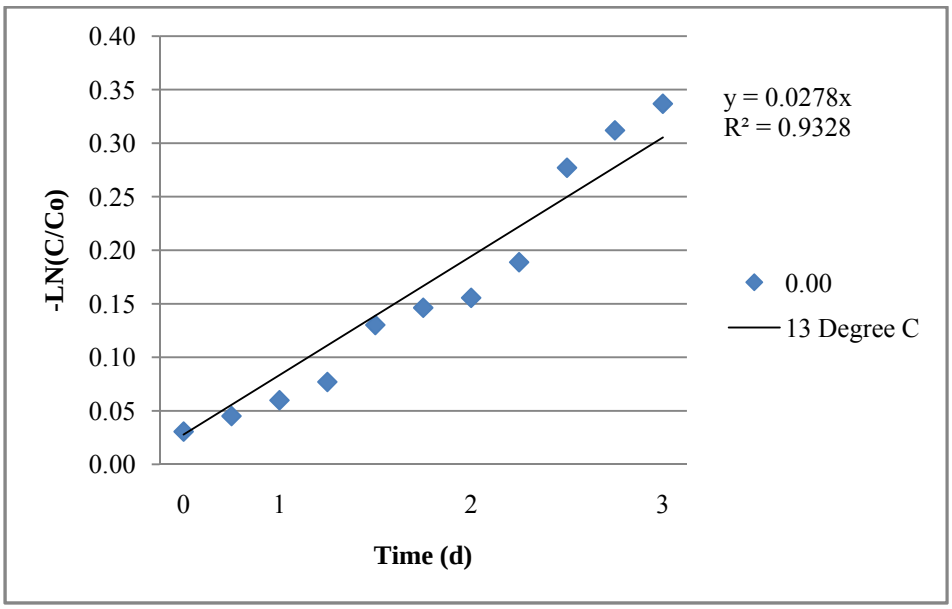
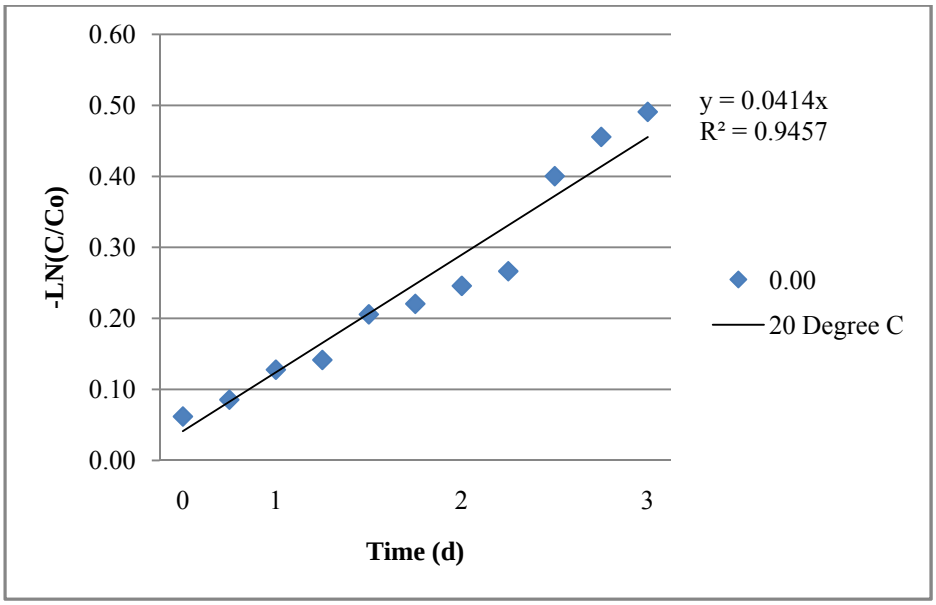
Appendix K

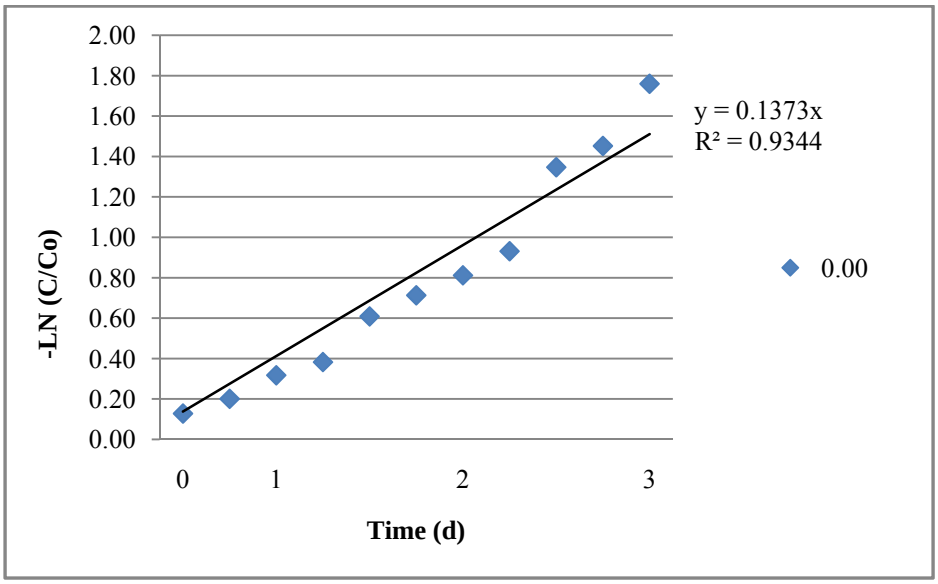
Determining Order of Reaction Kinetic from Data Plot



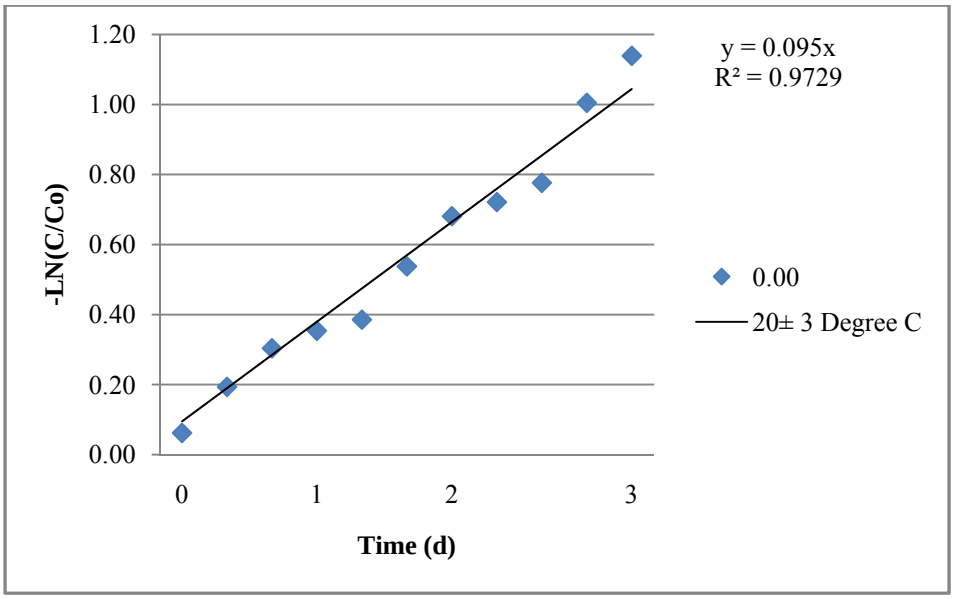
13 Degree C



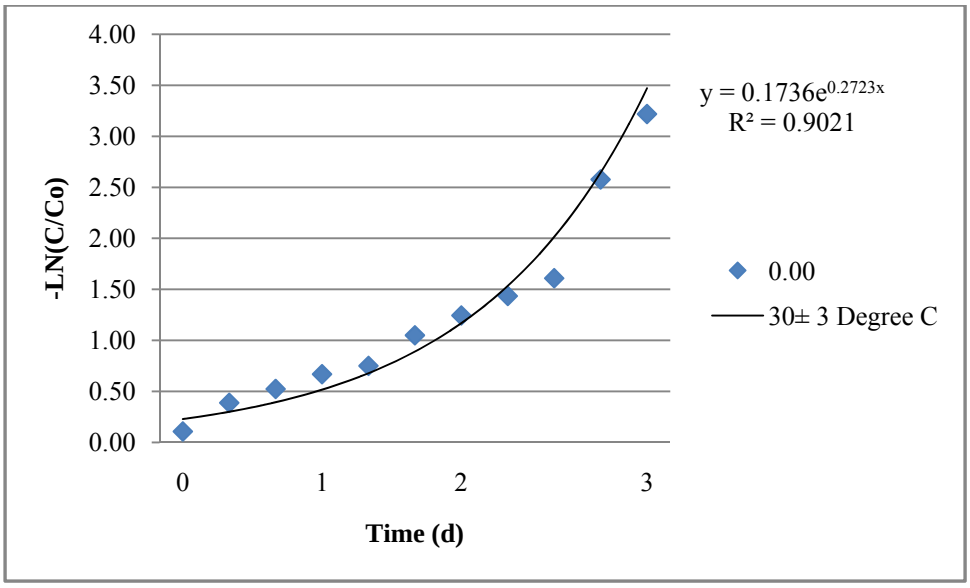
20 Degree C



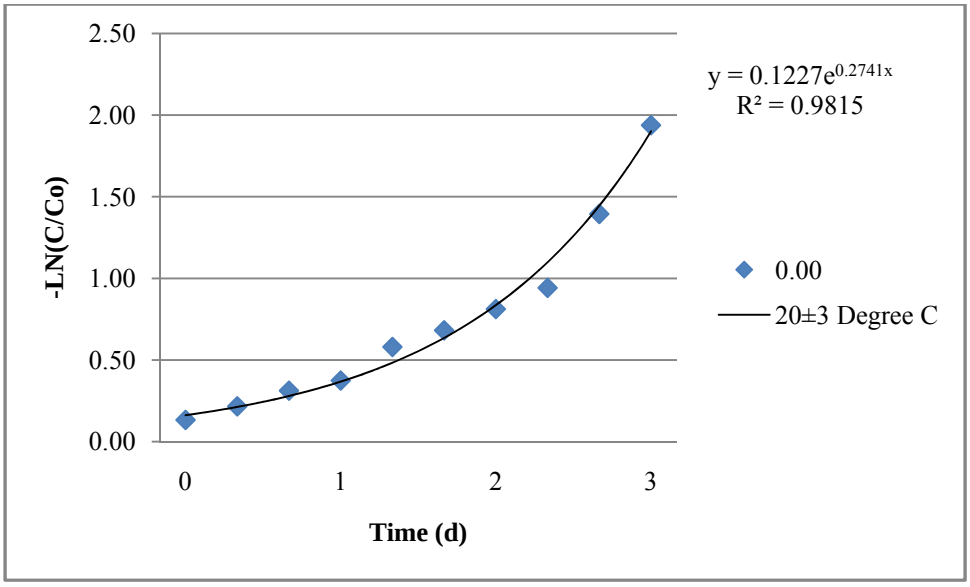
30 Degree C



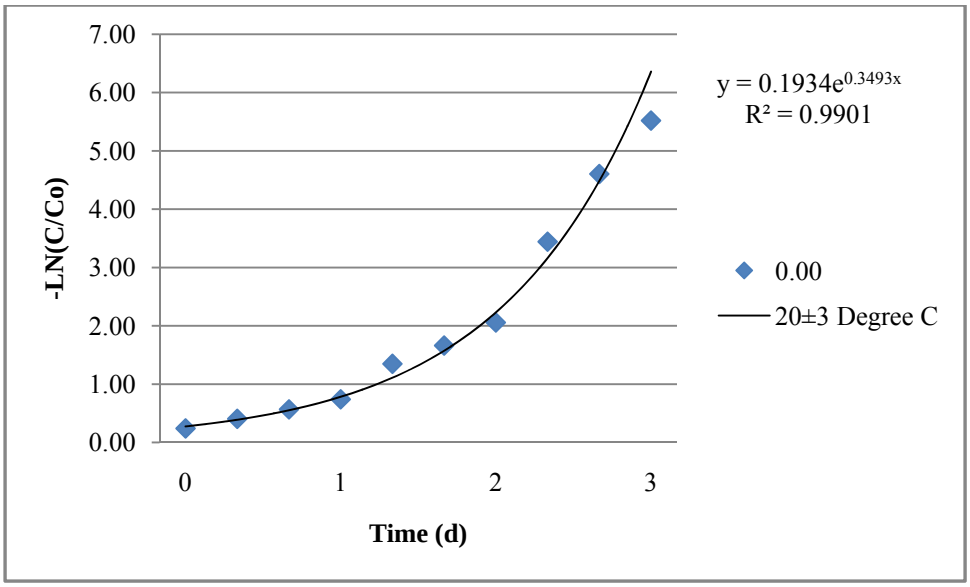
20±3 Degree C



30±3 Degree C



20±3 Degree C (Mixed Beads)



30±3 Degree C (Mixed Beads)

Appendix L

Comparison of Mean Concentrations for Steady State, Diurnal and Mixed Bead at Diurnal Regimes

The mean ammonia concentrations for the controlled tanks held at constant temperatures 13 & 30^{o}C were compared with the step-up temperature 13-30^{o}C, step-down temperature 30-13^{o}C, diurnal temperatures 20 ± 3^{o}C & 30 ± 3^{o}C were also compared to diurnal temperatures 20 ± 3^{o}C & 30 ± 3^{o}C with mixed beads.

