ABSTRACT

JONES, RICHARD DALE. The Removal of Fine Particulates and Dissolved Organic Matter Including the Off-flavor Compounds Geosmin and 2-Methylisoborneol from a Commercial Recirculating Aquaculture Facility. (Under the direction of Dr. Thomas Losordo).

The demand for quality seafood is ever increasing with the growth of the human population. Aquaculture has offset some of the pressures placed on wild stocks of fish. Over the years many new technologies have been created, applied and adapted to this industry to increase the production of a quality product as quickly and inexpensively as possible. This paper will focus on fish grown in recirculating aquaculture system (RAS). These systems treat and reuse 85% or more of the water each day. A major problem with these intensive culture systems is maintaining excellent water quality. As the percent water reuse increases in RAS the filtration of the system water, for optimal growth of the fish, becomes more difficult. We designed, installed and tested a multi-component side stream filtration loop at a commercial RAS fish farm for the removal/reduction of fine particulates and dissolved organic compounds. This side stream filtration loop consisted of UV sterilization, up-flow particulate filtration and ozone oxidation with capabilities for implementing the advanced oxidation process (AOP) utilizing an ozone peroxide mix. We tested several combinations of these filters (up-flow without media, up-flow filtration, up-flow with UV sterilization, up-flow filtration with ozone contact, up-flow filtration combined with UV sterilization and ozone contact and lastly up-flow filtration combined with UV sterilization and AOP) to determine which combination/treatment contributed to the highest fine particulate and dissolved organic compound removal. Chemical oxygen demand (COD) was used as the measure of fine particulate and dissolved organic compound concentration. COD removal rates were calculated using two different approaches (instantaneous removal and removal

over time). Calculating COD removal rates using the equation for removal over time showed differences between the different configurations. A statistical model was developed for predicting removal rates for different treatments using SAS. Additionally, from this model we compared the difference of least squares means to compare the removal rates between the different treatments. We found up-flow filtration with ozone contact had the highest removal rates compared to up-flow no media, up-flow filtration and up-flow filtration combined with UV sterilization and AOP with an predicted increase in the removal rate by 19.23 ± 7.42, 41.24 ± 10.14 and 20.88 ± 7.39 g/hr respectively. We expected the up-flow filtration combined with UV sterilization and AOP to have the highest removal rates. We presume that during the up-flow, UV with AOP treatments, the fine particulates and dissolved organic compounds found in the water were consuming all the ozone before the ozone reacted with the peroxide. Additionally, the consumption of ozone by the organics in the water caused for an overdose of peroxide which is counterproductive to the AOP. A further study was conducted for analysis of the removal of the dissolved organic off-flavor compounds geosmin and 2-methylisoborneol using the same treatment loop. The analysis of these off-flavor compounds resulted in inconsistent data. In turn the comparative analysis for the removal of these compounds between the treatments was determined inconclusive. Utilizing the AOP in RAS for the removal of off-flavor compounds and the reduction of fine and dissolve organic compound appears to be promising. More research needs to be conducted in order to determine whether this technology can be successfully implemented for water treatment in RAS.

The Removal of Fine Particulates and Dissolved Organic Matter including the Off-flavor Compounds Geosmin and 2-Methylisoborneol from a Commercial Recirculating Aquaculture Facility

by
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DEDICATION

To my family for all the love and support they have provided for me.

BIOGRAPHY

Rick Jones was born on October 9, 1977 in Boston, MA. He grew up in Marion, MA and attended Old Rochester Regional High School in Mattapoisett, MA. In 2001 he graduated Cum Laude from the University of Rhode Island in Kingston, RI with a Bachelors of Science Degree in Aquaculture and Fisheries Science with a minor in Microbiology and a minor in Biology. In August 2001 he participated in AmeriCorp volunteering with the fisheries department in the Great Smoky Mountains National Park. During the next four years he worked at the New England Aquarium in Boston, MA. In December of 2005 Rick moved to Raleigh, NC to work at the North Carolina State University (NCSU) Fish Barn. While working at the Fish Barn, Rick took several undergraduate engineering courses in order to qualify for acceptance in the Biological and Agricultural Engineering (BAE) masters program at NCSU. In 2008 Rick was accepted into BAE masters program with a focus in aquacultural systems engineering. In August 2010 Rick graduated with a Master's of Science in Biological and Agricultural Engineering with a minor in Civil Engineering under the guidance of Dr. Thomas Losordo.

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1.1.1 Off-flavor compounds

Introduction

The demand for seafood has increased with the ever expanding world population. Aquaculture has aided in the production of food fish to help offset the demand placed upon wild stocks. A major problem encountered in raising fish in pond culture and in Recirculating Aquaculture Systems (RAS) is the production of a fish with earthy and musty tastes. These off-flavors have directly cost the US catfish industry up to 60 million US dollars per year (Schrader, 2005). Off-flavored farm raised fish that have been sold at the seafood market have led to a negative perception farm raised fish. Additionally, consumers that have eaten farm raised fish that contain off-flavor are less likely to purchase farm raised fish again. Geosmin and 2-methylisoborneol (2-MIB) have been identified as the compounds that cause the earthy/musty off-flavor found in the flesh of some farmed raised fish and in drinking water supplies. Geosmin and 2-MIB are semi-volatile terpenoid compounds biosynthesized as second metabolites of cyanobacteria and actinomycetes (Guttman, 2008).

Identification of off-flavors

Persson (1995) indicates that Horsford and Jackson (1855) were the first in the scientific and technical literature to describe off-flavors found in drinking water. They studied the City of Boston water supply which reportedly had the off-flavor taste of

cucumber in the fall of 1854. This off-flavor would occur seasonably in the spring and fall. In 1881, the Boston water supply had severe off-flavor problems with the "cucumber taste" intensifying to a "fish oil" odor (Whipple, 1914). The cause of this off-flavor was never identified. Persson (1995) notes that it was not until 1897 that Jackson and Ellms had linked the off-flavor to and compiled a list of odorous algae and cyanobacteria.

A few years later in 1899, Whipple wrote the handbook Microscopy of Drinking Water. This book is considered the original handbook for studies of tastes and odors in drinking water (Persson, 1995). In it, the author relates the seasonal occurrences of off-flavors found in drinking water to seasonal blooms of *Synura uvella*. As it turns out *Synura uvella* was the algal organism excreting the compound that caused the "cucumber taste". Whipple made a significant contribution by developing a standard for describing odors that continue to be used today. The standard first uses a scale to describe the intensity of the odor (e.g. 0 = no odor; 5 = strong odor) and then describes the odors by name and provides descriptions (e.g. fishy – smell of cod liver oil).

