

ABSTRACT

GUERDAT, TODD C. A Large Scale Evaluation of Commercially Available Biological Filters for Recirculating Aquaculture Systems. (Under the direction of Dr. Thomas M. Losordo).

As aquaculture production systems increase in intensity to meet the demands of the marketplace, the need for environmentally friendly technology and management practices becomes increasingly important. The majority of previous studies have evaluated biological filter performance at the small, laboratory scale with artificial waste nutrients. Water from a commercial scale aquaculture facility may yield vastly different performance characteristics than those as determined previously in labs using artificial waste nutrients. Evaluating biological filters at the larger scale with aquaculture production-based nutrients is important in order to meet current and future industry requirements. Performance characteristics determined at the larger commercial scale would provide a more realistic model for proper sizing and selection of biofilters, thus aiding in the facility design process. There exist innate differences in water quality between commercial scale aquaculture production facilities and laboratory scale evaluations using artificial waste nutrients. As such, evaluations of biological filters at the commercial scale are needed. Total ammonia nitrogen (TAN) removal rates based on unexpanded biofilter media volume (volumetric TAN removal rate; VTR) offer a more realistic understanding of biological filter performance. VTR offers reporting of biofilter performance characteristics in a standardized form allowing simple comparisons between filter types. Greater accuracy of biological filter selection and sizing for commercial scale recirculating aquaculture systems (RAS) should result from evaluations conducted at the large production scale.

Three different commercially available biological filters were evaluated in triplicate on a 60 m³ tank-based Tilapia system under commercial warmwater growout conditions. This two-part study was performed at the North Carolina State University Fish Barn - a commercial scale research and demonstration recirculating aquaculture facility operated by the department of Biological and Agricultural Engineering. TAN removal rates were determined for the three types of biofilters for a range of TAN concentrations ranging from 0.13 to 1.20 g TAN m⁻³. For the first part of the study, TAN concentration was varied by feed rates and ammonium chloride additions, and limited by fish feeding response. Maximum feed rates were 65 kg feed d⁻¹ using a 40% protein diet. Average observed TAN removal rates (in g TAN/ m³ of unexpanded media per day $\pm$ standard deviation) for the three filters were 267.2 $\pm$ 123.3, 586.3 $\pm$ 284.2, and 667.4 $\pm$ 344.8 for the moving bed bioreactor, floating bead filter, and fluidized sand filter, respectively. These results are considerably lower than results previously published at the laboratory scale using artificial waste nutrients only.

Studies evaluating the impact of organic carbon on biological filters at the large-scale for aquaculture production are lacking. Understanding the performance characteristics of different biofilters under actual production conditions will be the only means by which recirculating system designers may properly select and size biological filters for commercial production use. Previous studies have determined the impact of organic carbon on biofilter performance at the small lab scale often using artificial waste nutrients in the evaluation. Evaluations under actual production conditions using real wastewater may produce vastly different results than previous lab scale studies using artificial nutrients. As such, the second part of this study is a preliminary step in evaluating the impact of organic carbon on

biological filters at the large scale under actual recirculating aquaculture production conditions. Daily TAN removal rates were determined based on biofilter media volume. Variability increased substantially in the volumetric TAN removal rate (VTR) for all three filters. VTR for all three filter types was reduced by 50% - 70%. The results demonstrate the importance of controlling the concentration of available organic carbon in a recirculating system.

The thesis presented here is a compilation of three papers written for submission to peer reviewed journals.

A Large Scale Evaluation of Commercially Available Biological Filters
for Recirculating Aquaculture Systems

by
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DEDICATION

The person to whom the real appreciation is due is my wife, Kate. Without her support, guidance, strength, understanding, and all around good lovin' I would never be where I am today. It is to her and our future as a family that I dedicate this and all future endeavors we both decide to undertake together.

BIOGRAPHY

Todd Guerdat grew up in Rochester, NY graduating high school in Penfield, NY. He then attended the University of North Carolina at Wilmington and Brunswick Community College graduating with a Bachelor of Science in Biology and an Associate of Science in Aquaculture, respectively. Upon the highly anticipated successful completion of the PhD program at North Carolina State University in Biological and Agricultural Engineering his goal is to further the aquaculture industry through sustainable, environmentally conscious and responsible practice and research. Todd feels that educating the farmers and general public alike is paramount to the success of such ambitious goals.

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Evaluating biological filtration performance in aquaculture: A review

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Abstract

Recirculating Aquaculture Systems (RAS) use biological filtration to reduce the concentration of ammonia and nitrite-nitrogen and make the recirculated water suitable for the cultured organisms. Biological filtration of aquaculture system water is one of the primary keys to reusing water in intensive tank-based aquaculture production systems. As aquaculture production systems increase in intensity to meet the demands of the marketplace, the need for environmentally friendly technology and management practices becomes increasingly important. The majority of previous studies have evaluated biological filter performance at the small, laboratory scale with artificial waste nutrients. Water from a commercial scale aquaculture facility may yield vastly different performance characteristics than those as determined previously in labs using artificial waste nutrients. Total ammonia nitrogen (TAN) removal rates determined in laboratory scale evaluations using ammonia as a sole substrate are higher than TAN removal rates in actual production conditions. Evaluating biological filters at the larger scale with aquaculture production-based nutrients is important in order to meet current and future industry requirements. Performance characteristics determined at the larger commercial scale would provide a more realistic model for proper

sizing and selection of biofilters, thus aiding in the facility design process. There exist innate differences in water quality between commercial scale aquaculture production facilities and laboratory scale evaluations using artificial waste nutrients. As such, evaluations of biological filters at the commercial scale are needed. Biofilter TAN removal rate is the primary performance characteristic of concern. TAN removal rates based on unexpanded biofilter media volume (volumetric TAN removal rate; VTR) offer a more realistic understanding of biological filter performance by providing a simpler comparison of TAN removed to volume of media required. Reporting of VTR as one standardized form of biofilter performance will allow simple comparisons between filter types. Greater accuracy of biological filter selection and sizing for commercial scale RAS should result from evaluations conducted at the large production scale. Large scale evaluations should also yield greater production, and fewer system failures. This paper reviews previous efforts to evaluate biological filter performance and highlights the need to perform these evaluations at the commercial scale using actual production based waste sources.

Keywords: **Biological filtration; Nitrification; Organic Carbon; Aquaculture**

1 . Introduction

With aquaculture as one of the fastest growing food producing sectors in the world and increasing pressures on fisheries, the industry has the potential to supply world market demands for seafood (FAO, 2005). As aquaculture production systems increase in intensity to meet the demands of the marketplace, the need for environmentally friendly technology

and management practices becomes increasingly important (Abeyasinghe et al., 1996; Timmons et al., 1998). Reducing waste effluent volume and concentration, reusing more water within the system, and containing and treating waste products all become larger issues as stocking densities and facility size increase. Recirculating aquaculture systems (RAS) rely heavily on biological filtration as a mechanism to reduce the effluent stream volume and make existing system water suitable for the cultured organisms (Losordo and Hobbs, 2000; Satoh et al., 2000; Chen et al., 2006).

Designers of intensive tank-based recirculating systems typically use one or more of numerous types of biological filter designs including trickling filters, submerged filters, fluidized bed filters, moving bed filters, and floating bead filters (van Rijn, 1996). Selecting which biological filter to use in RAS is dependent upon the culture organisms for which the system was designed and the potential system operator. A primary limiting factor in RAS is water quality (Losordo and Westers, 1994; Colt, 2006). In order to maintain the appropriate conditions within a system to meet the conditions required by the cultured species, a biological filter must be capable of processing the waste created by the cultured species. To correctly select the appropriate type and size of filter for the system, relevant and accurate filter performance design characteristics are required. The manufacturing and research communities alike struggle with the appropriate means by which filter performance is reported; as there are no standard conditions for aquaculture, there are no standard means of reporting performance characteristics (Colt et al., 2006; Drennan et al., 2006). Evaluations of biological filters are becoming more common. However biological filter performance studies are needed at the commercial scale since the majority of previous performance studies were

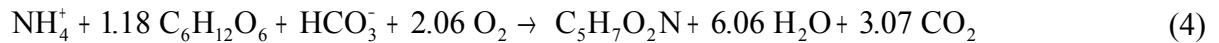
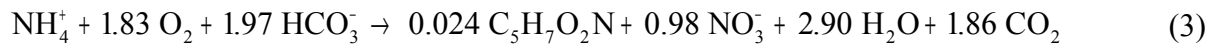
done at the lab or pilot scale. Differences between actual production culture conditions and laboratory conditions in terms of ammonia and organic carbon concentrations confound the understanding of actual operating characteristics for the biofilters evaluated. Knowing biological filter performance characteristics under actual production conditions will be the only means by which designers may properly select and properly size biological filters for commercial production facilities. The purpose of this paper is to review previous studies and establish the need for larger scale biological filter evaluations using actual production waste nutrients and reporting of such in standardized form.

2 . Biological Filtration Basics

2.1 Ammonia production and nitrification

Ammonia production has two main sources in RAS: cultured species and microbial processes. The primary source of ammonia is excretion by the cultured species as a direct result of metabolism. Additionally, to a lesser extent, aerobic heterotrophic oxidation of organic matter contributes to the ammonia production within RAS (Henze et al., 1995). Using biological filters, ammonia concentrations can be reduced through the nitrification process. Biological nitrification, the primary process occurring within biological filters, is an aerobic process by which autotrophic nitrifying bacteria oxidize ammonia-nitrogen ($\text{NH}_4^+\text{-N}$) to nitrate-nitrogen ($\text{NO}_3\text{-N}$) with nitrite-nitrogen ($\text{NO}_2\text{-N}$) as the intermediate component. The general half-reaction equations (Eqs. 1 and 2) show the basic conversions occurring in autotrophic nitrification (USEPA, 1984). When the chemical oxygen demand (COD) to

ammonia-nitrogen (N) ratio, COD/N is high enough, ammonia-nitrogen may also be consumed when organic waste products are assimilated by heterotrophic bacteria within the culture system and converted directly into microbial biomass (Ebeling et al., 2006; Schneider et al., 2007). Ebeling et al. (2006b) derived Eqs. 3 and 4 through stoichiometry to predict the autotrophic nitrification process summary and heterotrophic removal of ammonia-nitrogen in aerobic systems, respectively.



For every gram of ammonia-nitrogen ($\text{NH}_4^+\text{-N}$) converted to nitrate-nitrogen ($\text{NO}_3\text{-N}$) in the nitrification process, 4.18 g dissolved oxygen (DO) and 7.04 g alkalinity as CaCO_3 are consumed. As a result, 0.19 g of cellular biomass ($\text{C}_5\text{H}_7\text{O}_2\text{N}$) and 5.85 g carbon dioxide (CO_2) are produced. It is the first step (Eq. 1) of the nitrification process that produces hydrogen ions (H^+). Once in solution, CO_2 maintains equilibrium with carbonic acid (H_2CO_3). The combination of the two effectively makes nitrification an acid-producing process lowering the pH of the bulk solution. It is also worth noting that previous studies have shown the oxygen and alkalinity consumption, 4.57 g and 7.14 g respectively, to be slightly different than that which equation 3 predicts (Losordo and Westers, 1994; Timmons et al., 2002). Ammonia-nitrogen may also be consumed by heterotrophic bacteria when the

ratio of organic carbon to ammonia-nitrogen (C/N) is relatively high. By manipulating the C/N ratios in RAS it is possible to gain the added benefit of heterotrophic ammonia removal (Avnimelech, 1999). For every gram of ammonia-nitrogen converted to biomass, 15.17 g carbohydrates are required while 3.57 g alkalinity (as CaCO_3) and 4.71 g oxygen are consumed (Eq. 4). This results in the production of 8.07 g cellular biomass and 9.65 g CO_2 . That is a 12.7% (3% using 4.57 g O_2 consumption) increase in oxygen consumption with more than 40 times the biomass accumulation and 64.5% more CO_2 produced than autotrophic nitrification. Nitrification can be measured and quantified by the rate of removal of total ammonia nitrogen (TAN), the sum of NH_4^+ and NH_3 , from a system (Zhu and Chen 1999). The oxidation of ammonia effectively reduces the TAN concentration, part of which is toxic to fish (unionized ammonia; NH_3) (Meade, 1985), thereby providing a means for reuse of system water within a production system. Recirculating system designs must maximize the TAN removal rate and minimize the impact on the overall system. While using heterotrophic bacteria to remove the ammonia from the system consumes slightly less alkalinity, the overall process creates significantly more CO_2 requiring more alkalinity to serve as a pH buffer (e.g. sodium bicarbonate) and additional oxygen inputs, thus potentially increasing operating costs. CO_2 maintains equilibrium with carbonic acid (Eq. 5) in an aqueous solution. Increasing CO_2 production effectively lowers the pH of the bulk solution unless a suitable buffer is provided to maintain pH stability. It is also important to note that a biomass yield of more than 40 times that of autotrophic systems will create the additional need to manage sludge production. Currently, for the purposes of RAS it is more important to remove as much of the organic waste products as quickly as possible. In doing so, the

contribution by heterotrophic activity to the ammonia production will be minimized. Such an approach will also yield more efficient TAN removal by biological filters. Management strategies designed to promote autotrophic nitrification will maximize TAN removal, reduce biomass accumulation, minimize inputs, and maximize cost effectiveness.

Changes in the system will affect water quality parameters and potentially the ability of the biological filter to remove TAN. Operational changes may influence the performance of a biological filter. Typical changes in system operation include harvesting, changes in feed and feed rates, and changes in mechanical filtration. Inputs such as feed, inorganic carbon (e.g. bicarbonate), salt, etc., all have varying degrees of impact on the balance within the system. The variability (diurnally or hourly) of waste production affects the system water quality (Eding et al., 2006). Though feeding regimes vary by species, feeding schemes designed to reduce variability in the waste production will maintain more consistent water quality conditions. Creating conditions more similar to steady state will create a more stable biofilter. The TAN excretion rate is approximately 3% by weight of the daily feed rate (Wheaton et al., 1994). As feed inputs and/or protein content changes to maximize growth rates, the TAN production rate in the system will also increase. CO₂ production will also increase as a result of increased feed rates due to higher metabolism by the cultured species and heterotrophic bacteria within the system. As such, the rate of inputs such as oxygen and some form of alkalinity will be required to maintain oxygen levels within the filter or culture system and buffer the pH against changes. To reduce the amount of alkalinity required for pH buffering, degassing towers may be used to aid in the removal of CO₂ from the system water.

2.2 *Types of biological filters*

Two basic types of biological filters have been used in the recirculating aquaculture industry: suspended and attached growth. In a suspended growth filter, the microorganisms are freely suspended in the bulk solution providing direct contact with the solution and bacteria. Used extensively in wastewater treatment facilities, suspended growth systems are becoming more common in systems that depend on the dominance of heterotrophic bacteria in the water column. Attempts to incorporate suspended growth systems in intensive RAS have been made in the past. These systems, while more economical in design, are prone to potential washout and are neither as stable nor as capable of handling shock loading as an attached growth on a fixed biofilm system (Nogueira et al., 1998, 2002). These systems are finding uses with other cultured species such as marine shrimp in Active Suspension Ponds (ASP) (Avnimelech, 2006).

In an attached growth filter (also referred to as fixed-film), the bacteria create a layer, or biofilm on the media inside the filter. Once attached, the bacteria are immobile. These filters are more common in traditional “clear-water” recirculating systems (Chen et al., 2006; Malone and Pfeiffer, 2006). Fixed-film biological filters are available in a variety of forms for commercial aquaculture production purposes (Colt et al., 2006; Drennan et al., 2006). Malone and Pfeiffer (2006) categorize fixed-film filters into four main groups based on the means of oxygen delivery to the biofilm and handling of biofilm growth. These categories are: emerged, packed, expandable, and expanded. Emerged filters, typically trickling filters, are designed to maximize the oxygen availability to the biofilm. To allow for adequate oxygen availability, these filters have media with high porosity, thus relatively low specific

surface area per unit volume. This typically results in large reactor sizes. Packed bed filters are economical in nature and assume ample oxygen in the bulk solution is available. Water flows through a static bed of media usually comprised of rocks or shells. Such a design allows for biofouling and accumulation of solids if not managed properly. Expandable filters are characterized by an intermittent expansion of filter media. Such is the case with propeller washed and bubble washed bead filters. The media in the filters are static for a period of time and are periodically perturbed in such a way to control biofilm growth. Expanded beds, such as fluidized sand filters and moving bed reactors, utilize media which is constantly moving. In the case of a fluidized sand filter, the sand is fluidized by the water flowing up through the filter uniformly. This uniform flow effectively expands, or fluidizes the sand media. Media in a moving bed bioreactor is usually kept in motion with constant aeration.

Each group of filters has a set of advantages and disadvantages, depending on the system in which it could be used. Commercial scale RAS typically uses three of the four groups: trickling filters (emerged), floating bead filters (expandable), and moving or fluidized bed biofilters (expanded) (Losordo et al., 2000; Malone and Beecher, 2000; Summerfelt and Sharrer, 2004; Eding et al., 2006; Pfeiffer and Malone, 2006; Timmons et al., 2006). While packed beds are economical by design, they are often utilized in systems with low nutrient loading rates such as aquaria and crab shedding systems (Malone and Pfeiffer, 2006). Designs to improve performance and reduce biofouling and solids accumulation have been made, but packed bed filters generally are not appropriate for intensive aquaculture systems due to higher nutrient loading rates.

Each type of biological filter has different performance characteristics due to the unique design and variations in water quality parameters. The primary system conditions to consider are those that are important to the survival and growth of the cultured organism. Water quality parameters such as concentration of TAN and organic carbon, salinity, pH, temperature, dissolved oxygen, and alkalinity are among those to be considered when selecting a type and size of biological filter for a facility. Facility design characteristics such as maximum flow rate, TAN removal rate, head loss across the filter, physical size, as well as purchase price and operating cost are also important considerations when selecting the appropriate biofilters for the system.

2.3 Consideration of evaluation conditions

Losordo and Westers (1994) reported TAN as a primary limiting water quality factor in RAS. Evaluation of biological filters has commonly been performed at the laboratory scale under controlled conditions utilizing simulated aquaculture water (Losordo et al., 2000; Eding et al., 2006). Previous studies have focused on a specific set of water quality parameters such as total organic carbon (TOC) and TAN concentrations to determine the effect of the management and operation of biological filters, and ultimately aquaculture facilities. Lab scale evaluation studies should be viewed as precursors to larger pilot and commercial scale evaluations. Smaller test filters may have very different operating characteristics than large scale units due to differences in filter surface area to volume ratios. Nitrification occurring on the inside surface of the lab-scale biofilter container may contribute disproportionately to the overall nitrification process. As such, the biofilter performance may not scale linearly from small to large scale designs. Evaluation of

biological nitrification in an actual aquaculture facility under production feed rates and conditions will yield results more useful to designers of commercial scale systems. Using high TAN influent concentrations will yield higher TAN removal rates than those determined for lower influent TAN concentrations (Zhu and Chen, 1999, 2001; Losordo and Hobbs, 2000; Ling and Chen, 2005). TAN concentrations required in aquaculture operations often are lower than TAN concentrations that have been used in laboratory scale filter evaluations (Zhu and Chen, 1999; Losordo et al., 2000; Ling and Chen, 2005). Studies evaluating the rate of TAN removal using TAN as the sole substrate do not adequately represent actual production conditions and yield TAN removal rates different than those of actual production conditions. Ester et al. (1994) reported TAN removal rates for rotating biological contactors (RBC) for all three scales – lab, pilot, and commercial. The result was reduced nitrification performance as the size of the system and filter increased, respectively with commercial scale having the lowest TAN removal rate and lab scale showing the highest. Other studies have evaluated biofilter performance in industrial and domestic wastewater applications. The results of these studies are difficult to compare or use in aquaculture applications, since the TAN and organic carbon concentration in aquaculture facilities are significantly lower than in domestic or industrial applications (Zhu and Chen 1999, 2001; Losordo et al., 2000; Ling and Chen 2005).

Feed composition and assimilation by the cultured species will affect the TAN and organic carbon concentrations within the system. Typically, 20-30% of feed nutrients are assimilated by fish leaving the remainder for excretion and particulate accumulation within the system (Avnimelech, 2006). Uneaten feed particles that reside in the system too long

result in an accumulation of dissolved and particulate organic carbon. Organic carbon as low as 12 mg TOC / L indirectly reduces the nitrification rate (Abeyasinghe et al., 1996; Zhu and Chen, 2001; Nogueira et al., 2002; Tsuno et al., 2002; Ling and Chen, 2005; Michaud et al., 2006). A greater understanding of the effects of organic carbon on nitrification rate (at lower TAN concentrations) under actual aquaculture conditions would be of great benefit to designers and the industry. Currently, the primary means of removing organic carbon from the system is by mechanical filtration (Michaud et al., 2006). The means and efficiency by which solids removal is accomplished may have drastic effects on the overall system water quality. Reduced mechanical filtration efficiency will result in a direct reduction in TAN removal by the biological filter. Because organic carbon indirectly reduces the rate of TAN removal from the system, the practical implication is the need for larger biological filters in production systems (Leonard et al., 2002; Tsuno et al., 2002). From a short term perspective, maximizing solids removal from systems will yield higher TAN removal rates for biofilters and improved water quality conditions for the cultured organisms. Improving feed nutrient assimilation, feed composition and particle quality will provide long term improvements in water quality and possibly reduce initial costs and improve production.

Standards for RAS facility design do not exist. Actual production conditions are different from those in the laboratory studies. A review of the literature highlights the importance of evaluating biological filters in an environment more similar to an aquaculture production system, as the water from an actual aquaculture facility may yield very different performance characteristics than those determined previously in labs. Evaluating the TAN removal rate of commercially available biological filters in commercial scale aquaculture

production systems would also provide operational data useful in the selection and sizing of biological filters when designing aquaculture facilities.

3 . Environmental variables influencing biological filter performance

3.1 TAN

The effect of the TAN concentration on the TAN removal rate varies, depending on whether TAN is the sole substrate, or whether there are other influences such as excessive dissolved organic carbon. Zhu and Chen (1999) showed the modified Michaelis-Menten equation (Eq. 6) was sufficient in determining TAN removal in RAS when TAN was the sole substrate at low concentrations typical of RAS.

$$R = R_{\max} \frac{S - S_{\min}}{K_s + (S - S_{\min})} \quad (6)$$

Here, R is the removal rate of TAN (areal (m^2) or volumetric (m^3)) ($\text{mg}/\text{m}^x/\text{day}$), $R_{\max}$ is the maximum removal rate of TAN (mg/m^2 or m^3/day), S is the TAN concentration (mg/L), $S_{\min}$ is the minimum concentration required for nitrification (mg/L), and K_s is the half saturation constant for TAN (mg/L). The limitation of the minimum concentration required for nitrification to occur ($S_{\min} = 0.07 \text{ mg}/\text{L}$) gives rise to the need for the modification of the equation (Zhu and Chen, 1999).

When $S \ll K_s$, the equation may be simplified to a linear first order reaction model (Eq. 7) as determined by Ling and Chen (2005).

$$R = \frac{R_{\max}}{K_s}(S - S_{\min}) \quad (7)$$

Given sufficiently high TAN concentrations ($S \gg K_s$), equation 4 may be further simplified to a zero order reaction model (Wheaton et al., 1994). This means that the removal rate of TAN no longer increases as the TAN concentration is increased. As such, other factors become rate limiting. It is important to note that equations 4 and 5 were developed using synthetic ammonia as the sole substrate. The effects of other influences such as dissolved organic carbon are not considered in these equations.

TAN concentrations in commercial scale systems are typically significantly lower than those in lab scale studies, usually limited by the cultured species' tolerance. At the lower TAN concentrations (< 3.0 mg TAN / L) a linear relationship between TAN removal rate and TAN concentration exists in both lab and commercial scale systems (Ester et al., 1994; Ling and Chen, 2005). While each filter type may have a similar relationship between the TAN concentration and TAN removal rate, differences in system size and design as well as other variables confound the relationship for each type of biological filter. System variables such as organic carbon concentrations and means of mechanical filtration between lab and commercial scale studies create vastly different TAN removal rates, thus demonstrating the need for commercial scale evaluations of biofilter performance under

actual culture conditions (Ester et al., 1994; Malone and Beecher, 2000; Zhu and Chen, 2001).

3.2 *Organic carbon*

Organic carbon is derived from solid waste excreted by fish and also results from the degradation of uneaten feed and dead bacteria (Leonard et al., 2002; Steeby et al., 2004). Heterotrophic bacteria primarily degrade and metabolize dissolved and particulate organic matter. Autotrophic and heterotrophic bacteria occupy the space on biological filter media, thus creating a biofilm. The sharing of media surface causes competition for nutrients and oxygen between the two types of bacteria, resulting in a stratified biofilm structure (Nogueira et al., 2002). Faster growing heterotrophic bacteria ultimately occupy the outer layer of the stratified biofilm, where substrate concentration and detachment rates are both higher, resulting in the slower growing autotrophic nitrifying bacteria occupying the inner layer of the biofilm (Ohashi et al., 1995; Satoh et al., 2000; Nogueira et al., 2002; Lee et al., 2004; Chen et al., 2006; Michaud et al., 2006). Heterotrophic bacteria have been shown to have a maximum growth rate five times greater than that of autotrophic bacteria (Zhu and Chen, 2001). By covering the nitrifying bacteria, heterotrophic bacteria inhibit the diffusion of nitrogenous substrate and dissolved oxygen (DO) to the autotrophic nitrifying bacteria, thus negatively affecting the rate of nitrification (Nogueira et al., 2002; Chen et al., 2006). Higher levels of dissolved organic carbon-containing compounds result in decreased nitrification rates and associated undesirable affects on water quality (i.e., higher TAN concentrations).

Heterotrophic biomass and growth increase as the organic carbon to ammonia-nitrogen (C/N) ratio increases (Leonard et al., 2000; Zhu and Chen, 2001; Leonard et al., 2002; Nogueira et al., 2002; Ling and Chen, 2005; Michaud et al., 2006). Zhu and Chen (2001) demonstrated a 70% reduction in TAN removal rate at C/N = 1.0 as compared to C/N = 0. The same study showed no significant difference in reduction of the TAN removal rate in systems with C/N = 1.0 and 2.0. These results indicate that organic carbon has a significant impact on the TAN removal rate to a point, after which the effect is negligible. TAN removal rates from Zhu and Chen (2001) were similar to TAN removal rates observed in actual commercial scale operating conditions (Losordo et al., 2000). This implies that in actual production conditions organic carbon concentrations are high enough to effectively suppress the TAN removal rates in biofilters. By reducing the C/N ratio below the threshold, the TAN removal rate increases, thus providing water quality more suitable for aquaculture production (Zhu and Chen, 2001). It is also of note that Ling and Chen (2005) studied the effect of dissolved organic carbon expressed as a ratio of influent chemical oxygen demand (COD) to ammonia-nitrogen (N) and described the deleterious effects on nitrification in an exponential decay rate reaction model (Eq. 8) verifying a similar model developed by Carrera et al. (2004) (Eq. 9).

$$R = 0.67 + 2.27e^{(-1.38(\text{COD/N}))} \quad (8)$$

$$R_{\text{nitrification}} = 0.0323 + 0.334e^{(-1.66(\text{COD/N}))} \quad (9)$$

Here, R is the reaction rate of TAN removal ($\text{mg}/\text{m}^2/\text{day}$) (Eq. 8). Eq. 9 was developed using $10 \text{ mg TAN} / \text{L}$, a concentration significantly higher than that in commercial RAS. Caution must be taken to prevent over-predicting nitrification capacity when using this equation. Corrections should be made concerning substrate effects on nitrification for commercial scale purposes.

Heterotrophic bacteria concentrations are also linked to the amount of organic matter contained within the overall system. Feces, uneaten feed particles, and other sources of organic carbon should all be removed from the process flow prior to entering the biological filter. Leonard et al. (2002) found that the most effective means of eliminating heterotrophic bacteria from the system was to remove the particulate organic matter (POM) by way of mechanical filtration. Failing to remove the POM from the waste stream before the biological filter will result in accumulation in the biofilter, and heterotrophic bacterial growth will increase and successfully out-compete the autotrophic bacteria on the biofilter media (Leonard et al., 2002). Placement of the mechanical filter directly before the biological filter will result in maximum POM removal, ultimately improving the biological filter nitrification rate. An additional benefit of RAS is the potential to improve the feed conversion ratio (FCR), thus reducing organic waste and ultimately reducing heterotrophic bacteria concentrations (Losordo et al., 1998; Leonard et al., 2002). Reducing the organic carbon concentration in the system requires a system-wide approach. From using better quality feeds to proper selection and maintenance of mechanical filtration, the reduction of the organic carbon concentration in the system will yield more efficient RAS production.

3.3 *Alkalinity and pH*

Alkalinity, the primary source of inorganic carbon for autotrophic nitrifying bacteria, and pH are important factors that can affect the rate of nitrification (USEPA, 1985; Biesterfeld et al., 2003b). For every gram of TAN oxidized to nitrate, 4.18 g oxygen and 7.04 g alkalinity are consumed (Eq. 3). Carbonate alkalinity concentrations greater than 40 mg/L (as mg CaCO_3 / L) should be maintained to ensure the nitrification process occurs (Biesterfeld et al., 2003b). Alkalinity concentrations greater than 200 mg L^{-1} as CaCO_3 are recommended as a buffer for pH and to meet carbonate requirements of the nitrifying bacteria (Chen et al., 2006). Alkalinity additions are part of the daily inputs for intensive RAS that do not have an integrated denitrification system. As such, various sources of inorganic carbon are available, though sodium bicarbonate (NaHCO_3) and calcium carbonate (CaCO_3) are the most frequently used commercially. Each serves as both a source for inorganic carbon and pH buffer. Sodium bicarbonate is commonly used as a more conservative, safe alternative to calcium carbonate as the impact on the pH is not as rapid, therefore providing for a margin of error for alkalinity additions (Chen et al., 2006).

Optimum nitrification occurs between pH 6.7 and 7.0 for a TAN concentration of 0.37 mg/L, and pH 7.5 and 8.0 for a TAN concentration of 5.0 mg/L (Groeneweg et al., 1994). This implies that for optimum TAN removal rates in aquaculture production biofilters, the pH should not fall out of the ideal 6.7 – 7.0 range as desired TAN concentrations for aquaculture production systems are < 3.0 mg/L (Losordo et al., 2000; Ling and Chen, 2005). Maintaining a constant pH and alkalinity concentration in a production system becomes challenging when feed rates and biofilter performance may be pushed to the

system limits. Both H^+ ions (Eq. 1) and carbon dioxide (CO_2) (Eq. 3) are products of the nitrification process and play a role in the lowering of pH. To maintain pH levels above 6.7, a buffer is required to restore and maintain the pH to the appropriate range so as to prevent biofilter TAN removal efficiency inhibition.

3.4 *Effect of temperature*

The influence of temperature on nitrification in a fixed biofilm is more complicated than that of a suspended growth (Zhu and Chen, 2002). In a suspended growth, optimal biological reaction rates are observed as the system reaches an optimum temperature, above which rates decrease due to the denaturing of enzymatic proteins (Sawyer et al., 1994; Henze et al., 1995). A Monod-type relationship for steady-state substrate removal in a suspended growth under a single limiting substrate condition is generally described as:

$$R = \mu_{\max} \left(\frac{X_B}{Y_s} \right) \left(\frac{S}{K_s + S} \right) \quad (10)$$

where R is the rate of substrate removal ($g / m^3 / day$), $\mu_{\max}$ is the maximum specific growth rate ($1 / day$), X_B is the bacterial mass concentration ($g \text{ cell} / m^3$), Y_s is the bacterial mass yield per unit consumed substrate ($g \text{ cell} / g \text{ substrate}$), K_s is the half saturation constant (g / m^3), and S is the limiting substrate concentration (g / m^3) (Henze et al., 1995). By incorporating the equation used for estimating temperature effects on biological reaction

rates, the substrate removal rate (Eq. 10) can be corrected for temperature effects on biological reaction rates in a suspended growth (Henze et al., 1995).

$$\mu_{\max,20}(T) = \mu_{\max,20} e^{(\kappa(T-20))} \quad (11)$$

$$R = \mu_{\max,20} e^{(\kappa(T-20))} \left(\frac{X_B}{Y_S} \right) \left(\frac{S}{K_S + S} \right) \quad (12)$$

Here, $\mu_{\max}(T) = \mu$ at temperature T , $\mu_{\max,20} = \mu$ at 20 °C (1 / day), κ is the temperature coefficient, and T is the temperature (°C).

Addressing the effects of temperature, however, on a fixed film bioreactor is much more difficult. Equations to predict the effects of temperature and possible TAN and oxygen limitation for fixed film filters in RAS are more complicated as compared to suspended growth (Zhu and Chen, 2002). Nitrification kinetics in a fixed film reactor are subject to other temperature dependent phenomena and parameters (Fdz-Polanco et al., 1994). Zhu and Chen (2002) demonstrated that diffusional mass transport was pivotal to nitrification in a fixed film. The same study also showed that the impact of temperature on nitrification in a fixed film filter was less significant than estimated by Eq. 11. Ling and Chen (2005) also showed that the TAN removal rate was not significantly affected by temperature in cool water aquaculture systems (15 and 20 °C).

The dissolved oxygen (DO) may become a limiting factor in higher temperature systems due to the diffusion process limitation of mass flux into the fixed biofilm (Zhu and Chen, 2002). In stratified biofilms, competition for DO exists between the inner layer of autotrophic nitrifiers and outer layer of heterotrophic bacteria. The DO saturation

concentration in water decreases as temperature increases. As the thickness of the outer layer of the biofilm increases, diffusion of oxygen is inhibited by both the biofilm thickness and the additional consumption by the heterotrophs. These layers of heterotrophic bacteria reduce the mass flux of nutrients through the biofilm to the nitrifiers on the bottom of the stratified biofilm layer. Increased temperatures may further facilitate the layering effect of heterotrophic bacteria over the nitrifying autotrophs in systems with concentrations of increased organic carbon. When the nitrification rate in a fixed film filter is affected by oxygen limitation, temperatures between 14 and 27 °C is not a limiting factor on nitrification performance (Zhu and Chen, 2002). Thus, attention must be paid to organic carbon concentrations and heterotrophic interference in future evaluations of biological filters. Since commercial RAS operates at low TAN concentrations and high feed rates, the resulting organic loads play a much larger role in biofilter performance than temperature. The temperature range at which the system is operated still remains dependent upon culture species.

3.5 *Carbon dioxide*

Carbon dioxide (CO₂) production is a direct result of both microbial and macrobiological metabolism. The rate of metabolisms of both the cultured species and bacteria within the production system are not static. Metabolic activity is proportionate to feed rates. Any increase in metabolism will have a concurrent increase in the rate of oxygen utilization and CO₂ production. Unless the system is designed to maintain CO₂ concentrations at acceptable levels, the concentration within the system will rise with

corresponding higher rates of production. In a commercial scale recirculating salmonid culture system using fluidized sand filters, Summerfelt and Sharrer (2004) found that the biological filters were responsible for as much as 37% of the CO₂ production in the system. The implication of such is the need for CO₂ stripping directly after the biological filters as that will be the location at which the CO₂ concentration is the highest. Two exceptions to this are trickling filters and rotating biological contactors (RBC). Trickling filters may be designed to maximize CO₂ removal from the bulk solution. As the water trickles down through the filter media it degasses CO₂ (Eding et al., 2006). Ventilating the filter media by pumping air through the media stack from the bottom will effectively strip CO₂ from the system as it is degassed. Brazil (2006) demonstrated an average removal of CO₂ of 39% from the influent water using an RBC at the large production scale.

As previously mentioned, dissolved CO₂ maintains equilibrium with carbonic acid (H₂CO₃), effectively lowering the pH of the bulk solution. The effect of CO₂, coupled with the acid-producing process of nitrification requires higher alkalinity concentrations to maintain pH and carbonate concentration. Maintaining a commercial scale RAS at the limits of design is a tight balance of environmental conditions and inputs. Having a sufficiently high alkalinity concentration will help provide more stable operating conditions in the event of a possible system change.

4 . Reporting of evaluation studies results

4.1 Standardization

There exists a need for more realistic biofilter evaluation conditions as well as standards by which the results of such evaluations are reported and benchmarked. Drennan et al. (2006) noted that biofilter manufacturers report the performance characteristics of their respective filters in whichever way they deem appropriate. This has created individual testing and reporting criteria by each manufacturer for the purpose of evaluation and reporting of performance to the consumer. Commercial aquaculture is multifaceted, having a wide variety of system types as required by the cultured species and design limitations. A classification structure by which each system type may be described will provide a means of standardized comparison for facility operators, manufacturers and the research community alike. Malone and Pfeiffer (2006) suggested a biofilter rating system based on trophic levels and pH. The proposed system uses three main trophic levels with varying degrees of each as the basis by which volumetric TAN removal (VTR) is reported: oligotrophic (nutrient limited), mesotrophic (moderate nutrient limited), and eutrophic (nutrient enriched). Table 1 takes into account both ornamental and commercial food species production systems.

Table 1

A six-tiered biofiltration classification is suggested to serve the needs of the recirculating aquaculture community (it is based, primarily, on trophic levels; a specialized category was established to serve the acid oligotrophic needs of the freshwater ornamental fish industry) (Malone and Pfeiffer, 2006).

Class	Application	TAN/nitrite performance
		range (g-N m ⁻³)
Ultra-oligotrophic	Larval	0.0 – 0.1
Acidic-oligotrophic	Ornamental	0.1 – 0.3
Oligotrophic	Broodstock	0.1 – 0.3
Mesotrophic	Fingerling	0.3 – 0.5
Eutrophic	Growout	0.5 – 1.0
Hypereutrophic	Hardy growout	1.0 – 5.0
Acidic-hypereutrophic	Hardy growout	1.0 – 20

Given the wide variety of biofilter designs available in the industry today, and the differences associated with each system design, the time is now for the aquaculture community to develop standards for evaluation and reporting of biofilter performance.

4.2 Volumetric TAN conversion rate (VTR)

Perhaps one of the most important criteria to measure and report is the removal rate of TAN from system water by biofilters. Many studies discuss and report nitrification rates based upon media specific surface areas (Zhu and Chen, 1999, 2001; Ling and Chen, 2005; Eding et al., 2006; Chen et al., 2006). Biofilter media has been traditionally valued by its inherent specific surface area (SSA), with greatest value placed on the highest SSA. The purpose to such valuation is based on the fact that bacteria grow on the surface of the media. The theory behind such valuation is that the greater the SSA, the more bacteria are supported ideally translating to the most amount of TAN removed. In practice, the bacteria create a

biofilm that effectively covers the media, possibly layering over structural and topographical features of the media designed to increase surface area. This covering of the media topography essentially creates new media topography, reducing the actual media surface area used by the bacteria. As an example, expandable bed filters such as floating bead filters act as both packed and expanded bed filters based on the intermittent perturbation of the bed through backwashing. Such a design allows for possible covering of multiple beads in the bed reducing the amount of media surface used. Such limitations must be addressed by backwash frequency. As a result of reduced media surface area usage, manufacturers have begun to report the available surface area in addition to the SSA as an attempt to better report the actual performance of the media in a biological system. Given the right conditions, the heterotrophic bacteria may create enough biomass to effectively clog a biological filter (Michaud et al., 2006). Reporting the actual surface area used by the bacteria may predict more realistic expectations of media performance. Determining such information however would be almost impossible. Recently, it has been suggested that the reporting of biofilter nitrification rate measurements be based upon ammonia removal per unit of unexpanded biofilter media volume (Malone and Beecher, 2000; Drennan et al., 2006; Malone and Pfeiffer, 2006). The size of commercial scale biological filters becomes an important aspect in facility design and is a function of the volume of media contained within. In an effort to report biofilter performance in terms of TAN removal incorporating filter size, media volume, and actual media surface area used, it becomes apparent that reporting performance based on media volume would serve as the most informative means.