Ammonia Removal Rate at Steady State versus Step Temperatures

Mean ammonia concentrations for step temperature regimes 13-30^{o}C were compared to results from steady state 30^{o}C (Figure 1) regime. Mean concentrations for 30-13^{o}C and 13^{o}C steady state (Figure 2) were also compared. The mean concentration slope at step-up 13-30^{o}C was –m = 0.363 at R^2 = 0.973. Slope of mean concentration the steady state 30^{o}C was –m = 0.349 at R^2 = 0.986. There was no significant difference (P > 0.05) in the mean concentrations for 13-30^{o}C versus 30^{o}C.

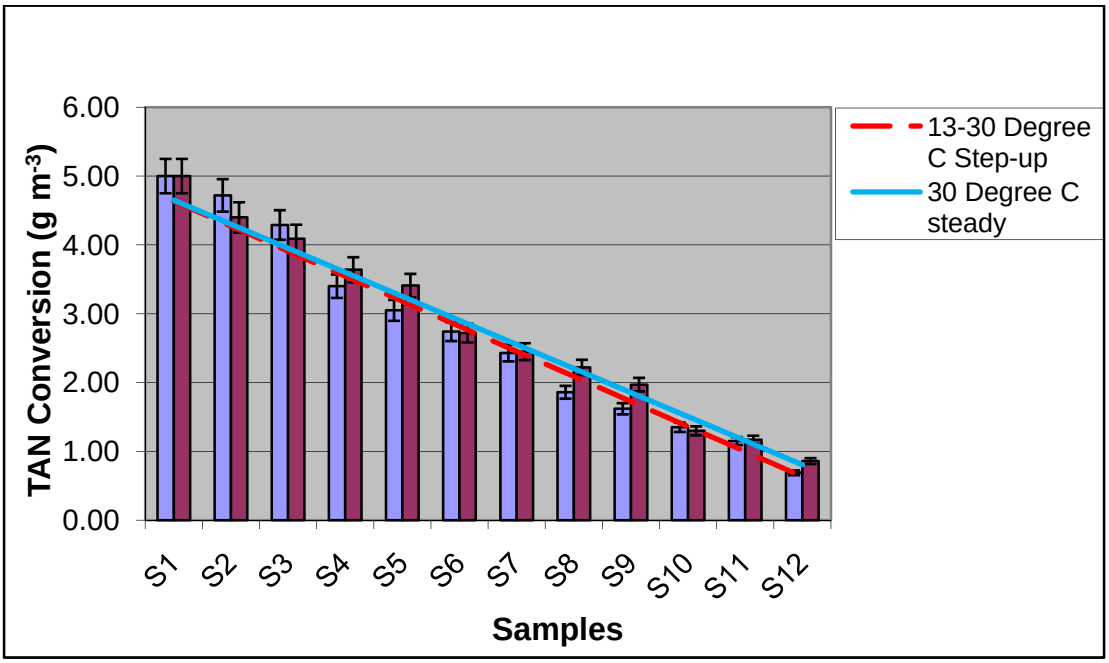


Figure 1: TAN mean concentration removal rates at 13-30 Vs 30^{o}C.

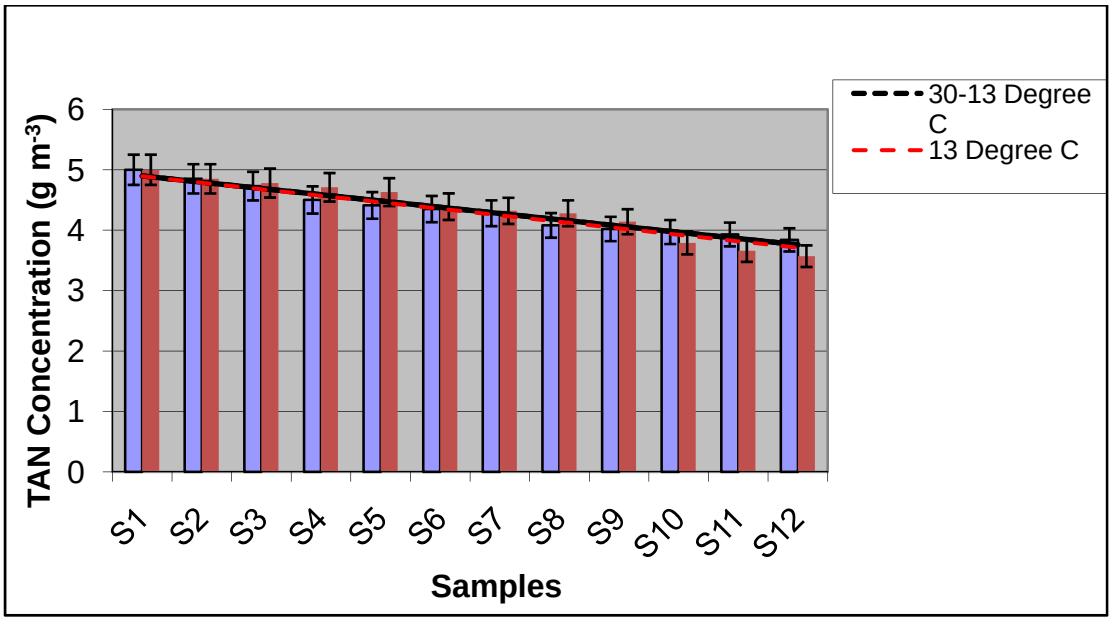


Figure 2: TAN mean concentration removal rates at 30-13 Vs 13^{o}C .

The step down 30-13^{o}C regime had a slope –m = 0.103 and R^2 = 0.968, while 13^{o}C had slope –m = 0.107 and R^2 = 0.930. There was no significant difference (P > 0.05) between the removal rates of 30-13^{0}C versus 13^{0}C. This may also suggest that the biofilter and the nitrifying bacteria in their entirety adapt to temperature changes. This may be explained also by Brownian kinetics which may suggest the decrease in reaction and metabolic process when temperature decreases to make up for the conversion rates. Considering the fact that the corresponding steady state temperature regimes do not differ in the removal rates with the step temperature regimes, it may be plausible to suggest that the bacteria do rebound and adapt their performance at a certain temperature regime. Further experiments to determine the biofilter performance, was done, comparing steady state to diurnal regimes in the following section.

Ammonia Removal Rate at 20 versus 20 ± 3°C Diurnal Regimes

Mean ammonia concentrations from the removal rate slope at 20°C steady state was compared with mean concentration at 20 ± 3°C (Figure 3) temperature regimes. The ammonia conversion rates for both treatments were plotted together to determine any difference in the conversion of ammonia. The mean ammonia concentration slope for diurnal 20 ± 3°C was -m = 0.285 and R^2 = 0.979. The conversion coefficient for the steady state 20°C was -m = 0.152 and R^2 = 0.963.

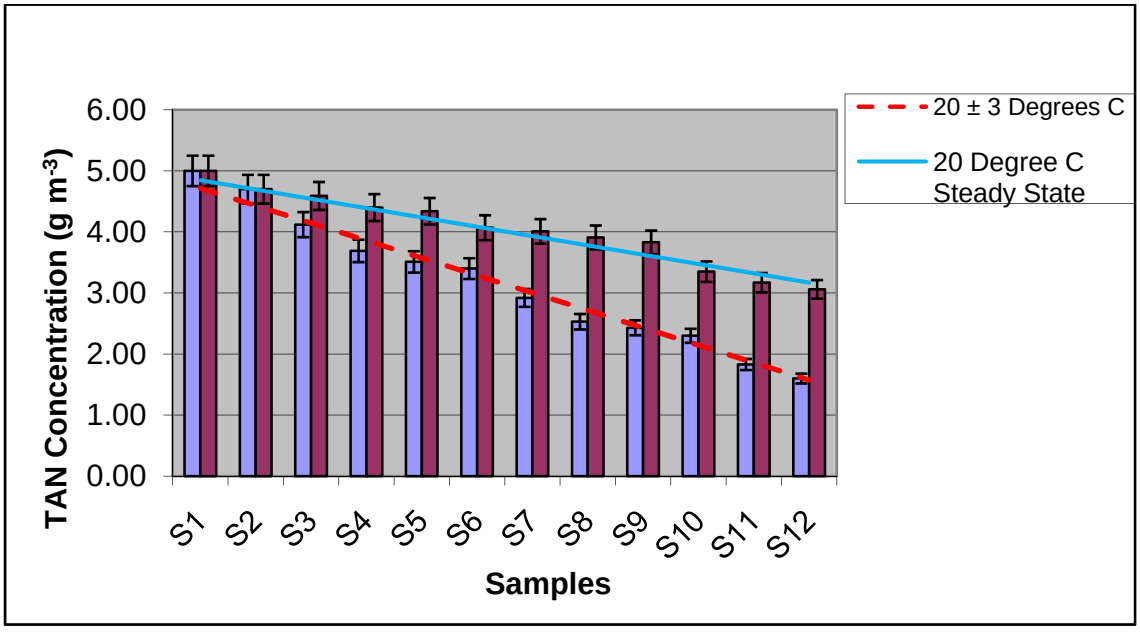


Figure 3: TAN mean concentrations at 20 Vs 20 ± 3°C diurnal temperature regimes.

The mean ammonia concentrations were significantly different ($P < 0.05$) for ammonia removal. This may suggest that biofilter beads acclimated at a steady state and subjected to diurnal temperature profiles may perform better than those at a steady state. A possible cause could be the net higher ammonia removal rate at the crest of the diurnal temperature profile. This may also be a result of the bacteria still in the growth phase of the biofilm consequently responding to

the temperature increase, which may have led to a resulting higher performance than the steady state regime.

Ammonia Conversion at 30 Steady State Vs 30 ± 3°C Diurnal Regime

Mean ammonia concentration using triplicate tanks at steady state temperature 30°C was compared to mean concentrations at 30 ± 3°C (Figure 4) diurnal regimes. The ammonia mean concentration removal rate for 30 ± 3°C was -m = 0.424 with R^2 = 0.965. The mean concentration removal rate for steady state 30°C was –m = 0.349 with R^2 = 0.986.