It was not until Adams (1929) that actinomycetes were described as causing earthy flavors in water. Less than a decade later, Thaysen and Pentlow (1936) were able to isolate the earthy odor from a pure culture of actinomycetes. In the mid 1960's, Gerber and Lechevalier (1965) identified this off-flavor compound caused by actinomycetes as geosmin, meaning earth odor. The authors used a pure culture of actinomycetes and isolated the compound by gas chromatography. A few years later, Gerber (1969) identified the off-flavor compound 2-MIB. These two compounds, geosmin and 2-MIB, have been positively identified as the main cause of earthy and musty off-flavors found in drinking water.

Off-flavors in fish

Geosmin and 2-MIB are the largest source of off-flavors found in aquacultured fish (Tucker, 2000). Occurrences of muddy flavors found in the flesh of fish have been documented as far back as 1558 in Conrad Gesener's treatise on fish (Persson, 1995). Over 350 years later Leger (1910) linked the occurrence of muddy off-flavors found in rainbow trout to a substance excreted by blooms of cyanobacteria, *Oscillatoria tenuis* (Tucker, 2000). Thaysen (1936) investigated the earthy off-flavor of salmon caught running up stream. He discovered that the earthy off-flavor found in the fish originated from excretions produced by actinomycetes. He was able to isolate the compounds excreted from the actinomycetes which he described as having a strong "manurial" odor. The author further notes that when the compound is diluted two parts in ten million it had an "earthy" odor similar to that found in the flesh of the tainted salmon. Thaysen and Pentelow (1936) conducted several experiments with this compound isolated from actinomycetes on rainbow and brown trout. They found that these fish can acquire an earthy off-flavor within an hour of being in water containing the off-flavor concentrate. Additionally, they discovered that it can take several days for these tainted fish to lose the earthy off-flavor when they are purged in clean water.

Fish can absorb geosmin and 2-MIB into their flesh through their gills, gut and skin (Howgate, 2004; Thaysen and Pentelow, 1936). However, most of the off-flavor compounds are passively taken up through the gills (Howgate, 2004), then transported through the blood and deposited in the tissues (Johnsen and Lloyd, 1992). This transport phenomenon is due to the hydrophobic nature of geosmin and 2-MIB, which is the driving force for bioaccumulation of these lypophilic compounds into the flesh of the fish (Schuurmann and

Klein, 1988). There has been a correlation of geosmin and 2-MIB uptake and depuration in fish tissues depending on water temperature and the fat content of the fish. Johnsen et al. (1996) suggests that at higher water temperatures, 2-MIB concentrations tend to increase in the fillet tissues of the fish. He also suggests that the depuration (removal) rate of 2-MIB increases with temperature; however, depuration rates are much slower than adsorption rates for similar temperatures. Johnsen and Lloyd (1992) found that fish with higher fat content had higher bioaccumulation of 2-MIB than leaner fish. They also found that the initial depuration rates where the same for fat and lean fish but that the fatter fish maintain a concentration of 2-MIB for a longer period of time.

Most literature on off-flavors found in fish pertains to catfish cultured in ponds in the southeastern US. However, off-flavors caused by geosmin and 2-MIB have been documented all over the world; Europe (Robertson et al., 2006; Thaysen and Pentelow, 1936; Papp, 2007), Asia (Baldia et al., 2003) and Australia (Percival et al., 2008). Additionally, many species of fish have been identified with off-flavor taints associated with geosmin and 2-MIB. These fish include largemouth bass (Schrader et. al., 2005), white sturgeon (Schrader et al., 2005), rainbow trout (Thaysen and Pentelow, 1936), catfish (Johnsen et al., 1996), northern pike (Persson, 1980), bream (Persson, 1980), tilapia (Papp, 2007), silver carp (Papp, 2007), grass carp (Papp, 2007), common carp (Papp, 2007) and barramundi (Percival et al., 2008). The earthy off-flavors of geosmin and 2-MIB are detectable at levels as low as10 ng/l in drinking water. However, detectable levels of off-flavors vary between different species of fish. Schrader et al. (2005) found that the detectable threshold concentration of geosmin in white sturgeon is 675 ng/kg where as in largemouth bass it is 23 ng/kg. He

attributed this variation to the stronger natural flavors of white sturgeon masking the earthy odors.

Management of off-flavor removal in aquaculture

Fish farmers need to produce high quality marketable fish free from off-flavors to build and maintain their market share and make the farm profitable. The nature of fish farming in ponds and RAS is conducive to optimal growing conditions for off-flavor producing cyanobacteria and actinomycetes in the water. Cyanobacteria and actinomycetes thrive in water with high nutrient and organic loading (Paerl and Tucker, 1995). Tucker (2000) describes three management practices used for minimizing off-flavors in cultured fish; manage around off-flavors episodes, manage cyanobacteria and actinomycetes growth, and purge fish with off-flavors. These management practices are applied in pond culture with limited success.

Management around off-flavor episodes

Managing harvests around off-flavor episodes involves growing fish to market size and delaying harvests until the fish are free from off-flavors. Delaying harvests will have economic impacts such as additional feed, electricity and labor costs and lost income due to setbacks in pond restocking until current pond is harvested.

Management of cyanobacteria and actinomycetes growth

Managing cyanobacteria and actinomycetes growth requires limiting nutrients available, incorporating planktivorous fishes or applying algaecides (Tucker, 2000). Limiting available nutrients for cyanobacteria and actinomycetes growth in pond culture is nearly impossible due to the large amounts of nutrients being added in the form of fish feed.

Tucker (2000) discusses decreasing nutrient levels by growing specific species of aquatic plants capable of nitrogen and phosphorous uptake thus reducing pond eutrophication. McVea and Boyd (1975) found growing water hyacinth in ponds stocked with tilapia reduced the growth of phytoplankton in the pond by decreasing the available nitrogen and phosphorous. However, no research was conducted to determine if the water hyacinth reduced the off-flavor producing cyanobacteria and actinomycetes.

Tucker (2006) experimented with polyculture of the planktivorous fish (fish that eat phytoplankton) silver carp, grown with channel catfish to determine if silver carp would be capable of reducing the number of off-flavor producing cyanobacteria. He found that the silver carp did not reduce the cyanobacteria population. Additionally, catfish harvested from the pond had earthy musty flavors associated with geosmin and 2-MIB.

Finally, the most practiced management procedure for reducing cyanobacteria and actinomycetes is the use of algaecides. Currently copper is the only algaecide approved for use in aquaculture. There is evidence that algae have exhibited some resistance to copper treatments (Smith et al., 2008). This raises some questions on the long term use of copper for cyanobacteria and actinomycetes management.