The volumetric TAN conversion rate (VTR) is the rate at which TAN is removed from the bulk solution through the biological filter and converted to either nitrite or nitrate-nitrogen. It can be used as one standardized measure for the performance of a biological filter. Malone and Beecher (2000) define VTR as:

$$\text{VTR} = \frac{(K_c)(Q_r)}{V_b}(\text{TAN}_I - \text{TAN}_E) \quad (13)$$

where K_c is the conversion factor ($1.44 \text{ min} \cdot \text{hr} \cdot \text{L}^{-1}$), Q_r is the flow rate through the filter (L/min), V_b is the total unexpanded volume of the filter media (m^3), and TAN_I and TAN_E are influent and effluent TAN concentration, respectively.

The individual nature of reporting biofilter performance characteristics as described above may not clearly answer the important questions of filter performance and might make it difficult for the consumer to make comparisons. Researchers and manufacturers alike have reported nitrification performance in terms of areal or volumetric TAN removal rate (ATR or VTR, respectively), or both. While both provide useful information, a means of reporting TAN removal performance in terms of media volume may be more realistic. It is likely that the amount of usable surface area on a given piece of biological filter media is reduced. The biofilm not only covers the media, but also stratifies in the presence of organic carbon, effectively covering the autotrophic nitrifying bacteria and creating a thicker biofilm. Given these effects, VTR will yield a more realistic means of reporting the biological filter performance. The volume of media is the primary factor used to describe the size of the filter

by the manufacturer and may be the most appropriate means of determining the proper size for a particular system. Colt et al. (2006) and Drennan et al. (2006) both suggest that VTR needs to be reported for different trophic operating conditions. Having the proper means of describing biofilter TAN removal rate is only the first of many steps in standardizing the reporting performance characteristics as described in greater detail in Colt et al. (2006) and Drennan et al. (2006).

5. Conclusions

The evaluation of biological filters at a large scale under commercial production conditions is the most effective means of establishing the performance characteristics of commercially available biofilters. Such evaluations have larger implications leading to reporting standards, design characteristics, and potential cost savings. Evaluating commercially available biofilters under standard trophic conditions will be most useful in creating a knowledge base of biological filter performance characteristics for the use of design and operation of RAS.

Results from the literature support the following conclusions:

- Future research should be conducted at a scale more similar to that of a large scale commercial RAS facility.
- Waste nutrient concentrations and composition used in lab scale evaluations are typically not representative of actual commercial RAS operating conditions. Biofilter performance at TAN and organic carbon concentrations indicative of actual production conditions are required for more realistic performance reporting. Organic waste composition must also

be more adequately represented in future evaluations to replicate actual production waste produced.

- The removal of particulate organic carbon will reduce the heterotrophic bacteria within a system, thus positively affecting biofilter nitrification performance.
- The effect of organic carbon has a greater effect on biofilter performance than temperature. As such, RAS designs must incorporate effective means of mechanical filtration to reduce organic carbon concentrations.
- Developing a standard by which performance of biological filters may be reported and compared is essential to the overall standardization push by the manufacturing and research communities.
- Evaluation studies reporting TAN removal rate in terms of VTR will yield a more realistic means of understanding biological filter nitrification performance characteristics.
- Proper maintenance of RAS yields improved water quality parameters. As such, the volume of the effluent stream from the facility and the potential environmental impact are reduced, as more system water is renovated and reused.

References

- Abeyasinghe, D.H., Shanableh, A., Rigden, B., 1996. Biofilters for water reuse in aquaculture. *Water Sci. and Tech.*, 34(11 pt 7), 253-260.
- Biesterfeld, S., Farmer, G., Russell, P., Figueroa, L., 2003. Effect of alkalinity type and concentration on nitrifying biofilm activity. *Water Env. Res.*, 75(3), 196-204.

- Carrera, J., Vicent, T., Lafuente, J., 2004. Effect of influent COD/N ratio on biological nitrogen removal (BNR) from high-strength ammonia industrial wastewater. *Process Biochem.* 39, 2035–2041.
- Chen, S., Ling, J., & Blancheton, J., 2006. Nitrification kinetics of biofilm as affected by water quality factors. *Aquacult. Eng.*, 34(3), 179-197.
- Colt, J. (2006). Water quality requirements for reuse systems. *Aquacult. Eng.*, 34(3), 143-156.
- Colt, J., Lamoureux, J., Patterson, R., & Rogers, G., 2006. Reporting standards for biofilter performance studies. *Aquacult. Eng.*, 34(3), 377-388.
- Drennan II, D.G., Hosler, K. C., Francis, M., Weaver, D., Aneshansley, E., Beckman, G., et al. 2006. Standardized evaluation and rating of biofilters. II. manufacturer's and user's perspective. *Aquacult. Eng.*, 34(3), 403-416.
- Ebeling, J.M., Timmons, M.B., Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. *Aquaculture* 257, 346-358.
- Eding, E.H., Kamstra, A., Verreth, J.A. J., Huisman, E.A., Klapwijk, A., 2006. Design and operation of nitrifying trickling filters in recirculating aquaculture: A review. *Aquacult. Eng.*, 34(3), 234-60.

- Groeneweg, J., Sellner, B., & Tappe, W., 1994. Ammonia oxidation in nitrosomonas at NH_3 concentrations near K_m : Effects of pH and temperature. *Water research*, 28(12), 2561-2566.
- Henze, M., Härremoes, P., Jansen, J.la C., Arvin, E., 1995. *Wastewater Treatment: Biological and Chemical Processes*. Springer Verlag, Berlin, Heidelberg, Germany, 383 pp.
- Losordo, T.M., Westers, H., 1994. System carrying capacity and flow estimation. In: Timmons, M.B., Losordo, T.M. (Eds.), *Aquaculture Water Reuse Systems: Engineering Design and Management*. Elsevier, pp. 9–60.
- Losordo, T.M., Hobbs, A.O., 2000. Using computer spreadsheets for water flow and biofilter sizing in recirculating aquaculture production systems. *Aquacult. Eng.*, 23(1), 95-102.
- Losordo, T.M., Hobbs, A.O., DeLong, D.P., 2000. The design and operational characteristics of the CP&L/EPRI fish barn: a demonstration of recirculating aquaculture technology. *Aquacult. Eng.* 22, 3–16.
- Malone, R.F., Beecher, L.E., 2000. Use of floating bead filters to recondition recirculating waters in warmwater aquaculture production systems. *Aquacult. Eng.*, 22(1), 57-73.
- Malone, R.F., Pfeiffer, T.J., 2006. Rating fixed film nitrifying biofilters used in recirculating aquaculture systems. *Aquacult. Eng.*, 34(3), 389-402.

- Meade, J.W., 1985. Allowable ammonia for fish culture. *Progressive Fish-Culturist*, 47, 135-145.
- Nogueira, R., Lazarova, V., Manem, J., Melo, L.F., 1998. Influence of dissolved oxygen on the nitrification kinetics in a circulating bed biofilm reactor. *Bioprocess Eng.* 19, 441.
- Nogueira, R., Melo, L.F., Purkhold, U., Wuertz, S., Wagner, M., 2002. Nitrifying and heterotrophic population dynamics in biofilm reactors: Effects of hydraulic retention time and the presence of organic carbon. *Water research*, 36(2), 469-481.
- Pfeiffer, T., Malone, R., 2006. Nitrification performance of a propeller-washed bead clarifier supporting a fluidized sand biofilter in a recirculating warmwater fish system. *Aquacult. Eng.*, 34(3), 311-321.
- FAO, 2005. Review of the state of the world marine fishery resources, 2005. FAO, Rome.
- Satoh, H., Okabe, S., Norimatsu, N., & Watanabe, Y., 2000. Significance of substrate C/N ratio on structure and activity of nitrifying biofilms determined by in situ hybridization and the use of microelectrodes. *Water Sci. and Tech.*, 41, 317-321.
- Sawyer, C.N., McCarty, P.L., Parkin, G.F., 1994. *Chemistry for Environmental Engineering*, 4th ed.. McGraw-Hill, New York, p. 658.
- Steeby, J.A., Hargreaves, J.A., Tucker, C.S., Kingsbury, S., 2004. Accumulation, organic carbon and dry matter concentration of sediment in commercial channel catfish ponds. *Aquacult. Eng.*, 30(1), 115-126.

- Summerfelt, S.T., Sharrer, M.J., 2004). Design implication of carbon dioxide production within biofilters contained in recirculating salmonid culture systems. *Aquacult. Eng.*, 32(1), 171-182.
- Timmons, M.B., Summerfelt, S.T., Vinci, B.J., 1998. Review of circular tank technology and management. *Aquacult. Eng.*, 18(1), 51-69.
- United States Environmental Protection Agency (US-EPA), 1984. Methods for the Chemical Analysis of Water and Wastewater, EPA-600/4-79-020. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory, Cincinnati, OH.
- van Rijn, J., Tal, Y., Schreier, H.J., 2006. Denitrification in recirculating systems: Theory and applications. *Aquacult. Eng.*, 34(3), 364-376.
- Wheaton, F.W., Hochheimer, J.N., Kaiser, G.E., Malone, R.F., Krones, M.J., Libey, G.S., Easter, C.C., 1994. Nitrification filter design methods. In: Timmons, M.B., Losordo, T.M. (Eds.), *Aquaculture Water Reuse Systems: Engineering Design and Management*. Elsevier, Amsterdam, pp. 101–171.
- Zhu, S., Chen, S., 1999. An experimental study on nitrification biofilm performances using a series reactor system. *Aquacult. Eng.*, 20(4), 245-259.
- Zhu, S., Chen, S., 2002. The impact of temperature on nitrification rate in fixed film biofilters. *Aquacult. Eng.*, 26(4), 221-237.

A large scale evaluation of commercially available biological filters for recirculating aquaculture systems

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Abstract

Three different commercially available biological filters were evaluated in triplicate on a 60 m³ tank-based Tilapia system under commercial warmwater growout conditions. The study was performed at the North Carolina State University Fish Barn - a commercial scale research and demonstration recirculating aquaculture facility operated by the department of Biological and Agricultural Engineering. TAN removal rates were determined for the three types of biofilters for a range of TAN concentrations ranging from 0.13 to 1.20 g TAN m⁻³. TAN concentration was varied by feed rates and ammonium chloride additions, and limited by fish feeding response. Maximum feed rates were 65 kg feed d⁻¹ using a 40% protein diet. Average observed TAN removal rates (in g TAN/ m³ of unexpanded media per day $\pm$ standard deviation) for the three filters were 267.2 $\pm$ 123.3, 586.3 $\pm$ 284.2, and 667.4 $\pm$ 344.8 for the moving bed bioreactor, floating bead filter, and fluidized sand filter, respectively. These results are considerably lower than results previously published at the laboratory scale using artificial waste nutrients only. This study highlights the need for future biofilter evaluations at the commercial scale using real aquaculture waste nutrients.

Keywords: Biological filtration; Nitrification performance; Aquaculture

1 . Introduction

Aquaculture production accounts for almost 50% of the seafood consumed worldwide (FAO, 2005). As the aquaculture industry continues to grow in response to demand for increased seafood production, the need for environmentally conscious operational practices and facility designs becomes more important (Abeyasinghe et al., 1996; Timmons et al., 1998). Reducing the volume of the effluent stream and reusing more water within the culture system are primary issues for recirculating aquaculture systems (RAS) as stocking densities and facility size increase. Recirculating aquaculture systems rely heavily on biological filtration as a mechanism to reduce the effluent stream volume and make existing system water suitable for the cultured organisms (Losordo and Hobbs, 2000; Satoh et al., 2000; Chen et al., 2006).

Water quality is a key limiting factor in RAS (Losordo and Westers, 1994; Colt, 2006). As a result of metabolism, ammonia is directly excreted by the cultured species. In solution, ammonia maintains equilibrium between an ionized (NH_4^+) and unionized (NH_3) form. Unionized ammonia-nitrogen (NH_3) is toxic to most aquacultured aquatic organisms and must be controlled within the production system (Mead, 1985). The concentrations of each are dependent upon pH and temperature (Henze et al., 1995; Hargreaves and Tucker, 2004). Biological filters are used to reduce the total ammonia nitrogen (TAN) concentration (the sum of NH_4^+ and NH_3). Nitrification, the biological process oxidizing ammonia to nitrate with nitrite as an intermediate component, effectively reduces the TAN concentration.

Biological nitrification is the main process occurring within biological filters. By reducing the TAN concentration, biological filters provide a means for reuse of system water within a production system. The concentration of TAN in the bulk solution is a key limiting factor in RAS (Losordo and Westers, 1994). Recirculating system designs must maximize the TAN removal rate to minimize the impact of TAN on the overall system. Biological filters with high TAN removal rates are an effective means of reducing the impact of TAN in RAS. In order to meet the increasing intensity of aquaculture production facilities, biological filters with superior performance characteristics are required. To date, the majority of biological filter performance evaluations have been performed at the small, laboratory scale under conditions not adequately representative of actual production conditions (Losordo et al., 2000; Eding et al., 2006). Biofilter evaluations at the larger pilot or commercial scales using actual waste nutrients will yield results more pertinent to actual aquaculture production conditions (Ester et al. 1994; Losordo et al., 2000; Brazil, 2006; Chen et al., 2006). TAN and organic carbon concentrations in commercial scale systems are typically considerably different than those in lab scale studies. TAN concentrations in commercial scale RAS are usually limited by the cultured species' tolerance and lower than those used in lab scale studies (Zhu and Chen, 1999; Losordo et al., 2000; Ling and Chen, 2005). Organic carbon concentration is related to feed assimilation and solids removal efficiency. The inhibitory effect of organic carbon on nitrification has been studied at the small scale and is well accepted (Zhu and Chen, 2001; Nogueira et al., 2002; Ling and Chen 2005; Michaud et al., 2006). The impact of organics on biofilter performance at the large production scale has not been studied as extensively. A comparison of organic carbon concentrations and the

associated TAN removal rates between lab scale studies and actual production conditions is needed. Additionally, large scale evaluations of filter performance will provide actual maintenance and operational characteristics unique to individual filter types. Understanding the associated operational and required maintenance characteristics of different filters is an important part of the selection process for facility operators and designers alike.

Several recent publications have begun to stress reporting standards for biofilter evaluations. Reporting biological filter performance characteristics in a standardized form is important in order to provide information to the industry for biofilter sizing and selection (Colt et al., 2006; Drennan et al., 2006). Standards such as TAN and nitrite conversion rates based on media volume will facilitate a simple and effective means for direct comparison. Volumetric TAN conversion rate (VTR) and volumetric nitrite conversion rate (VNR) have been proposed recently in the literature and hold great value in terms of creating reporting standards. Biofilter media is currently valued by its inherent specific surface area (SSA), with greatest value placed on the highest SSA. The reason for such valuation is based on the fact that bacteria create a visco-elastic layer, or biofilm on the surface of the media. The theory behind such valuation is that the greater the SSA, the more bacteria are supported ideally translating to the most amount of TAN removed. In practice, the bacteria create a biofilm that effectively covers the media, layering over structural and topographical features of the media designed to increase surface area. This covering of the media topography essentially creates new media topography, reducing the actual media surface area used by the bacteria. Estimating nitrification rates based on theoretical surface area can often be misleading. Substrate conversion rates based on the unexpanded volume of biofilter media

will provide a more realistic rate of conversion possible by the same media. Standardizing operating condition definitions will also provide data more useful to the industry as operating conditions are wide ranging by design. Malone and Pfeiffer (2006) suggested a biofilter rating system based on trophic levels and pH. The proposed system uses three main trophic levels with varying degrees of each as the basis by which volumetric TAN conversion (VTR) is reported: oligotrophic (nutrient limited – hatchery or broodstock systems), mesotrophic (moderate nutrient limited – fingerling systems), and eutrophic (nutrient enriched – growout systems). Agreement for both operating conditions and reporting standards within the research and manufacturing communities is imperative. Future studies, especially those focused on aquacultural engineering, should begin to report data according to these proposed reporting standards.

This study was conducted to investigate performance characteristics for three different types of commercially available biological filters under commercial warmwater aquaculture growout (eutrophic) conditions. The best fit statistical model suggested in this study is based on statistical analysis of two different variables typically used in reporting and predicting VTR at low TAN concentrations typical of commercial scale aquaculture.

2 . Materials and Methods

2.1 Experimental setup

This study was conducted at the commercial scale under actual production conditions. It should be noted that conditions in the production system environment are in constant flux. Such variability in operating conditions is mainly due to variation in feed rates based on

feeding response and maintaining water quality guidelines. As such, steady state conditions are difficult to attain. Samples were taken over a 180 day period and data used for this study were selected from the dataset based on pseudo-steady state conditions defined as constant feed rates for at least seven days and relatively stable TAN concentrations for at least three days. A return to pseudo-steady state conditions required time intervals as long as two weeks after changes in system operating conditions were implemented.

2.1.1 Culture system and biomass loading

The North Carolina State University Fish Barn was the site for this study. This facility is a commercial scale indoor recirculating aquaculture research and demonstration facility operated by the North Carolina State University department of Biological and Agricultural Engineering. A detailed review of the system design can be found in Losordo et al. (2000). One of two culture tanks was used as the main system in which *Tilapia* were grown as a source of waste nutrients. The culture tank was stocked with an average of approximately 5,000 fish during the study period. A total of three *Tilapia* cohorts were grown in the system during the time the filters were in operation, though only two cohorts were grown during the data collection period. The first cohort was used to condition the filters. The initial biomass for the first cohort was 1,115 kg, and the final biomass was 3,050 kg. The second cohort was grown for approximately two months once data collection began with a starting biomass of 2,470 kg and harvested at a biomass of 2,764 kg. The second cohort was affected by an illness which affected the final biomass as fish were lost to disease. The third cohort was moved into the system immediately after harvesting of the second with a

starting biomass of 2,531 kg and a final biomass of 5,500 kg. Daily feed rates were based on average fish biomass and increased incrementally to support maximum growth rates. A floating feed with 40% protein content was used throughout the entire study.

2.1.2 Filter system

As described by Losordo et al. (2000), water flowed out of the culture tanks and was mechanically filtered through a drum screen filter with 40 micron screens. The water flowed from the drum screen filter to a pumping sump from which nine biological filters were supplied (Fig. 1). Filter size for this study was based on the estimated volumetric nitrification capacity. The original design feed rates for a two tank system at the NCSU Fish Barn was approximately 100 kg d⁻¹ of 38% protein feed. The TAN excretion rate is typically estimated as 3% of the daily feed rate by weight (Wheaton et al, 1994). We estimated that the TAN produced would be removed over the nine filters evenly. Filter size was determined using manufacturer reported TAN removal rates. The TAN concentration was increased over the study period to provide TAN removal rates over a range of TAN loading rates.

This study evaluated the operational characteristics of three different types of biological filters, each type tested in triplicate. The filter types that were evaluated are listed below:

1. The Clearwater™ Low Space Bioreactor, model LSB25 (LSB; Aquatic Ecosystems, Inc., FL, USA). Each filter contained 0.71 m³ of Kaldnes biofilter media (Kaldnes North America Inc., RI, USA). Air is supplied to the filters through an octopus diffuser system at an average rate of 100.5 L/min. The low pressure air served as a

source for aeration and mixing, effectively operating each filter as a CSTR (completely stirred-tank reactor).

2. The PolyGeyser[®] Floating Bead Drop Filter, model DF15 (PG; Aquaculture Systems Technologies, LA, USA). Each filter contained 0.425 m³ of floating polyethylene beads. Air was supplied to the three filter air chambers at an average rate of 6.6 L min⁻¹. This rate of air flow provided for approximately thirteen backwash cycles per day for each filter.
3. The CycloBio[®] Fluidized Bed Biological filter: 61 cm (24 in) diameter (CB; Marine Biotech, Inc., MA, USA). Each filter contained 0.34 m³ of silica sand as a biofilter media. Sand properties were appropriate according to specifications provided by Marine Biotech.

Water leaving the filters was returned to sump #2 (Fig.1). The two sumps were connected to allow water from sump #2 to flow back to sump #1. A check valve prevented flow from sump #1 to sump #2. Water returned to the culture system was pumped from sump #1.

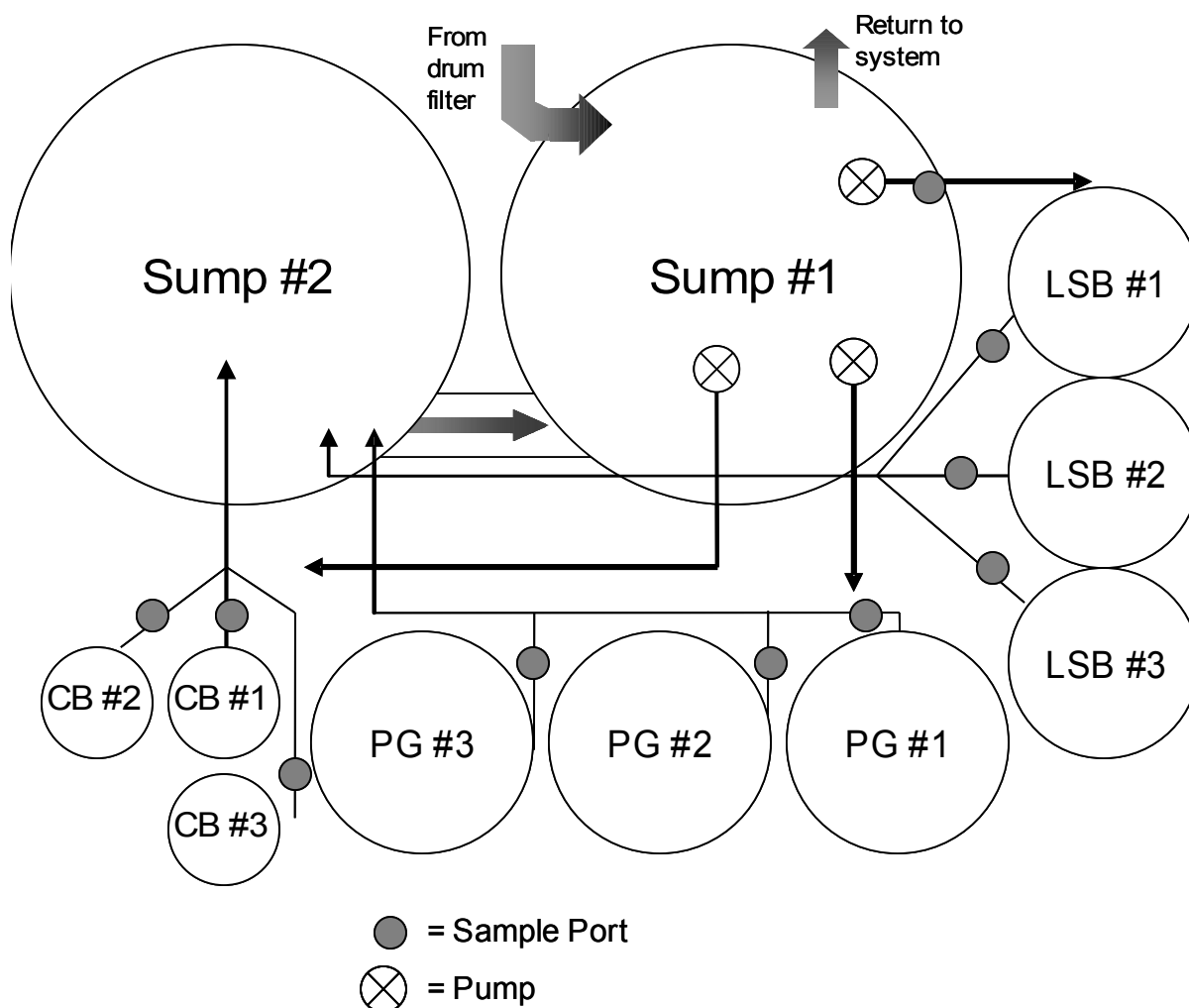


Fig. 1. A schematic of the experimental filter system layout. The system contained three filter types: fluidized sand (CB), floating bead (PG), and moving bed (LSB). All filters were evaluated in triplicate.

2.1.3 Pumps

Three pumps were used to supply effluent to each group of filters. All three filter systems were individually supplied by Goulds model series 3656 pumps. At the beginning of data collection the LSB and CB systems were each supplied by a 1.5 kW pump (Pumps # 1

and 2, respectively; model 53BF1G4D0). On day 59 of the sampling period a larger 2.24 kW pump (model 52BF1H4C0) was installed on the CB system to increase available pressure head with a strainer basket installed. Ultimately, the strainer basket did not suffice and was removed, leaving the replacement pump in place. The PG system was supplied by a 1.12 kW pump (Pump #2; model 55BF2F4B0) as the model DF15 filters required higher flow rates than the other two types of filters. All pumps received water from sump #1 at approximately 3.5 m below the surface to allow for uniform water quality supply to all filters. Mixing of the sump was achieved by the constant flow from the drum filter and biofilter effluents into sump #1.

2.1.4 Plumbing and flow rates

The filters were supplied by way of one manifold system for each group of filters. Flow measurements were taken using a Dynasonics® Transit Time Ultrasonic Flow Meter (TFXP Series; Racine Federated Inc., WI, USA). The inlet pipes from the manifold to the filters were lengthened to a minimum of 1.5 m to accommodate the requirement of a fully developed flow profile within the pipe and provide for more accurate flow measurements in this study. Inlet pipes for all three filter groups were of the same diameter for flow measurement purposes. Each filter was individually controlled using a ball valve directly in front of the filter inlet. Flow rates within each group were equalized with the total variation in flow rates between all three filters being no greater than 10%. Flow rates were recorded at the time of sampling and adjusted after sampling was complete, if required.

2.1.5 *Dosing system*

A dosing system was installed to periodically supplement the wastewater with ammonium chloride (NH_4Cl) using a Hannah Blackstone BL20-1 (HANNA instruments® Inc., RI, USA) positive displacement dosing pump. The pump was located directly above a 625 L polyethylene storage tank. Substrate was dosed into the effluent pipe of the drum screen filter to ensure proper mixing within the sump. Adjustment of the amount of substrate dosing was determined by the substrate concentration desired within the system, and was easily controlled using the adjustment knob on the dosing pump. Adjustment was required when higher TAN concentrations were required and based on feeding response. During harvesting periods ammonia was added to maintain biofilter activity as the culture system water was not circulated through the biofilter system.

Dosing of ammonium chloride began on day 30, and was maintained for the remainder of the study to ensure TAN concentrations were sufficiently high. A solution of 80 kg NH_4Cl / m^3 water was mixed in the storage tank. Changes in the dosing regime were followed by 10 – 14 day acclimation periods to allow filters ample time to compensate for the change in concentration. During the acclimation period samples were collected to monitor water quality conditions.

2.2 *Operation of biological filters*

All three filter groups were in full operation for a minimum of 194 days prior to the beginning of the data collection period. TAN loading rates were varied throughout the study

to obtain volumetric TAN removal rates (VTR) for a range of TAN concentrations. Feeding rates were adjusted to regulate the TAN concentration within the system. When feeding rates were not able to meet the TAN requirements for the study, dosing of ammonium chloride (NH_4Cl) at a rate proportionate to the existing TAN concentration was used to attain required TAN concentrations. Samples were taken when conditions were described as pseudo-steady state.

Maintenance of the filters was performed as needed. The PG filters required periodic flushing of accumulated material in the bottom of the filters through a valve. Since these filters were utilized for nitrification purposes only and not loaded with waste solids, they were flushed as necessary and not on a regular or routine schedule to minimize system water loss. The CB and LSB systems primarily required maintenance to ensure proper flow into the filters as each filter type was not able to handle large solids in the flow stream. Beads from the PG filters were found in the inlet screens and annuli of the LSB and CB filters, respectively throughout the duration of the study. Beads were also found in the culture tank and drum screen jet nozzles as system water was used for backwashing of the drum screen filter. There were several instances where both the LSB and CB filter system inlets were found to be almost completely blocked by beads from the PG filters and were consequently shut down for cleaning. There was also one instance where the inlets for the LSB and CB filters were blocked by feces from the culture system when the drum screen filter stopped functioning properly and allowed a bypass of the filter. As a modification, all possible locations for bead loss in the PG filters were sealed with 100% silicone or EPDM and foam gaskets to minimize bead loss.

During the acclimation process two of the CB filters required maintenance as the annulus for each was blocked by debris. Sand was removed and the annuli of the two CB filters were flushed to remove debris. The debris blocking the annuli was mainly beads from the PG filters and small tree parts from the trees surrounding the filter system. The sand from both CB filters also had pieces of cellophane which had to be removed. The cellophane was likely left over from the manufacturing process of the filters as the sand was carefully inspected before being added to the filters during the initial measurement. The sumps were consequently sealed more tightly so as to prevent further blocking. The sand was measured as it was removed from the filters to clear the blockages and it was found that approximately 12% of the initial sand volume had washed out of each filter. Calculations based on the volume of sand were based on the measured volumes for the remainder of the study thereafter.

2.3 Sampling and analysis

During the conditioning period prior to data collection, occasional samples were taken to monitor water quality. Twelve days prior to the start of data collection, regular samples were taken to ensure stable water quality conditions within the system. Sampling and water quality analysis were conducted as described below.

2.3.1 Water quality

Grab samples were taken at the pump #1 outflow sample port and each filter's respective sample port located directly after the filter exit (see Fig. 1). To eliminate possible

introduction of any settled material in the sample port into the water sample, water was wasted for 10 seconds prior to sampling. Sample bottles were then rinsed three times with water from the sample ports. This allowed for cleansing of the sample ports and bottles. Full sample bottles were immediately placed in an ice bath within an insulated container to stabilize the samples for transport to the Environmental Analysis Lab in the Department of Biological and Agriculture Engineering at North Carolina State University (approximately 9 km from study site).

All water samples were analyzed for total ammonia nitrogen (TAN), nitrite-nitrogen ($\text{NO}_2\text{-N}$), nitrate-nitrogen ($\text{NO}_3\text{-N}$), alkalinity (as mg CaCO_3/L), pH, dissolved oxygen (DO), temperature, chemical oxygen demand (COD), and total organic carbon (TOC). Samples were analyzed by automated analysis (Bran & Luebbe Digital Autoanalyzer III) for TAN by the salicylate method, $\text{NO}_2\text{-N}$ by the cadmium reduction method, and nitrite–nitrogen plus nitrate–nitrogen ($\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$) by the copper–cadmium reduction method (EPA, 1979). Chemical oxygen demand (COD) was analyzed by potassium dichromate – sulfuric acid digestion and colorimetric analysis using a HACH Dr/2010 spectrophotometer (Hach Method 8000. EPA Approved-Federal register, 1980). Analysis of TOC was conducted using a Teledyne Tekmar Apollo 9000 combustion TOC analyzer with auto-sampler via oxidation by combustion and IR detection (EPA, 1979).

Dissolved oxygen concentrations and temperatures were measured at the pump #1 outflow and outflow fitting of each filter using a portable oxygen meter (Yellow Springs Instruments, Model 55, OH, USA). Alkalinity and pH measurements were made using a bench-top pH meter (Thermo Fisher Scientific Inc., Accumet Basic with model 13-620-530

pH/ATC electrode, MA, USA). Alkalinity measurements were performed on site by potentiometric titration to end point pH 4.8 (EPA, 1979).

2.3.2 Volumetric TAN conversion rate

The volumetric TAN conversion rate (VTR) is defined as the daily amount of TAN converted to nitrite per unit volume of unexpanded media. VTR was calculated using the filter flow rates, non-expanded volume of media inside the filter, and the difference in TAN concentrations between the influent and effluent water for each filter. For this study, a modified form of the VTR equation from Colt et al. (2006) was used:

$$\text{VTR} = 1.44(Q_f) \frac{(\text{TAN}_I - \text{TAN}_E)}{V_m} \quad (1)$$

where VTR is measured as g TAN converted/ m³ filter media (unexpanded) per day, Q_f is the flow rate through the filter (L/min); V_m is the total unexpanded volume of the filter media (m³); and TAN_I and TAN_E are influent and effluent TAN concentration (g m⁻³), respectively.

2.3.3 Volumetric nitrite conversion rate

The volumetric nitrite conversion rate (VNR) is the overall daily amount of nitrite-nitrogen (NO₂-N) converted to nitrate-nitrogen (NO₃-N) per unit volume of unexpanded media volume. VNR is a function of both VTR as well as the apparent volumetric nitrite conversion rate (VNR_A) within the filter (Malone and Beecher, 2000), and are defined as:

$$\text{VNR}_A = 1.44(Q_f) \frac{(\text{NO}_2\text{-N})_I - (\text{NO}_2\text{-N})_E}{V_m} \quad (2)$$

$$\text{VNR} = \text{VTR} + \text{VNR}_A \quad (3)$$

where VNR and VNR_A are measured as g $\text{NO}_2\text{-N}$ removed/ m^3 filter media (unexpanded) per day, and $(\text{NO}_2\text{-N})_I$ and $(\text{NO}_2\text{-N})_E$ are the influent and effluent nitrite-nitrogen concentrations (g m^{-3}), respectively. Eq. 3 describes the nitrite conversion rate as nitrite is being produced when ammonia is converted to nitrite in the filters. Because of this, the apparent nitrite conversion may be estimated as near zero, while the filter may actually be removing nitrite (Malone and Beecher, 2000).

2.4 Statistics

For the statistical analysis of data from this study, various linear models for VTR were considered. These models included parameters for effects such as filter type and TAN concentration or loading rate, as described in section 3. Some degree of variable selection among these candidate factors was undertaken, either via F-tests comparing nested models, or via graphical assessment, to obtain the most descriptive and best fitting model, while striving to maintain parsimony. Errors of VTR about the model-based predictions were assumed normally distributed. The SAS software package (SAS, 2004), specifically PROC MIXED, was used to obtain the output needed for inference about the effects of the

experimental factors. A significance level of 5% was used for all tests of significance. The graphical figures in this manuscript were generated using SAS (SAS, 2004) and JMP (JMP, 2007) statistical software packages.

3 . Results and discussion

3.1 *Summary statistics*

Data was collected over a period of 180 days. From that dataset, 24 days were chosen based on pseudo-steady state conditions. A summary of the filter specific operating characteristics for the 24 days is provided in Table 1. Mass loading rates differ between filters due to design flow parameters specific to each filter. The substrate loading rate was calculated as:

$$LR_s = 1.44(Q_f) \frac{S_i}{V_M} \quad (4)$$

where LR_s = substrate loading rate (g m^{-3} (media - unexpanded) d^{-1}), S_i = influent substrate concentration (g m^{-3}), Q_f = flow into filter (L min^{-1}), and V_M = unexpanded volume of filter media (m^3). This equation effectively normalizes the substrate available to the bacteria contained within the filters for a more realistic comparison between filter types.

Table 1

Summary statistics of filter specific parameters for the CycloBio (CB), Low Space Bioreactor (LSB), and PolyGeyser (PG) filters. Statistics are based on observations during the 24 days of pseudo-steady state conditions.

Parameter	Mean $\pm$ Std. Dev; (Min., Max.)		
	CB	LSB	PG
Hydraulic Load Rate (L min ⁻¹)	253.4 $\pm$ 38.19 (179.9, 345.1)	358.6 $\pm$ 14.51 (326.6, 382.8)	505.1 $\pm$ 27.39 (457.2, 569.5)
TAN Load Rate (g m ⁻³ (media) d ⁻¹)	776.75 $\pm$ 388.1 (85.4, 1,710.4)	506.8 $\pm$ 205.7 (86.0, 943.0)	1,162.5 $\pm$ 493.3 (194.0, 2,345.1)
Nitrite-N Loading Rate (g m ⁻³ (media) d ⁻¹)	2,645.6 $\pm$ 2,119.7 (354.0, 7,364.5)	1535.7 $\pm$ 1,173.6 (226.9, 5,233.9)	3,568.7 $\pm$ 2,868.8 (483.9, 13,015.4)
VTR (g m ⁻³ (media) d ⁻¹)	667.4 $\pm$ 344.8 (85.4, 1,600.1)	267.2 $\pm$ 123.3 (65.9, 542.2)	586.3 $\pm$ 284.2 (48.0, 1,231.2)
VNR (g m ⁻³ (media) d ⁻¹)	1,294.5 $\pm$ 567.1 (448.0, 2501.3)	353.2 $\pm$ 208.4 (6.8, 699.1)	352.3 $\pm$ 259.9 (-50.6, 879.0)
Efficiency (% TAN removed)	87.1 $\pm$ 16.1 (43.3, 100.0)	53.1 $\pm$ 12.9 (29.0, 100.0)	49.4 $\pm$ 15.8 (9.7, 100.0)
Δ O ₂ (DO _{OUT} – DO _{IN}) (g m ⁻³)	-3.89 $\pm$ 1.01 (-5.8, -1.9)	-0.68 $\pm$ 0.35 (-2.0, 0.0)	-1.37 $\pm$ 0.57 (-2.2, -0.4)
Δ pH (pH _{OUT} – pH _{IN})	-0.03 $\pm$ 0.07 (-0.18, 0.08)	0.13 $\pm$ 0.07 (-0.06, 0.29)	0.02 $\pm$ 0.07 (-0.26, 0.20)

Water quality parameters monitored for the length of the study are summarized in Table 2. Variations in TAN and NO₂-N concentrations are expected per experimental design. Maximum TAN concentrations were limited by the feeding response. This was perhaps due to limitation by CO₂ as there was no CO₂ removal device installed on the system. To

maintain a system pH above 6.8, the minimum pH required for maximum nitrification in RAS (Groeneweg et al., 1994), alkalinity additions were significant. Increased alkalinity was used as a pH buffer in addition to providing a source of inorganic carbon for the autotrophic nitrifying bacteria in the form of sodium bicarbonate (NaHCO_3). Variations in pH and alkalinity were due to balancing the effects of feed rates, CO_2 production, and NaHCO_3 additions.

Table 2

Summary statistics for the culture system water quality parameters. The summary is based on the 24 days of pseudo-steady state conditions.