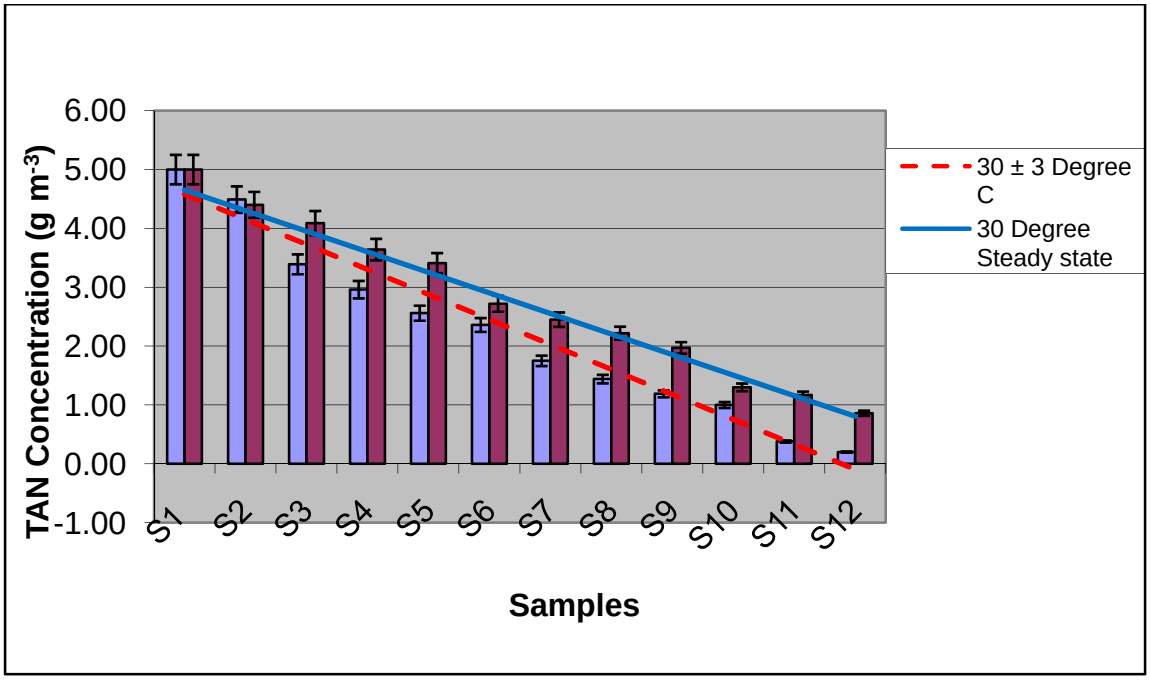


Figure 4: TAN mean concentrations at 30 Vs 30 ± 3°C diurnal temperature regimes

The mean concentration removal rates were significantly different ($P < 0.05$) for diurnal and steady state regime. This may again suggest that biofilter beads acclimated at a steady state and subjected to diurnal temperature profiles may perform better than those at a steady state.

Ammonia Removal Rate at 20 Vs 20 ± 3°C Diurnal Regimes (Mixed Beads)

The result of mean ammonia removal rates at 20°C steady state was compared with 20 ± 3°C mixed beads diurnal removal rate (Figure 5). Mean concentration removal rate slope for mixed beads at 20 ± 3°C was –m = 0.36 at R^2 = 0.977. The mean concentration removal rate at 20°C was –m = 0.152 at R^2 = 0.963.

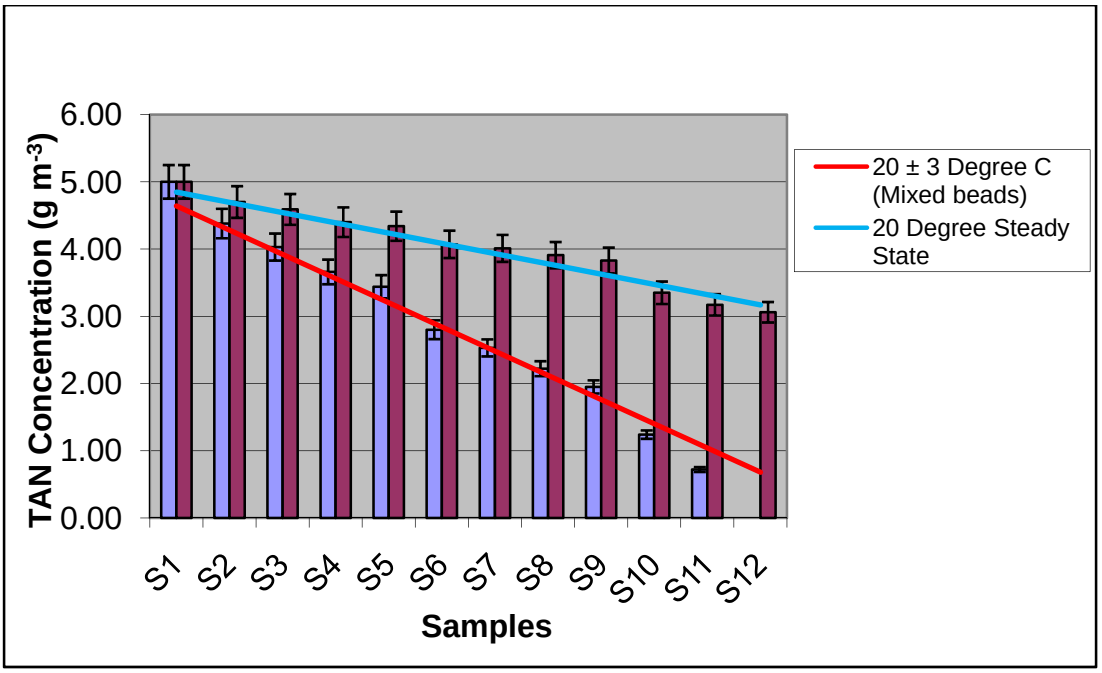


Figure 5: TAN mean concentrations at 20 Vs 20 ± 3°C diurnal temperature regimes using mixed beads.

There was a difference ($P < 0.05$) between the removal rates of the 20°C steady state versus the 20 ± 3°C mixed bead diurnal temperature regimes. This may suggest again that the average higher temperature amplitudes on the temperature profile curves accounts for the increase in mean removal rates by the biofilters.

Ammonia Removal Rate at 30 Vs 30 ± 3°C Diurnal Regime (Mixed Beads)

The mean concentration removal rate for 30°C steady state was compared with 30 ± 3°C diurnal regimes with mixed beads (Figure 6). The mean removal rate concentration for 30 ± 3°C was –m = 0.517 at R^2 = 0.950, while mean concentration removal rate for 30°C was –m = 0.349 at R^2 =0.986.

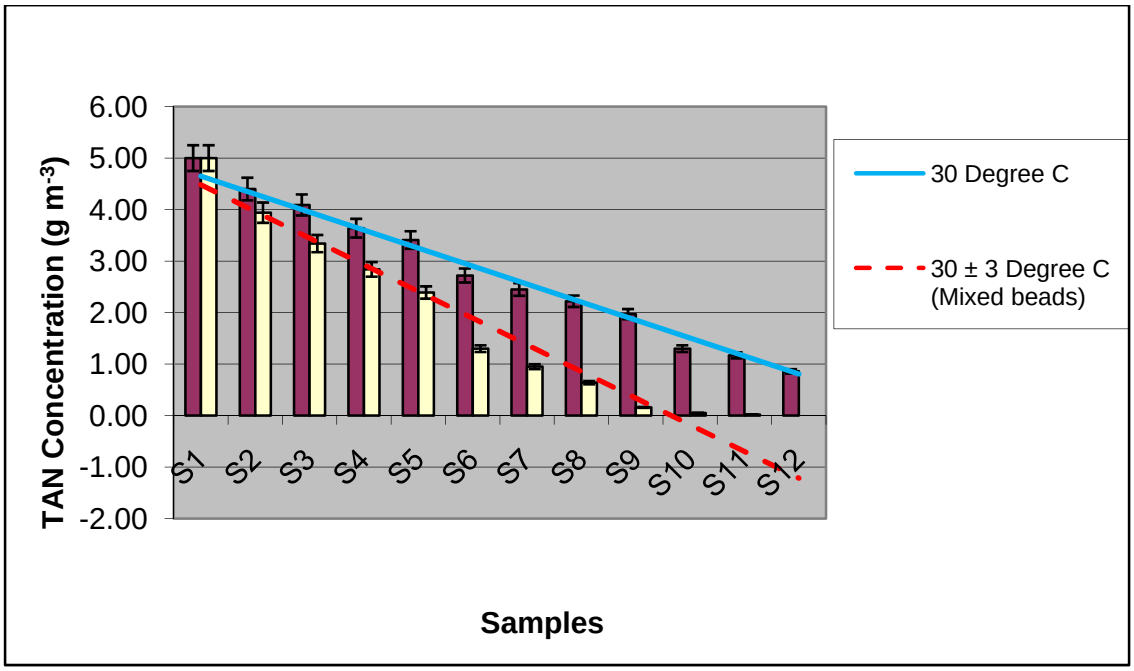


Figure 6: TAN mean concentrations at 30 Vs 30 ± 3°C diurnal temperature regimes using mixed beads.

The comparison between the ammonia conversions at 30 versus 30 ± 3°C were again significantly ($P < 0.05$) different. This also suggests that temperature increase may affect the rate at which the substrate ammonia is being converted in a biofiltration system. Further experiments were also performed to test the conversion rates between beads at diurnal temperature regime with mixed beads subjected to diurnal temperature regime in as shown in the next section.

Ammonia Removal Rate at 20 ± 3^{o}C Vs 20 ± 3^{o}C Diurnal Regimes (Mixed Beads)

Beads exposed to diurnal temperature regimes were compared to beads acclimated at three different temperature regimes 13, 20 and 30^{o}C, mixed and loaded in the biofilters. The mixed beads were exposed to diurnal temperature regimes 20± 3 and 30 ± 3^{o}C. The mean removal rates for 20 ± 3^{o}C diurnal regimes were compared to 20 ± 3^{o}C diurnal mixed beads mean concentration removal rates (Figure 7). Mean concentration removal rate for 20 ± 3^{o}C diurnal regime was –m = 0.285 with R^2 =0.979. The mean concentration removal rate for diurnal mixed bead at 20 ± 3^{0}C was –m = 0.360 with R^2 = 0.977. Although the data bars in the chart appear to have numeric differences, conversion rates were however compared for the diurnal 20 ± 3^{o}C to 20 ± 3^{o}C mixed beads diurnal regimes, there was also no significant difference ($P > 0.05$) in mean concentration removal rates. This may suggest that even though the beads were mixed, temperature is the probably the parameter accounting for the non difference in the conversion rates. The mixed and non-mixed were both at the same temperature fluctuation amplitudes. Further experiments were performed larger temperature fluctuation amplitudes as shown below.