Purging fish with off-flavors

As mentioned earlier, off-flavors can be purged from fish by transferring the fish into clean water, free of geosmin and 2-MIB compounds. This management technique would be difficult to accomplish in pond culture since there would need to be an extra pond with pristine water for purging off-flavored fish. Additionally, there would be a significant risk of fish mortalities due to the stress of handling and moving fish from one pond to another. On

the other hand the practice of purging fish grown in an RAS appears to be fairly common (Masser et al., 1999). To carry out off-flavor remediation at an RAS the farm would need another system for purging or have the capability to isolating and configuring the growout tank for purging. This setup would have additional cost associated with it such as capital cost of purging system, electricity (for pumps, blowers etc), space, additional feed cost and the risk of mortalities.

Management of off-flavor removal at drinking water facilities

Since the discovery of algae producing off-flavors in the late 1800's there has been a significant amount of research conducted in the removal of geosmin and 2-MIB in drinking water facilities. While it has been found that these compounds are not toxic, the sole basis that consumers judge the safety of water is typically by the taste (McGuire, 1995). According to McGuire (1999) there are three categories of treatments used at drinking water facilities for the control of earthy and musty off-flavors associated with geosmin and 2-MIB. They are oxidation, adsorption and the use of biological processes.

Oxidation

Chlorine is the most commonly used oxidant for the removal of the many different types of off-flavors that occur in drinking water. However, chlorine has no effect on the particular off-flavor compounds geosmin and 2-MIB (McGuire, 1999). Unlike chlorine, ozone is capable of oxidizing geosmin and 2-MIB (McGuire, 1999; Westerhoff, 2006). Although some research has found that ozonation itself does not readily remove geosmin and 2-MIB, ozone when combined with hydrogen peroxide (H_2O_2) (Park et al., 2006) or UV radiation (Meunier, 2006) has been shown to more easily degrade these substances.

Combining ozone with hydrogen peroxide, ozone with UV light or UV light with hydrogen peroxide catalyzes the formation of hydroxyl radicals which are much stronger oxidants than ozone by itself. The Advanced Oxidation Process (AOP) is the utilization of these hydroxyl radicals for oxidation. A very recent pilot scale study was conducted utilizing UV with ozone and UV with hydrogen peroxide for the degradation of geosmin and 2-MIB in RAS water (Klausen and Grønborg 2010). The water used for this study originated from a RAS trout farm. In preliminary tests the authors found the concentration of geosmin and 2-MIB varied from day to day in the RAS water. In order to ensure a concentration of these compounds in the water during the experimental tests the RAS water was spiked with geosmin and 2-MIB standards. The authors carried out batch experiments in reactors, using this water and the AOPs mentioned above. The authors observed degradation of both of these compounds under both treatments with the highest removal rate for both geosmin and 2-MIB in the UV and hydrogen peroxide treatment. Although they observed removal rates for these compounds, the rates were lower than that found in similar treatments of spiked tap water. Klausen and Grønborg (2010) attribute this to a high concentration of dissolved and particulate organic compounds found in RAS water compared to that of tap water. The dissolved and particulate organic compounds act as hydroxyl radical scavengers which compete for oxidation with geosmin and 2-MIB. Nonetheless, the AOP showed promise for the remediation of off-flavor compounds in RAS.

Adsorption

Adsorption practices are commonly used and have been proven effective at removing geosmin and 2-MIB. The process of adsorption physically removes geosmin and 2-MIB

from the water, where as oxidation and biological degradation alters the structure of the off-flavor compounds. The most common adsorbent used in this process is either Granulated Activated Carbon (GAC) or Powdered Activated Carbon (PAC) (McGuire, 1999; Ridal et al., 2001). Ellis and Korth (1993) had notable success using zeolite for geosmin and 2-MIB adsorption. Although the use of adsorbents is quite successful they are extremely expensive to use (McGuire, 1999), and have limited applications in aquaculture.

Biological processes

The biological process for the removal of off-flavor compounds involves using biofilters seeded with bacteria capable of metabolizing geosmin and 2-MIB. According to Ho et al. (2006) the use of biofiltration appears to be a suitable alternative for the removal of geosmin and 2-MIB since they are simple and require little maintenance and infrastructure. However, Huck et al. (1995) concluded that biodegradation as the primary method of removal for off-flavor compounds would probably not be suitable for drinking water due to inadequate off-flavor removal rates. Additionally, it is possible for biofilters to harbor off-flavor producing organisms which could lead to an increase of off-flavors production. In a study to determine the distribution of off-flavor compounds in RAS by Schrader and Summerfelt (2010), a few of their trials indicate a slight increase of geosmin concentration between the inlet and outlet of the biofilter. This could be an indication of off-flavor producing organisms living inside the biofilter.

Future Objectives

There has been much emphasis placed on the removal of geosmin and 2-MIB at drinking water treatment facilities. However, there has been limited off- flavor research

conducted on RAS. The technology that drinking water facilities utilize could be implemented in RAS if they are found to be cost effective. With the implementation of appropriate technologies within the growing system, efforts can be redirected from purging off-flavored fish to preventing these compounds from ever accumulating into the fish. As mentioned above, drinking water facilities use adsorption, biofiltration and oxidation for off-flavor removal. The use of adsorption technologies have been determined to be too large and too expensive for application in fish production. Additionally, biofiltration would be difficult to implement in an RAS since the biofilter would easily become contaminated with non-off-flavor metabolizing organisms. However, as noted by Klausen and Grønborg (2010), there is potential for eliminating or reducing off-flavor compounds through oxidation and the AOP. These technologies can easily be added to already existing RAS may prove to be an effective and economical solution to producing quality fish.

1.1.2 Removal of dissolved organic compounds in RAS

Many advancements in aquaculture design have occurred in the past three decades to enable the production of high quality farm-raised seafood quickly, inexpensively and sustainably. Accomplishing these goals is imperative for the future of aquaculture. One of the most important aspects in aquaculture is maintaining excellent water quality for optimal fish growth and health. Many species of aquatic animals are grown in Recirculating Aquaculture Systems (RAS). These systems treat and reuse 85% or more of the systems water. A problem that is faced in RAS is a buildup of fine and dissolved organic compounds in the systems water. This section of this chapter will discuss the causes of the buildup of fine and dissolved organic compounds in RAS and methods used to reduce these compounds in the systems water.

Fine and dissolved organic compound found in the water in RAS originate from fish waste, uneaten feed and micro fauna growing in the system (Chen et al., 1993). These compounds accumulate over time in RAS if the generation rate in the system is faster than the removal rate. With an increase of water reuse (less new water used), the removal of dissolved and particulate compounds becomes more problematic for the farmer. High levels of these compounds are detrimental to the fish's health (Bullock et al., 1994, Sharer and Summerfelt, 2007), decrease growth rates, and increase the biological oxygen demand in the systems water thus decreasing the oxygen available for fish respiration and decreasing the efficiency of nitrification in the biofilter (Michaud et al., 2006, Zhu and Chen, 2001). There are several common processes used in RAS for the removal of fine particulates and reduction

of dissolved organic compounds. They are microsreen filtration, granular media filters and oxidation.