Variable	Mean (Std. Dev.)	Minimum	Maximum
TAN concentration (g m^{-3})	0.69 (0.27)	0.13	1.20
Nitrite-N concentration (g m^{-3})	2.1 (1.52)	0.31	6.66
DO concentration (g m^{-3})	5.8 (0.80)	5.00	8.10
Temperature ($^{\circ}\text{C}$)	28.9 (2.27)	24.0	31.6
pH	7.16 (0.14)	6.85	7.48
Alkalinity (as $\text{g CaCO}_3 \text{ m}^{-3}$)	261.2 (69.59)	100.0	422.0
TOC (g m^{-3})	25.1 (15.5)	7.1	58.5
COD (g m^{-3})	80.4 (11.7)	51.0	98.0

3.2 TAN and VTR

VTR was calculated each day for each of the nine individual filters. For statistical analysis a mixed model appropriate to the experimental design performed was used. Two models were compared testing for linear dependence by VTR on TAN concentration or TAN loading rate (LR_{TAN}), respectively. These models included fixed effects for linear dependence on TAN concentration (Eq. 5) or TAN substrate loading rate (LR_{TAN}) (Eq. 6), with possible filter type-specific slopes, and independent, normally distributed random effects for day, individual filter and experimental error. The variance components for the random day effect were also type-specific, as there was considerably more variability in VTR for the CB filters. A least squares means analysis was used for each model to estimate the mean VTR for each filter type over the range of filter type specific operating conditions and compare the standard error associated with such estimates associated with each model. The least squares means analysis uses an average of the predictor variable specific to each filter type to estimate an average VTR for the three filter types. The least squares means analysis was used to show the difference in mean VTR values generated from the proposed linear model estimates and the observations of this study. The VTR under these two models, and the solution to each respective model, is then given by the following expressions:

$$VTR = \beta_0 + TAN(\beta_1 + CB(\beta_2) + LSB(\beta_3)) + CB(\beta_4) + LSB(\beta_5) + D + F_1 + E \quad (5)$$

$$\widehat{VTR} = -108.0 + TAN(1,007.3 + CB(824.2) + LSB(431.3)) + CB(244.5) + LSB(77.9) \quad (5.1)$$

$$\text{VTR} = \beta_0 + \text{LR}_{\text{TAN}}(\beta_1 + \text{CB}(\beta_2) + \text{LSB}(\beta_3)) + \text{CB}(\beta_4) + \text{LSB}(\beta_5) + D + F_i + E \quad (6)$$

$$\widehat{\text{VTR}} = -86.6 + \text{LR}_{\text{TAN}}(0.58 + \text{CB}(0.86) + \text{LSB}(0.54)) + \text{CB}(123.5) + \text{LSB}(78.9) \quad (6.1)$$

where VTR = Volumetric TAN removal rate (g m^{-3} (media - unexpanded) d^{-1}), $\widehat{\text{VTR}}$ = predicted VTR based on least squares estimates to Eqs. 5 and 6 as shown in Eqs. 5.1 and 6.1, respectively, β_0 = Y-intercept for the PG filter type (g m^{-3} (media - unexpanded) d^{-1}), TAN = TAN concentration (g m^{-3}), LR_{TAN} = TAN loading rate (g m^{-3} (media - unexpanded) d^{-1}), β_1 , β_2 , β_3 = slope parameters for the PG, CB, and LSB filter types, respectively, β_4 and β_5 = the difference in the Y-intercepts from PG for the CB and LSB filter types, respectively, the CB, LSB, and PG coefficients are chosen as either the numerals 0 or 1 to indicate which model is of interest, and D, F_i , and E are the random error components for day, individual filter, and experimental effects, respectively.

3.2.1 TAN concentration and VTR

The linear dependence of VTR on TAN concentration is shown in Fig.2. The relationships between TAN concentration and VTR for the CB and PG filters were very similar. The PG filters showed the possibility of out performing the CB filters at higher TAN concentrations. The CB filters demonstrated variability in VTR and problems maintaining fluidization levels of the sand at higher TAN concentrations ($> 0.80 \text{ g TAN/ m}^3$). The LSB filters showed steady VTR performance, though achieved the lowest TAN removal rates of the three filter types in this study. The relationship between VTR and TAN concentration is highly linear for the LSB and PG filter types ($R^2 = 0.92$ and 0.925 , respectively), and

moderately linear for the CB filters ($R^2 = 0.48$). There was a significant interaction effect between TAN concentration and filter type ($F = 44.88$, $p < 0.001$), thus demonstrating the need for filter type specific slopes ($\hat{\beta}_1, \hat{\beta}_2, \hat{\beta}_3$) in Eq. 5. The slope for the LSB filters ($\hat{\beta}_3$) differed significantly from the CB and PG slopes ($\hat{\beta}_1$ and $\hat{\beta}_2$, respectively) demonstrating LSB produced significantly lower VTR values than the CB and PG filters. Additionally, the CB and PG filters were not significantly different in terms of TAN removal using Eq. 5. Analysis using the least squares means method provided mean VTR estimates for each of the filter types based on Eq. 5.1 (Table 3). These estimates were the same as the observed means for the LSB and PG filters, and slightly higher than the observed mean for the CB filters. The estimate for the CB filters was based on 20 days of data as there four days when they were out of service and resuming pseudo-steady state operation. While the CB filters produced a higher mean VTR, the PG filters performed more consistently over the entire range of TAN concentrations for this study. The PG filters were able to close the gap between the CB filters at higher TAN concentrations, effectively eliminating any significant differences in VTR performance based on TAN concentration. Such consistency in VTR is very important in aquaculture production. Consistency in VTR performance may be seen in Fig. 2. The majority of data points for the PG and LSB filters were tightly grouped about the respective regression lines, as were the respective confidence intervals (Fig. 2). However, the data for the CB filters showed an increase in variability in VTR at TAN concentrations $> 0.8 \text{ g m}^{-3}$ (see Fig. 2 and standard error in Table 3).

Table 3

Model based estimates of mean VTR based on average TAN concentration using the Eq. 5 solution, Eq. 5.1. The average TAN concentration of 0.69 g m^{-3} was used for the LSB and PG filters, and 0.64 g m^{-3} for the CB filters.

Filter Type	VTR Estimate	Standard Error
	$(\text{g m}^{-3} \text{ d}^{-1})$	$(\text{g m}^{-3} \text{ d}^{-1})$
CB	704.6	67.16
LSB	267.2	39.72
PG	586.3	41.60

Analysis of several higher order relationships suggested possible non-linearity in the relationship between TAN concentration and VTR. The possibility of non-linearity is most likely due to the variation in the CB filters, and to a lesser extent the variation in the LSB filters at TAN concentrations $> 0.8 \text{ g m}^{-3}$. These two filter types showed a small tendency toward non-linearity while the PG filter type was much more of a linear relationship. Graphically, the linear relationship is visible for all three filters, with visible variation in VTR increasing for the CB filters above 0.8 g TAN m^{-3} and to a lesser extent for the LSB filters also. The linearity results for all three filter types are similar to previous studies evaluating biofilter performance at low TAN concentrations commonly found in aquaculture production systems. Ester et al. (1994) found that TAN removal rates for rotating biological contactors (RBC) were linear under commercial scale aquaculture conditions. Zhu and Chen (1999) modeled biofilter nitrification for a single-rate limiting substrate at a wide range of TAN concentrations in moving bed bioreactors. They demonstrated linear TAN removal at

low input TAN concentrations similar to conditions found in RAS. The same study developed a modified Michaelis-Menten model for TAN concentrations below 3 g m^{-3} as:

$$R = \frac{R_{\max}}{K_s} (S - S_{\min}) \quad (7)$$

where R = areal or volumetric TAN removal rate ($\text{mg m}^{-x} \text{ d}^{-1}$; areal ($x = 2$) or volumetric ($x = 3$)), $R_{\max}$ = maximum TAN removal rate ($\text{mg m}^{-x} \text{ d}^{-1}$), S is the TAN concentration (mg L^{-1}), $S_{\min}$ is the minimum concentration required for nitrification as determined in the study (mg L^{-1}), and K_s is the half saturation constant for TAN (mg L^{-1}). Nitrification is limited by a minimum TAN concentration ($S_{\min} = 0.07 \text{ mg L}^{-1}$) (Zhu and Chen, 1999). Modifying the Michaelis-Menten equation to account for $S_{\min}$ produces better TAN removal predictions. However, Eq. 7 requires knowledge of the maximum TAN removal rate for a particular biofilter, and may not account for nitrification inhibition within the filter at high organic loading rates (Malone et al., 2006). The same equation also assumes that adequate flow is provided to the filter and bacteria contained therein. Comparisons using bulk solution TAN concentrations to predict VTR typically assume ideal conditions inside the filters. The possibility of low TAN availability to the bacteria in a filter due to inadequate flow is possible in “real-world” applications and must be taken into consideration. A more effective means of evaluating biofilter performance would be to evaluate the relationship between VTR and the TAN substrate loading rate (LR_{TAN}). Such a relationship normalizes the comparison between filters accounting for availability of TAN to the bacteria. Evaluating the

relationship between LR_{TAN} and VTR allows for more accurate comparisons between filter types by accounting for hydraulic loading rates as well as TAN concentration.

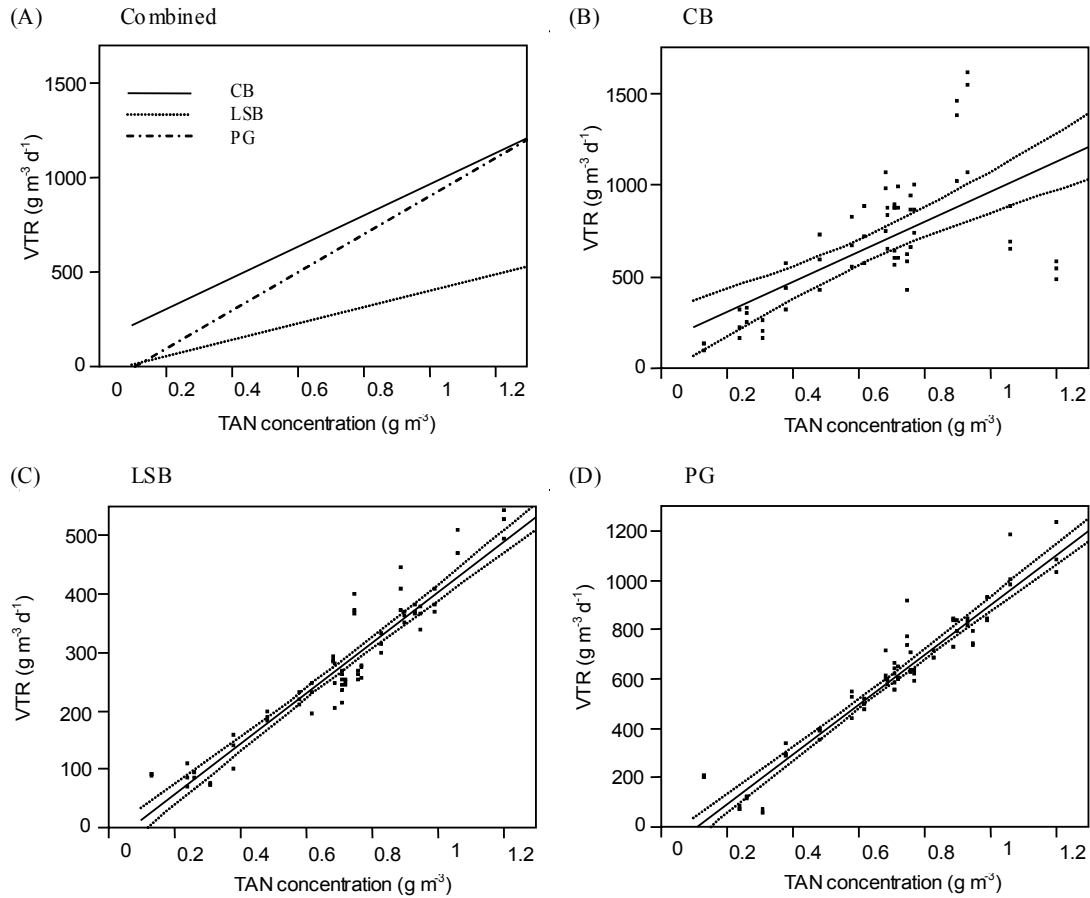


Fig. 2. Effect of TAN concentration ($g\ m^{-3}$) on VTR ($g\ m^{-3}\ media\ d^{-1}$). A graph of all three filters (A) compares the three filter types directly and corresponding regression lines. Graphs with 95% confidence intervals about the regression lines for filter types CB (B), LSB (C), and PG (D) are also shown.

3.2.2 *TAN loading rate and VTR*

By accounting for the influent flow rate, LR_{TAN} provides a different visualization of VTR for the three filter types from that of TAN concentration (Fig. 3). The CB filters continued to show the highest TAN removal rates of the three filters. Interestingly, the PG filters showed a considerable difference, comparatively. At $LR_{TAN} < 1,000 \text{ g m}^{-3} \text{ d}^{-1}$ the PG filters actually produced the lowest VTR for the study, though VTR for the LSB filters was similar. The comparison between the LSB and PG filters was limited by flow rate. The manufacturer suggested hydraulic loading rate for the LSB filters was 65% lower than that of the PG filters, but 30% lower for this study. The flow rate suggested by the manufacturer for the PG filters were almost twice the flow rates used for the study as the pump required to supply 3 PG filters at maximum manufacturer suggested flow rates would need to pump almost $3,000 \text{ L min}^{-1}$. Another important performance characteristic of note is TAN removal efficiency. The CB filters showed the highest removal efficiency followed by the LSB and PG filters, respectively (Table 1). Consequently, the hydraulic loading rates for the three filter types were oppositely ranked from high to low (PG, LSB, and CB, respectively). The CB and LSB filters were operated at the manufacturer suggested maximum flow rates while the PG filters were operated at approximately half the maximum suggested flow rate. Increasing the flow will decrease the efficiency per pass through the filters.

Using a statistical model with influent TAN concentration as the sole predictor (Eq. 5) does not predict VTR as well as one using LR_{TAN} as the predictor (Eq. 6). This model is a better fit for the linear representation of biological filter performance for all three filter types

($R^2 = 0.84, 0.92$, and 0.94 for CB, LSB, and PG, respectively). The interaction effect between LR_{TAN} and filter type was also found to be significant ($F = 28.50, p < 0.001$), again demonstrating the need for filter type specific slope parameters in the model. The slope for the CB filters ($\hat{\beta}_2$) differed significantly from the slopes for the PG and LSB filters ($\hat{\beta}_1$ and $\hat{\beta}_3$, respectively), and there was no significant difference between the slopes for the PG and LSB filters. Mean VTR estimates based on the Eq. 6 solution (Eq. 6.1) using the least squares means analysis showed estimates similar to Eq. 5.1 for all the filters (Table 4). The standard errors for the VTR estimates in Table 4 were considerably lower than those in Table 3. This further supports Eq. 6 as the more effective model for estimating mean VTR performance for the filters. Data points for all three filter types are more tightly grouped about the respective regression lines, as are the respective confidence intervals as compared to Fig. 2 (see Fig. 3). The smaller confidence intervals together with the lower standard error shows that using TAN loading rate as the sole predictor yields a much more suitable statistical model for biofilter VTR performance prediction. As a result, LR_{TAN} provided a more realistic understanding and prediction of VTR performance for this study.

Table 4

Model based estimates of mean VTR based on average LR_{TAN} values specific to each filter type from Table 1 and based on the Eq. 6 least squares estimate, Eq. 6.1.

Filter Type	VTR Estimate ($\text{g m}^{-3} \text{ d}^{-1}$)	Standard Error ($\text{g m}^{-3} \text{ d}^{-1}$)
CB	701.0	22.61
LSB	267.2	7.62
PG	586.3	14.03

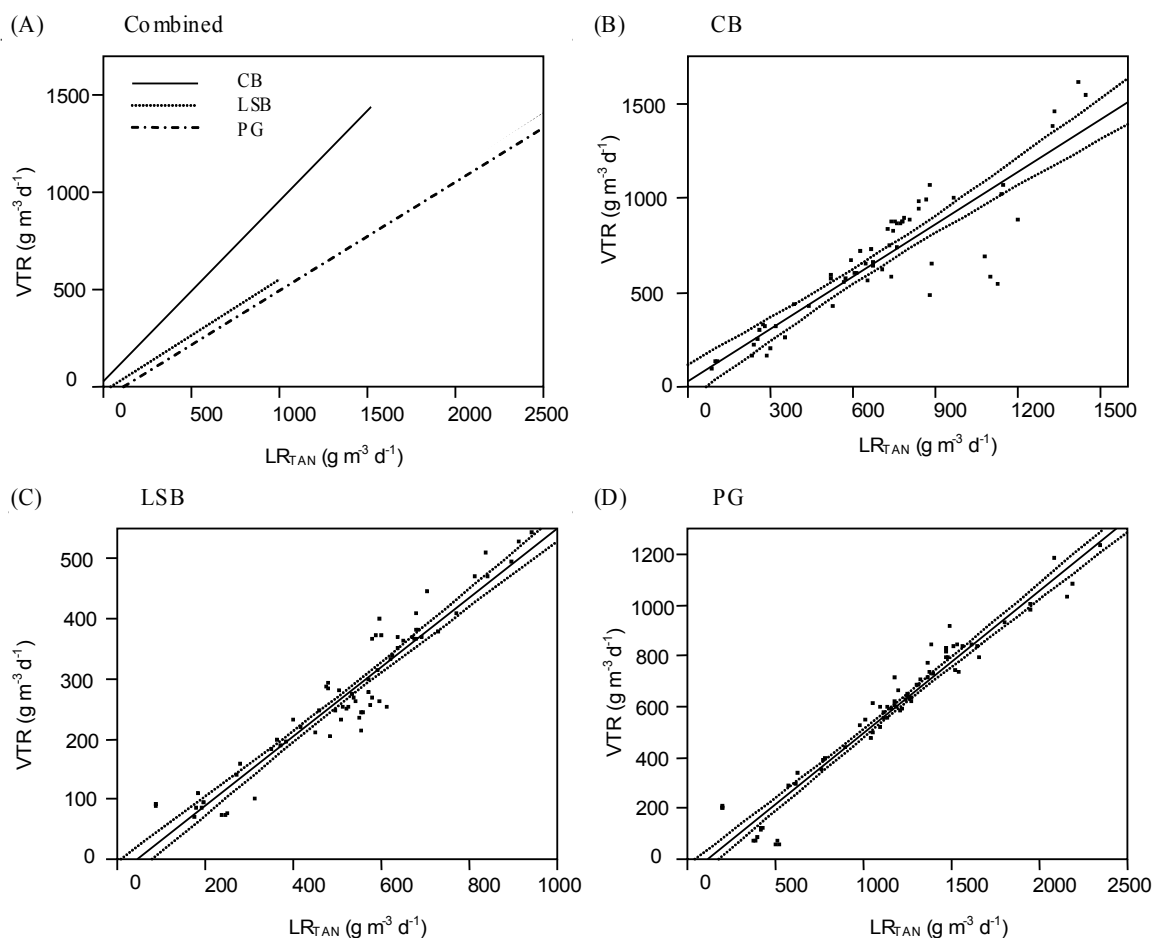


Fig. 3. Effect of TAN substrate loading rate on VTR. A graph of all three filters (A) compares the three filter types directly. Graphs with 95% confidence intervals about the regression line for filter types CB (B), LSB (C), and PG (D) are also shown.

3.2.3 Additional discussion

Monod style kinetics are typically used to describe the TAN conversion rate as the TAN loading rates increase (Malone et al., 2006). Graphical analysis of this relationship usually shows a first order, linear relationship at low TAN loading rates shifting to half-order

relationship at moderate to high TAN loading rates, and finally resulting in a zero-order relationship at high TAN loading rates. Analysis of Fig.3 shows a linear relationship, not a Monod relationship exists for the operating conditions for this study. This demonstrates that these filters were capable of operating at higher TAN loading rates than evaluated as an approach toward either half-order or zero-order relationships were not achieved. Evaluations at higher TAN concentrations will provide additional performance data, but the range of TAN used for this study is quite representative of RAS. TAN concentrations higher than 1.5 g m^{-3} are generally not recommended. Evaluations at lower TAN concentrations provide data more useful for RAS. TAN loading rates could be achieved at the same TAN concentrations by increasing the flow rates to each filter. Higher flow rates would be possible in the LSB and PG but not in the CB due to the fluidization height of the sand bed. Any increase in flow rate would result in loss of sand from the filter into the production system.

TAN conversion rates reported in this study show considerable difference from previous studies. Zhu and Chen (1999) reported TAN removal rates for a moving bed bioreactor at the lab scale using TAN as the sole substrate at concentrations substantially higher than this study. The resulting average VTR was 40% greater than the VTR reported for the LSB in this study. In a follow-up study evaluating the effects of organic carbon on TAN removal rate, the VTR reported at a combined ratio of $\text{C/N} = 1.0$ and 2.0 for the moving bed filter was 17% and 9% higher, respectively than that of the LSB filters in this study with a C/N ratio of 36.9 (Zhu and Chen, 2001). The same study showed that organic carbon inhibits the rate of TAN removal by 70%, and reported no significant difference in TAN removal rates between $\text{C/N} = 1.0$ and 2.0 . Ling and Chen (2005) reported TAN

removal rates for fluidized sand filters as well as floating bead filters at C/N = 0, 0.5, 1.0, and 2.0. Comparing the reported TAN removal rates for their study at the highest C/N ratio (2.0), VTR values were 63% and 637% higher than those reported for this study comparing the fluidized sand and floating bead filters, respectively. All of these studies were conducted at the lab scale using artificial waste nutrients. Results from these studies highlight the considerable differences between nitrification rates measured in large scale filters under fish culture conditions and small lab scale filters using artificial nutrients. Future biofilter evaluations should be conducted at a scale more representative of commercial scale using real waste nutrients. Additionally, such evaluations should be conducted at TAN concentrations more typical of actual production conditions.

Studies reporting TAN removal rates conducted at a scale more similar to that of commercial RAS have been lacking. A review of floating bead filter performance evaluations by Malone and Beecher (2000) shows that the VTRs demonstrated by the PG filters in this study were considerably higher than other previously reported studies. In a study performed at the pilot scale, VTR reported for a fluidized sand filter and a propeller washed floating bead filter were 78.3% and 82.4% less than those reported in the study presented here (Pfeiffer and Malone, 2006). The primary difference in the system design from the current study was the pre-filtration of the effluent prior to biofiltration. The influent water in the current study was mechanically filtered through a drum filter with 40 micron screens. The propeller washed bead filter served as the sole means of mechanical filtration for their study. As such, the TAN removal provided by the bead filter was largely secondary to solids removal. Failure to remove particulate organic matter (POM) from the influent

water to the biofilter increases the heterotrophic activity in the filter and results in reduced TAN removal rates (Leonard et al., 2002; Michaud et al., 2006). Greiner and Timmons (1998) reviewed microbead biofilter TAN removal rates under pilot scale production conditions removing only settleable solids while allowing suspended solids to travel to the filter. The TAN removal rates reported in the same range of TAN concentrations for this study were 18%, 34%, and 195% higher than those reported in this study for the fluidized sand, floating bead, and moving bed filters, respectively. The same study reported average TAN removal efficiency for the microbead filter of 8.6%, considerably lower than the efficiency reported in this study. It is also important to note that Timmons et al. (2006) recommends areal hydraulic loading rates ($\text{m}^3 (\text{m}^2 \text{ filter cross section})^{-1} \text{ d}^{-1}$) for the microbead filters 3%, 191%, and 162% higher than the equivalent flow rates used in this study for the CB, LSB, and PG filters, respectively. Timmons et al. (2006) also reported microbead TAN removal efficiencies between 11% and 29% for a variety of commercial scale operations. These lower efficiencies are most likely due to higher hydraulic loading rates. Standards in terms of operating conditions and subsequent reporting of results are necessary to provide accurate and actual comparison of biofilter performance. A direct comparison of the results from this study with the two studies mentioned above is difficult as differences in the methods of solids removal may yield different TAN removal rates.

Previous studies performed at the lab scale have also emphasized steady state conditions. Conditions whereby environmental variables as well as substrate loading and conversion are constant are considered steady state. In the production environment, true steady state conditions are nearly impossible to attain and maintain. Daily variation in feed

rates, chemical additions, and equipment operation are only a few factors responsible for reducing the stability of the system. The purpose of production is to maximize yield, and maximum yields are achieved by re-evaluating the rate of production on a regular basis. Making changes to improve and increase the rate of production are part of the daily operation in the production environment. Such changes are therefore responsible for the difficulty in attaining steady state conditions by “text book” definition. This study used a definition for pseudo-steady state conditions as constant feed rates for at least seven days and relatively stable TAN concentrations for at least three days prior to sampling. Pseudo-steady state conditions were used as an alternative to previously defined steady state conditions in an attempt to qualify samples as appropriate for analysis. Colt et al. (2006) proposes either graphical or statistical approaches toward defining steady state conditions, but recognizes the difficulty of such a definition for the larger production scale environments as well. Agreement within the research community on a definition for steady state or pseudo-steady state conditions is needed for future large commercial scale biofilter evaluations.

3.3 *Nitrite and VNR*

The main focus of this study was on TAN conversion under normal operating conditions at the commercial scale. As such, nitrite-N ($\text{NO}_2\text{-N}$) was not monitored in this study until day 68. Samples were analyzed for $\text{NO}_2\text{-N}$ on day 68 and for the remainder of the days sampled during the 180 day study period. $\text{NO}_2\text{-N}$ concentrations were monitored as a means to ensure proper biofilter functioning and to provide comparison with the water quality tests performed at the study site. VNR was calculated every day for each of the nine

individual filters for each of the 12 days in this study when the filters were determined to be at pseudo-steady state operating conditions. Overall, $\text{NO}_2\text{-N}$ concentrations in the culture tank were within acceptable levels throughout the study (see Table 2) except for day 99 when the $\text{NO}_2\text{-N}$ concentration was 6.66 g m^{-3} . Similarly, day 99 had the highest TAN concentration (1.2 g m^{-3}) for the 24 useable days. A comparison of the apparent VNR (VNRA) and the natural log of $\text{NO}_2\text{-N}$ loading rates ($\log(\text{LR}_{\text{NO}_2})$) showed considerable differences between the three filter types (Fig. 4). The CB filters showed a great deal of variability in VNRA, though the trend was positively increasing as $\log(\text{LR}_{\text{NO}_2})$ increased with no negative VNRA values. The LSB filters also showed a substantial amount of variability in VNRA and there were two days of net nitrite production observed (negative VNRA values). The linearity of the relationship between VNRA and $\log(\text{LR}_{\text{NO}_2})$ was low for the CB and LSB filters ($R^2 = 0.19$ and 0.12 , respectively). The comparison between $\log(\text{LR}_{\text{NO}_2})$ and VNRA is most notable for the PG filters as there was a consistent net nitrite production. A negative linear dependence of VNRA on $\log(\text{LR}_{\text{NO}_2})$ alone by the PG filters was markedly higher than the CB and LSB filters ($R^2 = 0.48$). Even when accounting for the production of nitrite by VTR, VNRA still should not be negative, though VNRA values near or slightly above zero are plausible (Malone and Beecher, 2006). When filters are operated at conditions similar to steady state, negative VNRA values would imply a net nitrite production. The production of nitrite by the PG filters is most likely due to capturing of organic solids by the media bed. Floating bead filters have been shown to act as both biological filters and physical filtration devices (Malone et al., 1993). As the system water is circulated through a floating bead filter, suspended solids are captured in the media bed

where biofiltration processes are occurring. Periodic cleaning (backwashing) of the media bed is required to release captured solids and restore hydraulic conductivity. The captured solids are degraded in the media bed between backwashing events effectively acting as a source of ammonia production. Retention times of the organic waste solids in the media bed due to the frequency of backwash events is probably the underlying reason for the observed increase in nitrite production in the PG filters. Given the strong TAN removal rates demonstrated by the PG filters, it is likely that the ammonia produced through organic waste solids degradation is converted to nitrite quickly in the media bed. For this study, the filters were backwashed at a frequency slightly less than every two hours. Manufacturer suggested times between backwash events ranged between 2 and 6 hours. Operating the filters with more time between backwash events would have increased the retention time, possibly increasing nitrite production. Pfeiffer and Malone (2006) showed a slight increase in VTR by increasing the backwash frequency in a propeller washed bead filter, though no report on VNR was made. In order to increase VNRA and decrease retention time backwash frequency will require further investigation.

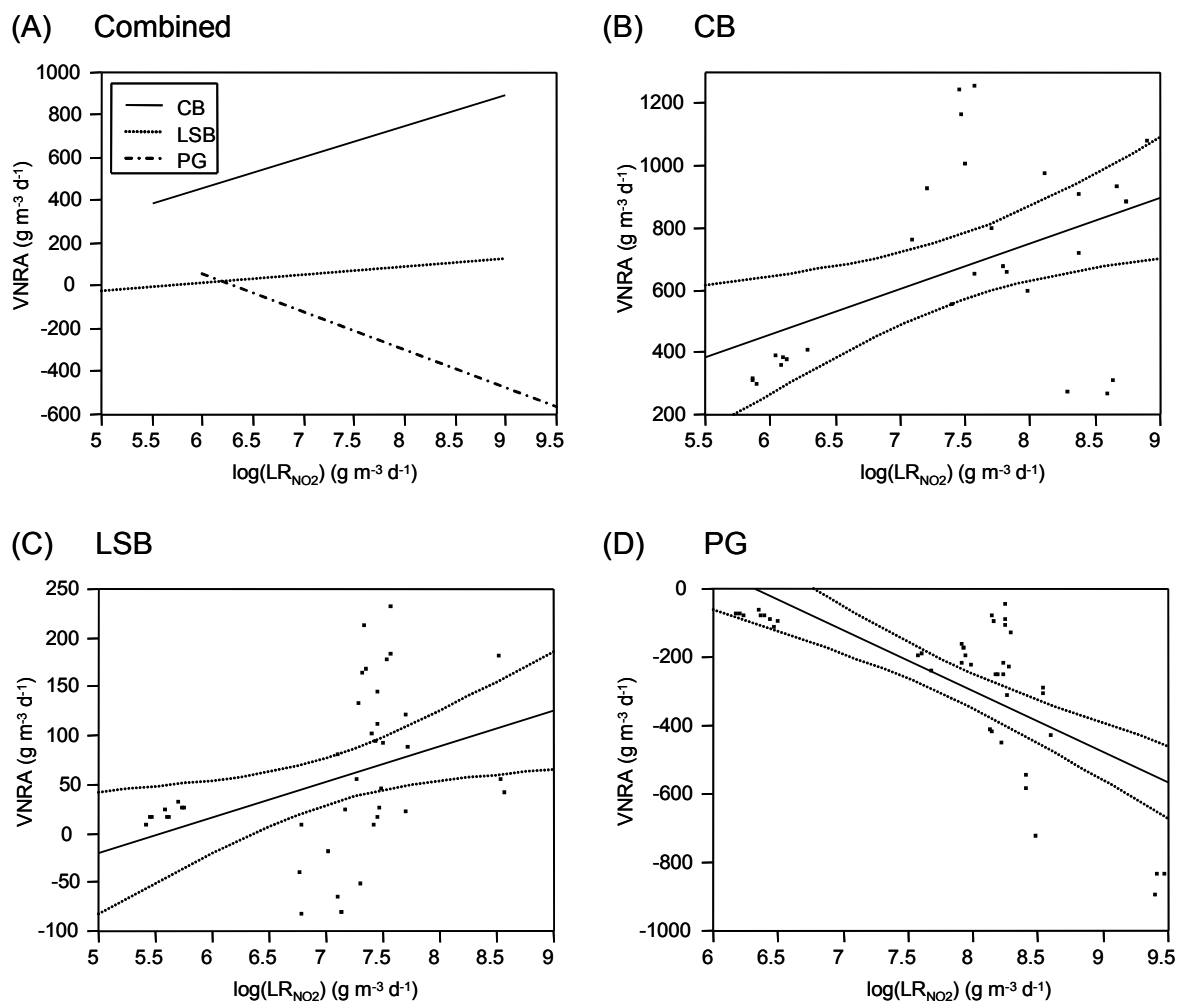


Fig. 4. Effect of log(LR_{NO2}) on the apparent nitrite removal rate (VNRA). A graph of all three filters (A) compares the three filter types directly. Graphs with 95% confidence intervals about the regression line for filter types CB (B), LSB (C), and PG (D) are also shown.

Several statistical models were considered for obtaining the best predictions of mean VNR values for the filters. The best fit model chosen included fixed effects for linear dependence on log transformations of LR_{TAN} and LR_{NO2}, with possible filter type-specific

slopes, and independent, normally distributed random effects for day, individual filter and experimental error. The least squares means analysis was also used as above to estimate mean VNR for each filter type based on the solution to this model. The VNR under this model is then given by the following expression:

$$\text{VNR} = \beta_0 + \log(\text{LR}_{\text{TAN}}) (\beta_1 + \text{CB}(\beta_2) + \text{LSB}(\beta_3)) + \log(\text{LR}_{\text{NO}_2}) (\beta_6 + \text{CB}(\beta_7) + \text{LSB}(\beta_8)) + \text{CB}(\beta_4) + \text{LSB}(\beta_5) + D + F_1 + E \quad (8)$$

$$\widehat{\text{VNR}} = -1,748.3 + \ln(\text{LR}_{\text{TAN}})(267.6 + \text{CB}(876.7) + \text{LSB}(216.7)) + \ln(\text{LR}_{\text{NO}_2})(31.5 + \text{CB}(275.2) + \text{LSB}(69.4)) + \text{CB}(-4,624.4) + \text{LSB}(290.1) \quad (8.1)$$

where VNR = Volumetric nitrite removal rate (g m^{-3} (media - unexpanded) d^{-1}), $\widehat{\text{VNR}}$ = predicted VNR based on model solution for Eq. 8 as shown in Eq. 8.1, $\log(\text{LR}_{\text{TAN}})$ = natural log of LR_{TAN} (g m^{-3} (media - unexpanded) d^{-1}), β_0 = Y-intercept for the PG filter type (g m^{-3} (media - unexpanded) d^{-1}), $\log(\text{LR}_{\text{NO}_2})$ = natural log of LR_{NO_2} (g m^{-3} (media - unexpanded) d^{-1}), $\beta_1, \beta_2, \beta_3$ = $\log(\text{LR}_{\text{TAN}})$ slope parameters for the PG, CB, and LSB filter types, respectively, $\beta_6, \beta_7, \beta_8$ = $\log(\text{LR}_{\text{NO}_2})$ slope parameters for the PG, CB, and LSB filter types, respectively, β_4 and β_5 = the difference in the Y-intercepts from PG for the CB and LSB filter types, respectively, the CB, LSB, and PG coefficients are chosen as either the numerals 0 or 1 to indicate which model is of interest, and D, F_1 , and E are the random error components for day, individual filter, and experimental effects, respectively.

Comparing VNR to $\log(\text{LR}_{\text{NO}_2})$ and $\log(\text{LR}_{\text{TAN}})$ separately, all three filter types showed positive VNR values (Figs. 5 and 6, respectively). VNR was variable for all three filter types (Table 1). The CB filters produced the highest nitrite removal rates in this study, followed by the LSB and PG filters, respectively. Linear dependence of VNR on $\log(\text{LR}_{\text{NO}_2})$ alone was moderate for all three filter types ($R^2 = 0.54, 0.59$, and 0.37 , for the CB, LSB, and PG filters, respectively). Linear dependence of VNR on $\log(\text{LR}_{\text{TAN}})$ alone was also moderate for all three filter types ($R^2 = 0.46, 0.81$, and 0.61 , for the CB, LSB, and PG filters, respectively). Using a model combining both of these effects provided more realistic estimation of the VNR performance for the filters.

For Eq. 8, the solution to which is Eq. 8.1, the interaction effect between $\log(\text{LR}_{\text{NO}_2})$ and filter type was significant ($F = 13.31, p = 0.0002$) as was the interaction effect between $\log(\text{LR}_{\text{TAN}})$ and filter type ($F = 15.7, p < 0.0001$). This demonstrated the need for the individual filter type specific slope parameters in the model for both variables. The CB filters differed significantly from the PG and LSB filters in all aspects of the model ($\hat{\beta}_2$ was significantly different from $\hat{\beta}_1$ and $\hat{\beta}_3$, $\hat{\beta}_4$ was significantly different from $\hat{\beta}_0$ and $\hat{\beta}_5$, and $\hat{\beta}_7$ was significantly different from $\hat{\beta}_6$ and $\hat{\beta}_8$). While the LSB filters produced the lowest mean VTR values for this study, there was no significant difference in VNR between the PG and LSB filters showing the need for improved VNR/VNRA performance by the PG filters.

Mean VNR estimates based on Eq. 8.1 derived from a least squares means analysis are shown in Table 5. These estimates provide a basis for comparison showing the respective

VNR performance for each filter type at mean values of the predictor variables specific to each filter type. The standard errors are relatively low demonstrating the adequacy of Eq. 8.1 in predicting mean VNR as well as relative consistency in VNR performance for each filter type.

Visualizing Eq. 8.1 is best achieved 3-dimensionally (Fig. 7). A 3-D analysis, generated using a smoothing spline interpolation (SAS, 2004), of the combined effects of $\log(\text{LR}_{\text{NO}_2})$ and $\log(\text{LR}_{\text{TAN}})$ on VNR demonstrates how both affected the VNR performance for each filter type. For all three filters there was a general positively increasing trend in VNR as both variables increased. The variability was more readily visible in the 3-D analysis for the CB filters, and the decline in VNR at higher LR_{TAN} and LR_{NO_2} in the PG filters was also apparent. The LSB filters showed the most consistent VNR performance as seen in the surface plot for these units.