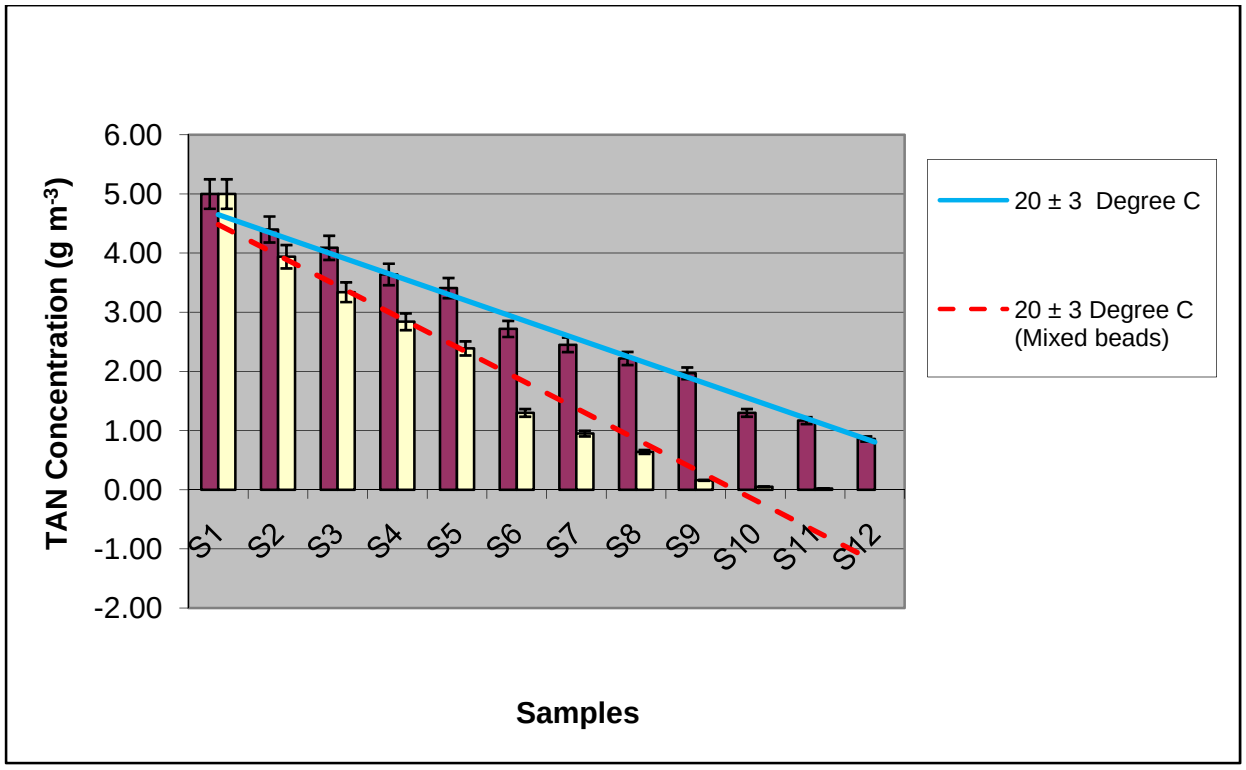


Figure 7: TAN mean concentrations removal rate at 20 ± 3°C transient Vs 20 ± 3°C diurnal mixed beads.

Ammonia Removal Rates at 30 ± 3°C Vs 30 ± 3°C Diurnal Regimes (Mixed Beads)

The mean concentration removal rate for 30 ± 3°C diurnal was also compared to 30 ± 3°C diurnal mixed beads removal rates (Figure 8). The mean concentration removal rate for 30 ± 3°C diurnal was -m = 0.424 with an R^2 = 0.965. The mean concentration removal rates for the mixed bead at 30 ± 3°C was -m = 0.517 and correlation coefficient R^2 =0.950. Although the data bars in the graph appear to have numeric differences, however conversion rates compared for the diurnal 30 ± 3°C to 30 ± 3°C mixed beads diurnal regimes were not significantly different (P > 0.05).

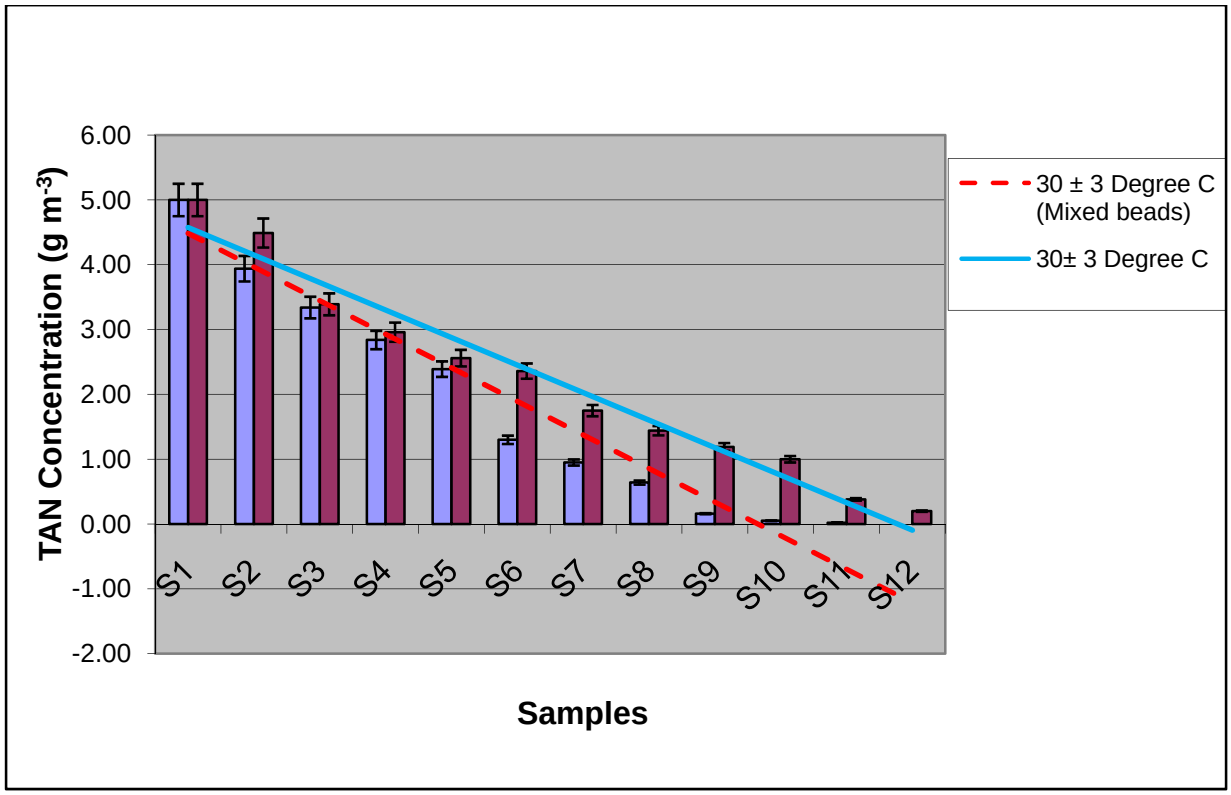


Figure 8: Ammonia conversion at 30 ± 3°C diurnal Vs 30 ± 3°C mixed bead diurnal regime.

The diurnal regime and the mixed beads at diurnal regime were exposed to the same temperature fluctuation amplitudes. This may suggest that even though the beads were mixed, temperature may be have affected the removal rates based on the reaction of the bacteria metabolic response to temperature change.

Appendix M

Installation, Configuration and Operation of Data Acquisition System (DAS) Boards

Three types of data acquisition boards were used in this project. A through description of installation, calibration, and operation for each of the boards follows.

1. PCIM-DAS1602/16 Analog and Digital I/O Board: Manufactured by Measurement Computing, performs multifunction measurement and control. The board is a PCI-compatible, plug and play board. Specific details of the configuration and calibration are illustrated in later sections of this chapter. The PCIM-DAS1602/16 Analog to Digital I/O Board (Figure 1)is also referred to as the A/D board.

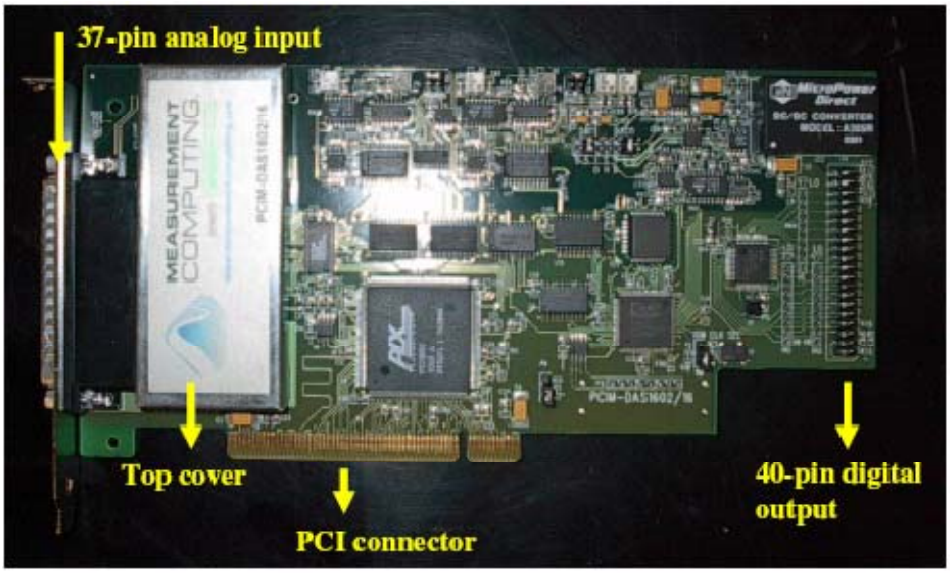


Figure 1: PCIM-DAS1602/16 Analog to Digital I/O Board with the top cover. The analog inputs and digital outputs are indicated using arrows. Switching the channel jumper from 8 differentials to 16 single ended channels was done by opening the top cover.

2. CIO-DIO24 Analog and Digital I/O Board:

Manufactured by Measurement Computing, is a multifunction measurement and control board. The board is a PCI-compatible, plug and play board (Figure 2). This board has 24 channels.

Figure 2: PCI-compatible, plug and play board

3. CIO-EXP 32 Multiplexer Board: This board is also manufactured by Measurement Computing, MA. One of the main functions of this board is processing of thermocouple signals. The installation, configuration, and calibration of this board are explained later. The CIO-EXP32 Multiplexer board (Figure 3) is also referred to as EXP32 board.

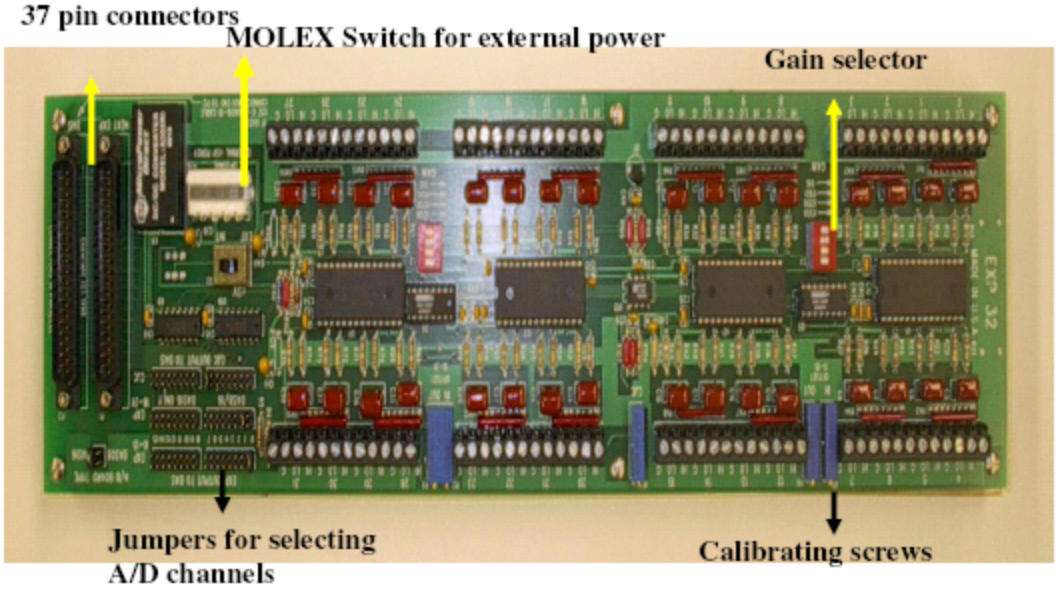


Figure 3: CIO-EXP 32 Multiplexer board was used in to measure temperatures values from tanks through thermocouples. Important components are shown using arrows.

PCIM-DAS1602/16 /CIO-DAS24 Analog and Digital I/O Boards:

Installation of A/D boards

The *InstaCal* software must be installed on the PC before installing the A/D board *InstaCal* has information that is needed for the PC to detect the A/D board. The following steps explain the installation process.

1. Turn the computer off and open the cover of the CPU.

2. Insert the **PCIM-DAS1602/16** board into an available PCI slot (Figure 4), as shown in the Figure below
3. Insert the **CIO-DAS24** board into an available ISA slot, as shown in the Figure below
4. Close the top of the computer and switch on the power.
5. A dialog box appears indicating that a new hardware has been detected. Follow the steps to run *InstaCal* software and to review the configuration of the boards. The PC may prompt you to restart before you begin configuration of the A/D board.