Microscreen filtration in RAS

The removal of solids from RAS with the use of microfiltration depends on the size of the particles. Larger suspended particles are typically removed from RAS utilizing this process. Microsreens are effective and commonly used for the removal of larger solids in RAS. Typically the microscreens used in RAS have a screen mesh size of 60 to 200 µm (Cripps and Bergheim, 2000) allowing for smaller particulates to flow through and accumulate in the system. More than 95% of the suspended solids found in RAS have a diameter less than 20 µm, making up 40 to 70% of the total weight of the suspended solids within the water column (Chen et al., 1993). According to Chen et al., (1993) and Cripps and Bergheim, (2000) the smaller particulates, 5 to 10 µm in size, are the most problematic in RAS for reasons mentioned above. Although microscreen filtration is efficient in the removal of larger particles, it is not an effective treatment for the removal of smaller particulates.

Granular media filters in RAS

Granular media filters or fixed bed filters are commonly used in RAS. Fixed bed filters are capable of further clarifying the water after it has passed through a microscreen filter. These filters remove suspended solids by flowing the system water through a fixed bed of media where the suspend solids either get trapped or adsorbed in the void spaces or surface of the media. These filters can be designed so that the water either flows upward, downward or horizontally through the media. Additionally, they can be designed to be

pressurized or at atmospheric pressure. Under normal operating conditions within fish culture systems, the media in these filters becomes clogged with collected particulates and biofilm that grows between backwashing cycles. Consequently the waste collected on the media needs to be removed. This is accomplished by mixing the bed of media to free the solids which are allowed to settle and flushed out of the filter. The bed of media is typically mixed with air, water or propeller. According to Malone and Beecher (2000) fixed bed filters can remove all of the suspended solids greater than or equal to 50 µm in diameter. Additionally, they can remove 40 – 50% of all the particulates 10 µm or smaller in a single pass. Chen et al. (1994) report that even with media 5 mm in size, the removal of particulates as small as 10 µm is possible in recirculating systems. These components are capable of significantly lowering the amount of suspended solids and fine particulates in the culture water of RAS.

Ozone oxidation and the advanced oxidation process in RAS

Ozone treatment in RAS is commonly used for the removal of dissolved and fine particulate organic compounds, disinfection, and nitrite oxidation (Otte and Rosenthal, 1979; Rueter and Johnson 1995; Summerfelt et al., 1997, Killops, 1986). Ozone is a strong oxidant that is lethal to fish at low dissolved concentrations. For that reason it is important to use caution when treating the water in RAS with ozone. As a safe guard, the Oxidation Reduction Potential (ORP) can be monitored in the culture water to protect against overdosing ozone. For freshwater systems an ORP reading of < 300 – 350 mV is considered safe (Bullock et al., 1997). Ozone treatment has been shown to be capable of reducing the effects of bacterial, viral and fungal infections such as Bacterial Gill Disease (BGD) (Bullock

et al., 1997), white spot syndrome baculovirus (Chang et al., 1998) and saprolegniasis (Forneris et al., 2003). Interestingly, Bullock et al., (1997) reports a reduction of mortality from BGD even though the ozone treatment did not stop the bacterium from colonizing on the fishes gills. The authors credit their findings to an improvement in water quality, more specifically a reduction in suspended solids due to ozone oxidation. They found that by adding 25 g of ozone per kg of feed significantly improved the water quality.

Ozone has been shown to reduce particulate and dissolved organic matter in RAS by inducing microflocculation. Rueter and Johnson (1995) reports that ozone applied to RAS water, decreases the particulates stability allowing for flocculation. The authors found that microflocculation induced by ozonation improved mass removal in a settling tube. Additionally, they found that mass removal was higher in a sand filter when ozone was applied to the water. Summerfelt et al., (1997) found similar results with an increase of solids removal with the application of ozone prior to microscreen filtration. They also attributed the increase in solids removal to microflocculation. Color found in RAS water is considered to be from a buildup of non-biodegradable organic compounds. Ozone is capable of breaking down these relatively inert organic compounds into smaller readily biodegradable compounds (Hozalski et al., 1999; Yavich et al., 2004). Summerfelt et al., (1997) and Christensen et al., (2000), in two different studies, found that ozonation reduced the color and Chemical Oxygen Demand (COD) in the water in RAS.

While ozone is a strong oxidant, hydroxyl radicals are even more powerful oxidants. The Advanced Oxidation Process (AOP) is the utilization of hydroxyl radicals for oxidation. These radicals are formed naturally when ozone reacts with organic compounds. However,

the formation can be enhanced by reacting hydrogen peroxide with ozone, ozone with UV light and UV light with hydrogen peroxide. There has been limited research in the use of AOP for the reduction of fine and dissolved organic compounds in RAS. Sharrer and Summerfelt (2007) and Summerfelt et al., (2009) report UV irradiation combined with ozonation is a highly affective process for disinfection in RAS. Summerfelt et al., (2009) found the combination of ozone followed by UV irradiation (dose rate of 100 mWs/cm^2) demonstrated heterotrophic bacteria removal efficiencies of nearly 100% and for ozone alone they found a removal efficiency of 56.3%. Sharrer et al., (2005) reports a 65 to 81% removal efficiency of heterotrophic bacteria with a UV dose of 78 and 150 mWs/cm^2 respectively. The study was conducted at the same location as the Summerfelt et al., (2009) study. Additionally, Summerfelt et al., (2009) indicate an improvement in water quality with a reduction color and total suspended solids with the combination of ozone and UV irradiation. A very recent study by Klausen and Grønborg (2010) investigated the affects of UV light with peroxide and UV light with ozone on the degradation of the dissolved organic compounds, geosmin and 2-MIB, on RAS water. They observed degradation of these compounds; however the degradation rate was significantly lower than observed in similarly treated spiked tap water. They attribute their findings to a higher concentration of organic and inorganic hydroxyl radical scavengers found in RAS water.

Further research needs

The use of microscreen filtration, fixed bed filtration, ozonation and AOP have been tested in RAS for the removal of fine particulate and dissolved organic compound removal. These technologies have their own benefits and limitations. Although there has been little in

the way of research in the area of AOP for the removal of fine particulates and dissolved organic compound in RAS there is a high potential for utilizing these technologies in a RAS. Many RAS use a combination of several of these technologies to maximize the removal of these compounds. Future research comparing removal rates of fine and dissolved organic compounds between these treatments remain to be addressed. Additionally, more studies utilizing AOP in RAS needs to be conducted to determine its potential for improving water quality. Maintaining excellent water quality at a RAS is paramount for producing a quality fish economically.