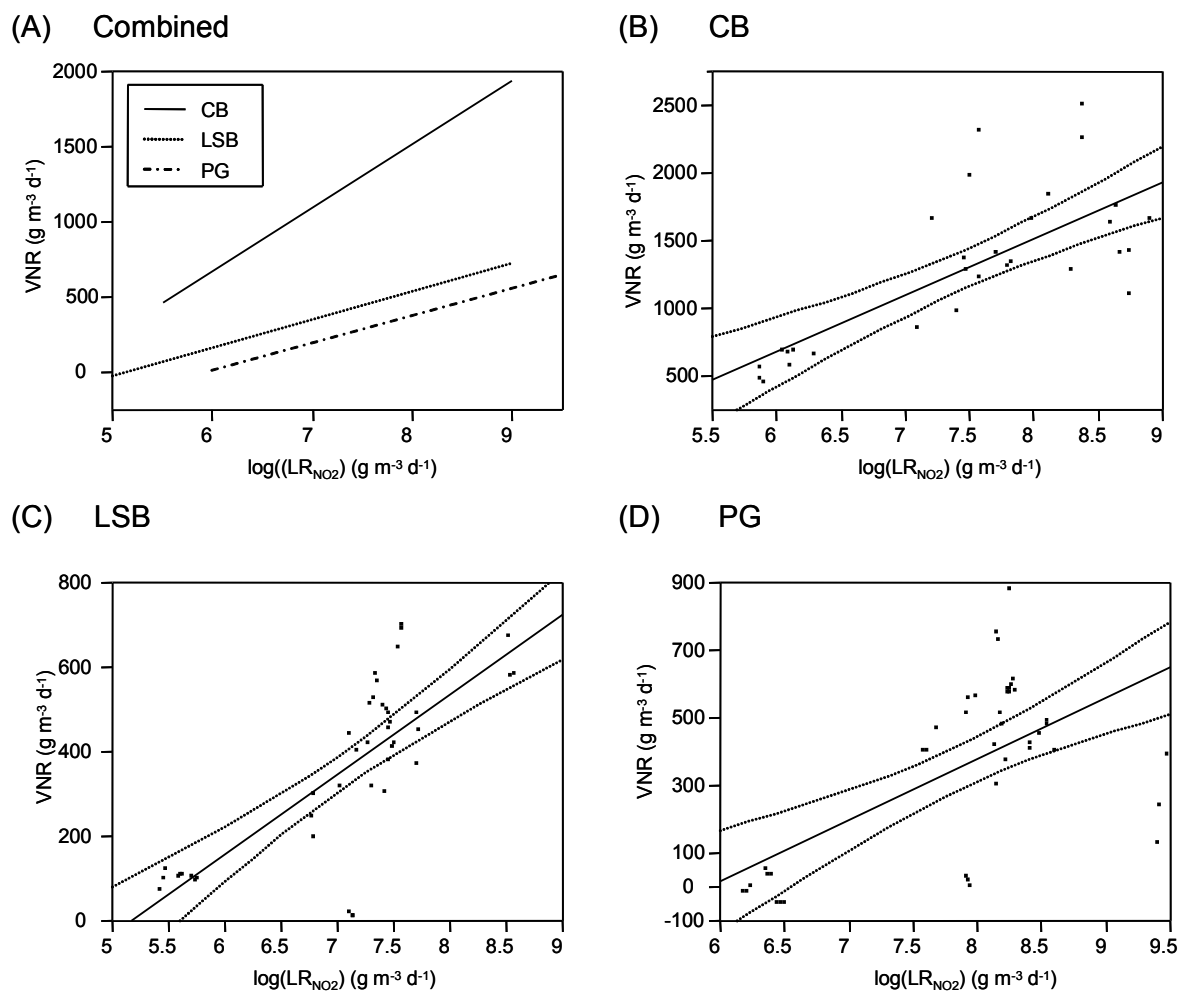


Fig. 5. Effect of $\log(LR_{NO_2})$ on the nitrite removal rate (VNR). A graph of all three filters (A) compares the three filter types directly. Graphs with 95% confidence intervals about the regression line for filter types CB (B), LSB (C), and PG (D) are also shown.

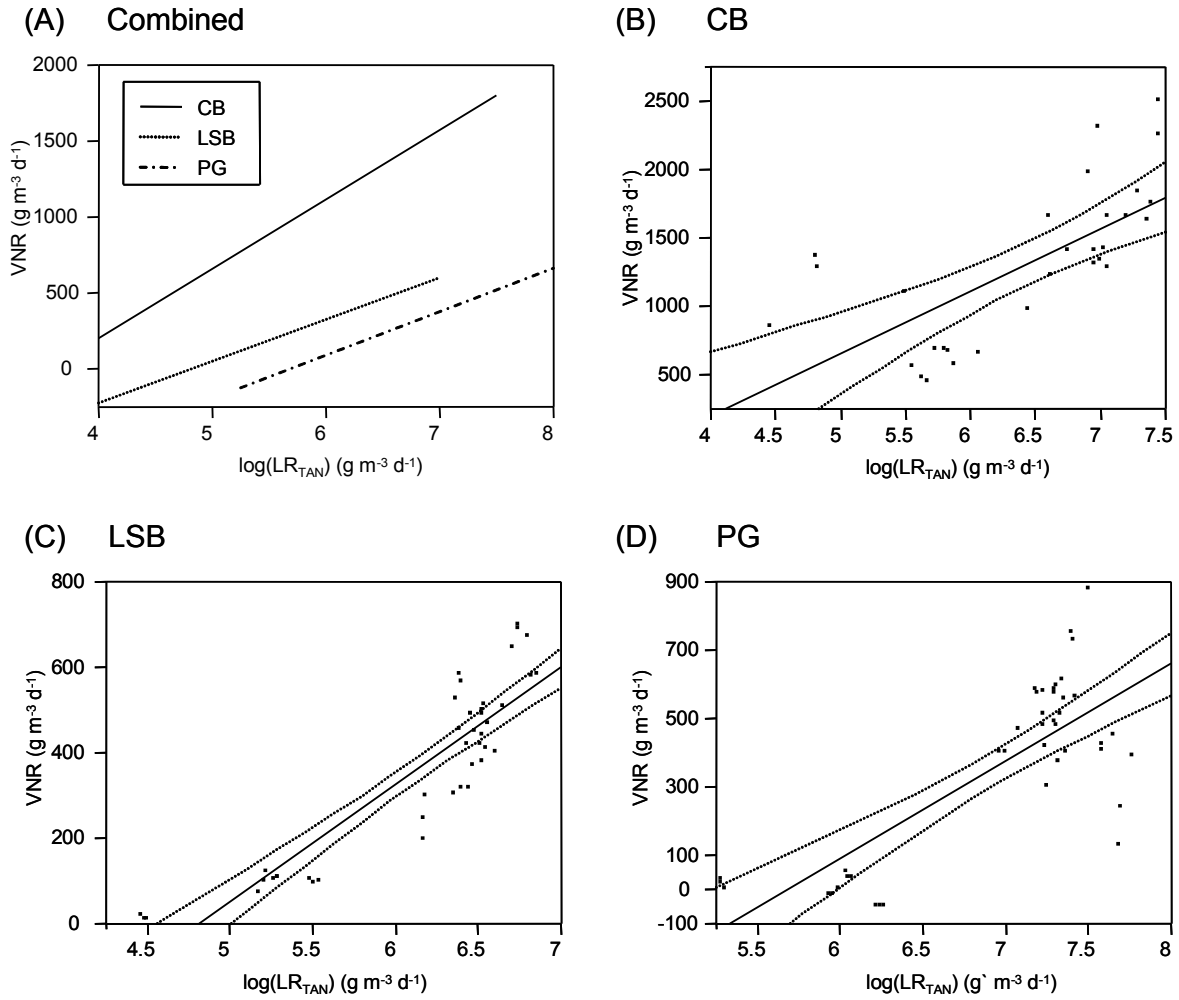


Fig. 6. Effect of $\log(LR_{TAN})$ on the nitrite removal rate (VNR). A graph of all three filters (A) compares the three filter types directly. Graphs with 95% confidence intervals about the regression line for filter types CB (B), LSB (C), and PG (D) are also shown.

Table 5

Model based estimates of mean VNR using mean values of $\log(\text{LR}_{\text{NO}_2})$ and $\log(\text{LR}_{\text{TAN}})$ specific to each filter type and based on the Eq. 8 least squares estimate, Eq. 8.1.

Filter Type	VNR Estimate ($\text{g m}^{-3} \text{ d}^{-1}$)	Standard Error ($\text{g m}^{-3} \text{ d}^{-1}$)
CB	1,294.5	208.03
LSB	353.2	25.22
PG	352.3	48.39

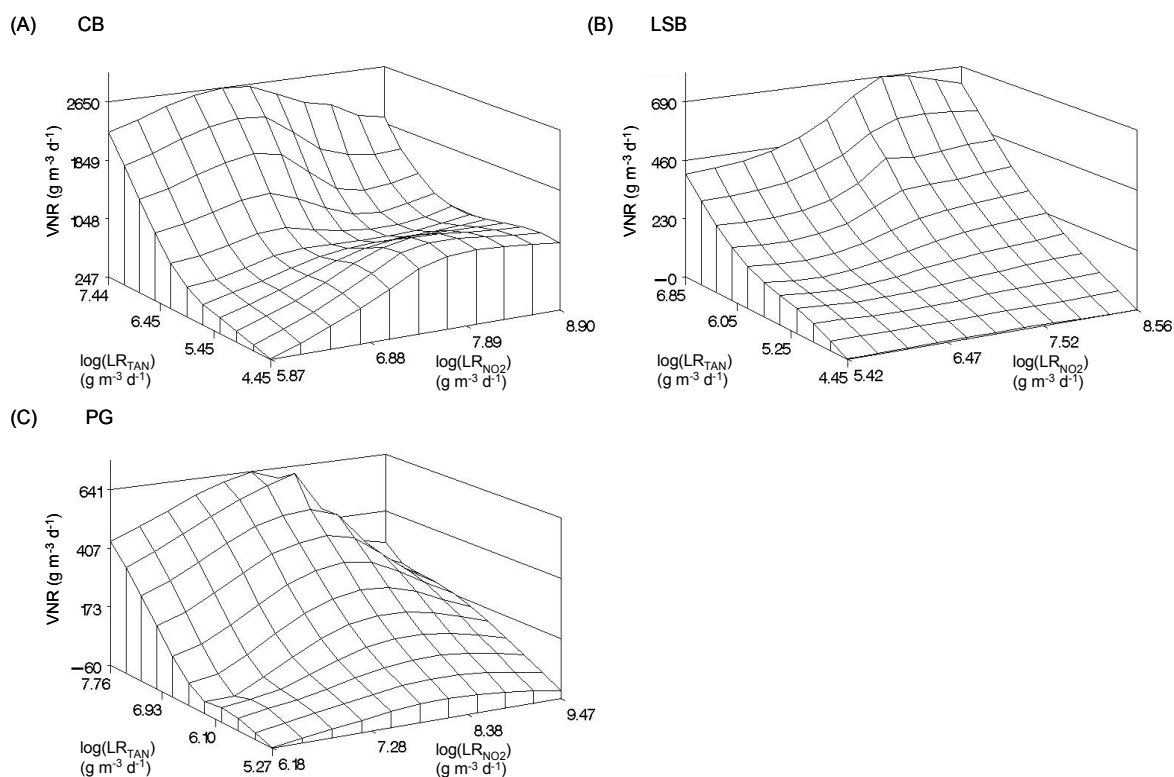


Fig. 7. A 3-dimensional analysis of the combined effects of $\log(\text{LR}_{\text{TAN}})$ and $\log(\text{LR}_{\text{NO}_2})$ on the nitrite removal rate (VNR) with a smoothing spline interpolation. The graphs for all three filters are based on the least squares estimate to Eq. 8 (Eq. 8.1): CB (A), LSB (B), and PG (C).

Positive VNR values are expected once VTR is taken into account based on Eq. 3. The realized nitrite removal rates of the filters are apparent in the VNRA data. VNRA is a measurement of what is experienced by the culture organisms within the system. Negative VNRA values imply a net production of nitrite, though nitrite may still be removed by the filters. Negative VNR and VNRA values should be cause for alarm implying a limitation of the filter's capacity to remove nitrite altogether. Only half of the usable sample days contained nitrite data. More data is needed to construct a suitable analysis of VNR performance related to each biofilter. VNR values are not widely published. VTR has been the primary focus of the majority of biofilter evaluation studies. As such, there is little information in the literature from which to draw reasonable VNR expectations and comparisons for each of the three filter types evaluated in this study. Additionally, the importance of VNRA should not be discounted. VNRA provides a measurement of the total $\text{NO}_2\text{-N}$ removed per pass through the filter. Negative VNRA values imply net nitrite production. Such implications have possible far reaching effects on production aquaculture systems. The PG filters demonstrated negative VNRA values over the course of the study once regular $\text{NO}_2\text{-N}$ measurements were taken. While the retention times of organic waste saolids is one possible reason for negative VNRA values as explained previously, another possibility is bacteria 'shifting'. A common sump through which all filters were supplied and recirculated was used for this study. The system was essentially a separate unit from the culture system as it recirculated on itself rather than directing filter effluent directly back into the culture system. The possibility of bacteria residing in a filter more suitable to the respective living conditions provides another possible explanation for different filter

performances. The possibility of numerous different bacterial populations located throughout the system is plausible based on the premise that bacteria grow where they are most successful. If the nitrite oxidizers were not as well suited for growing in the environment provided by the PG filters, then perhaps those bacteria were more effective at growing in another filter. This could possibly explain the consistent net nitrite production in the PG filters. This theory of course would also apply to the ammonia oxidizers and could potentially affect the results of biofilter evaluation studies where common source and effluent systems are used.

4. Conclusions

Evaluations at the large scale using actual waste nutrients provide a more suitable basis for sizing and selection of biofilters for RAS. TAN removal rates at the commercial scale using real fish waste are considerably lower than those as previously determined in lab scale studies. Conversely, due to the variability in operating conditions at the large production scale, the variability in VTR is considerably higher than that observed in lab scale studies. Reporting standards for biofilter performance studies should be followed for more realistic comparison and application in RAS. The relationship between VTR and influent TAN concentration provides an easy, though less appropriate comparison of biofilter performance as it relates to normal, commercial scale operating conditions per manufacturer operating guidelines. Statistical analysis of this relationship is markedly different than the relationship between VTR and TAN substrate loading rate. Using TAN loading rate as the predictor for VTR offers a greater understanding of TAN removal capacity for each filter

type. The substrate loading rate allows for better VTR prediction based on the amount of TAN available to the bacteria within the biofilter. Future biofilter evaluation studies should focus equally on VTR as well as VNR as a means of more wholly understanding biofilter performance at the large commercial scale under actual production conditions. Future studies should also account for possible increased separation of biofilters to provide more realistic understanding of bacterial dynamics within each filter type.

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References

- Abeyasinghe, D.H., Shanableh, A., Rigden, B., 1996. Biofilters for water reuse in aquaculture. *Water Sci. and Tech.*, 34(11 pt 7), 253-260.
- Akaike, H., 1974, "A New Look at the Statistical Model Identification," *IEEE Transaction on Automatic Control*, AC -19, 716 -723.

- Chen, S., Ling, J., Blancheton, J., 2006. Nitrification kinetics of biofilm as affected by water quality factors. *Aquacult. Eng.*, 34(3), 179-197.
- Colt, J., 2006. Water quality requirements for reuse systems. *Aquacult. Eng.*, 34(3), 143.
- Colt, J., Lamoureux, J., Patterson, R., Rogers, G., 2006. Reporting standards for biofilter performance studies. *Aquacult. Eng.*, 34(3), 377-388.
- Drennan II, D.G., Hosler, K. C., Francis, M., Weaver, D., Aneshansley, E., Beckman, G., et al., 2006. Standardized evaluation and rating of biofilters. II. manufacturer's and user's perspective. *Aquacult. Eng.*, 34(3), 403-416.
- Eding, E.H., Kamstra, A., Verreth, J.A. J., Huisman, E.A., Klapwijk, A., 2006. Design and operation of nitrifying trickling filters in recirculating aquaculture: A review. *Aquacult. Eng.*, 34(3), 234-60.
- Greiner, A.D., Timmons, M.B., 1998. Evaluation of the nitrification rates of microbead and trickling filters in an intensive recirculating tilapia production facility. *Aquacult. Eng.*, 18, 189-200.
- Groeneweg, J., Sellner, B. & Tappe, W., 1994. Ammonia oxidation in nitrosomonas at NH_3 concentrations near K_m : Effects of pH and temperature. *Water research* 28(12): 2561-2566.
- Henze, M., Härremoes, P., Jansen, J.la C., Arvin, E., 1995. *Wastewater Treatment: Biological and Chemical Processes*. Springer Verlag, Berlin, Heidelberg, Germany, 383 pp.

JMP version 7.0. 2007. SAS Institute Inc., Cary, NC, USA.

Losordo, T.M., Westers, H., 1994. System carrying capacity and flow estimation. In: Timmons, M.B., Losordo, T.M. (Eds.), *Aquaculture Water Reuse Systems: Engineering Design and Management*. Elsevier, pp. 9–60.

Losordo, T.M., & Hobbs, A.O., 2000. Using computer spreadsheets for water flow and biofilter sizing in recirculating aquaculture production systems. *Aquacult. Eng.*, 23(1), 95-102.

Losordo, T.M., Hobbs, A.O., DeLong, D.P., 2000. The design and operational characteristics of the CP&L/EPRI fish barn: a demonstration of recirculating aquaculture technology. *Aquacult. Eng.* 22, 3–16.

Malone, R.F., Chitta, B.S., Drennan, D.G., 1993. Optimizing nitrification in bead filters for warmwater recirculating aquaculture systems. In: Jaw-Kai Wang (Ed.), *Techniques for Modern Aquaculture*. ASAE Publication 02-93 (ISBN 0-9293355-40-7; LCCN 93-71584).

Malone, R.F., Beecher, L.E., 2000. Use of floating bead filters to recondition recirculating waters in warmwater aquaculture production systems. *Aquacult. Eng.*, 22(1), 57-73.

Malone, R.F., Pfeiffer, T.J., 2006. Rating fixed film nitrifying biofilters used in recirculating aquaculture systems. *Aquacult. Eng.*, 34(3), 389-402.

- Meade, J.W., 1985. Allowable ammonia for fish culture. *Progressive Fish-Culturist*, 47, 135-145.
- Michaud, L., Blancheton, J. P., Bruni, V. & Piedrahita, R., 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. *Aquacultural Engineering* 34(3): 224-233.
- Pfeiffer, T., Malone, R., 2006. Nitrification performance of a propeller-washed bead clarifier supporting a fluidized sand biofilter in a recirculating warmwater fish system. *Aquacult. Eng.*, 34(3), 311-321.
- FAO, 2005. Review of the state of the world marine fishery resources. FAO, Rome.
- SAS Institute Inc., 2004. SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2000-2004.
- Satoh, H., Okabe, S., Norimatsu, N., & Watanabe, Y., 2000. Significance of substrate C/N ratio on structure and activity of nitrifying biofilms determined by in situ hybridization and the use of microelectrodes. *Water Sci. and Tech.*, 41, 317-321.
- Timmons, M.B., Summerfelt, S.T., & Vinci, B.J., 1998. Review of circular tank technology and management. *Aquacult. Eng.*, 18(1), 51-69.
- United States Environmental Protection Agency (US-EPA), 1984. Methods for the Chemical Analysis of Water and Wastewater, EPA-600/4-79-020. U.S. Environmental Protection

Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

Wheaton, F.W., Hochheimer, J.N., Kaiser, G.E., Malone, R.F., Krones, M.J., Libey, G.S., Easter, C.C., 1994. Nitrification filter design methods. In: Timmons, M.B., Losordo, T.M. (Eds.), *Aquaculture Water Reuse Systems: Engineering Design and Management*. Elsevier, Amsterdam, pp. 101–171.

Zhu, S., Chen, S., 1999. An experimental study on nitrification biofilm performances using a series reactor system. *Aquacult. Eng.*, 20(4), 245-259.

Zhu, S., Chen, S., 2001. Effects of organic carbon on nitrification rate in fixed film biofilters. *Aquacultural Engineering* 25(1): 1-11.

Evaluating the effects of organic carbon on biological filtration performance in a large scale recirculating aquaculture system

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Abstract

Studies evaluating the impact of organic carbon on biological filters at the large-scale for aquaculture production are lacking. Understanding the performance characteristics of different biofilters under actual production conditions will be the only means by which recirculating system designers may properly select and size biological filters for commercial production use. Previous studies have determined the impact of organic carbon on biofilter performance at the small lab scale often using artificial waste nutrients in the evaluation. Evaluations under actual production conditions using real wastewater may produce vastly different results than previous lab scale studies using artificial nutrients. As such, this study is a preliminary step in evaluating the impact of organic carbon on three different commercially available biological filters at the large scale under actual recirculating aquaculture production conditions. The study was conducted at the North Carolina State University Fish Barn – a commercial scale research and demonstration recirculating aquaculture facility. The study was based on a 60 m³ Tilapia system with average daily feed rates of 45 kg using a 40% protein feed and an average biomass of 6,750 kg. The system was

dosed with sucrose ($C_{12}H_{22}O_{11}$) to increase the concentration of organic carbon in the system. The effect of elevated organic carbon concentrations on total ammonia nitrogen (TAN) removal rates was evaluated. Daily TAN removal rates were determined based on biofilter media volume. Variability increased substantially in the volumetric TAN removal rate (VTR) for all three filters. VTR for all three filter types was reduced by 50% - 70%. The results demonstrate the importance of controlling the concentration of available organic carbon in a recirculating system.

Keywords: Biological filtration; Organic Carbon; Nitrification; Aquaculture

1 . Introduction

Recirculating aquaculture relies heavily on biological filtration as the primary means of production system water renovation for reuse within the system (Losordo and Hobbs, 2000; Satoh et al., 2000; Chen et al., 2006). In recirculating aquaculture systems (RAS), biological nitrification is the primary process of interest occurring within biological filters. Nitrification is the aerobic process by which autotrophic nitrifying bacteria oxidize ammonia-nitrogen (NH_4^+-N) to nitrate-nitrogen (NO_3-N) with nitrite-nitrogen (NO_2-N) as the intermediate component. Nitrification can be measured and quantified by the rate of removal of total ammonia nitrogen (TAN), the sum of NH_4^+ and NH_3 , from a system (Zhu and Chen 1999). The oxidation of ammonia effectively reduces the TAN concentration, part of which is toxic to fish (unionized ammonia; NH_3) (Meade, 1985). Biological filtration renovates the system water to suitable condition for the cultured organisms, thereby providing a means for

reuse within a production system. Recirculating system designers must focus on minimizing the impact of TAN on the overall system and maximize the TAN removal rate.

When dissolved and/or particulate organic carbon concentrations (DOC and POC, respectively) are increased, inhibition of the nitrification process is observed due to competition between autotrophic and heterotrophic bacteria (Zhu and Chen, 2001; Leonard et al., 2002; Ling and Chen, 2005; Michaud et al., 2006). Autotrophic and heterotrophic bacteria occupy the space on biological filter media creating a biofilm. The sharing of media surface causes competition for nutrients and oxygen between the two types of bacteria, resulting in a stratified biofilm structure (Nogueira et al., 2002). Faster growing heterotrophic bacteria occupy the outer layer of the stratified biofilm where substrate concentration and detachment rates are both higher while the slower growing autotrophic nitrifying bacteria occupy the inner layer of the biofilm (Ohashi et al., 1995; Satoh et al., 2000; Nogueira et al., 2002; Lee et al., 2004; Chen et al., 2006; Michaud et al., 2006). Heterotrophic bacteria have a maximum growth rate five times greater than that of autotrophic bacteria (Zhu and Chen, 2001). By covering the nitrifying bacteria, heterotrophic bacteria inhibit the diffusion of nitrogenous substrate and dissolved oxygen (DO) to the autotrophic nitrifying bacteria, thus negatively affecting the rate of nitrification (Nogueira et al., 2002; Chen et al., 2006).

A ratio of organic carbon to ammonia-nitrogen (C/N) has been used as a measure of the availability and competition for organic carbon and ammonia. Higher C/N ratios allow heterotrophic bacteria to dominate the media space in the biofilters, effectively out-competing autotrophic bacteria for both space and oxygen. Reduction in TAN removal rates

as high as 70% have been reported at C/N ratios above 1.0 (Zhu and Chen, 2001; Ling and Chen, 2005). Improved feed quality and assimilation as well as better removal or finer mechanical filtration of waste solids are the two primary means of reducing particulate and dissolved organic concentrations in recirculating systems. Particulate organic matter (POM) in culture systems is comprised of uneaten feed particles, feces, and dead bacteria.

Accumulation of POM in biofilters has been shown to significantly reduce the TAN removal rates in recirculating systems (Leonard et al., 2002; Michaud et al., 2006). Incorporating particle filtration immediately prior to the biofilters will reduce the available POM and increase TAN removal rates. Improved feed quality and conversion will reduce the amount of POM and ultimately DOC in a recirculating system (Losordo et al., 1998; Leonard et al., 2002). Using feed which incorporates highly digestible nutrients and feed products that stimulates the production of a stable and solid fecal pellet will also contribute to reducing the overall C/N ratio in the system (Eding et al., 2006).

Studies evaluating the impact of organic carbon on biofilters at the large aquaculture production scale are lacking. Lab scale studies evaluating the impact of organics on TAN removal rates using artificial waste nutrients may not easily scale up to the larger commercial environment. Waste nutrient concentration and composition, system operating parameters, and organic/inorganic inputs vary widely between commercial facilities and are not adequately represented in the laboratory. This study provides data on biofilter performance under production conditions at the large scale. The objective of this study was to evaluate the impact of elevated organic carbon concentrations on the TAN removal rates of three

different commercially available biological filters as compared to normal operating conditions.

2 . Materials and Methods

2.1 Experimental setup

This study was conducted at the commercial scale under actual production conditions. The culture system, just prior to this study, was operated for 374 days to establish a bacterial population in the filters. During that period an evaluation was conducted on the same biofilters to measure and document performance characteristics under normal operating conditions (NOC) (Guerdat, 2008). The total organic carbon (TOC) concentration was increased by dosing sucrose into the system. Dosing rates were determined based on the mean TOC concentration observed under NOC. The NOC study was used as a basis for comparison in this study. Samples were taken over a 50 day period and data used for this study was selected based on pseudo-steady state conditions creating a 13 day dataset. Steady state conditions in the production environment are difficult to attain and maintain, as the system is in constant flux. Pseudo-steady state was defined for this study as constant organic carbon dosing and fish feed rates for a minimum of seven days. Time intervals as long as 14 days were used to allow for acclimation of the biofilters after changes in systems operations were implemented. Pseudo-steady state conditions were maintained during the acclimation and sampling periods.

2.1.1 Culture system and biomass loading

The North Carolina State University Fish Barn was the site for this study. This facility is a commercial scale indoor recirculating aquaculture research and demonstration facility operated by the North Carolina State University department of Biological and Agricultural Engineering. A detailed review of the system design may be found in Losordo et al. (2000). One of two 60 m³ culture tanks was used in which *Tilapia* were grown to serve as a source of waste nutrients for this biofilter study. The culture tank was stocked with an average of 5,000 fish during the 50 day study period. The average biomass for the study was 6,750 kg. Average daily feed rates were 45.0 kg ($\pm$ 9.7 kg, sd) using a 6.5 mm diameter 40% protein floating feed.

2.1.2 Filter system

Water flowed out of the culture tank and was mechanically filtered through a drum screen filter with 40 micron screens. The water flowed from the drum screen filter to a pumping sump (sump #1) from which nine biological filters were supplied (Fig. 1). Filter size for this study was based on the estimated volumetric nitrification capacity. The original design feed rates for a two tank system at the NCSU Fish Barn was approximately 100 kg d⁻¹ of 40% protein feed. The TAN excretion rate is usually estimated at 3% of the daily feed rate (Wheaton et al, 1994). Estimated TAN production was divided over the nine filters evenly and filter size was determined using manufacturer reported TAN removal rates.

This study focused on the evaluation of three different types of biological filters, each

type tested in triplicate. The filter types that were evaluated are listed below:

1. The Clearwater™ Low Space Bioreactor, Model LSB25 (LSB; Aquatic Ecosystems, Inc., FL, USA). Each filter contained 0.71 m³ of Kaldnes biofilter media (Kaldnes North America Inc., RI, USA). Air is supplied to the filters through an octopus diffuser system at an average rate of 100.5 L/min. The low pressure air serves as a source for aeration and mixing, effectively operating each filter as a CSTR (completely stirred-tank reactor).
2. The PolyGeyser® Floating Bead Drop Filter model DF15 (PG; Aquaculture Systems Technologies, LA, USA). Each filter contained 0.425 m³ of floating polyethylene beads. Air was supplied to the three filter air chambers at an average rate of 6.6 L min⁻¹. This rate of air flow provided for approximately thirteen backwash cycles per day for each filter.
3. The CycloBio® Fluidized Bed Biological filter: 61 cm (24 in) diameter (CB; Marine Biotech, Inc., MA, USA). The average media volume for each filter was 0.31 m³ of silica sand. Sand properties were appropriate according to specifications provided by Marine Biotech.

Water leaving the filters was returned to sump #2 (Fig.1). The two sumps were connected to allow water from sump #2 to flow back to sump #1. A check valve prevented flow from sump #1 to sump #2. Water returning to the culture system was pumped from sump #1.

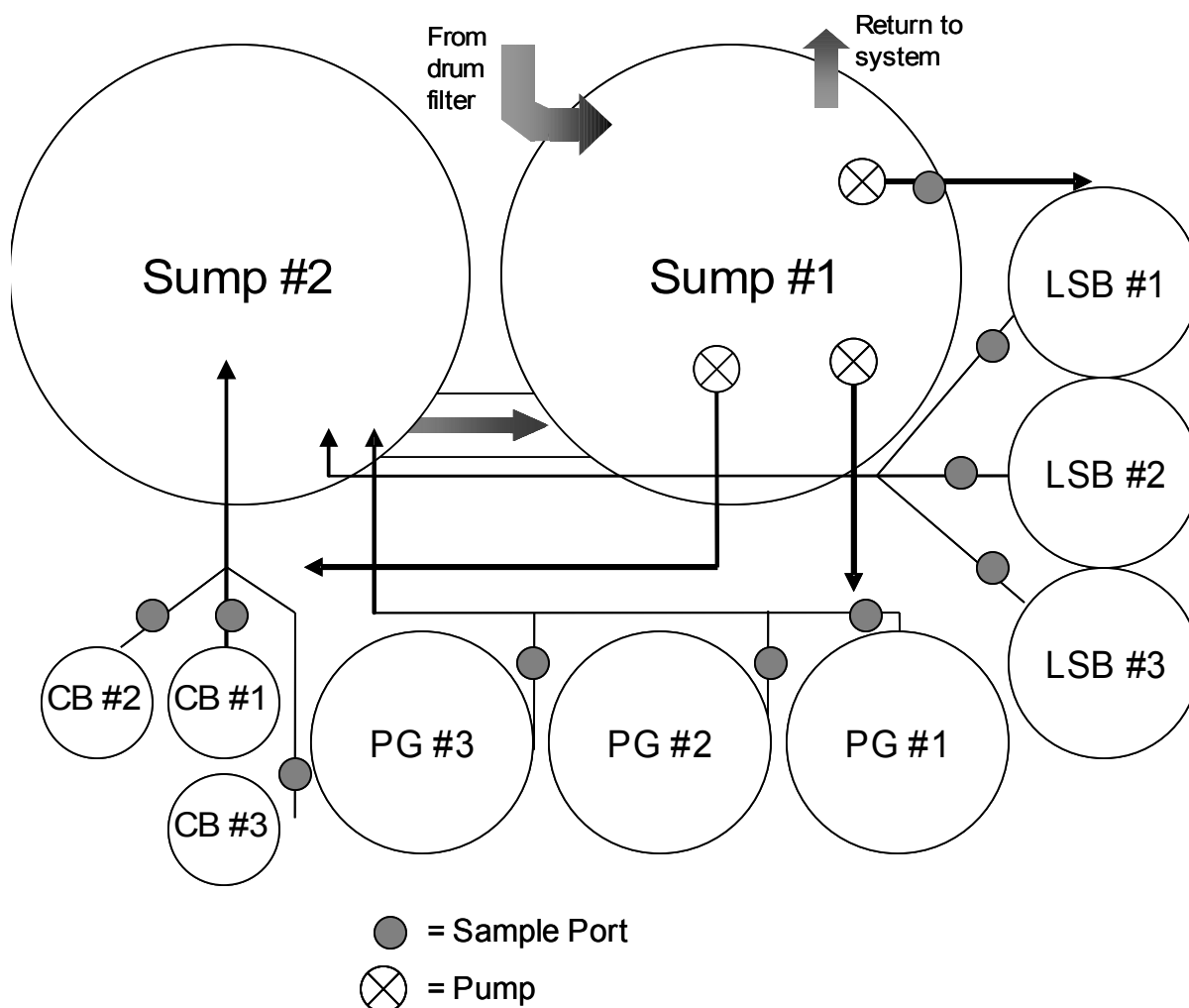


Fig. 1. A schematic of the experimental filter system layout. The system contained three filter types: fluidized sand (CB), floating bead (PG), and moving bed (LSB). All filters were evaluated in triplicate.

2.1.3 Pumps

Three pumps were used to supply effluent to each group of filters. All three filter systems were individually supplied by Goulds model series 3656 pumps. The LSB filter system was supplied by a 1.5 kW pump (Pump # 1; model 53BF1G4D0). The CB filter system was supplied by a larger 2.24 kW pump (Pump #3; model 52BF1H4C0) to increase available pressure head. The PG system was supplied by a 1.12 kW pump (Pump #2; model 55BF2F4B0) as the model DF15 filters required higher flow rates than the other two types of filters. All pumps received water from sump #1 at approximately 3.5 m below the surface to allow for uniform water quality supply to all filters. Mixing of the sump was achieved by the constant flow from the drum filter and biofilter effluents into Sump #1.

2.1.4 Plumbing and flow rates

The filters were supplied by way of one manifold system for each group of filters. Flow measurements were taken using a Dynasonics® Transit Time Ultrasonic Flow Meter (TFXP Series; Racine Federated Inc., WI, USA). The inlet pipes from the manifold to the filters were lengthened to a minimum of 1.5 m to accommodate the requirement of a fully developed flow profile within the pipe and provide for more accurate flow measurements in this study. Inlet pipes for all three filter groups were of the same diameter for flow measurement purposes. Each filter was individually controlled using a ball valve directly in front of the filter inlet. Flow rates within each group were equalized with the total variation

in flow rates between all three filters being no greater than 10%. Flow rates were recorded at the time of sampling and adjusted after sampling was complete, if required.

2.1.5 Dosing system

Organic carbon concentrations were manipulated by dosing sucrose ($C_{12}H_{22}O_{11}$). The mean TOC concentration previously determined under normal operating conditions was used as the basis for dosing rates. Sucrose has been used in previous biofilter evaluation studies to increase the dissolved organic carbon concentration within the system (Zhu and Chen, 2001; Ling and Chen 2005). The dosing system used to supplement the wastewater with sucrose was a Hannah Blackstone BL20-1 (HANNA instruments® Inc., RI, USA) positive displacement dosing pump wall-mounted directly above a 625 L polyethylene storage tank. Substrate was dosed into the effluent pipe of the drum screen filter to ensure proper mixing within the sump. Substrate dosing was adjusted at the pump and was determined by the substrate concentration desired within the system. The dosing rate was controlled using the adjustment knob on the dosing pump when higher TOC concentrations were required.

A solution of 160 kg sucrose/ m^3 water was mixed in the storage tank. Dosing of sucrose began on day 1 of this study to raise the TOC concentration 50% above NOC (established in Guerdat, 2008). This level of organic carbon adjustment will be referred to as the low level (OC_{Low}). Sucrose dosing was then increased on day 31 of the study to raise TOC concentration 100% above normal operating conditions. The resulting increase of TOC was 172%, significantly higher than the target. TOC concentrations were affected by increased feed rates which were adjusted at the same time as the sucrose dosing adjustment

resulting in higher TOC concentrations than intended. This level of organic carbon loading will be called the high level (OC_{High}). The resulting TOC concentration for the OC_{High} level was not adjusted due to the observed mean VTR values for the filters between the OC_{Low} and OC_{High} levels after the acclimation periods. The observed mean VTR values for the filters at the OC_{High} level were higher than those observed at the OC_{Low} level. For this study, it was hypothesized that greater VTR inhibition occurred at higher TOC concentrations. Since the opposite was observed, the TOC dosing rate was not changed. Acclimation periods of 10-14 days were allowed for filter compensation to TOC concentration changes. Water quality samples were monitored during the acclimation periods to ensure pseudo-steady state conditions were achieved.

2.2 *Operation of biological filters*

All three filter groups were in full operation for a minimum of 374 days prior to the beginning of the data collection period of this study. TAN removal rates were calculated for the two different organic carbon levels. Feeding rates were adjusted to regulate the TAN concentration in the system. Feeding rates were limited by water quality and adjusted upward based on fish growth. Data was used in this evaluation when conditions were defined as pseudo-steady state.

Maintenance and operation of the filters was specific to the filter type. The addition of sucrose to the system resulted in unstable operating performance of the CB filters. There was a dramatic increase in bacterial biomass on the sides of the filter vessels and in the fluidized sand bed. The increase in biomass created difficulty in maintaining stable

fluidization of the sand resulting in washout or bed collapse. Consequently, the CB filters were removed from the study on day 17 due to an inability to operate the filters without the fluidized bed collapsing. The data for this study are from samples of the CB filters in operation for a total of four days at pre-defined pseudo-steady state conditions before removal from the study. Samples taken during the acclimation period (days 1-13) showed considerable variability in operation and performance suggesting the filters may have been operating at maximum organic carbon loading rates under normal operating conditions at the beginning of the study.

Maintenance of the PG filters was specified by the manufacturer. The PG filters required periodic flushing of accumulated material contained in the bottom of the filters. These filters were utilized solely for nitrification purposes, unlike previous studies (Malone and Beecher, 2000; Pfeiffer and Malone, 2006) where floating bead filters were used only for solids removal or a combination of solids removal and nitrification. As such, the solids collected at the bottom of the PG filters were drained as necessary and not on a regular or routine schedule in an effort to minimize water loss. As previously noted in Guerdat (2008), bead loss from the PG filters was observed for this study also. Observation of bead loss in the previous study resulted in filter design modifications attempting to minimize bead loss. The modifications were effective, though beads continued to escape and ultimately caused problems with the inlet screens of the LSB filters.

The LSB filters required little to no maintenance. However, maintenance was performed on day 15 for one of the LSB filters. The inlet screens were plugged primarily

with beads from the PG filters resulting in reduced flow and a 1.5 to 3.0 cm thick bacterial layer covering approximately 50% of the inlet screen.

2.3 Sampling and analysis

Samples were collected during acclimation as well as pseudo-steady state conditions. Samples for this and the NOC study were collected and analyzed by the same means as described below.

2.3.1 Water quality

Grab samples were taken at the pump #1 outflow sample port and each filter's effluent sample port located directly after the filter outflow fitting (see Fig. 1). To eliminate possible introduction of any settled material into the water sample from the sample port, water was wasted for 10 seconds prior to sampling. Sample bottles were then rinsed three times with water from the sample ports. This allowed for cleansing of the sample ports and bottles. Full sample bottles were immediately transferred to an ice bath in an insulated container to stabilize the samples for transport to the Environmental Analysis Lab in the Department of Biological and Agriculture Engineering at North Carolina State University (approximately 9 km from study site).

All water samples were analyzed for total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2^- -N), nitrate-nitrogen (NO_3^- -N), alkalinity (as mg CaCO_3 / L), pH, dissolved oxygen (DO), temperature, chemical oxygen demand (COD), and total organic carbon (TOC). Samples were analyzed by automated analysis (Bran & Luebbe Digital Autoanalyzer III) for

TAN by the salicylate method, $\text{NO}_2\text{-N}$ by the cadmium reduction method, and nitrite–nitrogen plus nitrate–nitrogen ($\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$) by the copper–cadmium reduction method (EPA, 1979). Chemical oxygen demand (COD) was analyzed by potassium dichromate – sulfuric acid digestion and colorimetric analysis using a HACH Dr/2010 spectrophotometer (Hach Method 8000. EPA Approved-Federal register, 1980). Analysis of TOC was conducted using a Teledyne Tekmar Apollo 9000 combustion TOC analyzer with auto-sampler via oxidation by combustion and IR detection (EPA, 1979).

Dissolved oxygen concentrations and temperatures were measured at the pump #1 outflow and outflow fitting of each filter using a portable oxygen meter (Yellow Springs Instruments, Model 55, OH, USA). Alkalinity and pH measurements were made using a bench-top pH meter (Thermo Fisher Scientific Inc., Accumet Basic with model 13-620-530 pH/ATC electrode, MA, USA). Alkalinity measurements were performed on site by potentiometric titration to end point pH 4.8 (EPA, 1979).