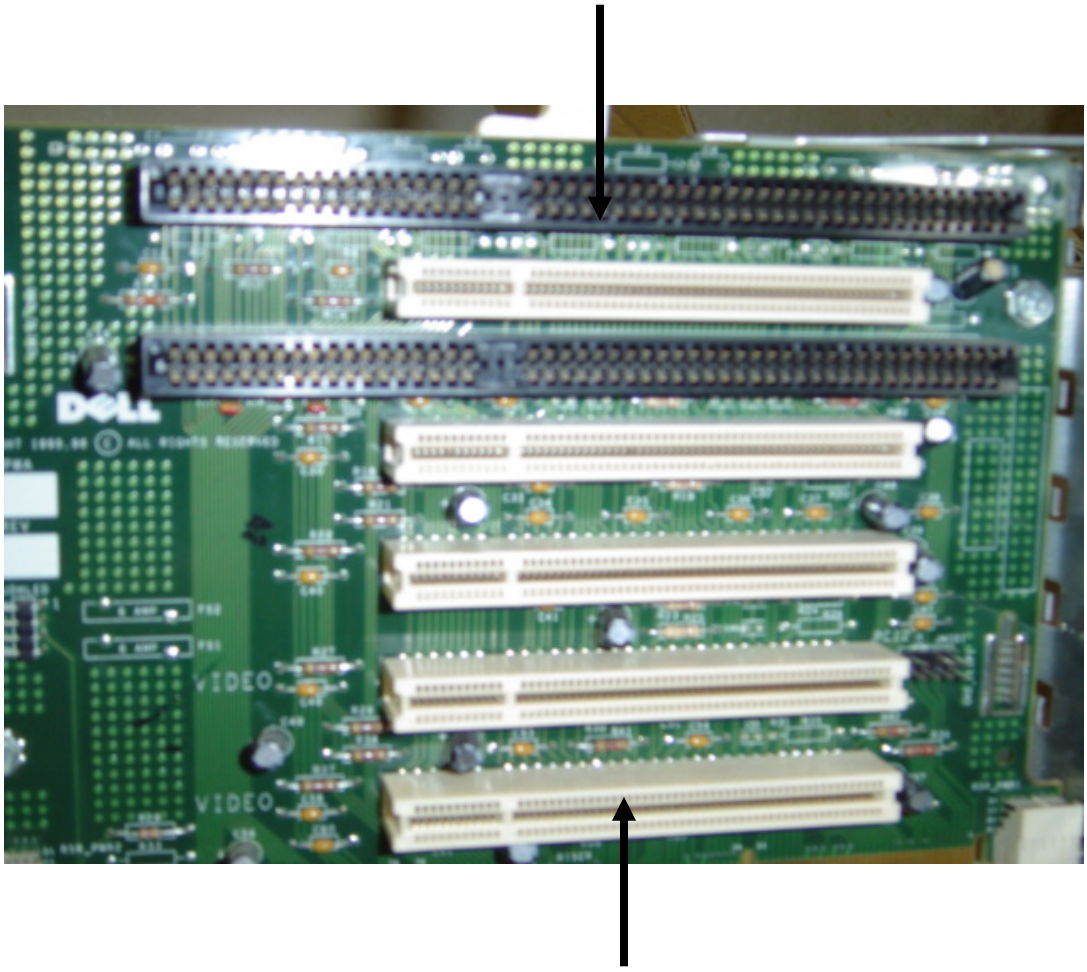


Figure 4: Available PCI and ISA slots are shown. The A/D boards were each inserted into one of the slots. When the computer is restarted, a dialog box appears indicating that the new hardware has been detected.

Connecting the Board for I/O Signals

The **PCIM-DAS1602/16** A/D board has a 37-pin male connector to receive analog inputs. The analog connector can be accessed from the rear of the PC on the back plate. To connect the analog connector, a female 37-pin D-type connector (Figure 5) may be used. The digital signals

are transmitted via a male 40-pin connector that is mounted on the side of the A/D board. The digital signals are brought to the back plate of the PC via a BP40-37 cable, manufactured by Measurement Computing Inc, MA.

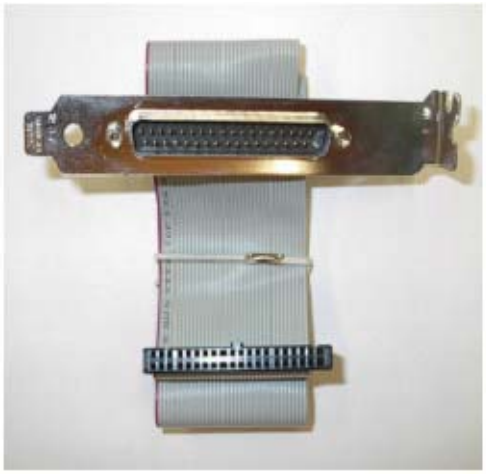


Figure 5: A BP40-37 cable manufactured my Measurement Computing Inc, MA, was used to bring digital signals from the rear of the board to the rear of the PC on the back plate.

Default Board Configuration

The A/D board is factory configured to default settings (Figure 6). It is advisable to check and change the configuration settings depending on the requirement and conditions imposed by the design. When used with an EXP32 board, the channel select switch must be changed to 16 single ended channels, rather than eight differential channels (Default setting). The following table shows default settings of the switches and jumpers.

Switch/Jumper description Default setting

Channel select switch 8 channels

A/D range select switch Bipolar

Trigger edge select switch Rising edge

Clock select jumper 1MHz

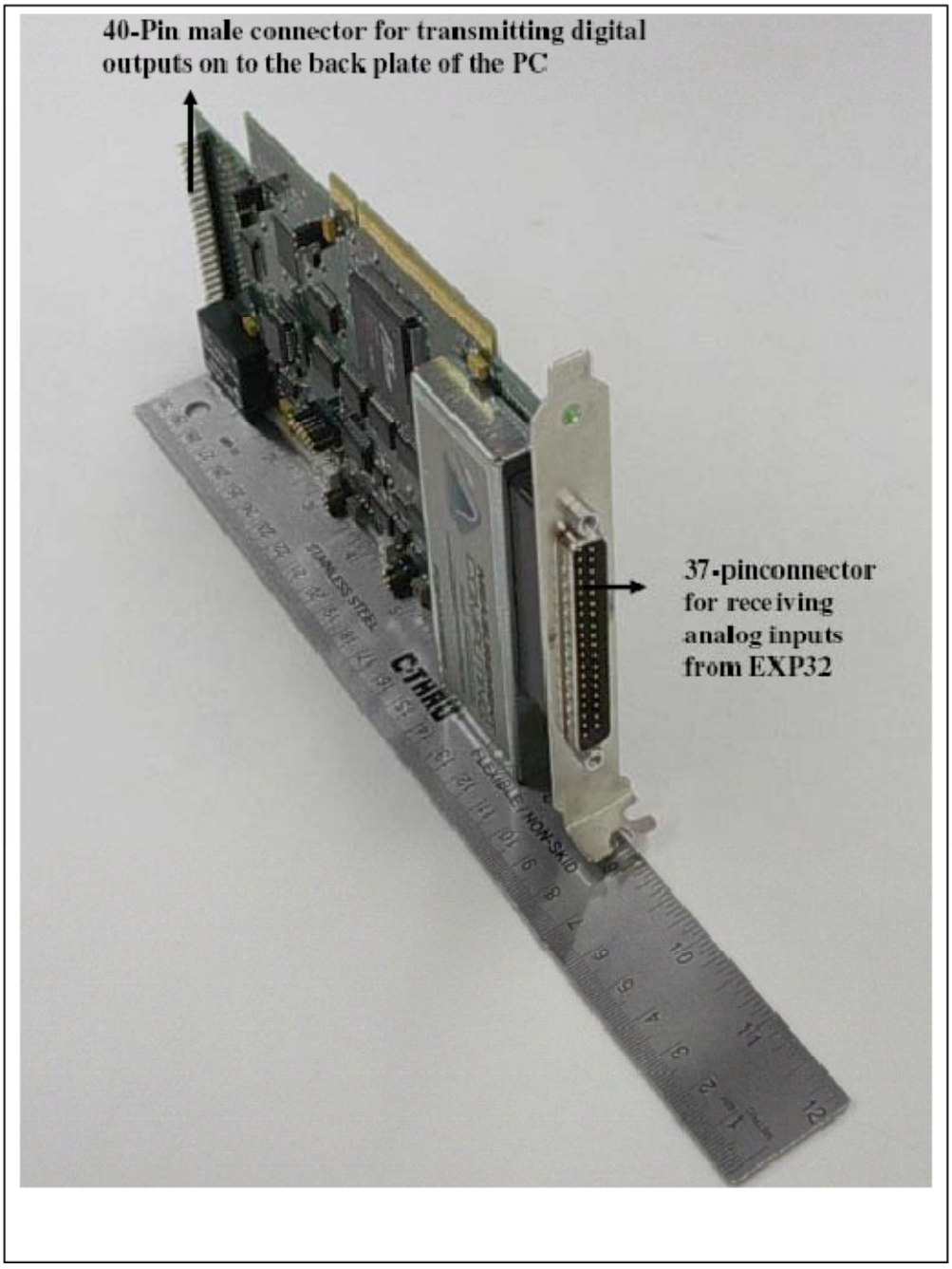


Figure 6: A side view of A/D board with analog inputs and digital outputs. A male 37- pin connector received the analog signals from EXP32 board. The digital signals were transmitted by BP40-37 cable, which brings the digital signals to the back on PC on the back plate.

The channel select switch and A/D range select switch are covered by the metal name plate on the A/D board. The name plate has to be removed using a screw driver to change the settings.

Channel Selection for the A/D Board

The A/D board (Figure 7) can be used with either eight differential or 16 single ended input channels. The board was set for 16 single ended options. Since, thermocouples were used for measuring temperatures, 16 single ended options reduced the electrical noise generated by the system.