The research described in the following chapters was designed to investigate the use of a series of filtration systems in under fish farming conditions, for the removal of off-flavors. The filtration test systems include: an ultraviolet sterilizer, an up-flow particulate filter, and a low head oxygenator (LHO) used as an ozone contactor. Ultraviolet sterilizers were utilized in an effort to inactivate microorganisms including off-flavor producing cyanobacteria and actinomycetes found in the water column. With the use of Ultraviolet sterilizers it was hypothesized that there would be a reduction in the amount of off-flavor compound being produced. The up-flow particulate filters were designed and used in an effort to remove fine solids from the water, in order to increase the potential for the oxidation of the off-flavor compounds in the water. Lastly LHOs will be used as the final treatment of the water. Ozone will be fed into the LHO's to provide a place for the oxidation of dissolved organic compounds including geosmin and 2-MIB. If a higher oxidation rate than achievable with ozone alone was required for reducing geosmin and 2-MIB, then hydrogen peroxide (H_2O_2) would be added to the water prior to the LHO's to enhance the oxidation process.

There will be significant beneficial economic impacts on the aquaculture industry if it is found that these technologies can be successfully utilized in fish production in RAS.

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CHAPTER 2

Removal of Fine Particulate and Dissolved Organic Matter in Recirculating Aquaculture Systems

2.1.0 Introduction

In an effort to produce fish economically, many improvements and adaptations in the design of Recirculating Aquaculture Systems (RAS) have occurred in the past two decades. With the use of these technologies, the production of farmed raised fish can be accomplished with a smaller volume of water when compared to open-pond culture methods. Additionally, RAS technology allows for better control of the water quality by filtering and re-using 85% or more of the water while exchanging only 5 - 15% of the system water per day. However, as the percentage of water re-use increases (less new water used), the potential for the buildup of fine particulates and dissolved organic compounds increases. This occurs as fish waste, uneaten feed and micro-fauna is produced within the culture system faster than they are removed from the system. Larger particles are typically removed with settling processes and mechanically with screens. Fine and dissolved compounds have been found to be more difficult to remove (Chen et al., 1994). Suspended solids found in RAS typically have a density similar to water (McMillan et al., 2003), making them difficult to be removed by settling. Additionally, many RAS use screen filters (typically drum screen filters) with a mesh size of 60 to 200 μm (Cripps and Bergheim, 2000) which allows smaller particulates to flow through. An accumulation of smaller particulates, 5 to 10 μm in diameter, and dissolved organic solids can cause detrimental affects to nitrifying biofilters, increase the

oxygen demand within the culture system, allow for the growth of non-beneficial microorganisms, cause gill damage, decrease growth rates and cause mortality (Chen et al., 1993; Cripps and Bergheim, 2000). Hence, there is a need to remove/minimize dissolved and suspended solids from RAS. Currently available methods for treating dissolved and suspended solids include fixed bed filtration, UV filtration, ozone oxidation and Advanced Oxidation Processes (AOP).

Fixed bed filtration has been found as an effective process in the removal of suspended solids and the reduction of turbidity in aquaculture. This type of filtration is accomplished by flowing water through a fixed bed of media. The flow can be directed in a downward or upward direction through the media which can be pressurized or remain at atmospheric pressure (gravity flow). The solid waste within the flowing water is filtered by the fixed bed and the solids are adsorbed on the media or captured within the void spaces between the media. Once the media becomes clogged with particulates or growth of bacteria, the bed is backwashed to remove the captured waste from the system. Malone and Beecher (2000) determined that floating bed filters can remove nearly all of the suspended solids greater than 50 µm and 40-50% of fine particulates less than 10 µm in a single pass. Chen et al., (1994), report that fixed bed filters can remove particulates down to 10 µm when applied to RAS even with media 5mm in diameter. These filters are capable of clarifying the water in RAS as the water is recycled through the media one or more times per hour.

Ultraviolet irradiation is commonly used in RAS and in aquaculture in general, for the destruction of algae and bacteria in an effort to reduce or eliminate pathogenic microorganisms (Losordo et al., 2009). UV irradiation works by damaging the

microorganisms DNA thus causing cell death or inactivity (Coonhill and Deering, 1969). The lethal dose of UV varies significantly between different microorganisms (Summerfelt, 2003). Liltved and Summerfelt (2007) indicate that a dose rate of 24,000 µWs/cm² is high enough to inactivate most pathogenic bacteria encountered in aquaculture production. Similarly, Wheaton (1977) indicates a dose rate of 26,400 µWs/cm² is high enough for 100% inhibition for most bacteria. While it is beneficial to inactivate pathogenic organisms to ensure fishes health, it is also beneficial to remove pathogenic and non-pathogenic microorganisms from the standpoint of reducing the Total Suspended Solids (TSS) and Chemical Oxygen Demand (COD) concentration in the production system. These reductions can reduce the waste load on the solids removal components and biofilter components.

Ozone has been used in aquaculture applications for a number of years for solids removal, the oxidation of Natural Organic Matter (NOM), nitrite oxidation and water disinfection (Otte and Rosenthal, 1979; Rueter and Johnson 1995; Summerfelt et al., 1997, Killops, 1986). The reduction of TSS and COD is accomplished by ozone induced microflocculation followed by sedimentation (Rueter and Johnson, 1995) or microscreen filtration (Summerfelt et al., 1997). Additionally, ozone has been found to enhance the removal of NOM by oxidizing these relatively inert Dissolved Organic Compounds (DOC) into smaller biodegradable compounds (Hozalski et al., 1999; Yavich et al., 2004). Color found in the water of RAS is considered to be the accumulation of these non-biodegradable DOC. Christensen et al. (2000) observed that treating RAS water with ozone significantly decreased water color with a measured decline in COD from 50 mg/l to 15 mg/l in the culture water. Ozone is an effective tool for the removal of fine particulate and dissolved organic

compounds in RASs. Additionally, AOP has been found to be effective treatment process for reducing COD in the (0-5 g/l) range (Andreozzi et al., 1999), which is well within the range found in RAS. AOP is the utilization of hydroxyl radicals which can be catalyzed from an ozone-peroxide mix. These hydroxyl radicals are a much stronger oxidant than ozone alone. There has been very little research on the use of AOP in RAS for the removal of dissolved and fine particulates.