2.3.2 Volumetric TAN conversion rate calculation

The volumetric TAN conversion rate (VTR) is defined as the daily amount of TAN converted to nitrite per unit volume of unexpanded media. VTR was calculated using the filter flow rates, non-expanded volume of media inside the filter, and the difference in TAN concentrations between the influent and effluent water for each filter. For this study the VTR equation from Colt et al. (2006) was used:

$$\text{VTR} = \frac{(K_c)(Q_f)}{V_m} (\text{TAN}_I - \text{TAN}_E) \quad (1)$$

where VTR is measured as g TAN converted/ m³ filter media per day, K_c is the conversion factor (1.44 min * hr * L⁻¹), Q_f is the flow rate through the filter (L/min), V_m is the total non-expanded volume of the filter media (m³), and TAN_I and TAN_E are influent and effluent TAN concentration, respectively.

2.3.3 Volumetric nitrite conversion rate calculations

The volumetric nitrite conversion rate (VNR) is the overall daily amount of nitrite-nitrogen ($\text{NO}_2\text{-N}$) converted to nitrate-nitrogen ($\text{NO}_3\text{-N}$) per unit volume of unexpanded media volume. VNR is a function of both VTR as well as the apparent volumetric nitrite conversion rate (VNR_A) across the filter (Malone and Beecher, 2000; Colt et al., 2006) and defined as:

$$\text{VNR}_A = \frac{(K_c)(Q_f)}{V_m} ((\text{NO}_2^- \text{-N})_I - (\text{NO}_2^- \text{-N})_E) \quad (2)$$

$$\text{VNR} = \text{VTR} + \text{VNR}_A \quad (3)$$

where $(\text{NO}_2\text{-N})_I$ and $(\text{NO}_2\text{-N})_E$ are the influent and effluent nitrite-nitrogen concentrations, respectively. Nitrite is being produced as ammonia is converted in the filters. Because of the

nitrite production, the apparent nitrite conversion may be near zero, although the filter may actually be removing nitrite (Malone and Beecher, 2000).

2.4 *Statistics*

Various linear models for VTR were considered for the statistical analysis of data from this study. These models included parameters for effects such as filter type and TAN concentration or loading rate, as described in section 3. Some degree of variable selection among these candidate factors was undertaken, either via F-tests comparing nested models, or via graphical assessment, to obtain the most descriptive and best fitting model, while striving to maintain parsimony. Errors of VTR about the model-based predictions were assumed normally distributed. The SAS software package (SAS, 2004), specifically PROC MIXED, was used to obtain the output needed for inference about the effects of the experimental factors. A significance level of 5% was used for all tests of significance. The graphical figures in this manuscript were generated using SAS (SAS, 2004) and JMP (JMP, 2007) statistical software packages.

3. Results and discussion

3.1 *Summary statistics*

Summary statistics for the normal operating conditions (NOC) study are provided in Tables 1 and 2 for the filter specific operating conditions and system water quality parameters, respectively. A complete description of the results from the NOC study may be found in Guerdat (2008). Mass loading rates differed according to experimental design and

hydraulic load rates specific to each filter type (see Table 1). TAN concentration was varied under NOC to attain TAN removal rates over a range of TAN loading rates (see Table 2).

Substrate loading rates were calculated as:

$$LR_S = \frac{K_C}{V_M}(S_I)(Q_F) \quad (4)$$

where LR_S = Substrate loading rate (g m^{-3} (media - unexpanded) d^{-1}), S = TAN or TOC, S_I = influent substrate concentration (g m^{-3}), Q_F = flow into filter (L min^{-1}), K_C = conversion factor ($1.44 \text{ min hr L}^{-1}$), and V_M = unexpanded volume of filter media (m^3). This equation effectively normalizes the substrate available to the bacteria contained within the filters for a more realistic comparison.

Table 1

Summary statistics of filter specific parameters for the CycloBio (CB), Low Space Bioreactor (LSB), and PolyGeyser (PG) filters at the normal operating conditions level. Statistics based on 24 days of pseudo-steady state conditions (Guerdat, 2008).

Parameter	Mean $\pm$ Std. Dev; (Min., Max.)		
	CB	LSB	PG
Hydraulic Load Rate (L min ⁻¹)	253.4 $\pm$ 38.19 (179.9, 345.1)	358.6 $\pm$ 14.51 (326.6, 382.8)	505.1 $\pm$ 27.39 (457.2, 569.5)
TAN Load Rate (g m ⁻³ (media) d ⁻¹)	776.75 $\pm$ 388.1 (85.4, 1,710.4)	506.8 $\pm$ 205.7 (86.0, 943.0)	1,162.5 $\pm$ 493.3 (194.0, 2,345.1)
Nitrite-N Loading Rate (g m ⁻³ (media) d ⁻¹)	2,645.6 $\pm$ 2,119.7 (354.0, 7,364.5)	1535.7 $\pm$ 1,173.6 (226.9, 5,233.9)	3,568.7 $\pm$ 2,868.8 (483.9, 13,015.4)
VTR (g m ⁻³ (media) d ⁻¹)	667.4 $\pm$ 344.8 (85.4, 1,600.1)	267.2 $\pm$ 123.3 (65.9, 542.2)	586.3 $\pm$ 284.2 (48.0, 1,231.2)
VNR (g m ⁻³ (media) d ⁻¹)	1,294.5 $\pm$ 567.1 (448.0, 2501.3)	353.2 $\pm$ 208.4 (6.8, 699.1)	352.3 $\pm$ 259.9 (-50.6, 879.0)
Δ O ₂ (DO _{OUT} – DO _{IN}) (g m ⁻³)	-3.89 $\pm$ 1.01 (-5.8, -1.9)	-0.68 $\pm$ 0.35 (-2.0, 0.0)	-1.37 $\pm$ 0.57 (-2.2, -0.4)
Δ pH (pH _{OUT} – pH _{IN})	-0.03 $\pm$ 0.07 (-0.18, 0.08)	0.13 $\pm$ 0.07 (-0.06, 0.29)	0.02 $\pm$ 0.07 (-0.26, 0.20)

Table 2

Summary statistics for the culture system water quality parameters at the normal operating conditions level. The summary is based on 24 days of pseudo-steady state conditions (Guerdat, 2008).

Variable	Mean (Std. Dev.)	Minimum	Maximum
TAN concentration (g m ⁻³)	0.68 (0.27)	0.13	1.20
Nitrite-N concentration (g m ⁻³)	2.1 (1.52)	0.31	6.66
DO concentration (g m ⁻³)	5.8 (0.80)	5.00	8.10
Temperature (°C)	28.9 (2.27)	24.0	31.6
pH	7.16 (0.14)	6.85	7.48
Alkalinity (as g CaCO ₃ m ⁻³)	261.2 (69.59)	100.0	422.0
TOC (g m ⁻³)	25.1 (15.5)	7.1	58.5
COD (g m ⁻³)	80.4 (11.7)	51.0	98.0

Summary statistics for the study reported in this paper were based on the 13 day dataset chosen from a 50 day sampling period based on pseudo-steady state conditions. The organic carbon study was performed at two different levels of organic carbon dosing (OC_{Low} and OC_{High}). Statistics for the specific biofilter operating conditions for the entire study period combining the two levels of organic carbon are provided in Table 3. Mass loading rates differed between filters due to feed rates, a reduction in TAN removal by the filters, and design flow parameters specific to each filter. Average TAN removal rates were significantly reduced overall as compared to normal operating conditions in each filter type after dosing sucrose into the system (see Figs. 5 and 9). Water quality parameters monitored

for the full length of the study are summarized in Table 4. Variation in TAN concentrations was due in part to feeding rates as well as the reduction in removal rates by the filters. Alkalinity additions considerably higher than normal operating conditions (106% higher) were required to maintain an appropriate system pH. The required increase in alkalinity was presumably due to increased heterotrophic activity which created a substantial increase in CO₂ production. The system was not fitted with a CO₂ stripping device; although CO₂ was removed by the LSB filters. As such, pH varied due to feed rates, CO₂ production, and sodium bicarbonate (NaHCO₃) additions.

The CB filters showed a high degree of instability once organic carbon was added to the system. That variability is represented by the larger confidence interval range about the VTR line for the effect of organic carbon in Fig. 3. Maintaining fluidization levels within each of the three filters was difficult as the biomass in the filters increased. The CB filters were prone to media washout and bed collapse. Flows to the filters required daily adjustment to compensate for fluidization levels. Flows were reduced to maintain fluidization of the bed and prevent media washout. When flows required to retain media in the filter were reduced to approximately 190 L min⁻¹ collapse of the fluidized sand beds occurred. The CB filters were shut down and eliminated from the study after day 17 with only 4 days of operation under pseudo-steady state system conditions and only under the OC_{Low} level.

Average TAN removal rates over the entire study (OC_{Low} and OC_{High} combined) for the CB, LSB, and PG filters were reduced by 78%, 58%, and 68%, respectively when compared to the data from the NOC study (see Fig. 5). Zhu and Chen (2001) reported a 70% decrease in TAN removal rates once the organic carbon/nitrogen ratio was $C/N \geq 1.0$. The

average total organic carbon to ammonia-nitrogen ratios (TOC/TAN) for the NOC and overall organic carbon studies were $C/N = 36.9$ and 79.4 , respectively. Each C/N ratio is considerably higher than those in the Zhu and Chen (2001) study; however the VTR results from this study agree with the approximate 70% reduction in TAN removal rates previously reported. The same study also reported a reduced potential for nitrification inhibition by the heterotrophic activity in the biofilters when the organic carbon concentration was sufficiently high ($C/N \geq 1.0$). While this study demonstrated a similar reduction percentage in VTR, it is important to note the C/N ratio for the NOC study was considerably higher than 1.0. The implication from the Zhu and Chen (2001) study was that organic carbon-free nitrification is reduced approximately 70% at C/N ratios higher than 1.0. The NOC study (Guerdat, 2008) was conducted at an average C/N ratio of 36.9 and still showed VTR values for the three filters considerably lower than similar values previously reported from lab scale evaluations. The organic carbon study reported herein demonstrated an average 70% reduction in VTR for the three filter types at an average C/N ratio more than twice that of the NOC study. The difference in VTR values for the three filters for this study was much less than the difference between filters under normal operating conditions (see Tables 1 and 3). Based on C/N ratios, the implication is that nitrification ceases to occur at high enough organic carbon concentrations. All of the studies evaluating the impact of organic carbon on nitrification have demonstrated a reduction in TAN removal rates. Understanding the basic underlying principle of inhibition is of much greater importance than knowing the exact concentration at which nitrification is completely inhibited. Systems designed to reduce the amount of organics available in the biofilters will yield higher TAN removal rates.

Table 3

Summary statistics of filter specific parameters for the CycloBio (CB), Low Space Bioreactor (LSB), and PolyGeyser (PG) filters for the entire organic carbon study. Statistics for the LSB and PG filters are based on the 13 days of pseudo-steady state conditions, while the statistics for the CB filters are based on 4 days of pseudo-steady state conditions.

Parameter	Mean $\pm$ Std. Dev; (Min., Max.)		
	CB	LSB	PG
Hydraulic Load Rate (L min ⁻¹)	213.3 $\pm$ 47.8 (152.9, 319.1)	369.5 $\pm$ 35.7 (292.2, 450.5)	476.8 $\pm$ 32.8 (411.9, 556.1)
TAN Load Rate (g m ⁻³ (media) d ⁻¹)	473.3 $\pm$ 142.3 (215.3, 743.2)	511.9 $\pm$ 285.0 (201.4, 1,240.8)	1,053.9 $\pm$ 587.4 (417.7, 2608.9)
Nitrite-N Loading Rate (g m ⁻³ (media) d ⁻¹)	238.2 $\pm$ 60.0 (172.0, 368.4)	256.3 $\pm$ 83.8 (139.0, 404.3)	526.6 $\pm$ 165.4 (345.5, 820.5)
VTR (g m ⁻³ (media) d ⁻¹)	146.0 $\pm$ 69.1 (39.7, 256.3)	112.9 $\pm$ 70.4 (-46.7, 238.3)	202.1 $\pm$ 161.6 (-128.4, 590.7)
VNR (g m ⁻³ (media) d ⁻¹)	277.4 $\pm$ 124.0 (79.4, 480.9)	155.2 $\pm$ 73.3 (-23.3, 279.4)	241.1 $\pm$ 195.4 (-60.1, 656.3)
Δ O ₂ (DO _{OUT} – DO _{IN}) (g m ⁻³)	-2.86 $\pm$ 0.69 (-3.7, -1.8)	-0.34 $\pm$ 0.49 (-1.1, 0.6)	-1.38 $\pm$ 0.69 (-2.4, -0.1)
Δ pH (pH _{OUT} – pH _{IN})	-0.06 $\pm$ 0.06 (-0.11, 0.05)	0.12 $\pm$ 0.03 (0.04, 0.17)	0.03 $\pm$ 0.06 (-0.10, 0.09)

Table 4

Summary statistics for the culture system water quality parameters for the full length of the organic carbon study. This summary is based on the 13 days of pseudo-steady state conditions.

Variable	Mean (Std. Dev.)	Minimum	Maximum
TAN concentration (g m ⁻³)	0.68 (0.38)	0.27	1.59
Nitrite-N concentration (g m ⁻³)	0.33 (.10)	0.23	0.49
Nitrate-N concentration (g m ⁻³)	65.87 (7.34)	56.08	78.77
TOC concentration (g m ⁻³)	54.0 (20.2)	7.0	74.4
COD concentration (g m ⁻³)	284.1 (54.1)	220.0	368.0
DO _{IN} concentration (g m ⁻³)	6.8 (0.83)	5.5	8.3
Temperature (°C)	23.9 (0.91)	22.9	25.7
pH	7.19 (0.12)	6.95	7.38
Alkalinity (as g CaCO ₃ m ⁻³)	538.2 (47.4)	448.0	596.0

The OC_{Low} level started on day 14 after beginning sucrose dosing. The 14 day acclimation period was assumed to be enough time as the filters demonstrated relatively quick acclimation to operating conditions changes from the NOC study. The length of time dedicated to running the filters at the OC_{Low} level was shortened by the removal of the CB filters. Upon removal of the CB filters, eleven additional days were allowed for the PG and LSB filters to adjust to the additional TAN loading rates. This resulted in only 4 days of OC_{Low} conditions. A summary of the filter specific operating conditions and system water quality parameters for OC_{Low} are shown in Tables 5 and 6, respectively. While the conditions pre-defined as pseudo-steady state were met for the OC_{Low} level, the biofilter performance characteristics for all three filters showed a high degree of variability suggesting conditions

other than steady state. VTR for the OC_{Low} period was inconsistent for all three filters. This was most likely due to insufficient adjustment time for the filters to the addition of sucrose. More data is needed to make adequate comparisons of biofilter performance characteristics between NOC and OC_{Low} conditions.

Table 5

Summary statistics of filter specific parameters during the OC_{Low} operating conditions for the CycloBio (CB), Low Space Bioreactor (LSB), and PolyGeyser (PG) filters. Statistics are based on 4 days of pseudo-steady state conditions.

Parameter	Mean $\pm$ Std. Dev; (Min., Max.)		
	CB	LSB	PG
Hydraulic Load Rate (L min ⁻¹)	213.3 $\pm$ 47.8 (152.9, 319.1)	373.8 $\pm$ 50.6 (292.2, 450.5)	467.6 $\pm$ 14.4 (450.9, 494.0)
TAN Load Rate (g m ⁻³ (media) d ⁻¹)	473.3 $\pm$ 142.3 (215.3, 743.2)	371.1 $\pm$ 115.7 (201.4, 584.3)	740.9 $\pm$ 201.2 (417.7, 957.4)
Nitrite-N Loading Rate (g m ⁻³ (media) d ⁻¹)	238.2 $\pm$ 60.0 (172.0, 368.4)	183.7 $\pm$ 27.8 (139.0, 241.2)	366.1 $\pm$ 17.2 (345.5, 395.1)
VTR (g m ⁻³ (media) d ⁻¹)	146.0 $\pm$ 69.1 (39.7, 256.3)	57.4 $\pm$ 32.7 (-22.0, 111.8)	70.2 $\pm$ 24.7 (15.0, 105.3)
VNR (g m ⁻³ (media) d ⁻¹)	277.4 $\pm$ 124.0 (79.4, 480.9)	100.7 $\pm$ 33.7 (29.2, 158.4)	41.5 $\pm$ 64.6 (-60.1, 109.2)
Δ O ₂ (DO _{OUT} - DO _{IN}) (g m ⁻³)	-2.86 $\pm$ 0.69 (-3.7, -1.8)	0.31 $\pm$ 0.28 (-0.2, 0.6)	-0.48 $\pm$ 0.29 (-0.9, -0.1)
Δ pH (pH _{OUT} - pH _{IN})	-0.06 $\pm$ 0.06 (-0.11, 0.05)	0.13 $\pm$ 0.02 (0.09, 0.15)	0.01 $\pm$ 0.04 (-0.04, 0.07)

Table 6

Summary statistics for the culture system water quality parameters under OC_{Low} conditions. The summary is based on the 4 days of pseudo-steady state conditions.

Variable	Mean (Std. Dev.)	Minimum	Maximum
TAN concentration (g m ⁻³)	0.48 (0.13)	0.27	0.63
Nitrite-N concentration (g m ⁻³)	0.24 (0.01)	0.23	0.26
Nitrate-N concentration (g m ⁻³)	74.8 (3.4)	70.7	78.8
TOC concentration (g m ⁻³)	37.3 (3.7)	33.1	43.1
COD concentration (g m ⁻³)	230.5 (7.0)	220.0	238.0
DO _{IN} concentration (g m ⁻³)	6.4 (0.47)	5.6	6.8
Temperature (°C)	24.2 (0.47)	23.5	24.8
pH	7.33 (0.04)	7.26	7.38
Alkalinity (as g CaCO ₃ m ⁻³)	519.5 (35.9)	470.0	554.0

The OC_{High} portion of the study began on day 28 of the study and was observed for 21 days. From the samples collected during that period, 9 days were used based on pseudo-steady state conditions. Table 7 shows the summary statistics for the biofilter operating conditions and performance characteristics. Table 8 shows the system water quality parameters for the OC_{High} level. VTR for the OC_{High} level was reduced by 49% and 56% for the LSB and PG filters, respectively as compared to the NOC operating conditions. The average TOC for the OC_{High} level was 173% higher than that of NOC conditions. The TOC concentration was not adjusted down to the target level because the VTR values for the filters were actually slightly higher than those determined during the OC_{Low} level. The ratio of total

organic carbon and ammonia-nitrogen (TOC/TAN) for the OC_{High} level of $C/N = 90.1$ was 144% higher than NOC conditions. The reduction in VTR at the OC_{High} level is lower than the combined effects of OC_{Low} and OC_{High} . While the LSB and PG filters were still significantly affected by the elevated organic carbon concentrations, these results suggest operating conditions more representative of pseudo-steady state for these filters.

Zhu and Chen (1999) reported an average VTR at a mean total organic carbon (TOC) to ammonia-nitrogen (C/N) ratio of 1.5 for a moving bed filter 172% higher than the average VTR for the LSB filter under OC_{High} conditions. At a C/N ratio of 2.0, Zhu and Chen (2001) reported an average VTR for a moving bed filter 127% higher than the average VTR for the LSB filters at the OC_{High} level. At $C/N = 2.0$, Ling and Chen (2005) reported the VTR for a floating bead filter 274% higher than the PG filters under OC_{High} conditions. Conversely, Pfeiffer and Malone (2006) provided VTR values for a propeller washed bead filter used primarily for solids removal 50% lower than that observed at the OC_{High} level for the PG filters. At an average C/N ratio for the OC_{High} level of 90.1, the implication is such that increased organic carbon available to the biofilter drastically decreases VTR. In the case of Pfeiffer and Malone (2006), the added reduction in VTR is most likely due to the increased availability of organics within the filter. The water flowing from the culture system was filtered down to 40 microns through the drum filter installed directly upstream of the filter system. The removal of solids reduced the organic loading to the filters and facilitated more efficient TAN removal rates.

Table 7

Summary statistics of filter specific parameters during the OC_{High} operating conditions for the CycloBio (CB), Low Space Bioreactor (LSB), and PolyGeyser (PG) filters. Statistics are based on 9 days of pseudo-steady state conditions.

Parameter	Mean $\pm$ Std. Dev; (Min., Max.)	
	LSB	PG
Hydraulic Load Rate (L min ⁻¹)	367.6 $\pm$ 27.8 (303.2, 421.3)	480.9 $\pm$ 37.7 (411.9, 556.1)
TAN Load Rate (g m ⁻³ (media) d ⁻¹)	574.5 $\pm$ 316.0 (309.7, 1,240.8)	1,192.9 $\pm$ 649.5 (641.8, 2,608.9)
Nitrite-N Loading Rate (g m ⁻³ (media) d ⁻¹)	288.5 $\pm$ 80.1 (167.9, 404.3)	597.9 $\pm$ 150.6 (353.2, 820.5)
VTR (g m ⁻³ (media) d ⁻¹)	137.6 $\pm$ 68.9 (-46.7, 238.3)	255.9 $\pm$ 163.0 (-128.4, 590.7)
VNR (g m ⁻³ (media) d ⁻¹)	179.4 $\pm$ 73.4 (-23.3, 279.4)	322.5 $\pm$ 170.2 (-48.2, 656.3)
Δ O ₂ (DO _{OUT} – DO _{IN}) (g m ⁻³)	-0.63 $\pm$ 0.21 (-1.1, -0.3)	-1.8 $\pm$ 0.31 (-2.4, -1.2)
Δ pH (pH _{OUT} – pH _{IN})	0.12 $\pm$ 0.04 (0.04, 0.17)	0.03 $\pm$ 0.06 (-0.10, 0.09)

Table 8

Summary statistics for the culture system water quality parameters under OC_{High} operating conditions. Statistics are based on the 9 days of pseudo-steady state conditions.

Variable	Mean (Std. Dev.)	Minimum	Maximum
TAN concentration (g m ⁻³)	0.76 (0.42)	0.40	1.59
Nitrite-N concentration (g m ⁻³)	0.38 (0.09)	0.24	0.49
Nitrate-N concentration (g m ⁻³)	61.9 (4.3)	56.1	68.0
TOC concentration (g m ⁻³)	68.5 (5.55)	61.5	78.4
COD concentration (g m ⁻³)	307.9 (48.5)	228.0	368.0
DO _{IN} concentration (g m ⁻³)	6.9 (0.9)	5.5	8.3
Temperature (°C)	23.8 (1.0)	22.9	25.7
pH	7.13 (0.09)	6.95	7.23
Alkalinity (as g CaCO ₃ m ⁻³)	546.4 (49.7)	448.0	596.0

Negative VTR values for all of the PG and LSB filters were observed on the last day of sampling. This was also the day with the highest TOC concentration (78.4 g m⁻³), but a mid range TAN concentration (0.40 g m⁻³). VTR for the filters during the preceding days showed variability, but did not show an obvious downward trend. These observations were attributed to variation of VTR at high organic carbon loading. More data is needed to derive conclusions on VTR performance at TOC concentrations above those observed during this study.

3.2 Volumetric TAN conversion rate

VTR was calculated each day for each of the individual filters while in operation. A mixed model was used appropriate to the experimental design performed in triplicate. This model included fixed effects for linear dependence on the natural log of the TAN and TOC loading rates (LR_{TAN} and LR_{TOC} , respectively) combined, with possible filter type-specific slopes, and independent, normally distributed random effects for day, individual filter and experimental error. The variance components for the random day effect were also type-specific, as there was considerable variability in VTR for all three filter types. Variable selection in these models was carried out using an Akaike Information Criterion (AIC) (Akaike, 1974). The AIC criterion is based on the error sum of squares and penalizes models as parameters are added. The model with the lowest AIC is chosen as the best fit model. The VTR under these models and the respective solutions are then given by the following expressions:

$$VTR = \beta_0 + \log(LR_{TAN})(\beta_1 + CB(\beta_2) + LSB(\beta_3)) + \log(LR_{TOC})(\beta_6 + CB(\beta_7) + LSB(\beta_8)) + CB(\beta_4) + LSB(\beta_5) + D + F_1 + E \quad (5)$$

$$\widehat{VTR} = -2,612.2 + \log(LR_{TAN})(228.4 + CB*(-4.3) + LSB*74.8) + \log(LR_{TOC})(114.2 + CB*246.3 + LSB*39.4) + CB*194.2 + LSB*1,847.6 \quad (5.1)$$

$$VTR = \beta_0 + \log(LR_{TAN})(\beta_1 + LSB(\beta_3)) + \log(LR_{TOC})(\beta_6 + LSB(\beta_8)) + LSB(\beta_5) + D + F_1 + E \quad (6)$$

$$\widehat{VTR} = -2,282.3 + \log(LR_{TAN})(264.8 + LSB(93.0)) + \log(LR_{TOC})(62.4 + LSB(-26.0)) + LSB(2,123.3) \quad (6.1)$$

where VTR = Volumetric TAN removal rate (g m^{-3} (media - unexpanded) d^{-1}), $\widehat{VTR}$ = predicted VTR based on the least squares estimates to Eqs. 5 and 6 as shown in Eqs. 5.1 and 6.1, respectively, β_0 = Y-intercept for the PG filter type (g m^{-3} (media - unexpanded) d^{-1}), TAN = TAN concentration (g m^{-3}), LR_{TAN} = TAN loading rate (g m^{-3} (media - unexpanded) d^{-1}), $\beta_1, \beta_2, \beta_3$ = LR_{TAN} slope parameters for the PG, CB, and LSB filter types, respectively, β_4 and β_5 = the difference in the Y-intercepts from PG for the CB and LSB filter types, respectively, $\beta_6, \beta_7, \beta_8$ = LR_{TOC} slope parameters for the PG, CB, and LSB filter types, respectively, the CB, LSB, and PG coefficients are chosen as either the numerals 0 or 1 to indicate which model is of interest, and D, F_i , and E are the random error components for day, individual filter, and experimental effects, respectively.

3.2.1 Effect of TAN and TOC loading rates on VTR for OC_{Low} and OC_{High} levels combined

Evaluating the data for the entire organic carbon study, Eq. 5 was used as a statistical model to describe mean VTR performance. The linear dependence of VTR on $\log(LR_{TAN})$ for the entire study is shown in Fig.2. Using the coefficient of determination (R^2) as a means of understanding the linearity between VTR and $\log(LR_{TAN})$, R^2 values for the CB, LSB, and PG filters were 0.07, 0.33, and 0.39, respectively. There was a considerable amount of variation in VTR for the CB filters accounting for the low R^2 value. Elevated variance in VTR for all three filter types was observed as compared to the NOC study. Confidence

intervals about the regression lines represent the variable nature of the relationship (Fig. 2). The linear dependence on $\log(\text{LR}_{\text{TOC}})$ by VTR is shown in Fig. 3. There was a considerable amount of variation in the linear relationship between VTR and LR_{TOC} for the LSB and PG filters ($R^2 = 0.11$ and 0.14 , respectively). However, the CB filters showed much less variation, indicating a strong effect by LR_{TOC} on VTR in the CB filters ($R^2 = 0.81$), though this was a positive association implying increased VTR with increasing LR_{TOC} . The operation and performance of the CB filters was negatively affected once organic carbon was dosed into the system. The relationship between $\log(\text{LR}_{\text{TOC}})$ and VTR shows that there was a likely interaction between increased TOC and the change in VTR performance for the CB filters.

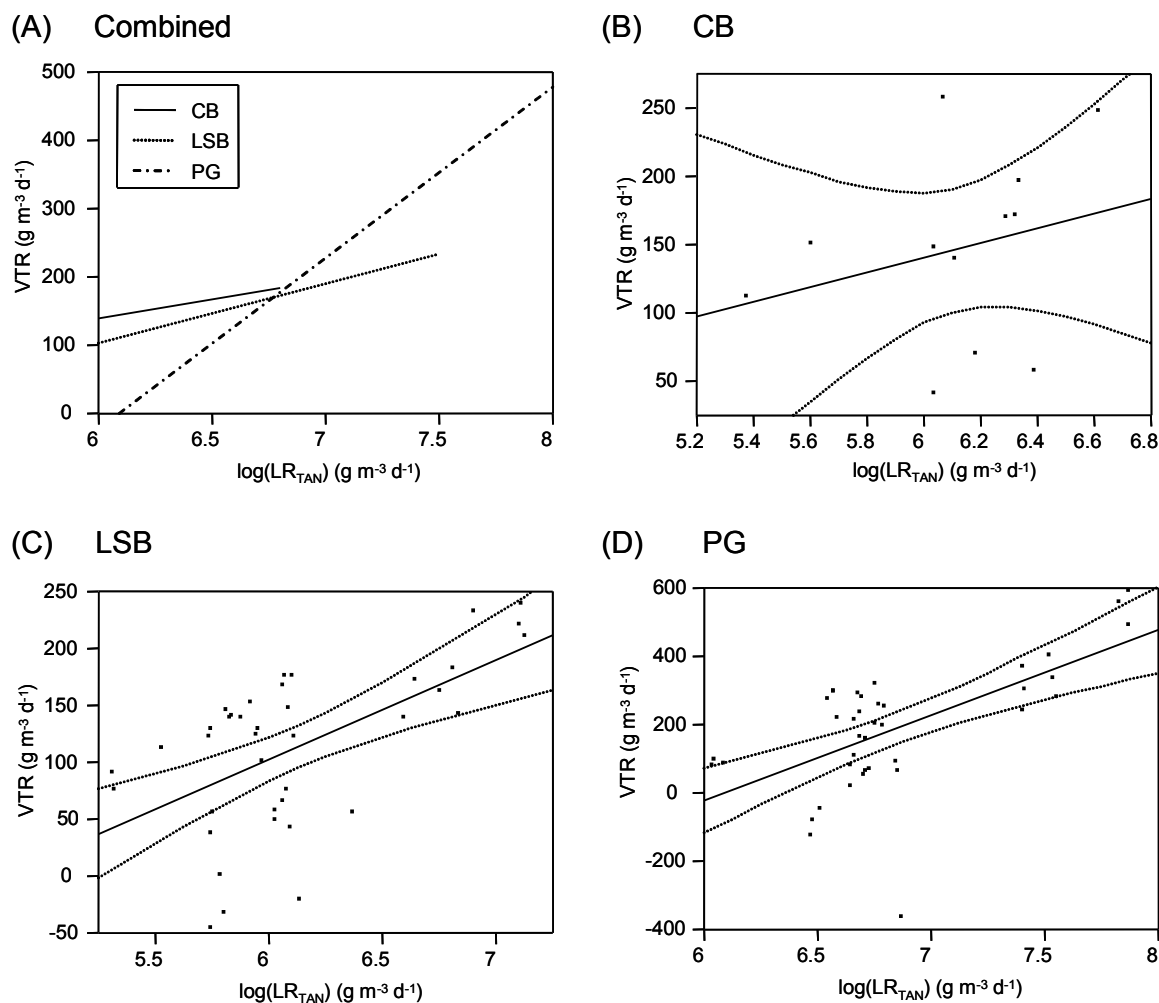


Fig. 2. The effect of $\log(LR_{TAN})$ on VTR (g m⁻³ media d⁻¹) for the entire organic carbon study (OC_{Low} and OC_{High} levels combined). A graph of all three filters (A) compares the filter types directly with corresponding regression lines. Graphs with 95% confidence intervals about the regression lines for filter types CB (B), LSB (C) and PG (D) are also shown. The data is based on 13 days for the combination of the OC_{Low} and OC_{High} levels.

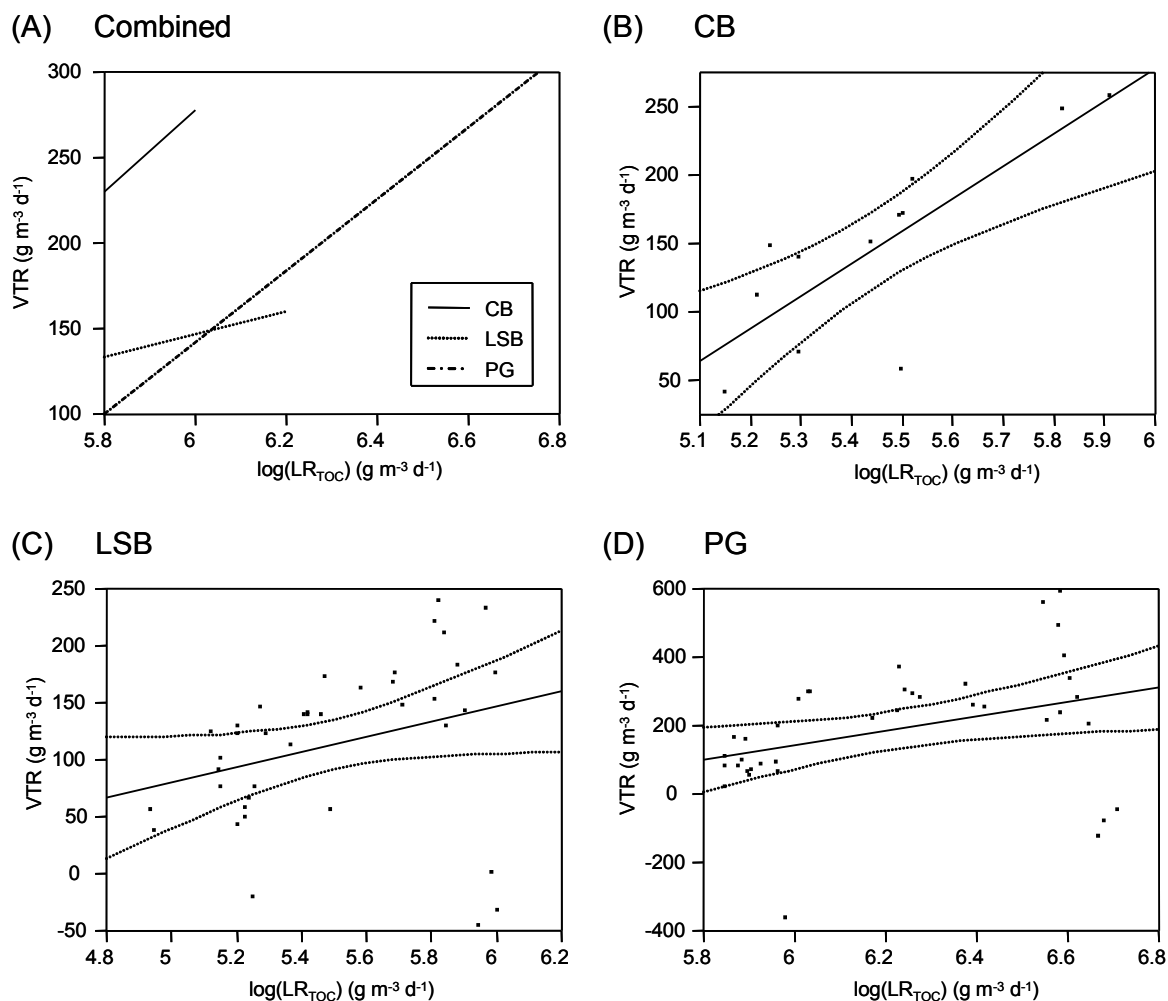


Fig. 3. The effect of $\log(LR_{TOC})$ on VTR (g m⁻³ media d⁻¹) for the entire organic carbon study (OC_{Low} and OC_{High} levels combined). A graph of all three filters (A) compares the filter types directly with corresponding regression lines. Graphs with 95% confidence intervals about the regression lines for filter types CB (B), LSB (C) and PG (D) are also shown. The data is based on 13 days for the combination of the OC_{Low} and OC_{High} levels.

The p-value for the interaction effect of $\log(LR_{TAN})$ and filter type was not significant at the 0.05 level, though it was still necessary to include the term in the model (Eq. 5) ($F = 3.18$, $p = 0.1064$). Slopes for $\log(LR_{TAN})$ that were specific to filter type provided a better fit

and resulted in a lower AIC criterion than a model with a single slope for all three filter types. Filter type specific slopes were included in the model as these effects had p-values close to 0.05, and they provided a better fit model than main effects alone. Additionally, there was a significant difference between the slope for the interaction effect between $\log(\text{LR}_{\text{TAN}})$ and VTR for the CB ($\hat{\beta}_2$) and PG filters ($\hat{\beta}_1$). The p-value for the difference was slightly insignificant for the slope parameters between the LSB ($\hat{\beta}_3$) and PG ($\hat{\beta}_1$) filters ($p = 0.0554$). However, there was not a significant difference in the CB and LSB slopes ($\hat{\beta}_2$ and $\hat{\beta}_3$, respectively).

Similarly, the interaction effect of $\log(\text{LR}_{\text{TOC}})$ and VTR was not significant at the 0.05 level, however it was also required for more appropriate mean VTR predictions in Eq. 5 based on the AIC criterion ($F = 2.15$, $p = 0.1672$). The p-value for the difference in the LSB and CB slope parameters ($\hat{\beta}_8$ and $\hat{\beta}_7$, respectively) for the interaction effect between $\log(\text{LR}_{\text{TOC}})$ and VTR was also only slightly insignificant ($p = 0.0793$). There was no significant difference between $\hat{\beta}_6$ and $\hat{\beta}_7$ nor $\hat{\beta}_6$ and $\hat{\beta}_8$.

Combined together, these two interaction effects provided the best fit model predicting VTR for this study by accounting for the amount of TAN and TOC available to the biofilter as the combination produced the lowest AIC criterion. Visualizing Eq. 5.1 is

best achieved 3-dimensionally (Fig. 4). A 3-D analysis, using a smoothing spline interpolation (SAS, 2004), of the combined effects of $\log(\text{LR}_{\text{TAN}})$ and $\log(\text{LR}_{\text{TOC}})$ on VTR demonstrates how both variables affected the VTR performance for each filter type. For the LSB and PG filters there was a general negative trend as LR_{TAN} and LR_{TOC} increased. The CB filters showed a slight positive change in VTR on the $\log(\text{LR}_{\text{TAN}})$ axis and a steady positive trend on the $\log(\text{LR}_{\text{TOC}})$ axis. The surface of the plots implies variability in VTR over both axes.