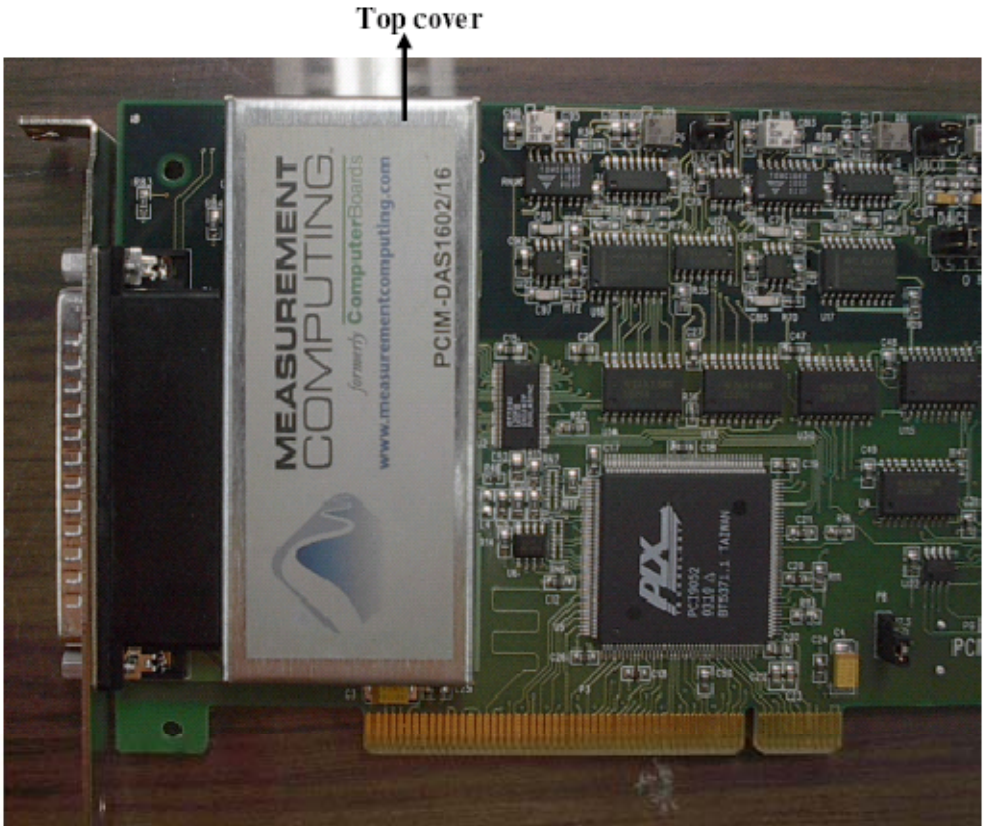


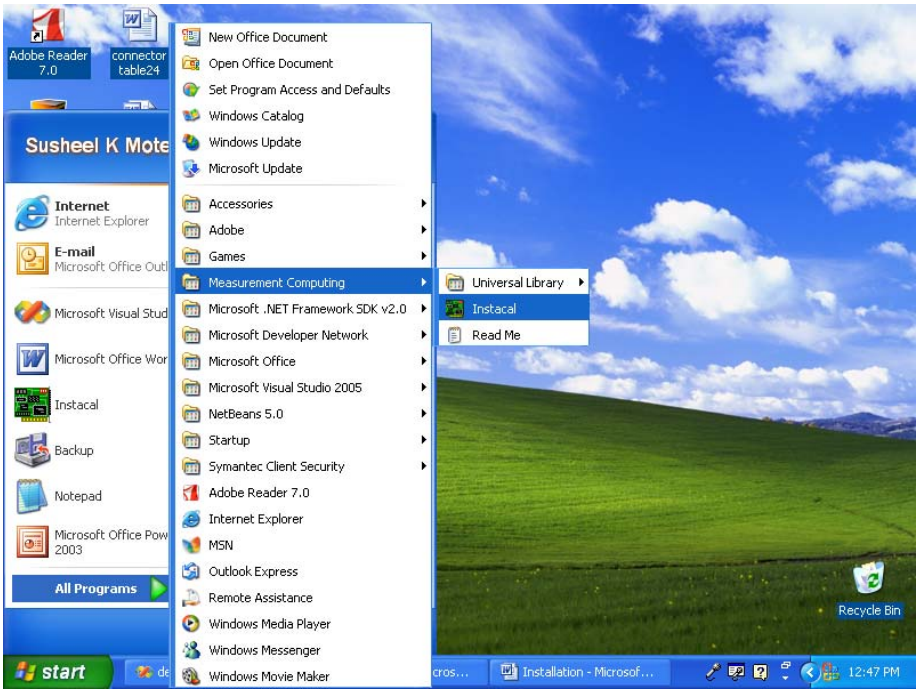
Figure 7: The top cover of the A/D board was removed to change the jumper switch from 8 differential channels to 16 single ended channels to reduce electrical noise.

Addition of EXP32 Board to A/D Board

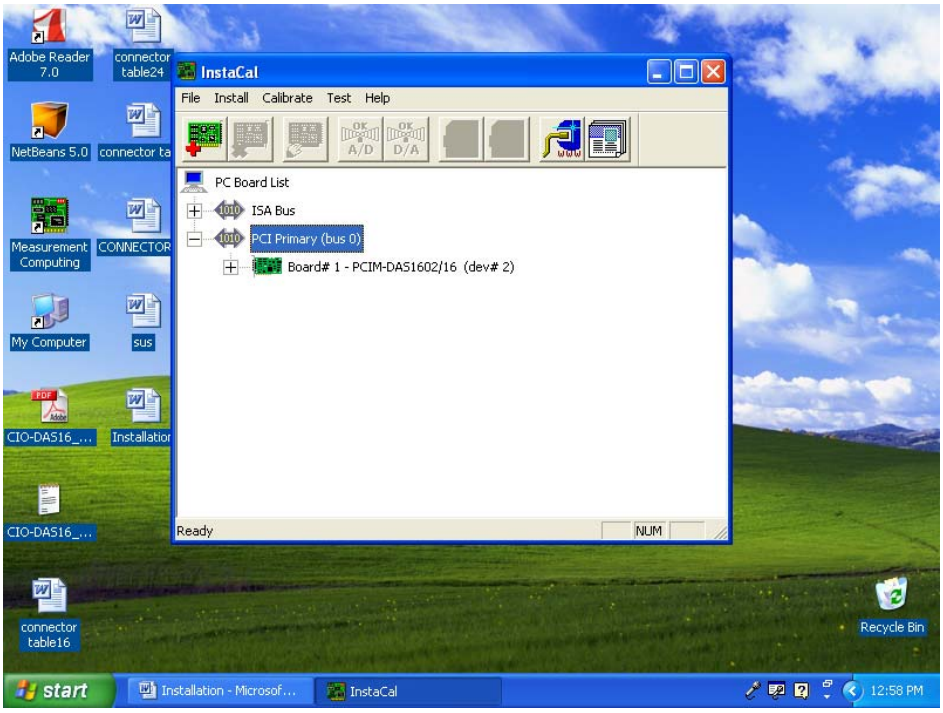
This section explains the steps for adding data acquisition devices to the A/D board.

The steps are:

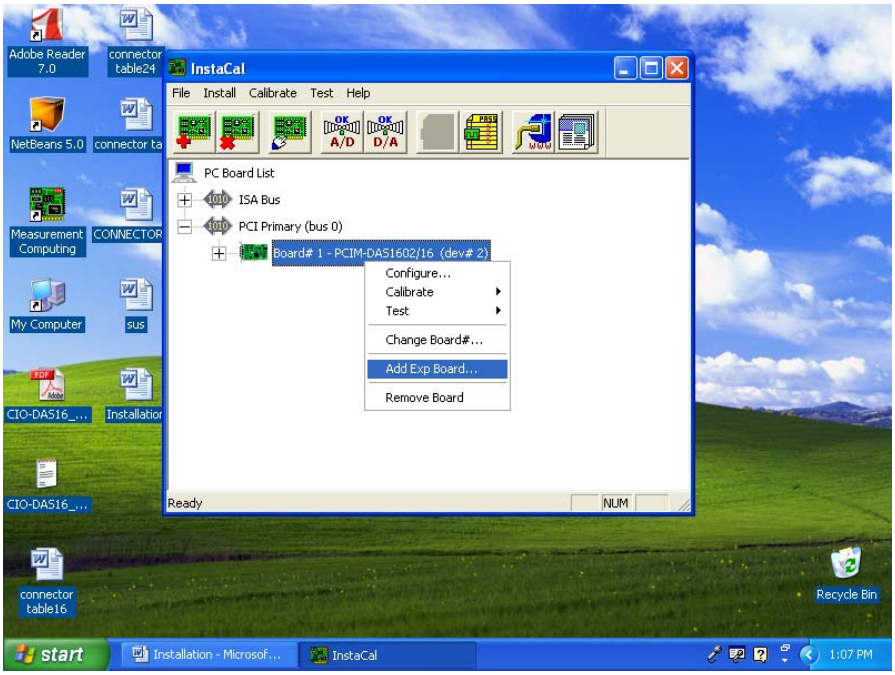
1. Open *Instacal* software as shown below



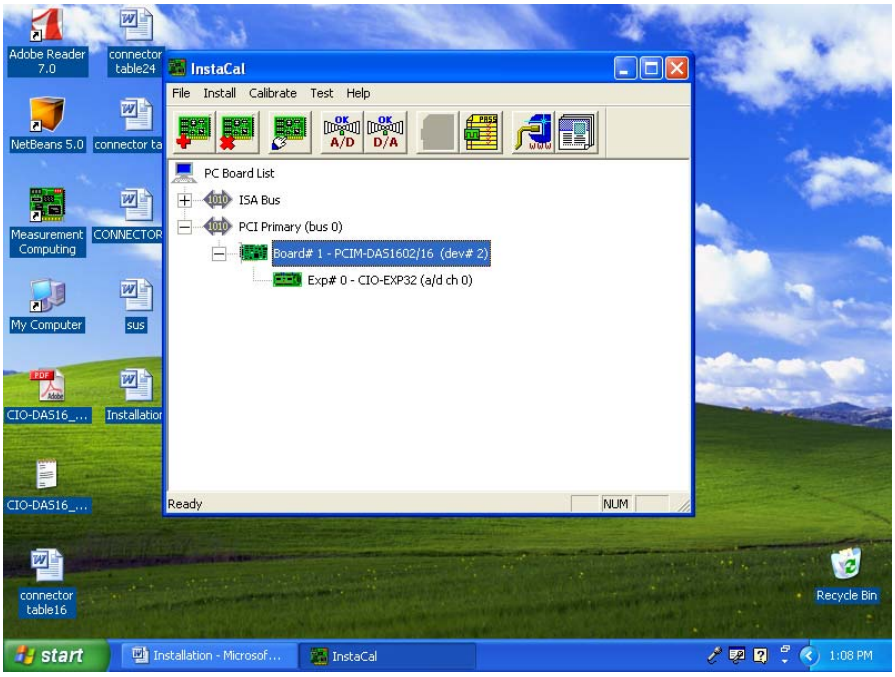
2. Double click on the *Instacal* icon. If PC has ISA and PCI slots the PCIM-DAS16 board is connected to PCI slot, the following screen appears.



3. For our program EXP32 is connected to channel 0 of PCIM-DAS16. So right click on the board and click on the Add Exp Board to add expansion board. The following screen appears.



4. Select the board needed for data acquisition. For this project EXP32 boards are selected. After that screen will appear like this.



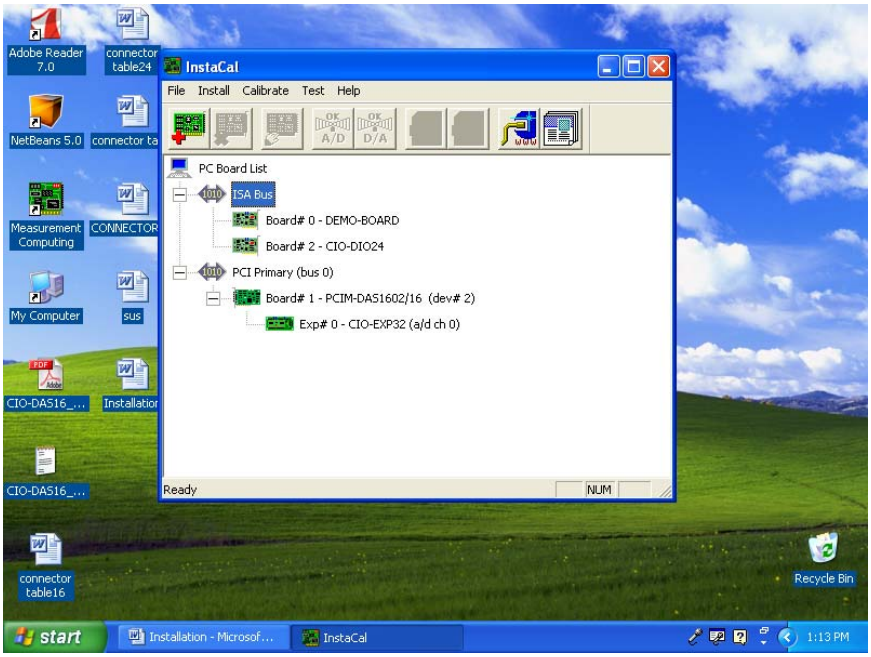
5. Connect the EXP32 board and analog connector to the A/D board with a special 37-conductor cable, CEXP2DAS16-10 (Figure 8), manufactured by Measurement Computing, Inc, MA.

Figure 8: A CEXP2DAS16-10 cable was used to connect EXP32 with the A/D board.

The cable connects the A/D board from the rear of the PC. One end marked MUX was

Connected to EXP32, and other end connected the A/D board.

6. Process control program for 36 tankss need three CIO-DIO 24 channel boards. When these boards are connected to ISA/PCI slot, right click on the ISA/PCI Bus to add these boards and click on add board option. After that the screen will appear like this.



CIO-EXP32 Multiplexer Board: This is a 32-channel (0-31) external board that can be mounted either on a bench top or secured in a water proof case.