Therefore, the objective of this study was to evaluate the effects of the AOP along with UV filtration, up-flow particulate filtration to reduce fine and dissolved organic compounds from a commercial sturgeon farm in North Carolina. The farm utilized a state-of-the-art RAS treatment layout. An experimental treatment loop was added. We hypothesized that the experimental loop would aid in the reduction of fine and dissolved organic compounds. The experimental treatment loop consists of UV filtration, up-flow particulate filtration, ozone contact, AOP (ozone – peroxide mix) and a Moving Bed Biofilter (MBB). This work was conducted in an effort to determine and quantify the effect of each of these filtration components used (where possible) singularly and in various combinations.

2.2.0. Materials and methods

This research was conducted at the LaPaz, LLC fish farm located in Happy Valley, NC. The LaPaz, LLC Building 1 has twelve 68 m^3 (18,000 gallon) fish culture tanks. The twelve tanks are divided into six recirculating aquaculture system Pods (henceforth referred to as RAS Pods). Three species of sturgeon are currently grown at this facility: Atlantic, Russian, and Siberian. These fish are farmed for their highly valued caviar and meat. One of these RAS Pods was used to conduct this research on fine and dissolved organic compound

removal. The RAS Pod consists of two growout tanks, both of which were stocked with Atlantic sturgeon, *Acipenser oxyrinchus*.

2.2.1. RAS Pod and treatment loop design

The design of the experimental Pod and the other 5 RAS Pods can be seen in Figure 2.2.1. Water flows by gravity to a treatment system for each Pod consisting of a 60 micron drum screen filter (model 803, Hydrotech, Water Management Technologies, Baton Rouge, LA) for suspended solids removal, and a custom designed and locally fabricated MBB containing 10 m^3 of K1 Kaldnes biofilter media (Miljøtekolobi A.S., Tønsberg, Norway) for the conversion of ammonia to nitrate via the biological process of nitrification. The MBB media is mixed with air supplied by a regenerative blower (model DR656D72X, AMETEK, Kent, OH) through 12 flexible membrane disc diffusers (model 01798, Environmental Dynamic Inc., Columbia, MO) located on the bottom of the MBB. The treated water is then pumped with two 3 hp pumps (Goulds Pumps, model 3656, Seneca Falls, NY) to two oxygen saturator columns (custom designed by LaPaz and built by Spears Manufacturing Co., Sylmar, CA) for the addition of pure oxygen and return to the two growout tanks at a rate of approximately 2,270 lpm (600gpm), 1,135 lpm for each tank (300 gpm).

2.2.2 Fine and dissolved organic remediation loop

The recirculating system that was developed (by LaPaz, LLC in conjunction with NC State University) for the culture of Atlantic sturgeon was not sufficient to remove the fine and dissolved solids that accumulated within the culture system and caused extreme turbidity within the water column. An experimental filtration system, developed by the authors in consultation with LaPaz, LLC, was added to the RAS pod as a side-stream process in an

effort to determine the requirements to remove fine and dissolved organic compounds that built up within the culture system under normal operating conditions. Water from the primary pumps (labeled 1 in Figure 2.2.1) for the RAS was diverted to the experimental treatment loop (see Fig. 2.2.2a and 2.2.2b). Effluent from the treatment loop returns to the beginning of the MBB. Water was pumped to the experimental loop at a rate of approximately 738 lpm (195 gpm). The experimental treatment loop was developed with three identical treatment systems operating in parallel to each other. Each treatment system consisted of an ultraviolet (UV) sterilization unit (120 Watt high output sterilizer COM5120HO-2U, Emperor Aquatics, Pottstown, PA), a custom built up-flow fixed bed particulate filter, a Low Head Oxygenator (LHO) used as an ozone contactor (LHO, Water Management Technologies, Baton Rouge, LA) and a custom built MBB (main system nitrifying biofilter, also used as the sump for the experimental loop). A dosing system was installed to dose Hydrogen Peroxide between the up-flow particulate filters and the LHOs. The three components; Up-flow particulate, UV and LHO, were placed in series with a flow rate of 246 lpm (65 gpm) pumped from the MBB. As noted previously, these three component filter systems were built in triplicate and operated in parallel so that the treatment loop treated a total of 738 lpm (195 gpm).

2.2.3 Filter system components

The UV sterilizers used in this research have an effective manufacturer rated dose rate of 30,000 μWs/cm² at 265 lpm (70 gpm). As noted earlier this dose rate is considered high enough to kill most bacteria and algae found in aquaculture.

The up-flow particulate filters were designed at NC State and custom fabricated from 1135.5 liter (300 gallon) conical tanks (62343 unit, Norwesco, Bonifacius, MN) (See Fig 2.2.3). Each tank was filled with 0.75 m^3 of floating K1 Kaldnes media. Water enters the conical tank below the kaldnes media through a perforated manifold. This manifold was constructed from a 50 mm (2 inch) PVC pipe with 6 mm (¼ inch) wide slots cut every 13 mm (½ inch) along the pipe. The slots were placed facing up towards the media to disperse the flow of water throughout the static bed. The water flows up through the bed of media to the top of the tank where a perforated drain manifold is located. The drain manifold was constructed from a 76 mm (3 inch) (diameter) high density polyethylene perforated screen tube. Under normal operation, over time, the Kaldnes media becomes clogged with captured fine particulate solids and some biological growth. The captured solids and biosolids are removed by mixing the bed of Kaldnes media with low pressure air followed by draining the freed settled solids from the cone at the bottom of the filter tank. The air for backwashing the filter was supplied from the blower end of a Craftsman portable wet-dry vacuum (17761 model, Sears Holding Corp., Hoffman Estates, IL) through the 50 mm (2 inch) perforated manifold located below the media. Prior to backwashing the filters, the flow of water is turned off by closing a ball valve. The drain for the up-flow particulate filter was constructed of three 50 mm (2 inch) PVC pipes in the shape of a T with 8 mm (5/16 inch) holes drilled onto the underside of the horizontal pipes and around the peripheral of the vertical pipe. As noted previously, these filters were designed to remove the fine particulate matter from the water to decrease the load of organic compounds in the water. While it would have been ideal to have the UV sterilization components after the up-flow filters

were not pressure capable and flowed by gravity back to the fish culture system's biofilter. As such, the UV sterilizers were placed in front of the up-flow particulate filters.