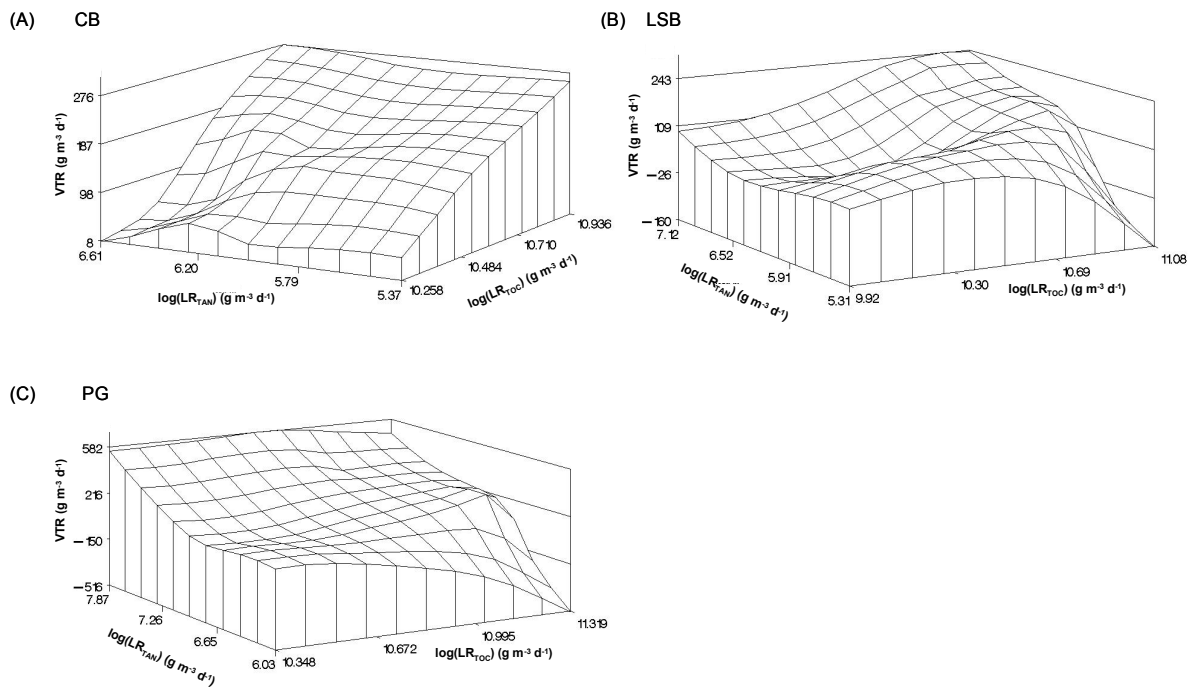


Fig. 4. A 3-dimensional analysis of the combined effects of $\log(\text{LR}_{\text{TAN}})$ and $\log(\text{LR}_{\text{NO}_2})$ on VTR. The graphs for all three filters are based on data provided by the least squares estimate to Eq. 5 (Eq. 5.1) using a smoothing spline interpolation: CB (A), LSB (B), and PG (C).

As a means of comparison, Fig 5 shows the VTR differences in the NOC and the organic carbon studies. The relationship between LR_{TAN} and VTR for the NOC study was highly linear ($R^2 = 0.84, 0.92,$ and 0.94 for CB, LSB, and PG, respectively). The same relationship for the organic carbon study showed a decrease in linearity as the impact of organic carbon presumably increased the variability in VTR ($R^2 = 0.10, 0.37,$ and 0.44 for the CB, LSB, and PG filters, respectively). VTR for the CB, LSB and PG filters was reduced by 78%, 58%, and 68%, respectively for the organic carbon study as compared to normal operating conditions.

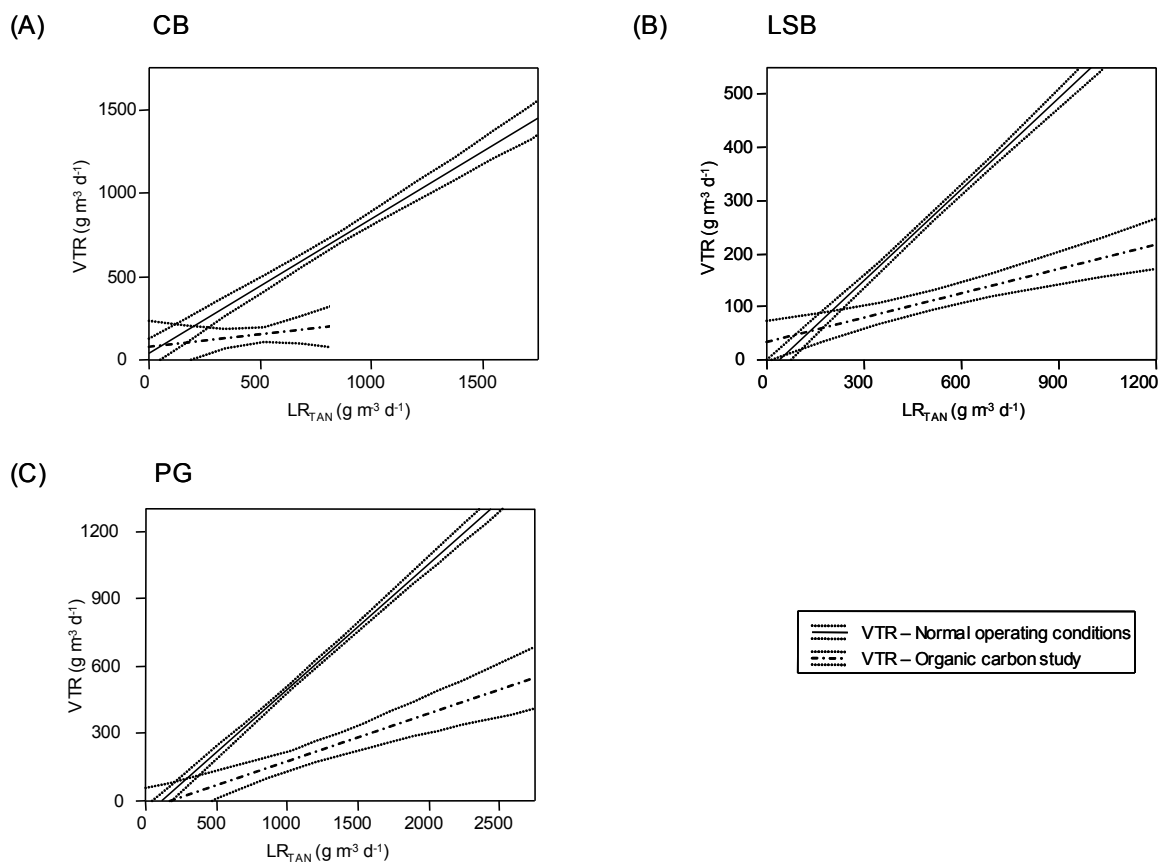


Fig. 5. Difference in the effect of TAN load rate (LR_{TAN}) on VTR between normal operating conditions and elevated organic carbon concentration with 95% confidence intervals about the regression lines for the three filter types: CB (A), LSB (B), and PG (C). The data is based on the 13 day dataset combining OC_{Low} and OC_{High} levels.

3.2.2 VTR analysis for the OC_{High} level

The OC_{High} level was based on 9 days of pseudo-steady state operating conditions. Due to operational instability, the CB filters were removed from the study. The CB filters were not in operation for the OC_{High} portion of the study as the elevated levels of organic carbon negatively affected stability and operation. Eq. 6 was developed to describe mean VTR performance for the OC_{High} dataset.

The linear dependence of VTR on $\log(LR_{TAN})$ for is shown in Fig.6. R^2 values for the relationship between VTR and $\log(LR_{TAN})$ were 0.41 and 0.54 for the LSB and PG filters, respectively. There was still variation in VTR for both of the filter types, though the variation was reduced using the OC_{High} dataset as these conditions were considered more representative of pseudo-steady state. The variability in VTR was higher than that in the NOC study.

The linear dependence on $\log(LR_{TOC})$ by VTR is shown in Fig. 7. Similar to the previous section, the linear relationship between VTR and LR_{TOC} for the LSB and PG filters was relatively low ($R^2 = 0.18$ and 0.13 , respectively). There was a negative relationship between $\log(LR_{TOC})$ and VTR for the OC_{High} level. As the LR_{TOC} increased, the VTR decreased, demonstrating the negative impact of organic carbon on VTR.

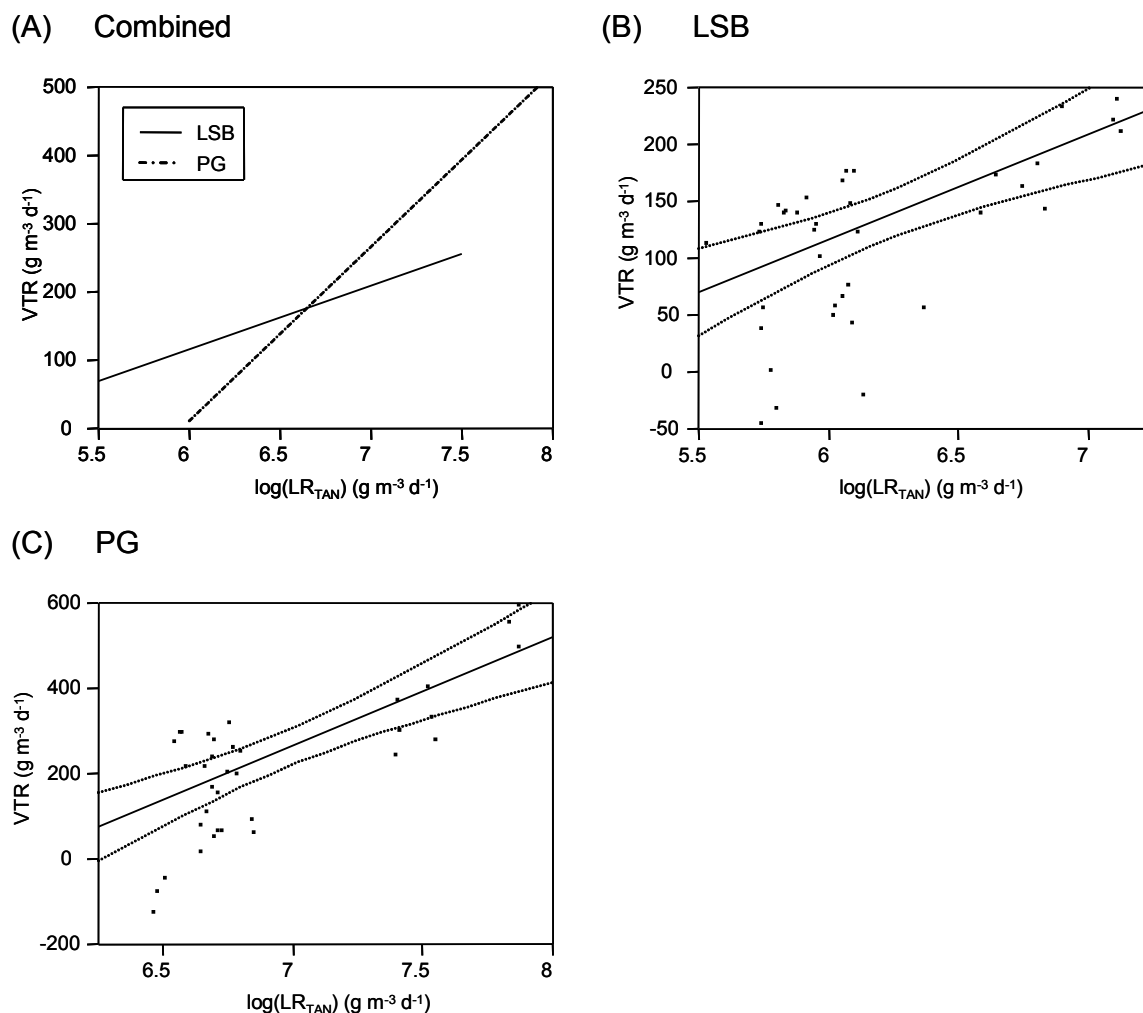


Fig. 6. The effect of $\log(LR_{TAN})$ on VTR (g m⁻³ media d⁻¹) for the OC_{High} level. A graph of all three filters (A) compares the filter types directly with corresponding regression lines. Graphs with 95% confidence intervals about the regression lines for filter types CB (B), LSB (C) and PG (D) are also shown. The data is based on 13 days for the combination of the OC_{Low} and OC_{High} levels.

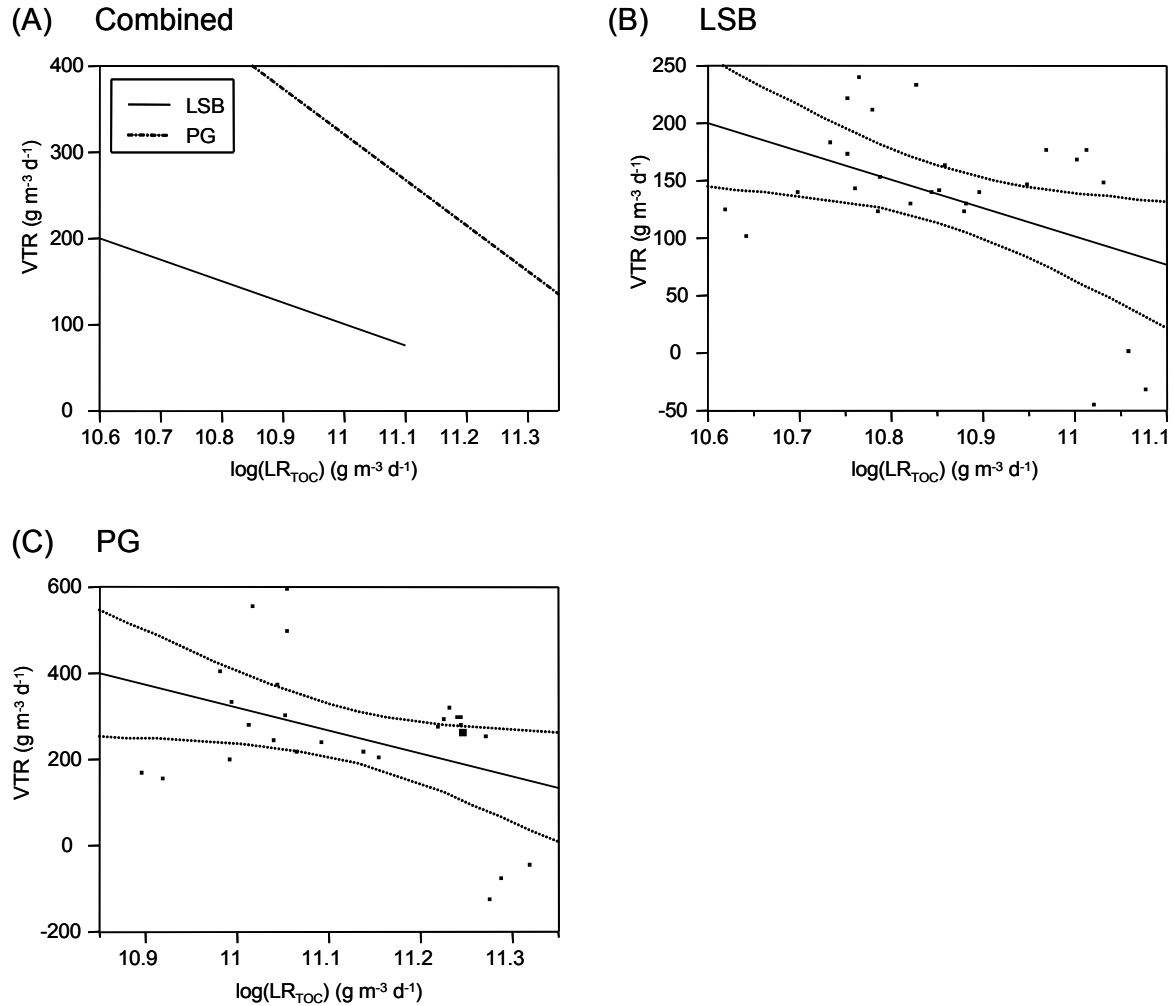


Fig. 7. The effect of $\log(LR_{TOC})$ on VTR ($g\ m^{-3}\ media\ d^{-1}$) for the OC_{High} level. A graph of all three filters (A) compares the filter types directly with corresponding regression lines. Graphs with 95% confidence intervals about the regression lines for filter types CB (B), LSB (C) and PG (D) are also shown. The data is based on 13 days for the combination of the OC_{Low} and OC_{High} levels.

The statistical analysis for the OC_{High} level was similar to the previous section. Filter type specific slopes were included in Eq. 6 as the interaction effect of $\log(LR_{TAN})$ and filter type was slightly insignificant at the 0.05 level and considered necessary for predicting VTR

($F = 3.46$, $p = 0.0837$). While the p-value for the difference between the slopes for the LSB and PG filters ($\hat{\beta}_3$ and $\hat{\beta}_1$, respectively) was not significant at the 0.05 level, but the modeling choice was made to keep the interaction in the model based on the AIC criterion.

The interaction effect of $\log(\text{LR}_{\text{TOC}})$ and VTR was also not significant at the 0.05 level, however it was required for more appropriate mean VTR predictions in Eq. 6 based on the AIC criterion ($F = 0.10$, $p = 0.7495$). The degree of difference in the LSB and PG slope parameters ($\hat{\beta}_8$ and $\hat{\beta}_6$, respectively) was enough to keep filter type specific slopes for LR_{TOC} and filter type in the model.

Combined together, these two interaction effects provided the best fit model predicting VTR for the OC_{High} level. Eq. 6 accounts for the amount of TAN and TOC available to the biofilter by using the respective substrate loading rates in predicting mean VTR for the filters. The least squares estimate to Eq. 6, (Eq. 6.1) is best visualized using a 3-dimensional graph demonstrating the combined effects of each variable on VTR (Fig. 8) with a smoothing spline interpolation (SAS, 2004). For the LSB and PG filters there was a general negative trend in VTR as LR_{TAN} and LR_{TOC} increased. The wavy surface of the graph further demonstrates the variability in VTR for both filters..

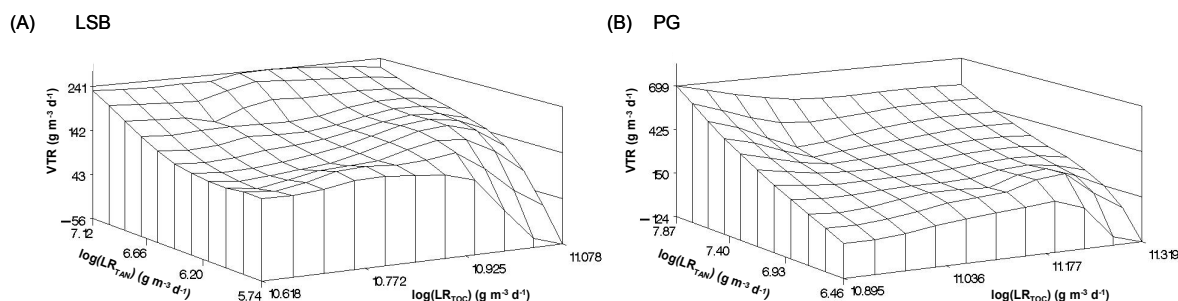


Fig. 8. A 3-dimensional analysis of the combined effects of $\log(\text{LR}_{\text{TAN}})$ and $\log(\text{LR}_{\text{NO}_2})$ on VTR. The graphs for all three filters are based on data provided by the solution to Eq. 5 (Eq. 5.1) using a smoothing spline interpolation: CB (A), LSB (B), and PG (C).

Comparing the NOC study with the results from the OC_{High} level, VTR was reduced by 49% and 56% for the LSB and PG filters, respectively (Fig. 9). The impact of organic carbon on VTR was slightly less than the comparison combining the OC_{Low} and OC_{High} levels, as the OC_{Low} data produced highly variable VTR values for all three filter types.

The net effect of increased concentrations of organic carbon is that of nitrification performance inhibition and increased variability in TAN removal rates. Competition for dissolved oxygen, media space, and nutrients between the heterotrophic and autotrophic bacterial communities reduces the TAN removal performance of biofilters (Zhu and Chen, 2001; Nogueira et al., 2002). The data from the normal operating conditions and organic carbon studies demonstrate considerable differences in nitrification rates for biofilters at the large commercial scale from that of lab scale evaluations. Organic carbon concentrations higher than previous lab scale studies were observed in both studies. Consequently, as the C/N ratio increased VTR decreased in all filters evaluated. The implication of this research clearly demonstrates the need for the removal of organics prior to biofiltration to maximize TAN removal in RAS.

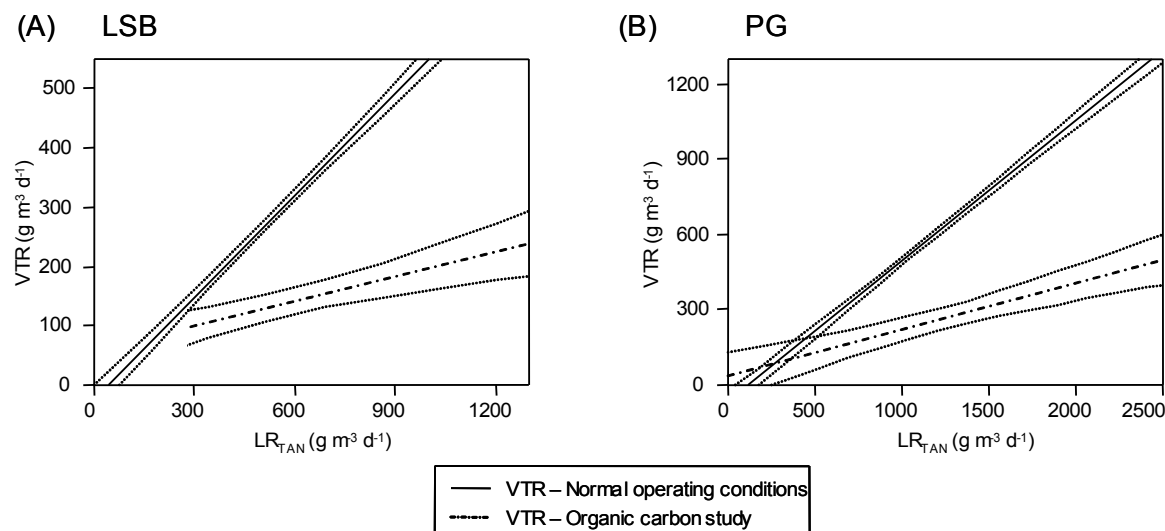


Fig. 9. Difference in the effect of TAN load rate (LR_{TAN}) on VTR between normal operating conditions and OC_{High} level of elevated organic carbon concentrations with 95% confidence intervals about the regression lines for the two filter types: LSB (A), PG (B). The organic carbon data is based on the 9 days for the OC_{High} level.

3.2.3 Organic carbon availability

At elevated concentrations of organic carbon, the difference in TAN removal rates decreased between three different types of filters evaluated in a study by Ling and Chen (2005). The results of their study demonstrated significant differences in TAN removal rates at $C/N = 0$ for all three filter types evaluated, and no significant differences between the three filter types at $C/N = 2.0$. This indicates that the indirect effect of organic carbon on nitrification in biofilters is the equalization of individual performance characteristics between filter types. The same study also showed an exponential decay in TAN removal rate in a floating bead filter with increasing COD/N ratios. As the COD/N ratio approaches 3 the

TAN removal rate exponentially decreases and remains unchanged and maintains a minimum value at COD/N ratios greater than 3 (Carrera et al., 2004; Ling and Chen, 2005). This suggests organic carbon at sufficiently high concentrations effectively eliminate differences in TAN removal rates between filters until something else becomes a limiting factor. The differences in VTR values between the filters for the organic carbon study are considerably smaller than those in the NOC study. Increasing organic carbon in the system may effectively equalize filter VTR performance. Heterotrophic activity is limited by biologically available organic carbon in the system, both dissolved and particulate. Increasing the availability of DOC in the system increases the heterotrophic activity to a point, after which the limiting factor is available particulate organic carbon (POC) (Leonard et al., 2002; Michaud et al., 2006). Improved feed assimilation and mechanical filtration will reduce the availability of both dissolved and particulate organic carbon. Reducing the availability of organic carbon effectively reduces heterotrophic activity and nitrification inhibition within a biofilter. Future studies quantifying the impact will need to differentiate between total organic carbon (TOC) in the system and biologically available carbon. The measure of organic carbon in terms of availability for degradation is commonly misrepresented in a C/N ratio. Creating a ratio more representative of degradable organic carbon and ammonia-nitrogen will allow for better understanding of potential impacts on nitrification.

Previous studies have used ratios comparing the amount of ammonia-nitrogen to COD (COD/N) or TOC (C/N) as a means of comparing the amount of organic carbon in a system and relating that to nitrification rates in biofilters (Ohashi et al., 1995; Satoh et al., 2000; Carrera et al., 2004; Ling and Chen, 2005; Zhu and Chen, 2001). Such measures are

feasible only in controlled studies using artificial waste nutrients to measure TAN removal rates. Actual production conditions are subject to profoundly different organic inputs in the form of feed, feces, and dead organisms. Ratios produced using actual water from large scale production systems are markedly higher than those found at the lab scale as seen in this and the NOC studies. Total organic carbon in a system measured via TOC or COD analysis show all organic carbon in the system and does not account for that which is unavailable or not readily degradable by heterotrophic bacteria. Biochemical oxygen demand (BOD) would be a more appropriate measure of available organics and the potential for nitrification inhibition. BOD is a measure of the oxygen required by the micro-organisms to oxidize the biologically available organic matter and ammonia in the sample (Henze et al., 1995). The industry standard for BOD measurements is a 5 day BOD analysis (BOD_5). Running a BOD analysis for 5 days at 20°C allows the major part of the biodegradable material to be oxidized. Modifying the analysis to account for oxidation of only organic carbon yields a carbonaceous BOD_5 ($CBOD_5$) analysis (APHA, 1998). $CBOD_5$ is a more specific measure of the biologically degradable organic carbon in the system as the oxidation of ammonia is blocked thus making it a more appropriate measure to be used in future studies. Relating VTR to a ratio of carbonaceous BOD_5 / ammonia-nitrogen ($CBOD_5/N$) will provide a measure more appropriate to the production environment than COD/N and C/N .

Negative VTR values for the PG and LSB filter types were observed on the final day of sampling in this study. While the conditions were considered pseudo-steady state, these results may show the possibility of complete heterotrophic inhibition of nitrification. To gain a full understanding of the maximum sustainable level of organic carbon in a system, more

data over a longer time period with elevated organic carbon concentrations is needed. The type of organic carbon used in future studies should be considered also. Sucrose carbon is easily degraded by the heterotrophic bacteria, thus creating an artificial heterotrophic advantage. Utilizing increased feed inputs, a soluble mixture derived from feed, or reduced mechanical filtration efficiency to adjust the level of organic carbon in the system could provide results that are more similar to actual culture conditions.

4. Conclusions

The results from this study support the following conclusions:

- By increasing organic carbon in the system, variability in VTR for all three filter types increases, ultimately reducing TAN removal rates by 50% to 70%.
- Increased organic carbon concentrations reduce the difference in TAN removal rates between individual filter types.
- Future studies evaluating organic carbon effects on biofilter nitrification capacity should consider using measures more applicable to the production environment. Using a CBOD₅/N ratio to describe the amount of organic carbon in the system and the potential effects on VTR will yield more useful results than that of COD/N or C/N ratios.
- Reducing mechanical filtration efficiency and increasing feed rates will produce greater concentrations of actual organic waste nutrients in the system. Using this or other feed based sources of organic carbon for future evaluation studies will produce a more realistic understanding of heterotrophic interaction at the commercial scale.

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References

- APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C.
- Carrera, J., Vicent, T., Lafuente, J., 2004. Effect of influent COD/N ratio on biological nitrogen removal (BNR) from high-strength ammonia industrial wastewater. *Process Biochem.* 39, 2035–2041.
- Chen, S., Ling, J., Blancheton, J., 2006. Nitrification kinetics of biofilm as affected by water quality factors. *Aquacultural Engineering* 34(3): 179-197.
- Colt, J., Lamoureux, J., Patterson, R., Rogers, G., 2006. Reporting standards for biofilter performance studies. *Aquacultural Engineering* 34(3): 377-388.

- Eding, E. H., Kamstra, A., Verreth, J. A. J., Huisman, E. A., Klapwijk, A., 2006. Design and operation of nitrifying trickling filters in recirculating aquaculture: A review. *Aquacultural Engineering* 34(3): 234-260.
- Guerdat, T.C., 2008. A large scale evaluation of commercially available biological filters for recirculating aquaculture systems. Not published currently. 19pp.
- Henze, M., Härremoes, P., Jansen, J. la C., Arvin, E., 1995. Wastewater Treatment: Biological and Chemical Processes. Springer Verlag, Berlin, Heidelberg, Germany, 383 pp.
- JMP version 7.0, 2007. SAS Institute Inc., Cary, NC, USA.
- Lee, L. Y., Ong, S. L., Ng, W. J., 2004. Biofilm morphology and nitrification activities: Recovery of nitrifying biofilm particles covered with heterotrophic outgrowth. *Bioresource technology* 95(2): 209-214.
- Leonard, N., J. P. Guiraud, E. Gasset, J. P. Cailleres and J. P. Blancheton. 2002. Bacteria and nutrients - Nitrogen and carbon - In a recirculating system for sea bass production. *Aquacultural Engineering* 26(2): 111-127.
- Ling, J., Chen, S. L., 2005. Impact of organic carbon on nitrification performance of different biofilters. *Aquacultural Engineering* 33(2): 150-162.

Losordo, T. M., Masser, M. P., Rakocy, J., 1998. Recirculating Aquaculture Tank Production Systems: An Overview of Critical Considerations. SRAC 451, 6 pp.

Losordo, T. M. and A. O. Hobbs. 2000. Using computer spreadsheets for water flow and biofilter sizing in recirculating aquaculture production systems. *Aquacultural Engineering* 23(1): 95-102.

Losordo, T.M., Hobbs, A.O., DeLong, D.P., 2000. The design and operational characteristics of the CP&L/EPRI fish barn: a demonstration of recirculating aquaculture technology. *Aquacult. Eng.* 22, 3–16.

Malone, R.F., Beecher, L.E., 2000. Use of floating bead filters to recondition recirculating waters in warmwater aquaculture production systems. *Aquacult. Eng.* 22, 57-73.

Meade, J. W., 1985. Allowable ammonia for fish culture. *Progressive Fish-Culturist* 47, 135-145.

Michaud, L., J. P. Blancheton, V. Bruni and R. Piedrahita. 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. *Aquacultural Engineering* 34(3): 224-233.

- Nogueira, R., Melo, L. F., Purkhold, U., Wuertz, S., M. Wagner, 2002. Nitrifying and heterotrophic population dynamics in biofilm reactors: Effects of hydraulic retention time and the presence of organic carbon. *Water research* 36(2): 469-481.
- Ohashi, A., Virajde Silva, D.G., Mobarry, B., Manem, J.A., Stahl, D.A. Rittman, B.E., 1995. Influence of substrate C/N ratio on the structure of multi-species biofilms consisting of nitrifiers and Heterotrophs. *Water Sci. Technol.* 32, 75-84.
- Pfeiffer, T. and R. Malone. 2006. Nitrification performance of a propeller-washed bead clarifier supporting a fluidized sand biofilter in a recirculating warmwater fish system. *Aquacultural Engineering* 34(3): 311-321.
- SAS Institute Inc., 2004. SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2000-2004.
- Satoh, H., Okabe, S., Norimatsu, N., Watanabe, Y., 2000. Significance of substrate C/N ratio on structure and activity of nitrifying biofilms determined by in situ hybridization and the use of microelectrodes. *Water Science and Technology* 41, 317-321.
- United States Environmental Protection Agency (US-EPA), 1984. *Methods for the Chemical Analysis of Water and Wastewater*, EPA-600/4-79-020. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

Zhu, S., Chen, S., 1999. An experimental study on nitrification biofilm performances using a series reactor system. *Aquacultural Engineering* 20(4): 245-259.

Zhu, S., Chen, S., 2001. Effects of organic carbon on nitrification rate in fixed film biofilters. *Aquacultural Engineering* 25(1): 1-11.

APPENDIX

Appendix 1

1. SAS programs

1.1 Normal operating conditions

```

options formdlm="_";

data vtr;
  input day DO Temp pH TAN NO2 TOC COD FilterType $ FilterNumber TAN_LR
  VTR NO2_LR VNRA;
  VNR = vtr+vnra;
  cod_n = cod/tan;
  tanc=tan-.6892; label tanc="centered TAN";
  tanlrc=tan_lr-817.6; label tanlrc="centered TAN_LR";
  lvtr=log(vtr);
  eff=(vtr/tan_lr)*100;
  ltanlr=log(tan_lr);
  ltan=log(tan);
  lno2lr=log(no2_lr);
cards;

Data removed for publication purposes

;

/*
proc print data=vtr;
run;

*/

ods html;

proc sort data=vtr;
  by filtertype;
run;

data vtrcomp_all;
  set vtr;
  if tan_lr>943.0 then delete;
  if tan_lr<194.0 then delete;
run;

data vtrcomp_cb1sb;
  set vtr;
  if tan_lr<86.0 then delete;
  if tan_lr>943.0 then delete;
run;

```

```

data vtrcomp_cbpbg;
    set vtr;
    if tan_lr<194.0 then delete;
    if tan_lr>1710.4 then delete;
run;

```

```

data vtrcomp_lsbg;
    set vtr;
    if tan_lr<194.0 then delete;
    if tan_lr>943.0 then delete;
run;

```

```

proc means data=vtr;
    title 'Means';
    *by filtertype;
    var tan ph do temp no2 toc cod;
run;

```

```

proc means data=vtr ;
    by filtertype;
    var tan_lr vtr no2_lr vnr eff;
run;

```

```

proc sort data=vtr;
    by filtertype;
run;

```

```

symbol value=dot i=r1;
proc gplot;
    title 'Comparison';
    *by filtertype;
    *plot lvtr*tan = filtertype;
    *plot lvtr*tan_lr = filtertype;
    *plot vtr*tan = filtertype;
    *plot vtr*tan_lr = filtertype;
    plot vnr*no2_lr = filtertype;
    plot vnr*no2 = filtertype;
run;

```

```

symbol value=dot i=r1;
/*
proc gplot;
    plot vtr*cod_n = filtertype;
run;
*/

```

```

*proc mixed data=vtr method=type3;
proc mixed data=vtr;
  title "Modeling VTR as a function of TAN concentration";
  *by filtertype;
  class filtertype day filternumber;
  *model lvtr=tan|filtertype /solution outp=two ddfm=kr  noint;
  model vtr=tan|filtertype /solution outp=two ddfm=kr;
  *model vtr=tanc|filtertype /solution outp=two ddfm=kr  noint;
  random filternumber(filtertype);
  random day /group=filtertype;
  lsmeans filtertype / bylevel;
  *estimate "diff between slopes for LSB and CB (TAN)" tanc*filtertype 1
-1 0;
  *estimate "diff between slopes for LSB and CB (TAN)" tan*filtertype 1 -1
0;
  *estimate "diff between slopes for PG and CB (TAN)" tan*filtertype 1 0
-1;
  *estimate "diff between slopes for LSB and PG (TAN)" tan*filtertype 0 -1
1;
run;
/*
proc mixed data=vtr;
  title "Modeling VTR as a quadratic function of TAN concentration";
  *by filtertype;
  class filtertype day filternumber;
  *model lvtr=tan|filtertype /solution outp=two ddfm=kr  noint;
  model vtr=tan|filtertype tan*tan tan*tan|filtertype /solution outp=two
ddfm=kr;
  *model vtr=tanc|filtertype /solution outp=two ddfm=kr  noint;
  random filternumber(filtertype);
  random day /group=filtertype;
  lsmeans filtertype / diffs adj=bon bylevel;
  *estimate "diff between slopes for LSB and CB (TAN)" tanc*filtertype 1
-1 0;
  *estimate "diff between slopes for LSB and CB (TAN)" tan*filtertype 1 -1
0;
  *estimate "diff between slopes for PG and CB (TAN)" tan*filtertype 1 0
-1;
  *estimate "diff between slopes for LSB and PG (TAN)" tan*filtertype 0 -1
1;
run;
*/
/*
proc mixed data=vtr;
  title "Modeling VTR as a log function of TAN concentration";
  *by filtertype;
  class filtertype day filternumber;
  *model lvtr=tan|filtertype /solution outp=two ddfm=kr  noint;
  model vtr=ltan|filtertype /solution outp=two ddfm=kr;
  *model vtr=tanc|filtertype /solution outp=two ddfm=kr  noint;
  random filternumber(filtertype);
  random day /group=filtertype;
  lsmeans filtertype / diffs adj=bon;

```

```

    *estimate "diff between slopes for LSB and CB (TAN)" tan*c*filtertype 1
-1 0;
    *estimate "diff between slopes for LSB and CB (TAN)" tan*filtertype 1 -1
0;
    *estimate "diff between slopes for PG and CB (TAN)" tan*filtertype 1 0
-1;
    *estimate "diff between slopes for LSB and PG (TAN)" tan*filtertype 0 -1
1;
run;

proc gplot data=two;
    plot resid*pred=filtertype;
run;
*/
/*
proc gplot data=two;
    by filtertype;
    plot vtr*tan;
run;
*/
proc mixed data=vtr;
    title "Modeling VTR as a function of TAN Loading Rate";
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=tan_lr|filtertype /solution outp=two ddfm=kr;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel ;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cbldb;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cbpg;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_lsdbpg;

run;
/*
proc mixed data=vtr;
    title "Modeling VTR as a quadratic function of TAN Loading Rate";
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=tan_lr|filtertype tan_lr*tan_lr|filtertype /solution outp=two
ddfm=kr ;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel diffs adj=bon;

```

```

lsmeans filtertype / diffs  adj=bon bylevel om=vtrcomp_cb1sb;
lsmeans filtertype / diffs  adj=bon bylevel om=vtrcomp_cbpg;
lsmeans filtertype / diffs  adj=bon bylevel om=vtrcomp_ls1bpg;

run;
*/
/*
proc mixed data=vtr;
  title "Modeling VTR as a log function of TAN Loading Rate";
  class filtertype day filternumber;
  *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
  model vtr=ltanlr|filtertype /solution outp=two ddfm=kr ;
  *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
  random filternumber(filtertype);
  random day /group=filtertype;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
  lsmeans filtertype / bylevel diffs adj=bon;
run;

proc gplot data=two;
  plot resid*pred=filtertype;
run;
*/
/*
proc mixed data=vtr;

  title "Modeling VNR as a function of TAN_LR";
  class filtertype day filternumber;
  model vnr=tan_lr|filtertype /solution outp=two ddfm=kr ;
  random filternumber(filtertype);
  random day /group=filtertype;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
  *estimate "diff between slopes for LSB and CB (NO2_LR)"
no2_lr*filtertype 1 -1 0;
  lsmeans filtertype / bylevel ;
  *lsmeans filtertype / diffs  adj=bon bylevel om=vtrcomp_cb1sb;
  *lsmeans filtertype / diffs  adj=bon bylevel om=vtrcomp_cbpg;
  *lsmeans filtertype / diffs  adj=bon bylevel om=vtrcomp_ls1bpg;

run;

proc mixed data=vtr;

  title "Modeling VNR as a log function of TAN_LR";
  class filtertype day filternumber;
  model vnr=ltanlr|filtertype /solution outp=two ddfm=kr ;
  random filternumber(filtertype);

```

```

    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (NO2_LR)"
no2_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel ;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cb1sb;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cbpg;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_ls1bpg;

run;

proc mixed data=vtr;

    title "Modeling VNR as a quadratic function of TAN_LR";
    class filtertype day filternumber;
    model vnr=tan_lr|filtertype tan_lr*tan_lr|filtertype/solution outp=two
ddfm=kr ;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (NO2_LR)"
no2_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel ;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cb1sb;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cbpg;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_ls1bpg;

run;

proc mixed data=vtr;

    title "Modeling VNR as a log function of NO2_LR";
    class filtertype day filternumber;
    model vnr=lno2lr|filtertype /solution outp=two ddfm=kr ;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (NO2_LR)"
no2_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel ;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cb1sb;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cbpg;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_ls1bpg;

run;

proc mixed data=vtr;

    title "Modeling VNR as a quadratic function of NO2_LR";
    class filtertype day filternumber;