Powering the Board

The board requires a 5VDC. The power source can be either internal or external. The A/D board via a 37-conductor cable supplies internal power, which is adequate for two EXP32 boards. If internal power is used, the +5V switch (S3) on the board should be set to INT (Figure A. 7). The current setup used for this board is internal power. When three or more EXP32 boards are used, external power may be required. The Personal Computer (PC) connected via a CPCPOWER- 10 MOLEX cable (manufactured by Measurement Computing) supplies the external power (Figure 9). To connect this cable, the computer has to be opened after turning off the power. One end of the MOLEX cable is connected to the expansion power connectors in the PC, while other end is connected to the EXP32 connector labeled “OPTIONAL EXTERNAL +5V POWER” The +5V switch (S3) on the EXP32 board should be set to EXT.

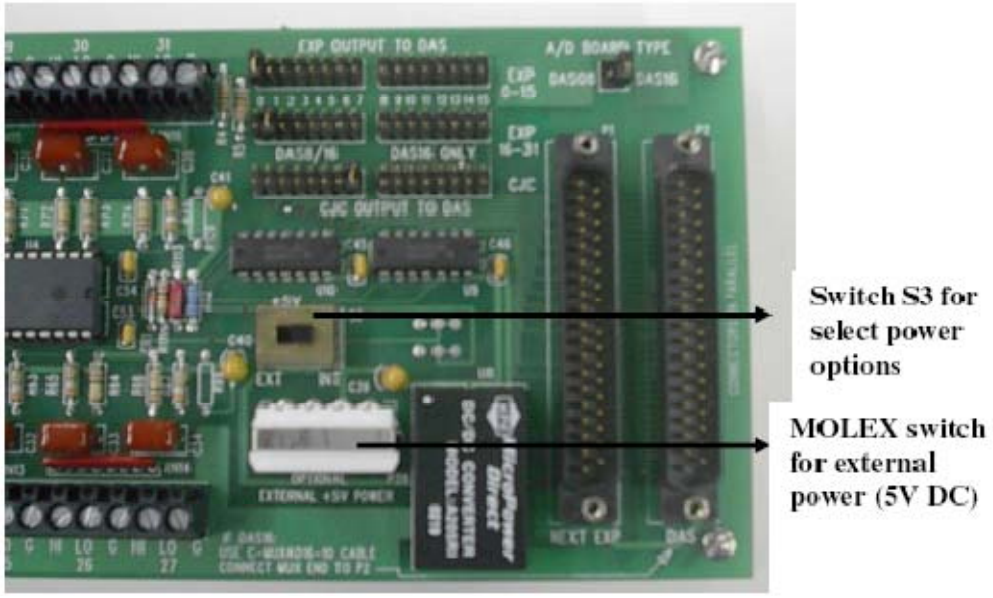


Figure 9: Internal power (5V DC) was selected for this project. External power is required when two or more EXP32 boards are used.

Right Connections

The EXP32 board has two connectors that are identical 37D connectors, P1 and P2 (Figure 10). The first connector (P2) is used to connect the EXP32 with an A/D board. Because the pin relationships are not same between the EXP32 and A/D board, a special 37-conductor cable, CEXP2DAS16-10 (manufactured by Measurement Computing Inc, MA) is required. One end (labeled MUX) of the CEXP2DAS16-10 cable (Figure 11) connects the first connector (P2) on the EXP32 and the other end connects the A/D board's analog connector on the back plate of the PC. The second D37 connector (P1) is used only when

More than one EXP32 boards are used.

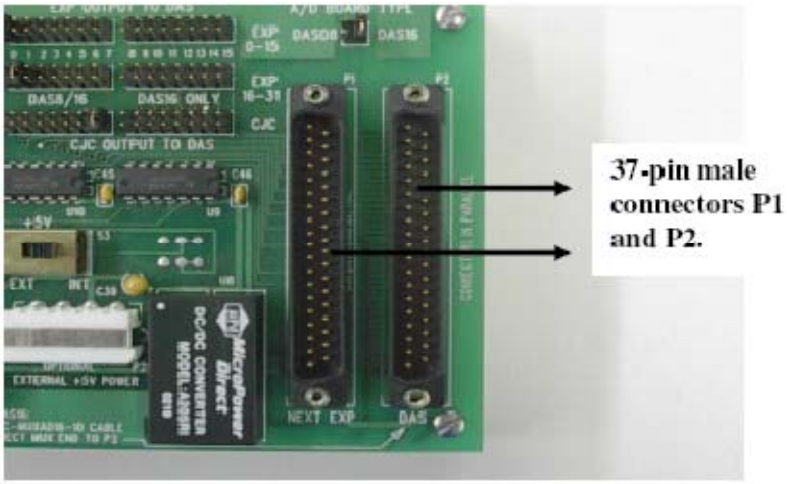


Figure 10: Male 37-pin connector P2 was used by CEXP2DAS16-10 to connect EXP32 with A/D board. P1 may be used when more than one EXP32 boards are used to capture data.

Figure 11: A CEXP2DAS16-10 cable was used to connect EXP32 with the A/D board. The cable connects the A/D board from then rear of the PC. One end marked MUX was connected to EXP32 and other end connected the A/D board

Channel Selection

The EXP32 has 32 channels (0-31) for input. These are divided into two sections of 16 channels each. Section 1 is connected to the jumper block labeled “EXP 0-15” and section 2 is connected to jumper block labeled “EXP 16-31” (Figure 12). Each section of 16 inputs is read by one channel of the A/D board. For this project channels 0-15 of EXP32 are multiplexed into channel 0 of the A/D board and channels 16-31 are multiplexed into channel 1 of the A/D board. Figure A.10 shows the sections 1 and 2 and corresponding jumpers set to channels 0 and 1 for the A/D board.

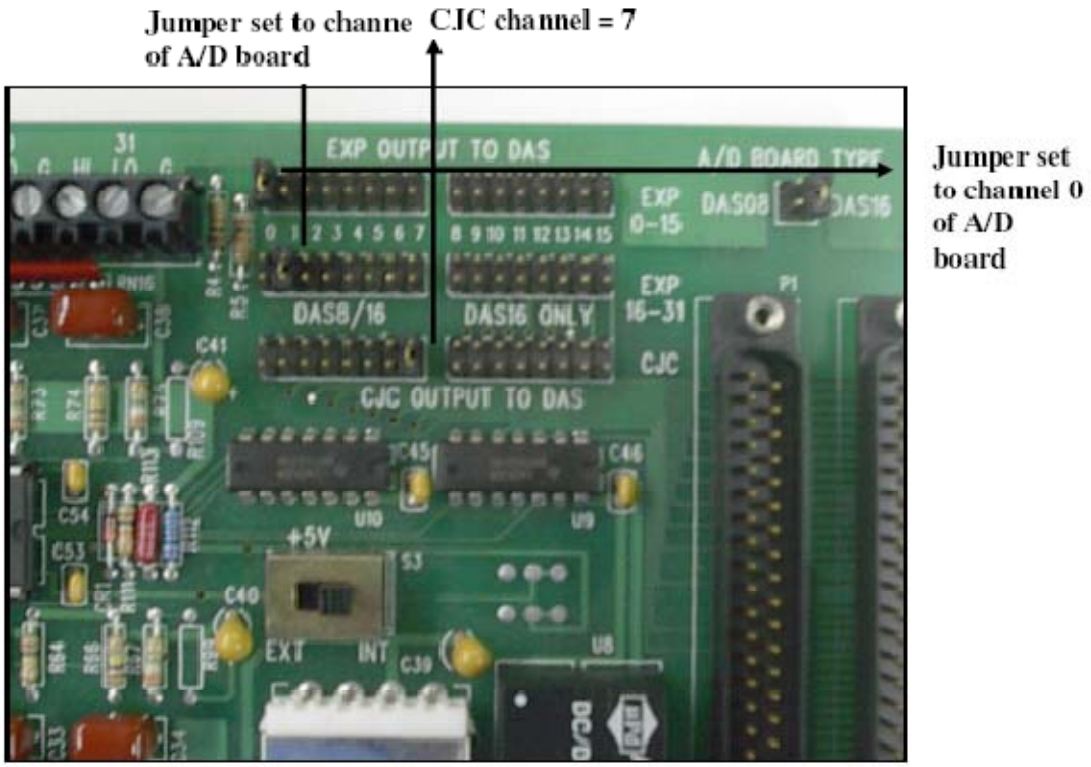


Figure 12: Jumper block for first fifteen channels 0-15 was connected to channel 0 of A/D board and jumper block for channels 16-31 was connected to channel 1 of A/D board.

The channel inputs are screw terminals capable of accepting 12-22 AWG wire and each channel consists of a screw terminal for signal high, signal low and ground. All inputs from signal source are floating differential, requiring two wires, signal high and signal low. The ground is not used for temperature measurements using thermocouples. However, all grounds (G pads) are bridged using solder.

Cold Junction Compensation (CJC)

The EXP32 board has a semiconductor temperature sensor to measure the temperature of the screw terminals on the board. This is cold junction temperature. When thermocouples are used to measure the temperatures, cold junction temperatures are needed for accurate temperature measurement. The CJC jumper has to be set on the corresponding channel. For this project, the CJC jumper was set to channel 7 (the default configuration).

Selecting the Gain

For each section of 16 input channels, there is a bank of four DIP switches, located in the central portion of each section. These four switches control the amplification factor of the thermocouple input signals. One or more switches (Figure 12 can be turned on or off simultaneously depending on the desired amplification (gain). It is important to note that these gains are additive. A gain of 100 was selected for this project.

Configuring EXP32

Each channel of the EXP32 has to be configured (Figure 13) before measuring temperature using thermocouples. This can be done by bridging the V, G, and C pads on the etch side (underside) of the EXP32 by soldering the pads. It is important to use solder provided by the board manufacturer only, since the solder contains flux core and is water-soluble. The A/D board has to be configured to 16 single-ended to be compatible with EXP32.

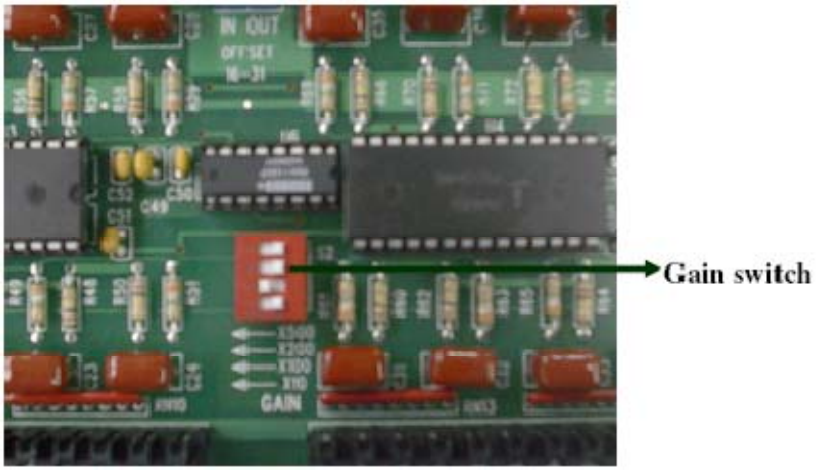


Figure 12: A gain of 100 was selected for this project by turning on the second switch. The gains were additive across switches and ranged from 10 to 810.

Calibration of EXP32

Calibration of EXP32 requires *InstaCal* software supplied by board manufacturer. *InstaCal* is the installation, calibration, and testing software for boards manufactured by Measurement Computing Inc, MA. Ideally, *InstaCal* and *Universal Library* software must be loaded before adding any boards to the PC. *Instacal* is compatible with Windows 3.x, Windows 95, Windows 98, Windows 2000, Windows ME, and Windows XP.