The LHO component was utilized to deliver and dissolve ozone into the process water for the oxidation of fine particulates and dissolved organic compounds including micro-organisms that were not destroyed by UV light or removed by the up-flow filters. The LHOs were designed by the manufacturer for flow rates of up to 284 lpm (75 gpm) each. Ozone gas for this study was produced from gaseous oxygen with an ozone generator that was rated by the manufacturer to produce 22 g / hr from pure oxygen gas (Model K2, (22 g/hr), Reclaim Filters and Systems Inc., Wake Forest, NC). The concentration of ozone produced in this study was analyzed with a Teledyne ozone monitor (Model 454H, Teledyne Technologies Incorporated, San Diego, CA). The total gas (ozone) flow rate was monitored with a digital flow meter (XFM Digital Mass Flow Meter, Aalborg Instruments and Controls, Inc., Orangeburg, NY). The flow of ozone could be individually controlled and was split evenly between the three LHOs through the use of rotameters (7530 model, King Instrument Company, Garden Grove, CA), one feeding each LHO. The dissolved ozone concentration was measured at the bottom of the LHOs with Hach Chemical Companies mid range (0 – 0.75 mg/l) and high range (0 – 1.5 mg/l) ozone reagent ampules (Hach Company, Loveland, CO).

Hydrogen Peroxide injection was accomplished by metering the chemical into the top of the LHO. The H_2O_2 was delivered at a known rate with a dosing pump (BL20 Hanna Instruments, Woonsocket, RI). The dose rate was calculated and set to obtain a ratio 0.354 g H_2O_2 per g O_3 delivered.

The MBB used as the sump for the experimental loop is described above (see 2.2.1 RAS Pod and treatment loop design).

The water flow rate to each filter group (in triplicate) was evenly divided and monitored with a digital flow meter and totalizer (Signet 8150 Total Flow Meter 3-8150.090-1, George Fischer Signet LLC, Schaffhausen, Switzerland) located in front of the UV sterilization components. As mentioned above the target flow rates for each filter set was 246 lpm (65 gpm). Sampling was conducted at an inlet port prior to the UV sterilizers and outlet port after the LHOs.

2.2.4. Test methodology

During the filter trials, the fish growout tanks were separated from the primary treatment system (Drum-screen filter, MBB, and oxygentation) and the experimental filter loop described above (Figure 2.2.2a). The growout tanks were separated from the primary treatment loop by stopping all flow to and from the fish culture tanks by closing all the influent manifold valves and shutting down one of the main systems pumps. The system configuration was implemented to reduce the volume of water being treated by the experimental treatment loop and as an effort to stop or significantly reduce the largest source of organic waste generated within the system (fish metabolites and uneaten feed released in the tanks). Splitting the system in this manner decreased the volume of water to be treated to about 10% of the total normal system volume, which, allowed the experimental treatment system to effect a change in water quality more rapidly than if the system was connected to the fish culture tanks. While the growout tanks were separated from the RAS filter system, pure oxygen was bubbled into the tanks through diffusers. Dissolved Oxygen (D.O.) levels

were monitored to insure proper fish culture conditions were maintained. Feed was withheld from the tanks prior to and during these experimental trials in order to keep the D.O. levels high and the ammonia levels low in the growout tanks during the experiments.

The study was begun by conducting a number of preliminary ranging experiments (appendix A). That is, we ran short studies to determine the rate at which the experimental loop could change the quality of the water within the MBB and experimental treatment loop.

2.2.5. Calculating removal rates

Chemical Oxygen Demand (COD) was used as a measure of the concentration fine and dissolved organic compounds in the water within the culture system. Inlet and outlet samples were taken to obtain what we will refer to as "instantaneous COD removal rates". That is, these were estimates of the COD removal rates at the time and location of that particular sample. These instantaneous COD removal rates were calculated as:

$$COD_{(in\text{-}out)} \text{ removal rate} = [COD_{in(ti)} - COD_{out(ti)}] \times \text{flow rate} \qquad (2.1)$$

(mg/h) (mg/l) (l/h)

Additionally, removal rates over a specified period of time were estimated from the change in COD during the experimental trial. This measure of COD was calculated as:

$$COD_{(\Delta t)} \text{ removal rate} = [(COD_{(ti)} - COD_{(ti+1)}) \times V] / \Delta \text{ time} \qquad (2.2)$$

(mg/h) (mg/l) (l) (h)

Where V is the volume of the treatment system in liters.

2.2.6. Experimental trials

The final experimental trials commenced in October, 2009. As noted above, from data collected in the ranging experiments we determined that an experimental trial period of

8 hours was appropriate. Six samples were collected at each measurement time, three from the inlet port just prior to the UV sterilizer furthest downstream and the other three from the effluent flowing out of the LHOs (see figure 2.2.2a for sample locations). The sample times varied slightly over the 8 hrs of the different trials. During the first 7 trials, samples were collected at time 0, 2, 4, 5, 6, 7 and 8 hrs. In the last 15 trials, samples were collected at time 0, 1, 2, 3, 4, 6 and 8 hrs. During each sampling, the water flow rate (instantaneous) and total flow (volume of water treated between samples) were recorded. For trials using ozone, the ozone flow rate, total gas flow, ozone gas concentration g/m^3 and ozone concentration in the water were recorded. Table 2.2.1 provides a description of the filter system configurations for the experimental trials.

2.2.7. Chemical oxygen demand analysis

All COD analysis was conducted at the North Carolina State University Environmental Analysis Laboratory using ultra low range (0 to 40 mg/l) COD test vials (2415815, Hach Company, Loveland, CO). The test vials were analyzed using the Milton Roy Spectronic 401 spectrophotometer (Spectronic Instruments, Inc., Rochester, NY). All samples were acidified to a pH below 2.0 with 19.2 N sulfuric acid and stored in coolers with ice immediately after collection. Upon arrival, the samples were stored in a refrigerator (4° C) until analyses. All samples were analyzed within 96 hrs of collection. See appendix B for spectrophotometer and COD analysis protocol.

2.3.0. Results and discussion

Results of these experiments were analyzed in three ways. A statistical model was created using the mixed procedure in SAS (SAS version 9.1.3, SAS Institute Inc, North

Carolina) to predict COD removal rates for each treatment and to compare and contrast the treatment effects. Additionally, the data was analyzed to determine percent COD removal at specific times during the test period. And finally, the degradation rate of the COD concentration in the water and estimated removal rates are presented graphically below.

2.3.1. Removal predictions by the model

The COD removal rates for the combinations of treatments were analyzed statistically to determine if there was a significant difference in COD removal rates between the trials. As mentioned earlier, COD removal rates were calculated two different ways; $COD_{(in-out)}$ (an instantaneous measure of the removal rate in the filter) and $COD_{(\Delta t)}$ (a measured removal rate over a defined period of time) There was no significance at the $\alpha = 0.05$ level for a difference in COD removal rates for the different treatments when COD removal was calculated using $COD_{(in-out)}$. However, when the COD removal rates were calculated using $COD_{(\Delta t)}$ there was significance ($\alpha = 0.05$) in the removal rates between the different treatments (described below).