```

```

    model vnr=no2_lr|filtertype no2_lr*no2_lr|filtertype /solution outp=two
ddfm=kr ;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (NO2_LR)"
no2_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel ;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cb1sb;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cbpg;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_lsbg;

run;

```

```

proc mixed data=vtr;

    title "Modeling VNR as a function of TAN_LR and NO2_LR";
    class filtertype day filternumber;
    model vnr=tan_lr|filtertype no2_lr|filtertype/solution outp=two
ddfm=kr ;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (NO2_LR)"
no2_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cb1sb;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cbpg;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_lsbg;

run;
*/

```

```

proc mixed data=vtr;

    title "Modeling VNR as a log function of TAN_LR and NO2_LR";
    class filtertype day filternumber;
    model vnr=ltanlr|filtertype lno2lr|filtertype/solution outp=vnr
ddfm=kr ;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (NO2_LR)"
no2_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cb1sb;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cbpg;
    *lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_lsbg;

run;

```

```

goptions reset=global gunit=pct border cback=white
        colors=(black blue green red)
        ftext=swiss ftitle=swissb htitle=6 htext=4;

proc g3grid data=vnv out=smooth2;
    by filtertype;
    grid ltanlr*lno2lr=vnv / smooth=.05 spline ;
run;

proc g3d data=smooth2;
    by filtertype;
    plot ltanlr*lno2lr=vnv / ctop=black cbottom=gray caxis=black grid
side rotate=30;
run;

/*
proc mixed data=vtr;

    title "Modeling VNR as a function of NO2_LR and TAN_LR";
    class filtertype day filternumber;
    model vnr=no2_lr|filtertype tan_lr|filtertype /solution outp=two ddfm=kr
;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (NO2_LR)"
no2_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel diffs adj=bon;
    lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cbldb;
    lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_cbpg;
    lsmeans filtertype / diffs adj=bon bylevel om=vtrcomp_lsbg;

run;

proc gplot data=two;
    plot resid*pred=filtertype;
    plot vnr*pred=filtertype;
run;

proc mixed data=vtr;

    title "Modelling VNR as a function of NO2 concentration and VTR";
    class filtertype day filternumber;
    model vnr=NO2|filtertype vtr|filtertype /solution outp=three ddfm=kr ;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;

```

```

*estimate "diff between slopes for LSB and CB (NO2)" NO2*filtertype 1 -1
0;
lsmeans filtertype/diffs adj=bon;
run;

```

```

proc mixed data=vtr;
  title "Modelling VNR as a function of NO2";
  class filtertype day filternumber;
  *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
  model vtr=NO2|filtertype /solution outp=two ddfm=kr ;
  *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
  random day filternumber(filtertype);
  random day /group=filtertype;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
  estimate "diff between slopes for LSB and CB (NO2)" NO2*filtertype 1 -1
0;
  lsmeans filtertype/diffs adj=bon;
run;

```

```

proc glm data=vtr;
  title "modelling vnr as a function of no2_lr";
  class filtertype day filternumber;
  *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
  model vnr=no2_lr|filtertype day filternumber /solution ;
  *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
  random day filternumber filtertype;
  *random day /group=filtertype;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
  estimate "diff between slopes for LSB and CB (NO2_lr)" no2_lr*filtertype
1 -1 0;
  *lsmeans filtertype/diffs adj=bon;
run;

```

```

proc mixed data=vtr;
  title "modelling vnr as a function of no2_lr";
  class filtertype day filternumber;
  *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
  model vnr=no2_lr|filtertype /solution outp=two ddfm=kr ;
  *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
  random day filternumber(filtertype);
  random day /group=filtertype;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
  estimate "diff between slopes for LSB and CB (TAN_lr)" no2_lr*filtertype
1 -1 0;
  lsmeans filtertype/diffs adj=bon;
run;

```

```

proc gplot data=two;
  plot resid*pred=filtertype;

```

```

run;

proc glm data=vtr;
  title 'Modeling VNR as function of NO2 concentration';
  *by filtertype;
  class filtertype day filternumber;
  *model lvtr=tan|filtertype day filternumber /solution;
  *model vtr=tan_lr|filtertype day filternumber /solution;
  *model vtr=tan_lr|filtertype tan|filtertype day filternumber /solution;
  model vnr=no2|filtertype day filternumber /solution;
  *model vtr=day filternumber tan_lr|filtertype/solution;
  random day filternumber;
  estimate "diff between slopes for LSB and CB (NO2)" no2*filtertype 1 -1
0;
  *estimate "diff between slopes for pg and CB(TAN)" tan*filtertype 1 0 -1;
  *estimate "diff between slopes for LSB and pg(TAN)" tan*filtertype 0 -1
1;
  *estimate "diff b/t FT params for lsb and cb(TAN)" filtertype 1 -1 0;
  *estimate "diff b/t FT params for pg and cb(TAN)" filtertype 1 0 -1;
  *estimate "diff b/t FT params for lsb and pg(TAN)" filtertype 0 -1 1;
  **estimate "diff b/t slopes for lsb and cb(TAN_LR)" tan_lr*filtertype 1
-1 0;
  **estimate "diff b/t slopes for pg and cb(TAN_LR)" tan_lr*filtertype 1 0
-1;
  **estimate "diff b/t slopes for lsb and pg(TAN_LR)" tan_lr*filtertype 0
-1 1;

run;
/*
proc glm data=vtr;
  *by filtertype;
  class filtertype day filternumber;
  model lvtr=tan_lr|filtertype day filternumber /solution;
  *model vtr=tan_lr|filtertype day filternumber /solution;
  *model vtr=tan_lr|filtertype tan|filtertype day filternumber /solution;
  *model vtr=tan|filtertype day filternumber /solution;
  *model vtr=day filternumber tan_lr|filtertype/solution;
  random day filternumber;
  *estimate "diff between slopes for LSB and CB (TAN)" tan*filtertype 1 -1
0;
  *estimate "diff between slopes for pg and CB(TAN)" tan*filtertype 1 0 -1;
  *estimate "diff between slopes for LSB and pg(TAN)" tan*filtertype 0 -1
1;
  estimate "diff b/t slopes for lsb and cb(TAN_LR)" tan_lr*filtertype 1
-1 0;
  estimate "diff b/t slopes for pg and cb(TAN_LR)" tan_lr*filtertype 1 0
-1;
  estimate "diff b/t slopes for lsb and pg(TAN_LR)" tan_lr*filtertype 0 -1
1;
  estimate "diff b/t FT params for lsb and cb(TAN_LR)" filtertype 1 -1 0;
  estimate "diff b/t FT params for pg and cb(TAN_LR)" filtertype 1 0 -1;
  estimate "diff b/t FT params for lsb and pg(TAN_LR)" filtertype 0 -1 1;

```

```

run;

proc glm data=vtr;
  *by filtertype;
  class filtertype;
  model vtr=in_tan|filtertype / solution;
  estimate "diff between slopes for LSB and CBV" in_tan*filtertype 1 -1 0;
run;

proc mixed data=vtr;
  by filtertype;
  title 'Modeling VTR as a function of DO and TAN Loading Rate';
  class Day FilterType FilterNumber;
  *model VTR=TAN|FilterType cod cod_n toc tan_lr/solution;
  *model VTR=tan|filtertype/solution;
  model VTR=tan_lr|filtertype DO /solution;
  random Day FilterNumber;
  *estimate "diff between slopes for LSB and CB (TAN)" tan*filtertype 1 -1
0;
  *estimate "diff between slopes for pg and CB(TAN)" tan*filtertype 1 0 -1;
  *estimate "diff between slopes for LSB and pg(TAN)" tan*filtertype 0
-1 1;
  estimate "diff b/t slopes for lsb and cb(TAN_LR)" tan_lr*filtertype 1
-1 0;
  estimate "diff b/t slopes for pg and cb(TAN_LR)" tan_lr*filtertype 1 0
-1;
  estimate "diff b/t slopes for lsb and pg(TAN_LR)" tan_lr*filtertype 0
-1 1;
  *estimate "diff b/t FT params for lsb and cb(TAN_LR)" filtertype 1 -1
0;
  *estimate "diff b/t FT params for pg and cb(TAN_LR)" filtertype 1 0 -1;
  *estimate "diff b/t FT params for lsb and pg(TAN_LR)" filtertype 0 -1
1;
  lsmeans filtertype/diffs adj=bon;
run;

proc mixed data=vtr;
  title 'Modeling VTR as a function of DO and TAN concetration';
  class Day FilterType FilterNumber;
  *model VTR=TAN|FilterType cod cod_n toc tan_lr/solution;
  *model VTR=tan|filtertype/solution;
  model VTR=tan|filtertype DO/solution;
  random Day FilterNumber;
  estimate "diff between slopes for LSB and CB (TAN)" tan*filtertype 1 -1
0;
  estimate "diff between slopes for pg and CB(TAN)" tan*filtertype 1 0 -1;
  estimate "diff between slopes for LSB and pg(TAN)" tan*filtertype 0 -1 1;
  *estimate "diff b/t FT params for lsb and cb(TAN)" filtertype 1 -1 0;
  *estimate "diff b/t FT params for pg and cb(TAN)" filtertype 1 0 -1;
  *estimate "diff b/t FT params for lsb and pg(TAN)" filtertype 0 -1 1;

```

```
*estimate "diff b/t slopes for lsb and cb(TAN_LR)" tan_lr*filtertype 1
-1 0;
*estimate "diff b/t slopes for pg and cb(TAN_LR)" tan_lr*filtertype 1 0
-1;
*estimate "diff b/t slopes for lsb and pg(TAN_LR)" tan_lr*filtertype 0
-1 1;
lsmeans filtertype/diffs adj=bon;
run;
*/

ods html close;
```

1.2 Organic carbon study

```

options formdlm="_";

data vtr;
  input day DO Temp pH Alk TAN NO2 NO3 COD TOC FilterType $ FilterNumber
  VTR VNRA TAN_LR NO2_LR COD_LR TOC_LR;
  VNR = vtr+vnra;
  cod_n = cod/tan;
  *tanc=tan-.6777; label tanc="centered TAN";
  *tanlrc=tan_lr-817.6; label tanlrc="centered TAN_LR";
  lvtr=log(vtr);
  ltan=log(tan);
  ltanlr=log(tan_lr);
  ltoclr=log(toc_lr);
  ltanlr2=log(tan_lr*tan_lr);
cards;
Data removed for publication purposes
;

*proc print data=vtr;
*run;

ods html;

proc sort data=vtr;
  by filtertype;
run;

data vtrcomp_cblsb;
  set vtr;
  if tan_lr<215.3 then delete;
  if tan_lr>743.2 then delete;
run;

data vtrcomp_cbpg;
  set vtr;
  if tan_lr<417.7 then delete;
  if tan_lr>743.2 then delete;
run;

data vtrcomp_lsbpg;
  set vtr;
  if tan_lr<417.7 then delete;
  if tan_lr>1240.8 then delete;
run;

data OClow;
  set vtr;

```

```

        if day>4 then delete;
run;

data OChigh;
    set vtr;
    if day<5 then delete;
run;

data ochigh_lsbgp;
    set ochigh;
    if tan_lr<641.8 then delete;
    if tan_lr>1240.8 then delete;
run;

proc means data=vtr;
    title 'Means of Overall OC operating conds';
    var tan ph do temp no2 toc cod alk ;
run;

proc means data=vtr;
    title 'Means of Overall OC operating conds';
    by filtertype;
    var tan_lr vtr no2_lr vnr cod_lr toc_lr;
run;

proc means data=OClow;
    title 'Means of OClow';
    var tan ph do temp no2 toc cod alk ;
run;

proc means data=OClow;
    title 'Means of OClow';
    by filtertype;
    var tan_lr vtr no2_lr vnr cod_lr toc_lr;
run;

proc means data=OChigh;
    title 'Means of OChigh';
    var tan ph do temp no2 toc cod alk ;
run;

proc means data=OChigh;
    title 'Means of OChigh';
    by filtertype;
    var tan_lr vtr no2_lr vnr cod_lr toc_lr;
run;

proc sort data=vtr;
    by filtertype;
run;

```

```

symbol value=dot i=rl;
proc gplot data =vtr;
  title "Comparison";
  *by filtertype;
  *plot lvtr*tan = filtertype;
  *plot lvtr*tan_lr = filtertype;
  *plot vtr*tan = filtertype;
  *plot vtr*tan_lr = filtertype;
  *plot vtr*ltanlr = filtertype;
  *plot vtr*toc_lr = filtertype;
  *plot vnr*no2_lr = filtertype;
  *plot vnr*no2 = filtertype;
  plot vtr*ltanlr=filtertype;
  plot vtr*ltoclr=filtertype;
run;

symbol value=dot i=rl;
/*
proc gplot;
  plot vtr*cod_n = filtertype;
run;
*/
/*
proc nlin  data=vtr;
  title "Non-Linear exponential decay model of VTR dependence on COD/N";
  by filtertype;
  parms a=500 b=40 k=-0.5;
  model vtr =a + b*exp(k*cod_n) ;
  output out=pred p=yhat student=resid l95m=lower u95m=upper;run;

proc gplot data=pred;
  plot resid*yhat=filtertype;
  *plot vtr*p.=filtertype;
run;

*proc mixed data=vtr method=type3;
proc mixed data=vtr;
  title "Modeling VTR as a function of TAN concentration";
  *by filtertype;
  class filtertype day filternumber;
  *model lvtr=tan|filtertype /solution outp=two ddfm=kr  noint;
  model vtr=tan|filtertype /solution outp=two ddfm=kr  noint;
  *model vtr=tanc|filtertype /solution outp=two ddfm=kr noint;
  random filternumber(filtertype);
  random day /group=filtertype;
  lsmeans filtertype/diffs  adj=bon ;
  *estimate "diff between slopes for LSB and CB (TAN)" tanc*filtertype 1
-1 0;
  *estimate "diff between slopes for LSB and CB (TAN)" tan*filtertype 1 -1
0;

```

```

    *estimate "diff between slopes for PG and CB (TAN)" tan*filtertype 1 0
-1;
    *estimate "diff between slopes for LSB and PG (TAN)" tan*filtertype 0 -1
1;
run;

proc gplot data=two;
    plot resid*pred=filtertype;
    plot vtr*pred=filtertype;
run;

proc gplot data=two;
    by filtertype;
    plot vtr*tan;
run;
*/
/*
proc mixed data=ochigh;
    title "Modeling VTR as a function of log(tan_lr*tan_lr)";
    *by filtertype;
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=ltanlr2|filtertype /solution outp=two ddfm=kr ;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel diffs adj=bon e;
run;
*/
/*
proc mixed data=vtr;
    title "Modeling VTR as a function of TAN Loading Rate";
    *by filtertype;
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=tan_lr filtertype /solution outp=two ddfm=kr ;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel ;
run;

proc mixed data=vtr;
    title "Modeling VTR as a quadratic function of TAN Loading Rate";
    *by filtertype;

```

```

class filtertype day filternumber;
*model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
model vtr=tan_lr|filtertype tan_lr*tan_lr|filtertype /solution outp=two
ddfm=kr ;
*model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
random filternumber(filtertype);
random day /group=filtertype;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
lsmeans filtertype / bylevel diffs adj=bon e;
run;

```

```

proc mixed data=vtr;
title "Modeling VTR as a log function of TAN Loading Rate";
*by filtertype;
class filtertype day filternumber;
*model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
model vtr=ltanlr|filtertype /solution outp=two ddfm=kr ;
*model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
random filternumber(filtertype);
random day /group=filtertype;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
lsmeans filtertype / bylevel ;
run;

```

```

proc mixed data=vtr;
title "Modeling VTR as a function of TAN Loading Rate and TOC loading
rate";
*by filtertype;
class filtertype day filternumber;
*model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
model vtr=tan_lr filtertype toc_lr /solution outp=two ddfm=kr ;
*model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
random filternumber(filtertype);
random day /group=filtertype;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
lsmeans filtertype / bylevel ;
run;

```

```

proc mixed data=vtr;
title "Modeling VTR as a function of tan_lr*tan_lr and toc_lr*toc_lr";
*by filtertype;
class filtertype day filternumber;
*model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;

```

```

    model vtr=tan_lr|filtertype tan_lr*tan_lr|filtertype toc_lr|filtertype
    toc_lr*toc_lr|filtertype /solution outp=two ddfm=kr ;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
    tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
    tan_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel diffs adj=bon e;
run;
*/

```

```

proc mixed data=vtr;
    title "Modeling VTR as a log function of TAN Loading Rate and a log
    function of TOC Load Rate";
    *by filtertype;
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=ltanlr|filtertype ltoclr|filtertype /solution outp=allvtr
    ddfm=kr ;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    estimate "diff between slopes for LSB and CB (ltanlr)" ltanlr*filtertype
    1 -1 0;
    estimate "diff between slopes for LSB and CB (ltoclr)" ltoclr*filtertype
    1 -1 0;
    lsmeans filtertype / bylevel ;
run;

```

```

goptions reset=global gunit=pct border cback=white
    colors=(black blue green red)
    ftext=swiss ftitle=swissb htitle=6 htext=4;

```

```

proc g3grid data=allvtr out=smooth1;
    by filtertype;
    grid ltanlr*ltoclr=vtr / smooth=.01 spline ;
run;

```

```

proc g3d data=smooth1;
    by filtertype;
    plot ltanlr*ltoclr=vtr / ctop=black cbottom=gray caxis=black grid
    side rotate=30;
run;

```

```

/*
proc mixed data=vtr;

```

```

    title "Modeling VTR as a log function of TAN Loading Rate and a log
function of TOC Load Rate - no TOC intxn";
    *by filtertype;
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=ltanlr|filtertype ltoclr filtertype /solution outp=two ddfm=kr
;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel ;
run;

```

```

proc mixed data=vtr;
    title "Modeling VTR as a function of TOC Load Rate";
    *by filtertype;
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=toc_lr filtertype /solution outp=two ddfm=kr ;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel ;
run;

```

```

proc mixed data=vtr;
    title "Modeling VTR as a quadratic function of TOC Loading Rate";
    *by filtertype;
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=toc_lr|filtertype toc_lr*toc_lr|filtertype /solution outp=two
ddfm=kr ;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel diffs adj=bon e;
run;

```

```

proc mixed data=vtr;
  title "Modeling VTR as a log function of TOC Loading Rate - no intxn";
  *by filtertype;
  class filtertype day filternumber;
  *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
  model vtr=ltoclr filtertype /solution outp=two ddfm=kr ;
  *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
  random filternumber(filtertype);
  random day /group=filtertype;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
  lsmeans filtertype / bylevel ;
run;

```

```

proc mixed data=ochigh;
  title "Modeling VTR as a function of TAN Loading Rate - OChigh only";
  class filtertype day filternumber;
  *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
  model vtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
  *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
  random filternumber(filtertype);
  random day /group=filtertype;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
  lsmeans filtertype / bylevel diffs adj=bon e;
run;

```

```

proc mixed data=ochigh;
  title "Modeling VTR as a quadratic function of TAN Loading Rate - OChigh
only";
  class filtertype day filternumber;
  *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
  model vtr=tan_lr|filtertype tan_lr*tan_lr|filtertype /solution outp=two
ddfm=kr ;
  *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
  random filternumber(filtertype);
  random day /group=filtertype;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
  lsmeans filtertype / bylevel diffs adj=bon e;
run;
*/
/*
proc mixed data=ochigh;

```

```

    title "Modeling VTR as a log function of TAN Loading Rate - OChigh
only";
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=ltanlr|filtertype /solution outp=two ddfm=kr ;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel ;
run;

proc mixed data=ochigh;
    title "Modeling VTR as a function of TAN Loading Rate and TOC loading
rate - OChigh only";
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=tan_lr|filtertype toc_lr|filtertype /solution outp=two ddfm=kr
;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel diffs adj=bon;
run;

proc mixed data=ochigh;
    title "Modeling VTR as a function of tan_lr*tan_lr and toc_lr*toc_lr -
OCHIGH";
    *by filtertype;
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=tan_lr|filtertype tan_lr*tan_lr|filtertype toc_lr|filtertype
toc_lr*toc_lr|filtertype /solution outp=two ddfm=kr ;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
    lsmeans filtertype / bylevel diffs adj=bon e;
run;
*/

```

```

proc mixed data=ochigh ;
  title "Modeling VTR as a log function of TAN Loading Rate and a log
function of TOC Load Rate - OCHIGH";
  *by filtertype;
  class filtertype day filternumber;
  *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
  model vtr=ltanlr|filtertype ltoclr|filtertype /solution outp=ochighvtr
ddfm=kr ;
  *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
  random filternumber(filtertype);
  random day /group=filtertype;
  *estimate "diff between slopes for LSB and CB ltanlr" ltanlr*filtertype
1 -1 0;
  *estimate "diff between slopes for LSB and CB ltoclr" ltoclr*filtertype
1 -1 0;
  lsmeans filtertype / bylevel ;
run;

proc g3grid data=ochighvtr out=smooth2;
  by filtertype;
  grid ltanlr*ltoclr=vtr / smooth=.01 spline ;
run;

proc g3d data=smooth2;
  by filtertype;
  plot ltanlr*ltoclr=vtr / ctop=black cbottom=gray caxis=black grid
side rotate=30;
run;

/*
proc mixed data=ochigh;
  title "Modeling VTR as a log function of TAN Loading Rate and a log
function of TOC Load Rate no TOC intxn- OCHIGH";
  *by filtertype;
  class filtertype day filternumber;
  *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
  model vtr=ltanlr|filtertype ltoclr filtertype/solution outp=two
ddfm=kr ;
  *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
  random filternumber(filtertype);
  random day /group=filtertype;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
  *estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
  lsmeans filtertype / bylevel;
run;

proc mixed data=ochigh;

```

```

title "Modeling VTR as a log function of TOC Load Rate - OCHIGH";
*by filtertype;
class filtertype day filternumber;
*model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
model vtr=ltocl_r|filtertype /solution outp=two ddfm=kr ;
*model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
random filternumber(filtertype);
random day /group=filtertype;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
lsmeans filtertype / bylevel;
run;

proc mixed data=ochigh;
title "Modeling VTR as a quadratic function of TOC Loading Rate -
OCHIGH";
*by filtertype;
class filtertype day filternumber;
*model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
model vtr=toc_lr|filtertype toc_lr*toc_lr|filtertype /solution outp=two
ddfm=kr ;
*model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
random filternumber(filtertype);
random day /group=filtertype;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tan_lr*filtertype 1 -1 0;
lsmeans filtertype / bylevel diffs adj=bon e;
run;
*/

/*
proc gplot data=two;
plot resid*pred=filtertype;
plot vtr*pred=filtertype;
run;
*/

/*
proc mixed data=vtr;

title "Modeling VNR as a function of NO2_LR and VTR";
class filtertype day filternumber;
*model vnr=no2_lr|filtertype vtr|filtertype /solution outp=two ddfm=kr ;
model vnr=no2_lr|filtertype vtr|filtertype /solution outp=two ddfm=kr ;
random filternumber(filtertype);
random day /group=filtertype;

```

```

    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (NO2_LR)"
no2_lr*filtertype 1 -1 0;
    lsmeans filtertype/diffs adj=bon ;
run;

proc gplot data=two;
    plot resid*pred=filtertype;
    plot vnr*pred=filtertype;
run;

proc mixed data=vtr;

    title "Modelling VNR as a function of NO2 concentration and VTR";
    class filtertype day filternumber;
    *model vnr=NO2|filtertype vtr|filtertype /solution outp=three ddfm=kr ;
    model vnr=NO2|filtertype vtr|filtertype /solution outp=three ddfm=kr ;
    random filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    *estimate "diff between slopes for LSB and CB (NO2)" NO2*filtertype 1 -1
0;
    lsmeans filtertype/diffs adj=bon;
run;

proc gplot data=three;
    plot resid*pred=filtertype;
    plot vnr*pred=filtertype;
run;

proc mixed data=vtr;
    title "Modelling VNR as a function of NO2";
    class filtertype day filternumber;
    *model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
    model vtr=NO2|filtertype /solution outp=two ddfm=kr ;
    *model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
    random day filternumber(filtertype);
    random day /group=filtertype;
    *estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
    estimate "diff between slopes for LSB and CB (NO2)" NO2*filtertype 1 -1
0;
    lsmeans filtertype/diffs adj=bon;
run;
*/
/*
proc glm data=ochigh;
    title "Modeling VTR as a function of TAN and TOC load rate - OChigh
only";

```

```

*by filtertype;
class filtertype day filternumber;
*model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
model VTR=tan_lr|filtertype toc_lr day filternumber /solution ;
*model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
random day filternumber filtertype;
*random day /group=filtertype;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
lsmeans filtertype/pdiff adj=bon;
run;

proc glm data=vtr;
title "Modeling VTR as a function of TAN and TOC load rate";
*by filtertype;
class filtertype day filternumber;
*model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
model VTR=tan_lr|filtertype toc_lr day filternumber /solution ;
*model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
random day filternumber filtertype;
*random day /group=filtertype;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
lsmeans filtertype/pdiff adj=bon;
run;
*/
/*
proc mixed data=vtr;
title "modelling vnr as a function of no2_lr";
class filtertype day filternumber;
*model lvtr=tan_lr|filtertype /solution outp=two ddfm=kr ;
model vnr=no2_lr|filtertype /solution outp=two ddfm=kr ;
*model vtr=tanlrc|filtertype /solution outp=two ddfm=kr noint;
random day filternumber(filtertype);
random day /group=filtertype;
*estimate "diff between slopes for LSB and CB (TAN_lr)"
tanlrc*filtertype 1 -1 0;
estimate "diff between slopes for LSB and CB (TAN_lr)" no2_lr*filtertype
1 -1 0;
lsmeans filtertype/diffs adj=bon;
run;

proc gplot data=two;
plot resid*pred=filtertype;
run;

proc glm data=vtr;
title 'Modeling VNR as function of NO2 concentration';
*by filtertype;
class filtertype day filternumber;
*model lvtr=tan|filtertype day filternumber /solution;

```

```

*model vtr=tan_lr|filtertype day filternumber /solution;
*model vtr=tan_lr|filtertype tan|filtertype day filternumber /solution;
model vnr=no2|filtertype day filternumber /solution;
*model vtr=day filternumber tan_lr|filtertype/solution;
random day filternumber;
estimate "diff between slopes for LSB and CB (NO2)" no2*filtertype 1 -1
0;
*estimate "diff between slopes for pg and CB(TAN)" tan*filtertype 1 0 -1;
*estimate "diff between slopes for LSB and pg(TAN)" tan*filtertype 0 -1
1;
*estimate "diff b/t FT params for lsb and cb(TAN)" filtertype 1 -1 0;
*estimate "diff b/t FT params for pg and cb(TAN)" filtertype 1 0 -1;
*estimate "diff b/t FT params for lsb and pg(TAN)" filtertype 0 -1 1;
**estimate "diff b/t slopes for lsb and cb(TAN_LR)" tan_lr*filtertype 1
-1 0;
**estimate "diff b/t slopes for pg and cb(TAN_LR)" tan_lr*filtertype 1 0
-1;
**estimate "diff b/t slopes for lsb and pg(TAN_LR)" tan_lr*filtertype 0
-1 1;

run;

```

```

proc glm data=vtr;
  *by filtertype;
  class filtertype day filternumber;
  model lvtr=tan_lr|filtertype day filternumber /solution;
  *model vtr=tan_lr|filtertype day filternumber /solution;
  *model vtr=tan_lr|filtertype tan|filtertype day filternumber /solution;
  *model vtr=tan|filtertype day filternumber /solution;
  *model vtr=day filternumber tan_lr|filtertype/solution;
  random day filternumber;
  estimate "diff between slopes for LSB and CB (TAN)" tan*filtertype 1 -1
0;
  *estimate "diff between slopes for pg and CB(TAN)" tan*filtertype 1 0 -1;
  *estimate "diff between slopes for LSB and pg(TAN)" tan*filtertype 0 -1
1;
  estimate "diff b/t slopes for lsb and cb(TAN_LR)" tan_lr*filtertype 1
-1 0;
  estimate "diff b/t slopes for pg and cb(TAN_LR)" tan_lr*filtertype 1 0
-1;
  estimate "diff b/t slopes for lsb and pg(TAN_LR)" tan_lr*filtertype 0 -1
1;
  estimate "diff b/t FT params for lsb and cb(TAN_LR)" filtertype 1 -1 0;
  estimate "diff b/t FT params for pg and cb(TAN_LR)" filtertype 1 0 -1;
  estimate "diff b/t FT params for lsb and pg(TAN_LR)" filtertype 0 -1 1;

run;

```

```

proc glm data=vtr;
  *by filtertype;
  class filtertype;

```

```

    model vtr=in_tan|filtertype / solution;
    estimate "diff between slopes for LSB and CBV" in_tan*filtertype 1 -1 0;
run;

proc mixed data=vtr;
  by filtertype;
  title 'Modeling VTR as a function of DO and TAN Loading Rate';
  class Day FilterType FilterNumber;
  *model VTR=TAN|FilterType cod cod_n toc tan_lr/solution;
  *model VTR=tan|filtertype/solution;
  model VTR=tan_lr|filtertype DO /solution;
  random Day FilterNumber;
  *estimate "diff between slopes for LSB and CB (TAN)" tan*filtertype 1 -1
0;
  *estimate "diff between slopes for pg and CB(TAN)" tan*filtertype 1 0 -1;
  *estimate "diff between slopes for LSB and pg(TAN)" tan*filtertype 0
-1 1;
  estimate "diff b/t slopes for lsb and cb(TAN_LR)" tan_lr*filtertype 1
-1 0;
  estimate "diff b/t slopes for pg and cb(TAN_LR)" tan_lr*filtertype 1 0
-1;
  estimate "diff b/t slopes for lsb and pg(TAN_LR)" tan_lr*filtertype 0
-1 1;
  *estimate "diff b/t FT params for lsb and cb(TAN_LR)" filtertype 1 -1
0;
  *estimate "diff b/t FT params for pg and cb(TAN_LR)" filtertype 1 0 -1;
  *estimate "diff b/t FT params for lsb and pg(TAN_LR)" filtertype 0 -1
1;
  lsmeans filtertype/diffs adj=bon;
run;

proc mixed data=vtr;
  title 'Modeling VTR as a function of DO and TAN concetration';
  class Day FilterType FilterNumber;
  *model VTR=TAN|FilterType cod cod_n toc tan_lr/solution;
  *model VTR=tan|filtertype/solution;
  model VTR=tan|filtertype DO/solution;
  random Day FilterNumber;
  estimate "diff between slopes for LSB and CB (TAN)" tan*filtertype 1 -1
0;
  estimate "diff between slopes for pg and CB(TAN)" tan*filtertype 1 0 -1;
  estimate "diff between slopes for LSB and pg(TAN)" tan*filtertype 0 -1 1;
  *estimate "diff b/t FT params for lsb and cb(TAN)" filtertype 1 -1 0;
  *estimate "diff b/t FT params for pg and cb(TAN)" filtertype 1 0 -1;
  *estimate "diff b/t FT params for lsb and pg(TAN)" filtertype 0 -1 1;
  *estimate "diff b/t slopes for lsb and cb(TAN_LR)" tan_lr*filtertype 1
-1 0;
  *estimate "diff b/t slopes for pg and cb(TAN_LR)" tan_lr*filtertype 1 0
-1;
  *estimate "diff b/t slopes for lsb and pg(TAN_LR)" tan_lr*filtertype 0
-1 1;

```

```
    lsmeans filtertype/diffs adj=bon;  
run;  
*/  
ods html close;
```

2. SAS output

2.1 Normal operating conditions

Means

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN	216	0.6891667	0.2695099	0.1300000	1.2000000
pH	207	7.1630435	0.1414691	6.8500000	7.4800000
DO	189	5.8428571	0.8012718	5.0000000	8.1000000
Temp	189	28.8809524	2.2726770	24.0000000	31.6000000
NO2	126	2.0785714	1.5245567	0.3100000	6.6600000
TOC	216	25.0720833	15.5320261	7.1000000	58.5000000
COD	144	80.4375000	11.6593363	51.0000000	98.0000000

Means

The MEANS Procedure

FilterType=cb

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	60	776.7450000	388.0664775	85.4000000	1710.40
VTR	60	667.3533333	344.8297666	85.4000000	1600.10
NO2_LR	30	2645.55	2119.67	354.0000000	7364.50
VNR	30	1294.48	567.0731150	448.0000000	2501.30
eff	60	87.0892161	16.0850896	43.3340870	100.0000000

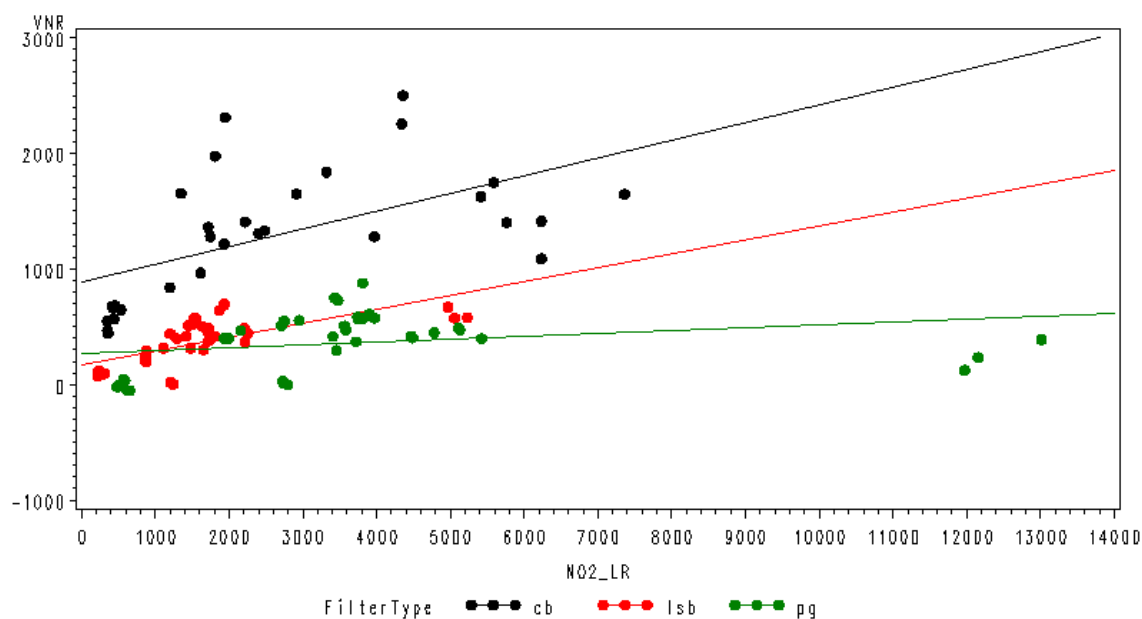
FilterType=lsb

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	72	506.8083333	205.7208109	86.0000000	943.0000000
VTR	72	267.1750000	123.3277652	65.9000000	542.2000000
NO2_LR	42	1535.70	1173.64	226.9000000	5233.90
VNR	42	353.2309524	208.3546946	6.8000000	699.1000000
eff	72	53.0892839	12.8736971	29.0309446	100.0000000

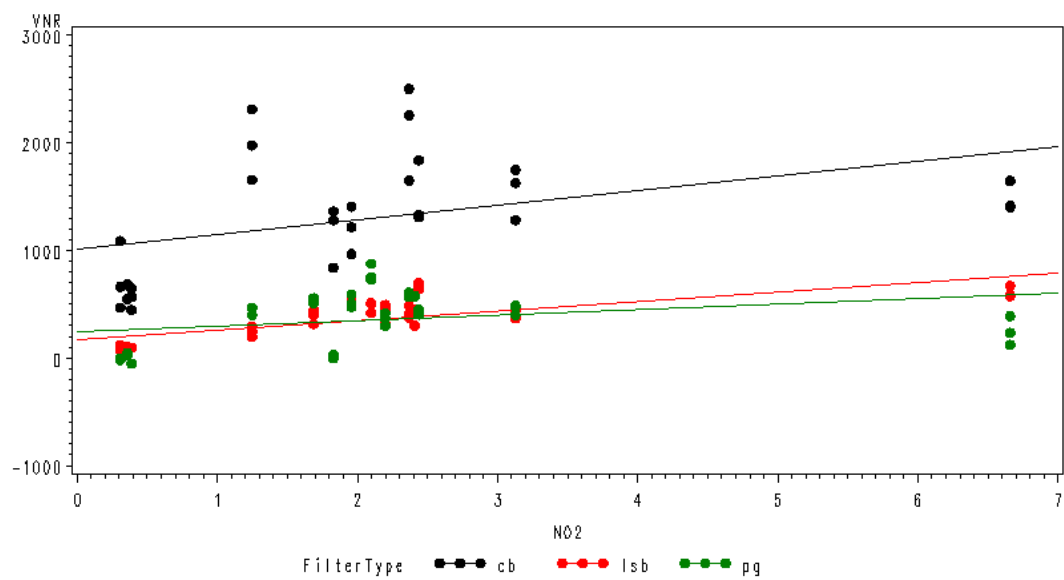
FilterType=pg

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	72	1162.48	493.2459235	194.0000000	2345.10
VTR	72	586.2597222	284.2094014	48.0000000	1231.20
NO2_LR	42	3568.72	2868.81	483.9000000	13015.40
VNR	42	352.2785714	259.8902541	-50.6000000	879.0000000
eff	72	49.4268029	15.7631998	9.6715696	100.0000000

Comparison



Comparison



Modeling VTR as a function of TAN concentration

The Mixed Procedure

Model Information	
Data Set	WORK.VTR
Dependent Variable	VTR
Covariance Structure	Variance Components
Group Effect	FilterType
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	Levels	Values
FilterType	3	cb lsb pg
day	24	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
FilterNumber	3	1 2 3

Dimensions	
Covariance Parameters	5
Columns in X	8

Dimensions	
Columns in Z	81
Subjects	1
Max Obs Per Subject	216

Number of Observations	
Number of Observations Read	216
Number of Observations Used	204
Number of Observations Not Used	12

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	2568.69095911	
1	2	2313.78252547	0.00005217
2	1	2313.72654667	0.00000185
3	1	2313.72468853	0.00000000

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	Group	Estimate
FilterNum(FilterTyp)		4561.44
day	FilterType cb	57091
day	FilterType lsb	266.23

Covariance Parameter Estimates		
Cov Parm	Group	Estimate
day	FilterType pg	3943.85
Residual		3293.95

Fit Statistics	
-2 Res Log Likelihood	2313.7
AIC (smaller is better)	2323.7
AICC (smaller is better)	2324.0
BIC (smaller is better)	2324.7