The software is stored in a default folder called **Measurement Computing**. After installation of *InstaCal* , the board properties can be changed by double clicking on the board name. For the present project, the board properties were selected as:

1. CJC: Channel 7

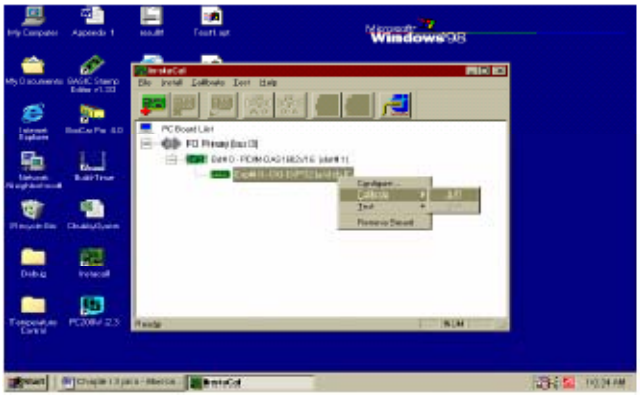
2. EXP32 channels 0-15: channel 0 of A/D board, 16-31 to channel 1 of A/D board

3. Power options: +5VDC External

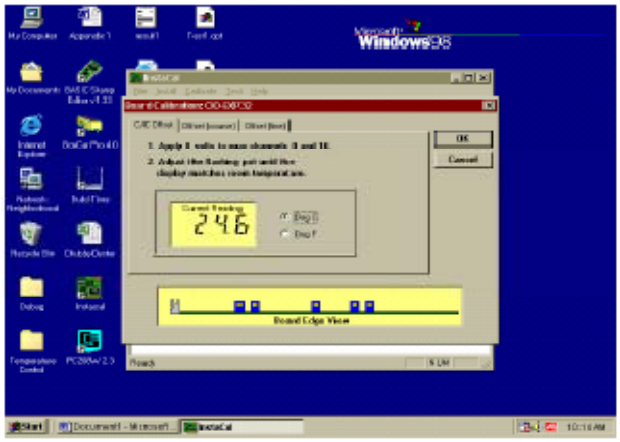
4. Gain: 100

The calibration process for EXP32 is simple. The following steps explain the steps for calibration

a. Open *Instacal* software and right click on the EXP32 board as shown below.



b. Select the A/D option and the following screen appears for calibrating the EXP32 board. Follow the instructions and set the CJC temperature to the room temperature by adjusting the calibrating screws



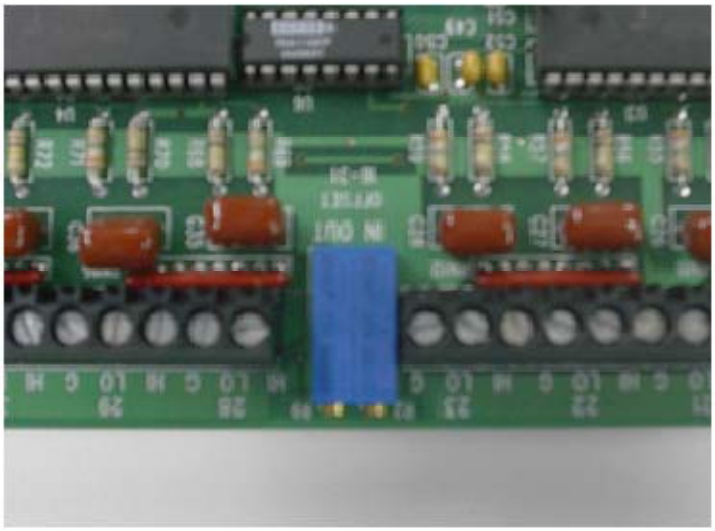


Figure 13: Then calibrating screws were turned either clockwise or anti-clock wise depending on whether the measured temperature was higher or lower than actual room temperature.

After calibration, the EXP32 was ready for measure the temperatures. This simple process, coupled with regular calibration of thermocouples allowed for accurate measurement of temperatures within ± 0.5 °C.

CONNECTOR TABLE:

Pin out of a BP40-37 when connected to the 40-pin digital connector

Board	*Pin #on board (40 pins)*	*Pin # on 37 female Connector*	*Port*	*Color code for 8-conductor cable from the relays*	*Controls*
CIO-DIO24					
1	36	37	A10	A11 (Red)	Pump 1
1	34	36	A11	A12 (Green)	Heater 1
1	32	35	A12	A13 (Brown)	Pump 2
1	30	34	A13	A14 (Blue)	Heater 2
1	28	33	A14	A15 (Orange)	Pump 3
1	26	32	A15	A16 (White)	Heater 3
1		19	Ground	Ground (Black)	
1	24	31	A16	A17 (Red)	Pump 4
1	22	30	A17	A18 (Green)	Heater 4
1	19	10	B10	B11 (Brown)	Pump 5
1	17	9	B11	B12 (Blue)	Heater 5
1	15	8	B12	B13 (Orange)	Pump 6
1	13	7	B13	B14 (White)	Heater 6
1		11	Ground	Ground (Black)	
1	11	6	B14	B15 (Red)	Pump 7
1	9	5	B15	B16 (Green)	Heater 7
1	7	4	B16	B17 (Brow)	Pump 8
1	5	3	B17	B18 (Blue)	Heater 8
1	20	29	C10	C11 (Orange	Pump 9
1	18	28	C11	C12 (White)	Heater 9
1		13	Ground	Ground (Black)	
1	16	27	C12	C13 (Red)	Pump 10
1	14	26	C13	C14 (Green	Heater10
1	12	25	C14	C15 (Brown	Pump 11
1	10	24	C15	C16 (Blue)	Heater 11
1	8	23	C16	C17 (Orange)	Pump 12
1	6	22	C17	C18 (White)	Heater 12
1	19	19	Ground	Ground (Black)	

CIO-DAS16

2	36	37	A0	A1 (Red)	Pump 13
2	34	36	A1	A2 (Green)	Heater 13
2	32	35	A2	A3 (Brown)	Pump 14
2	30	34	A3	A4 (Blue)	Heater 14
2	28	33	A4	A5 (Orange)	Pump 15
2	26	32	A5	A6 (White)	Heater 15
2		19	Ground	Ground (Black)	
2	19	10	B0	B1 (Red)	Pump 16
2	17	9	B1	B2 (Green)	Heater 16
2	15	8	B2	B3 (Brown)	Pump 17
2	13	7	B3	B4 (Blue)	Heater 17
2	11	6	B4	B5 (Orange)	Pump 18
2	9	5	B5	B6 (White)	Heater 18
2		11	Ground	Ground (Black)	
2	20	29	C0	C1 (Red)	Pump 19
2	18	28	C1	C2 (Green)	Heater 19
2	16	27	C2	C3 (Brown)	Pump 20
2	14	26	C3	C4 (Blue)	Heater 20
2		13	Ground	Ground (Black)	

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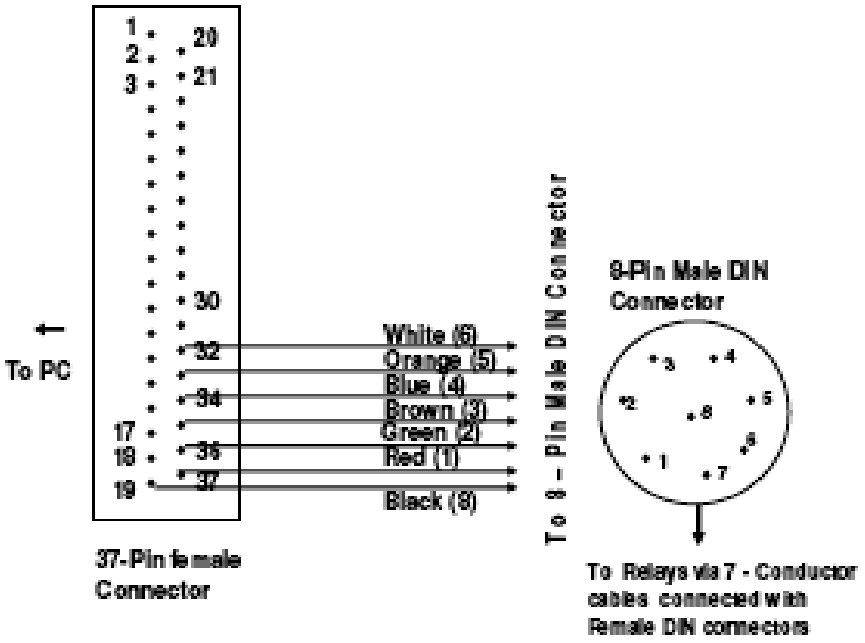
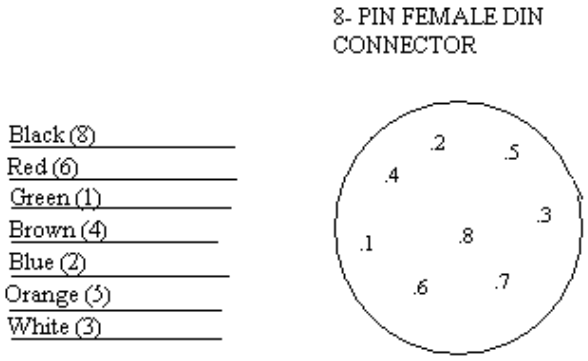
3	36	37	A10	A21 (Red)	Pump 21
3	34	36	A11	A22 (Green)	Heater 21
3	32	35	A12	A23 (Brown)	Pump 22
3	30	34	A13	A24 (Blue)	Heater 22
3	28	33	A14	A25 (Orange)	Pump 23
3	26	32	A15	A26 (White)	Heater 23
3		19	Ground	Ground (Black)	
3	24	31	A16	A27 (Red)	Pump 24
3	22	30	A17	A28 (Green)	Heater 24
3	19	10	B10	B21 (Brown)	Pump 25
3	17	9	B11	B22 (Blue)	Heater 25
3	15	8	B12	B23 (Orange)	Pump 26
3	13	7	B13	B24 (White)	Heater 26
3		11	Ground	Ground (Black)	
3	11	6	B14	B25 (Red)	Pump 27
3	9	5	B15	B26 (Green)	Heater 27
3	7	4	B16	B27 (Brown)	Pump 28
3	5	3	B17	B28 (Blue)	Heater 28
3	20	29	C10	C21 (Orange)	Pump 29
3	18	28	C11	C22 (White)	Heater 29
3		13	Ground	Ground (Black)	
3	16	27	C12	C23 (Red)	Pump 30

3	14	26	C13	C24 (Green)	Heater30
3	12	25	C14	C25 (Brown)	Pump 31
3	10	24	C15	C26 (Blue)	Heater 31
3	8	23	C16	C27 (Orange)	Pump 32
3	6	22	C17	C28 (White)	Heater 32
3	19	19	Ground	Ground (Black)	





The above figure is used for the connection between male and female DIN

Appendix N

Letter of Permission

Department of Biological and Agricultural Engineering,
Louisiana State University,
Baton Rouge, LA 70803.

February 25, 2009
Dr. Michael B. Timmons
Department of Biological and Environmental Engineering,
Cornell University,
302 Riley-Robb Hall,
Ithaca, New York, 14853

Dear Dr. Timmons,

I am currently a PhD candidate in the Department of Biological and Agricultural Engineering at Louisiana State University. I am preparing my dissertation and would like to request permission to reproduce material from your published book entitled "Recirculating Aquaculture Systems 2nd Edition" published and copyrighted by Cayuga Aqua Ventures. I look forward to hearing from you at your earliest convenience.

Sincerely,

Milton Saidu.

Vita

Milton Maada-Gomoh Saidu was born in Njala, Moyamba District, Sierra Leone, West Africa. He received a high school diploma from Bo Government Secondary School, Sierra Leone, West Africa. He received the degree of Bachelor of Science in physics with a minor in education and mathematics from University of Sierra Leone in 2001. As an undergraduate in his senior year, he taught as an intern physics teacher at a community college for one semester and also served as a Minister of Energy and Power in the student union government. He enrolled in the Louisiana State University graduate school master's program, in the College of Engineering in 2001, in the Department of Construction Management and Industrial Engineering. He will receive the degree of Doctor of Philosophy in the summer of 2009.