Solution for Fixed Effects						
Effect	FilterType	Estimate	Standard Error	DF	t Value	Pr > t
Intercept		-107.96	55.7727	18.4	-1.94	0.0685
TAN		1007.33	53.9041	22	18.69	<.0001
FilterType	cb	244.50	154.58	25.5	1.58	0.1260
FilterType	lsb	77.8692	71.1430	14.5	1.09	0.2916
FilterType	pg	0	.	.	.	.
TAN*FilterType	cb	-183.10	205.76	20.7	-0.89	0.3838
TAN*FilterType	lsb	-575.99	60.7606	33.1	-9.48	<.000

Solution for Fixed Effects						
Effect	FilterType	Estimate	Standard Error	DF	t Value	Pr > t
pe						1
TAN*FilterType	pg	0	.	.	.	.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
TAN	1	21.4	118.74	<.0001
FilterType	2	21.3	1.44	0.2580
TAN*FilterType	2	30.8	44.88	<.0001

Least Squares Means								
Effect	FilterType	Margins	By Level	Estimate	Standard Error	DF	t Value	Pr > t
FilterType	cb	WORK.VTR	Yes	704.58	67.1593	22.4	10.49	<.0001
FilterType	lsb	WORK.VTR	Yes	267.17	39.7155	6.03	6.73	0.0005
FilterType	pg	WORK.VTR	Yes	586.26	41.5999	7.23	14.09	<.0001

Modeling VTR as a function of TAN Loading Rate

The Mixed Procedure

Model Information	
Data Set	WORK.VTR
Dependent Variable	VTR
Covariance Structure	Variance Components
Group Effect	FilterType
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	Levels	Values
FilterType	3	cb lsb pg
day	24	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
FilterNumber	3	1 2 3

Dimensions	
Covariance Parameters	5
Columns in X	8

Dimensions	
Columns in Z	81
Subjects	1
Max Obs Per Subject	216

Number of Observations	
Number of Observations Read	216
Number of Observations Used	204
Number of Observations Not Used	12

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	2410.92820874	
1	3	2119.63742983	0.00008098
2	1	2119.56030185	0.00000185
3	1	2119.55864835	0.00000000

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	Group	Estimate
FilterNum(FilterTyp)		37.6042
day	FilterType cb	21762
day	FilterType lsb	835.86

Covariance Parameter Estimates		
Cov Parm	Group	Estimate
day	FilterType pg	4167.41
Residual		767.68

Fit Statistics	
-2 Res Log Likelihood	2119.6
AIC (smaller is better)	2129.6
AICC (smaller is better)	2129.9
BIC (smaller is better)	2130.5

Solution for Fixed Effects						
Effect	FilterType	Estimate	Standard Error	DF	t Value	Pr > t
Intercept		-86.5576	34.5970	24.7	-2.50	0.0193
TAN_LR		0.5788	0.02720	25	21.28	<.0001
FilterType	cb	123.48	46.6271	62.1	2.65	0.0102
FilterType	lsb	78.9032	38.9136	37.1	2.03	0.0498
FilterType	pg	0	.	.	.	.
TAN_LR*FilterType	cb	0.2761	0.04250	54.5	6.50	<.0001
TAN_LR*FilterType	lsb	-0.03651	0.04183	50	-0.87	0.387

Solution for Fixed Effects						
Effect	FilterType	Estimate	Standard Error	DF	t Value	Pr > t
pe						0
TAN_LR*FilterType	pg	0	.	.	.	.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
TAN_LR	1	79.4	1386.77	<.0001
FilterType	2	60.4	3.54	0.0352
TAN_LR*FilterType	2	53	28.50	<.0001

Least Squares Means								
Effect	FilterType	Margins	By Level	Estimate	Standard Error	DF	t Value	Pr > t
FilterType	cb	WORK.VTR	Yes	700.97	22.6079	82.8	31.01	<.0001
FilterType	lsb	WORK.VTR	Yes	267.18	7.6174	16.3	35.07	<.0001
FilterType	pg	WORK.VTR	Yes	586.26	14.0299	23.1	41.79	<.0001

Modeling VNR as a log function of TAN_LR and NO2_LR

The Mixed Procedure

Model Information	
Data Set	WORK.VTR
Dependent Variable	VNR
Covariance Structure	Variance Components
Group Effect	FilterType
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	Levels	Values
FilterType	3	cb lsb pg
day	14	11 12 13 14 15 16 17 18 19 20 21 22 23 24
FilterNumber	3	1 2 3

Dimensions	
Covariance Parameters	5
Columns in X	12
Columns in Z	51

Dimensions	
Subjects	1
Max Obs Per Subject	216

Number of Observations	
Number of Observations Read	216
Number of Observations Used	114
Number of Observations Not Used	102

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	1449.52769803	
1	3	1300.71559509	0.00374343
2	2	1298.06088217	0.00185549
3	2	1296.76008986	0.00065045
4	1	1296.32515801	0.00011912
5	1	1296.25129696	0.00000572
6	1	1296.24803320	0.00000002
7	1	1296.24802429	0.00000000

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	Group	Estimate
FilterNum(FilterTyp)		616.90
day	FilterType cb	429450
day	FilterType lsb	4789.32
day	FilterType pg	28664
Residual		3716.75

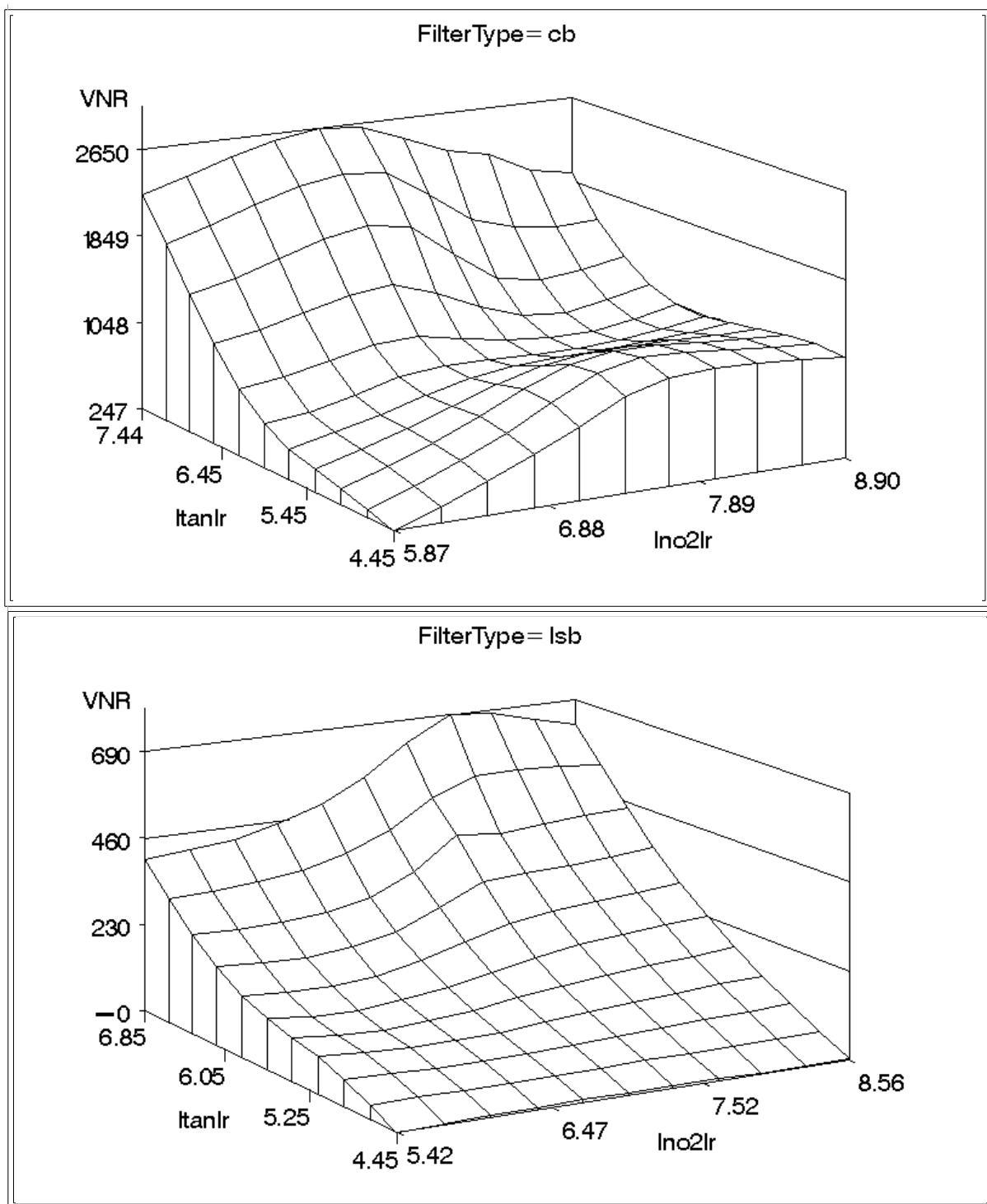
Fit Statistics	
-2 Res Log Likelihood	1296.2
AIC (smaller is better)	1306.2
AICC (smaller is better)	1306.9
BIC (smaller is better)	1307.2

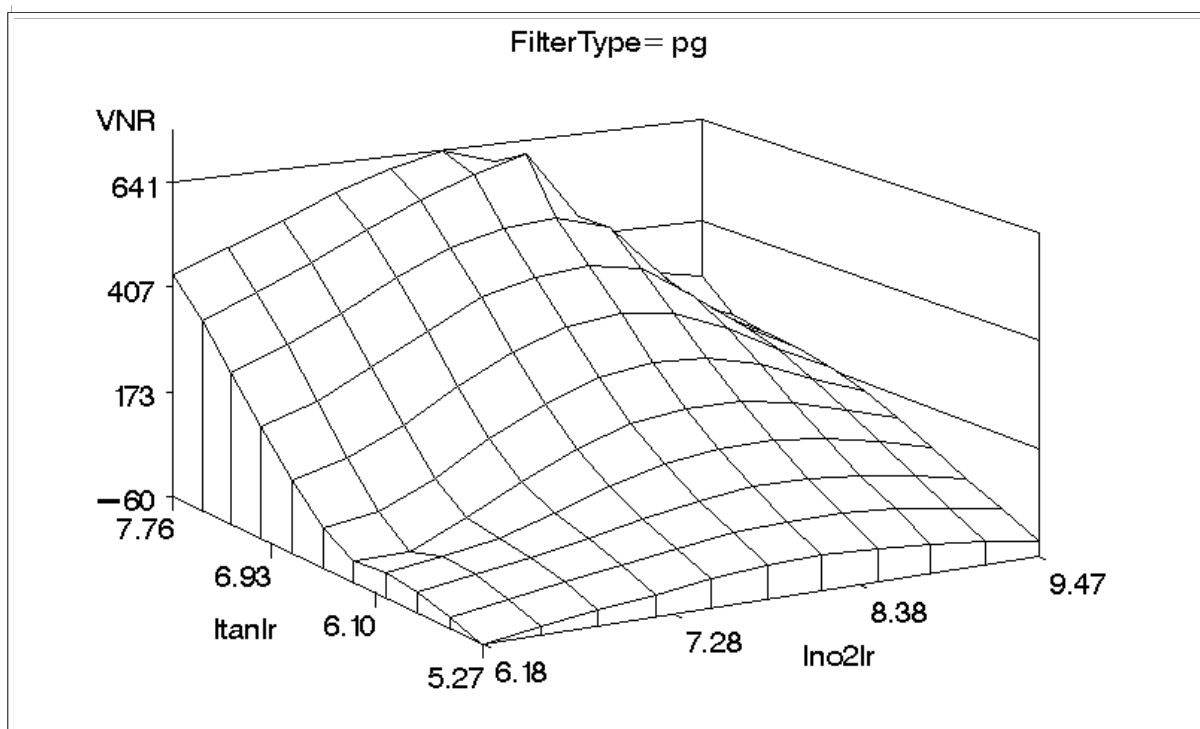
Solution for Fixed Effects						
Effect	FilterType	Estimate	Standard Error	DF	t Value	Pr > t
Intercept		-1748.27	471.42	11.4	-3.71	0.0032
ltanlr		267.59	94.4796	11.1	2.83	0.0161
FilterType	cb	-4624.39	862.63	43.4	-5.36	<.0001
FilterType	lsb	290.05	511.06	15.3	0.57	0.5786

Solution for Fixed Effects						
Effect	FilterType	Estimate	Standard Error	DF	t Value	Pr > t
FilterType	pg	0	.	.	.	.
Itanlr*FilterType	cb	609.08	143.83	30.8	4.23	0.0002
Itanlr*FilterType	lsb	-50.8669	103.65	15.5	-0.49	0.6305
Itanlr*FilterType	pg	0	.	.	.	.
Ino2lr		31.5167	75.9600	11	0.41	0.6862
Ino2lr*FilterType	cb	243.71	80.3046	13.6	3.03	0.0091
Ino2lr*FilterType	lsb	37.8561	83.3193	15.3	0.45	0.6560
Ino2lr*FilterType	pg	0	.	.	.	.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Itanlr	1	36.4	82.31	<.0001
FilterType	2	27.7	21.05	<.0001
Itanlr*FilterType	2	25.4	15.70	<.0001
Ino2lr	1	18.3	18.56	0.0004
Ino2lr*FilterType	2	21.3	13.31	0.0002

Least Squares Means								
Effect	FilterType	Margins	By Level	Estimate	Standard Error	DF	t Value	Pr > t
FilterType	cb	WORK.VTR	Yes	1294.48	208.03	6.59	6.22	0.0006
FilterType	lsb	WORK.VTR	Yes	353.23	25.2234	9.82	14.00	<.0001
FilterType	pg	WORK.VTR	Yes	352.28	48.3893	12.5	7.28	<.0001





2.2 Organic carbon study

Means of Overall OC operating conds

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN	117	0.6776923	0.3801506	0.2700000	1.5900000
pH	117	7.1930769	0.1207338	6.9500000	7.3800000
DO	117	6.7615385	0.8277354	5.5000000	8.3000000
Temp	117	23.9307692	0.9148640	22.9000000	25.7000000
NO2	117	0.3346154	0.1011702	0.2300000	0.4900000
TOC	117	58.8607692	15.3141372	33.1400000	78.4400000
COD	117	284.0769231	54.0738884	220.0000000	368.0000000
Alk	117	538.1538462	47.4437263	448.0000000	596.0000000

Means of Overall OC operating conds

The MEANS Procedure

FilterType=cb

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	12	473.2771876	142.2685357	215.3000000	743.2141810
VTR	12	145.9917861	69.1368899	39.7000000	256.3000000
NO2_LR	12	238.2011500	59.9532604	172.0000000	368.4000000
VNR	12	277.3919428	123.9792462	79.4000000	480.9032936
COD_LR	12	249056.71	52382.91	186879.50	375854.50
TOC_LR	12	38730.98	8095.61	28497.20	56158.90

FilterType=lsb

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	39	511.9026951	285.0080350	201.4000000	1240.80
VTR	39	112.9335326	70.4162354	-46.7000000	238.3000000
NO2_LR	39	256.2831159	83.7636694	139.0000000	404.3000000
VNR	39	155.1550770	73.2746198	-23.3000000	279.4000000
COD_LR	39	217914.89	50039.10	134317.00	320595.00
TOC_LR	39	44967.34	12513.83	20233.00	64713.10

FilterType=pg

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	39	1053.85	587.3787721	417.7000000	2608.90
VTR	38	202.1170442	161.6236172	-128.4000000	590.7000000
NO2_LR	39	526.5709115	165.4106329	345.5117910	820.5000000
VNR	38	241.1316172	195.3613662	-60.0890071	656.3000000
COD_LR	39	280995.44	61208.60	200771.10	385117.30
TOC_LR	39	58259.34	16684.18	31205.60	82334.20

Means of OClow

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN	36	0.4825000	0.1332747	0.2700000	0.6300000
pH	36	7.3300000	0.0447852	7.2600000	7.3800000
DO	36	6.3750000	0.4668282	5.6000000	6.8000000
Temp	36	24.1500000	0.4675162	23.5000000	24.8000000
NO2	36	0.2375000	0.0131747	0.2300000	0.2600000
TOC	36	37.2600000	3.6890626	33.1400000	43.1200000
COD	36	230.5000000	6.9713700	220.0000000	238.0000000
Alk	36	519.5000000	35.8676137	470.0000000	554.0000000

Means of OClow

The MEANS Procedure

FilterType=cb

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	12	473.2771876	142.2685357	215.3000000	743.2141810
VTR	12	145.9917861	69.1368899	39.7000000	256.3000000
NO2_LR	12	238.2011500	59.9532604	172.0000000	368.4000000
VNR	12	277.3919428	123.9792462	79.4000000	480.9032936
COD_LR	12	249056.71	52382.91	186879.50	375854.50
TOC_LR	12	38730.98	8095.61	28497.20	56158.90

FilterType=lsb

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	12	371.1420924	115.6580773	201.4000000	584.3000000
VTR	12	57.4173143	32.6810880	-22.0000000	111.8000000
NO2_LR	12	183.6951267	27.8345882	139.0000000	241.2000000
VNR	12	100.6956671	33.6856825	29.2000000	158.4000000
COD_LR	12	178350.51	26053.86	134317.00	221685.70
TOC_LR	12	28853.11	5214.27	20233.00	39995.50

FilterType=pg

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	12	740.9175471	201.1624408	417.7000000	957.4000000
VTR	11	70.1497891	24.6895653	15.0222518	105.3000000
NO2_LR	12	366.0471291	17.2263397	345.5117910	395.1000000
VNR	11	41.5001321	64.6025722	-60.0890071	109.2000000
COD_LR	12	222907.42	10463.59	207158.20	243109.10
TOC_LR	12	35990.51	3354.40	31205.60	41075.50

Means of OHigh

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN	81	0.7644444	0.4207256	0.4000000	1.5900000
pH	81	7.1322222	0.0898471	6.9500000	7.2300000
DO	81	6.9333333	0.8949860	5.5000000	8.3000000
Temp	81	23.8333333	1.0424730	22.9000000	25.7000000
NO2	81	0.3777778	0.0929247	0.2400000	0.4900000
TOC	81	68.4611111	5.5514359	61.5000000	78.4400000
COD	81	307.8888889	48.5046390	228.0000000	368.0000000
Alk	81	546.4444444	49.7473617	448.0000000	596.0000000

Means of OChigh

The MEANS Procedure

FilterType=cb

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	0	.	.	.	.
VTR	0	.	.	.	.
NO2_LR	0	.	.	.	.
VNR	0	.	.	.	.
COD_LR	0	.	.	.	.
TOC_LR	0	.	.	.	.

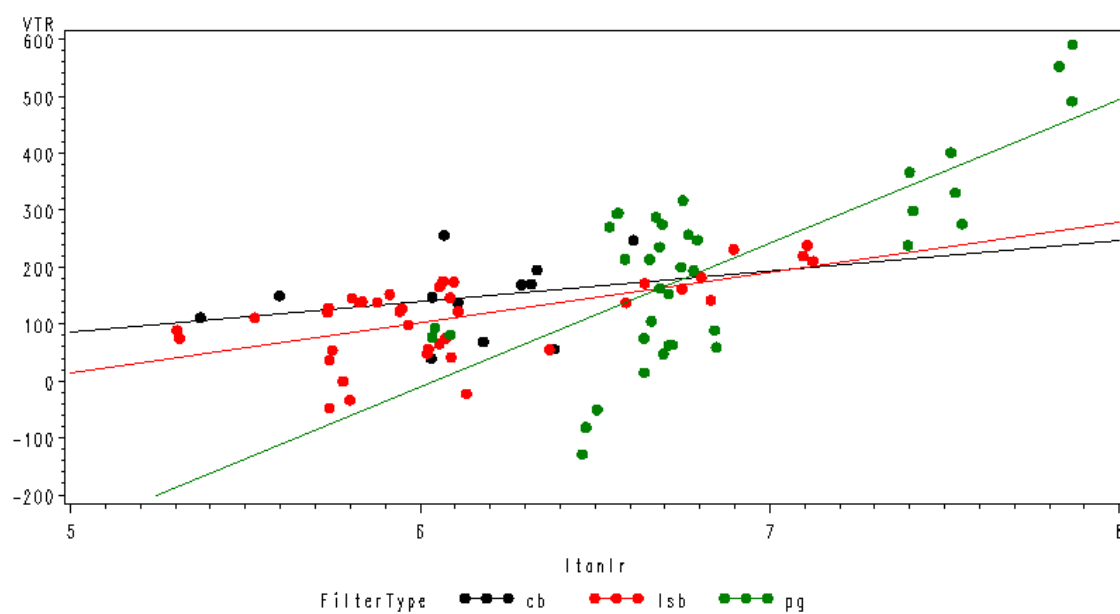
FilterType=lsb

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	27	574.4629630	315.9932158	309.7000000	1240.80
VTR	27	137.6074074	68.8504954	-46.7000000	238.3000000
NO2_LR	27	288.5444444	80.0887408	167.9000000	404.3000000
VNR	27	179.3592593	73.4163064	-23.3000000	279.4000000
COD_LR	27	235499.06	48257.81	142949.00	320595.00
TOC_LR	27	52129.22	6652.49	40882.70	64713.10

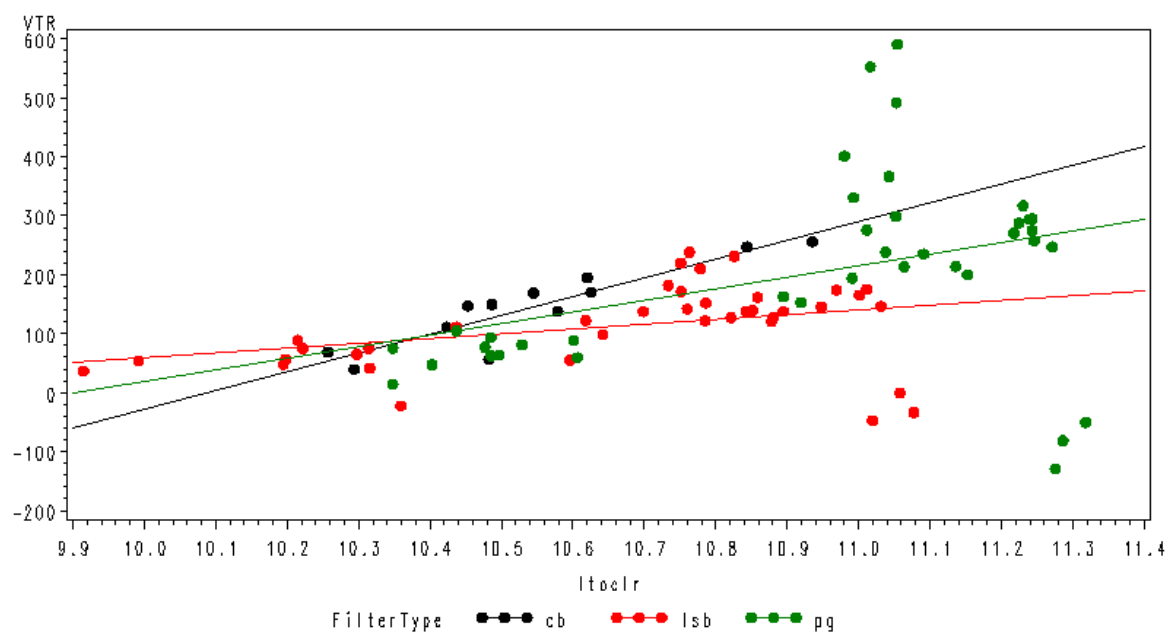
FilterType=pg

Variable	N	Mean	Std Dev	Minimum	Maximum
TAN_LR	27	1192.94	649.4954990	641.8000000	2608.90
VTR	27	255.8814815	163.0018760	-128.4000000	590.7000000
NO2_LR	27	597.9148148	150.6138969	353.2000000	820.5000000
VNR	27	322.4629630	170.2294970	-48.2000000	656.3000000
COD_LR	27	306812.33	56390.10	200771.10	385117.30
TOC_LR	27	68156.59	8454.31	53915.70	82334.20

Comparison



Comparison



Modeling VTR as a log function of TAN Loading Rate and a log function of TOC Load Rate

The Mixed Procedure

Model Information	
Data Set	WORK.VTR
Dependent Variable	VTR
Covariance Structure	Variance Components
Group Effect	FilterType
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	Levels	Values
FilterType	3	cb lsb pg
day	13	1 2 3 4 5 6 7 8 9 10 11 12 13
FilterNumber	3	1 2 3

Dimensions	
Covariance Parameters	5
Columns in X	12

Dimensions	
Columns in Z	48
Subjects	1
Max Obs Per Subject	117

Number of Observations	
Number of Observations Read	117
Number of Observations Used	89
Number of Observations Not Used	28

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	943.81010926	
1	2	845.59067017	0.00032102
2	1	845.45813669	0.00004182
3	1	845.44207684	0.00000133
4	1	845.44159930	0.00000000

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	Group	Estimate
FilterNum(FilterTyp)		159.91
day	FilterType cb	920.80

Covariance Parameter Estimates		
Cov Parm	Group	Estimate
day	FilterType Isb	3043.03
day	FilterType pg	11648
Residual		733.96

Fit Statistics	
-2 Res Log Likelihood	845.4
AIC (smaller is better)	855.4
AICC (smaller is better)	856.3
BIC (smaller is better)	856.4

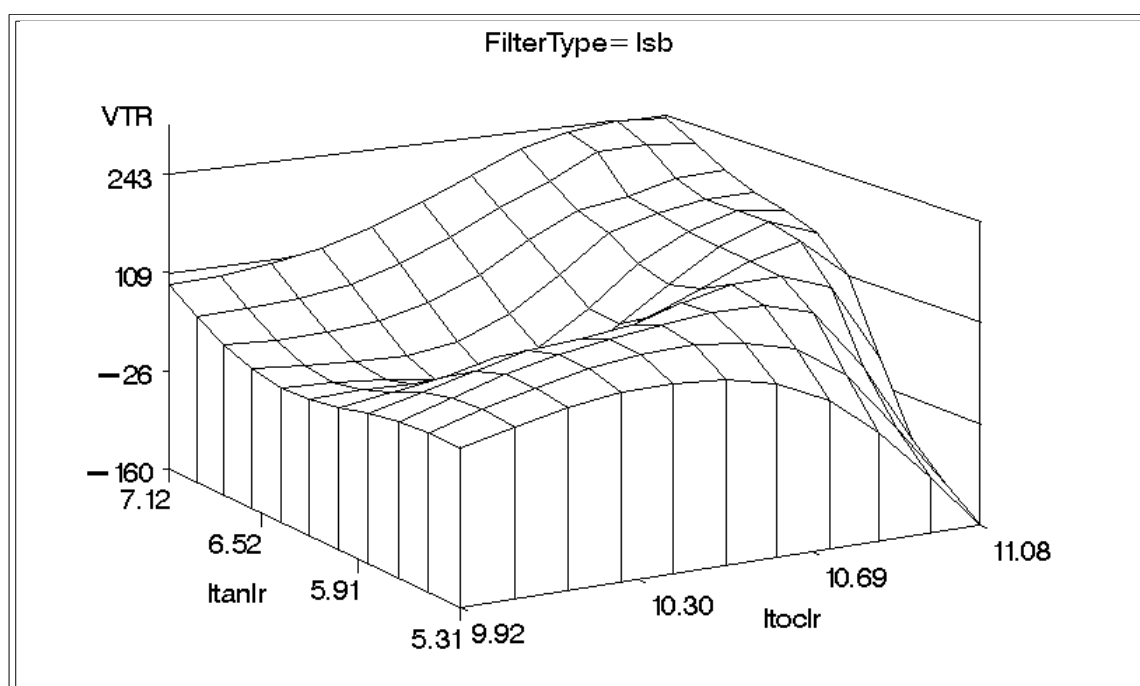
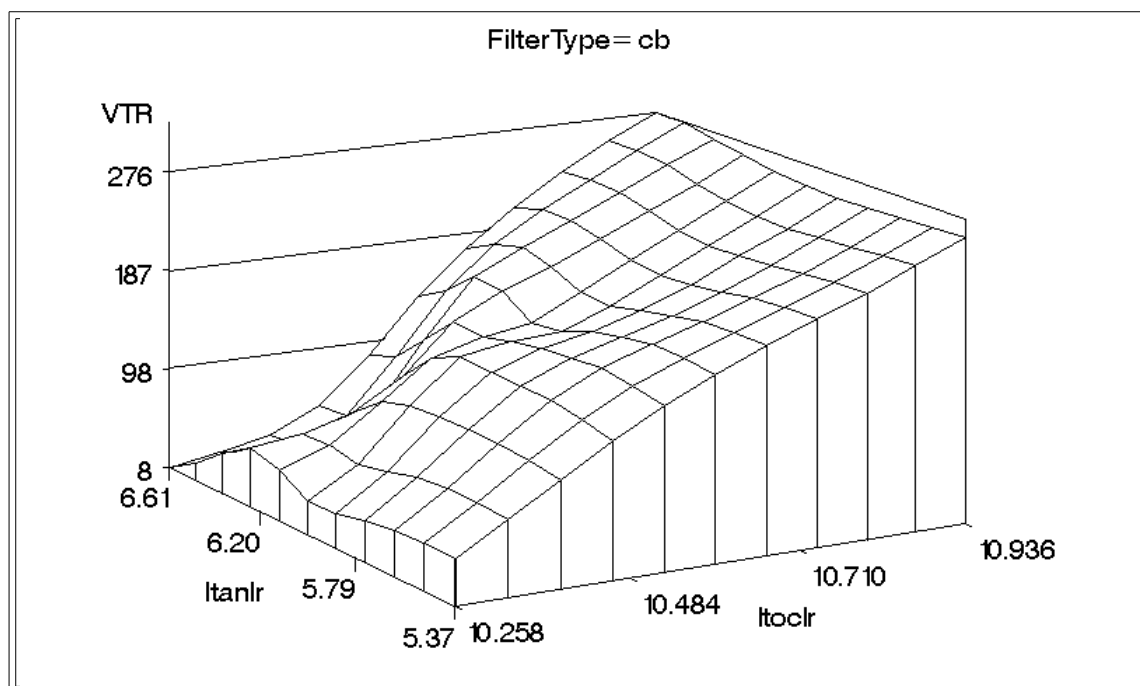
Solution for Fixed Effects						
Effect	FilterType	Estimate	Standard Error	DF	t Value	Pr > t
Intercept		-2612.24	1025.24	15.2	-2.55	0.0221
Itanlr		228.44	66.4326	10.9	3.44	0.0056
FilterType	cb	194.24	1250.35	23.6	0.16	0.8779
FilterType	Isb	1847.56	1111.64	20.6	1.66	0.1117
FilterType	pg	0	.	.	.	.
Itanlr*FilterType	cb	-232.71	89.3487	9.41	-2.60	0.0276

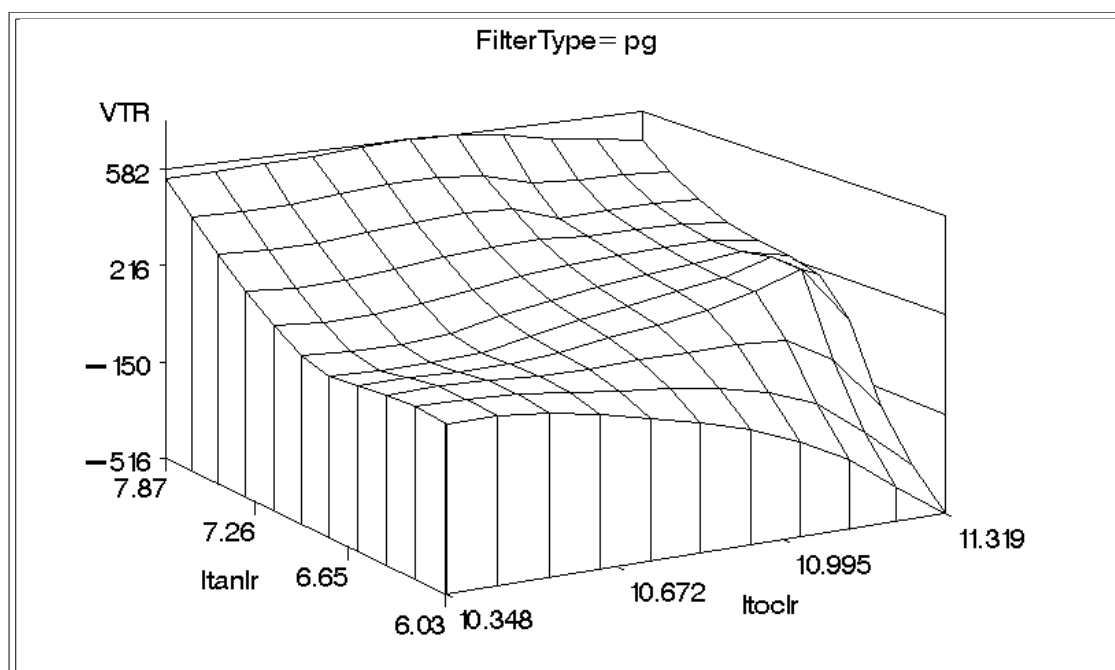
Solution for Fixed Effects						
Effect	FilterType	Estimate	Standard Error	DF	t Value	Pr > t
Itanlr*FilterType	lsb	-153.64	74.4734	16.3	-2.06	0.0554
Itanlr*FilterType	pg	0	.	.	.	.
Itoclr		114.21	95.7956	13	1.19	0.2545
Itoclr*FilterType	cb	132.06	129.24	13.4	1.02	0.3249
Itoclr*FilterType	lsb	-74.8447	105.84	18.7	-0.71	0.4882
Itoclr*FilterType	pg	0	.	.	.	.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Itanlr	1	12.4	9.81	0.0083
FilterType	2	23.7	2.73	0.0860
Itanlr*FilterType	2	6.73	3.18	0.1064
Itoclr	1	17.1	8.54	0.0095
Itoclr*FilterType	2	10.1	2.15	0.1672

Estimates					
Label	Estimate	Standard Error	DF	t Value	Pr > t
diff between slopes for LSB and CB (ltanlr)	-79.0663	68.5777	3.94	-1.15	0.3140
diff between slopes for LSB and CB (ltoclr)	206.90	97.7305	5.91	2.12	0.0793

Least Squares Means								
Effect	FilterType	Margins	By Level	Estimate	Standard Error	DF	t Value	Pr > t
FilterType	cb	WORK.VTR	Yes	153.07	19.7205	3.24	7.76	0.0034
FilterType	lsb	WORK.VTR	Yes	112.93	17.4986	12.9	6.45	<.0001
FilterType	pg	WORK.VTR	Yes	198.63	31.1274	11.4	6.38	<.0001





Modeling VTR as a log function of TAN Loading Rate and a log function of TOC Load Rate - OCHIGH

The Mixed Procedure

Model Information	
Data Set	WORK.OCHIGH
Dependent Variable	VTR
Covariance Structure	Variance Components
Group Effect	FilterType
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	Levels	Values
FilterType	2	lsb pg
day	9	5 6 7 8 9 10 11 12 13
FilterNumber	3	1 2 3

Dimensions	
Covariance Parameters	4
Columns in X	9

Dimensions	
Columns in Z	24
Subjects	1
Max Obs Per Subject	81

Number of Observations	
Number of Observations Read	81
Number of Observations Used	54
Number of Observations Not Used	27

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	573.84592305	
1	3	513.83821497	0.00028928
2	1	513.76901106	0.00001456
3	1	513.76579657	0.00000005
4	1	513.76578584	0.00000000

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	Group	Estimate
FilterNum(FilterTyp)		258.38
day	FilterType lsb	2789.47

Covariance Parameter Estimates		
Cov Parm	Group	Estimate
day	FilterType pg	13227
Residual		880.76

Fit Statistics	
-2 Res Log Likelihood	513.8
AIC (smaller is better)	521.8
AICC (smaller is better)	522.7
BIC (smaller is better)	520.9

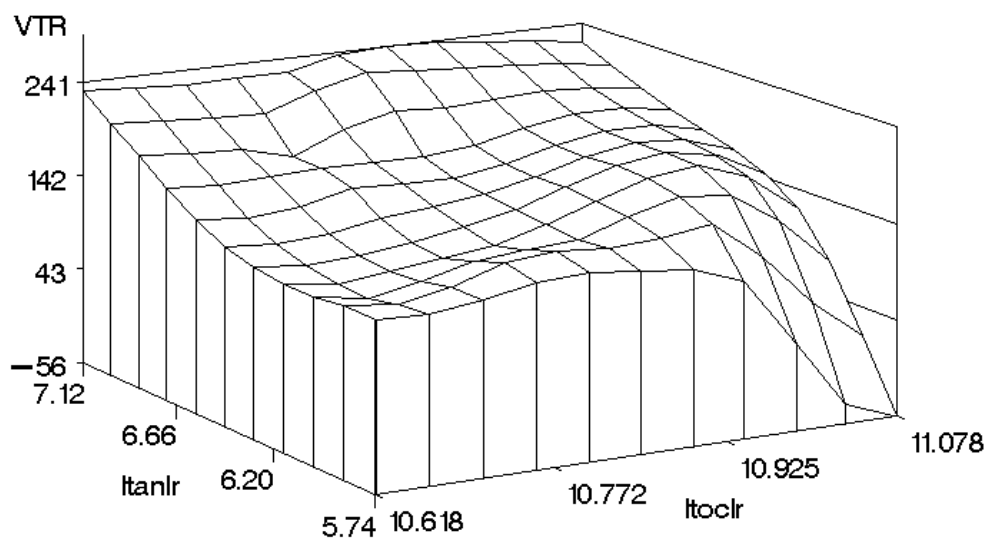
Solution for Fixed Effects						
Effect	FilterType	Estimate	Standard Error	DF	t Value	Pr > t
Intercept		-2282.25	2867.62	33.7	-0.80	0.4317
Itanlr		264.76	83.1151	9.96	3.19	0.0098
FilterType	lsb	2123.29	3264.77	38.4	0.65	0.5193
FilterType	pg	0	.	.	.	.
Itanlr*FilterType	lsb	-171.79	92.3344	14.2	-1.86	0.0837
Itanlr*FilterType	pg	0	.	.	.	.
Itoclr		62.4293	239.72	31	0.26	0.796

Solution for Fixed Effects						
Effect	FilterType	Estimate	Standard Error	DF	t Value	Pr > t
						3
Itoclr*FilterType	lsb	-88.4558	275.02	37.7	-0.32	0.7495
Itoclr*FilterType	pg	0	.	.	.	.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Itanlr	1	14.2	15.01	0.0016
FilterType	1	38.4	0.42	0.5193
Itanlr*FilterType	1	14.2	3.46	0.0837
Itoclr	1	37.7	0.02	0.8954
Itoclr*FilterType	1	37.7	0.10	0.7495

Least Squares Means								
Effect	FilterType	Margins	By Level	Estimate	Standard Error	DF	t Value	Pr > t
FilterType	lsb	WORK.OCHIGH	Yes	137.61	20.7048	8.55	6.65	0.0001
FilterType	pg	WORK.OCHIGH	Yes	255.88	39.8554	7.65	6.42	0.0002

Modeling VTR as a log function of TAN Loading Rate and a log function of TOC Load Rate — OCHIG
FilterType= lsb



Modeling VTR as a log function of TAN Loading Rate and a log function of TOC Load Rate — OCHIG
FilterType= pg