Calculating removal rates based on sampling the inflow and outflow provides a view of the filtration processes during a moment in time. This measurement, at a specific moment in time, might not represent the true removal rate during the time of interest to the researcher or designer. Using this method, tests the change of COD in the filtration loop with a low retention time of approximately 5 mins which may lead to variable and / or inaccurate conclusions. In this study, calculating COD removal rates using the change in COD concentration over time ($COD_{(\Delta t)}$) within the treatment loop (including the MBB) rather than the difference between COD in and out ($COD_{(in-out)}$) proved a much better method for

comparing the different treatment configurations. Analysis of the $COD_{(\Delta t)}$ removal rates (with a retention time of 1 or 2 hours depending on sampling regimes) demonstrated that there was a statistical difference in COD removal rates between the system configurations. Analysis of the $COD_{(in-out)}$ removal rate showed no difference in COD removal between the treatments and would have lead us to an inaccurate conclusion.

A fixed effects model was created using SAS for predicting $COD_{(\Delta t)}$ removal rates for the treatments. The empirical $COD_{(\Delta t)}$ removal rate model:

$$\text{COD Removal Rate} = 3.52 * [\text{COD}] + \beta_{(\text{Treatment})} - 66.35 \qquad (2.3)$$

Table 2.3.1. $COD_{(\Delta t)}$ removal rate model variables.

$\beta_{(\text{no media})}$	-9.46
$\beta_{(\text{Up-flow})}$	-31.47
$\beta_{(\text{Up-flow and UV})}$	-1.30
$\beta_{(\text{Up-flow and Ozone})}$	9.77
$\beta_{(\text{Up-flow, UV and Ozone})}$	-11.11
$\beta_{(\text{Up-flow, UV and Ozone with Peroxide})}$	0
[COD]	COD concentration (mg / L)
COD Removal Rate	(g / h)

The difference of least squares means (Diff. LSM) was used to compare the different treatment against each other (see table 2.3.2). The significance of the $COD_{(\Delta t)}$ removal rates between the treatments can be compared in the table by observing the Diff. LSM between the intersecting treatments. For example, the LSM $COD_{(\Delta t)}$ removal rate for the Up-flow filter and Ozone combination has a significantly higher removal rate than the Up-flow only, by 41.24 ± 10.14 g/hr.

Results from the model developed to predict COD removal rates suggests that the most effective treatment for removing fine and dissolved organic compounds was the Up-flow and ozone combination treatment. According to the difference of LSM the Up-flow with ozone treatment increased the $COD_{(\Delta t)}$ removal rate by 41.24 ± 10.14 g/hr beyond what the up-flow filter alone could accomplish. Additionally, the model predicted that the up-flow and ozone treatment would outperform the up-flow filter with no media and the up-flow, UV filtration and ozone contact combination by 19.23 ± 7.98 and 20.88 ± 7.39 g/hr, respectively. Although the difference of LSM for up-flow and ozone contact compared to, up-flow and UV filtration, and up-flow, UV filtration with AOP was not considered statistically different at $\alpha = 0.05$ the up-flow and ozone contact treatment had a higher $COD_{(\Delta t)}$ removal rate in both cases with increase in removal rates of 11.07 ± 7.22 g/hr and 9.77 ± 7.70 g/hr respectively.

Given that up-flow (fixed bed) filtration has been shown to be an effective method for fine particulate removal (Chen et al., 1994) and ozone treatment has demonstrated excellent dissolved organic compound oxidation (Christensen et al., 2000), we expected that this combination would provide an effective treatment. Additionally, we also expected that the treatment using up-flow, UV filtration with AOP would provide even higher COD removal rates. This was hypothesized as the ozone - peroxide mix should catalyze the formation of hydroxyl radicals (HO•) which is a much stronger oxidant than ozone alone. The optimal dose rate of hydrogen peroxide to ozone for the maximum production of hydroxyl radicals is 0.354 g H_2O_2 per gram of ozone (Crittenden et al., 2005). Crittenden et al. (2005)

additionally indicated that a dose rate higher or lower than this could be detrimental to hydroxyl radical concentrations by scavenging hydroxyl radicals via the reactions:

$O_3 + HO\bullet \rightarrow HO_2\bullet + O_2$ (Ozone overdose) (2.4)

$H_2O_2 + HO\bullet \rightarrow HO_2\bullet + H_2O$ (Peroxide overdose) (2.5)

The authors also noted that, "residual H_2O_2 can be more problematic than ozone because hydrogen peroxide is more stable than ozone".

The up-flow, UV filtration with AOP trials were carried out with the assumption of a dissolved ozone concentration of 0.75 mg/l. This concentration was measured during the up-flow and ozone contact trials. A dose rate of H_2O_2 was setup to inject 0.354 grams H_2O_2 per gram of ozone based on an ozone concentration of 0.75 mg/l. However, during these AOP trials the ozone concentration measured in the water at the base of the LHO contactors was never detectable. We may have never obtained a concentration of residual ozone due to a higher concentration of organic matter in the treatment water. Christensen et al., (2000), observed similar results in a study dosing ozone in a RAS for modeling color destruction as a function of feed rate. Similarly to our up-flow, UV filtration with AOP the authors never detected residual ozone. They also attributed their finding to a high concentration of organic compounds in the water. Since we were dosing H_2O_2 for an assumed ozone concentration of 0.75 mg/l, we suspect that system may have been overdosed with H_2O_2 during the trial period. As noted above, an overdose of H_2O_2 would be detrimental to hydroxyl radical production and as a result this treatment was probably not as effective as it potentially could have been.

Results from the model obtained for predicting removal rates indicates that the up-flow treatment with no media would provide higher $COD_{(\Delta t)}$ removal rates than the up-flow filter with media with a difference in LSM of 22.01 ± 8.78 g/hr. This was unexpected and counter intuitive since the up-flow filter without media essentially has no filtration capabilities except for the possibility of settling within the filter or somewhere within the system. The observed COD removal rates from the up-flow filter with no media may be a result of the up-flow filter media being removed just prior to the first experimental trial with no media. Removing the media from the filter caused an increase of suspend solids in the treatment system from biofilm shedding from the media and trapped solids found in and around the media. We believed that, in the first trial after removing the media, the solids that were released during the media removal process, settled in the up-flow filters causing a decrease in COD over the 8 hr treatment period.

The $COD_{(\Delta t)}$ removal rate model predicts that combining UV filtration with up-flow filtration increases the removal rate by 30.14 g/hr according to the difference in LSM. An increase of COD removal rate was expected with the addition of UV filtration. This demonstrates that the dose rate of 30,000 $\mu Ws/cm^2$ was sufficient for microorganism death and or inactivity. Similar results were observed by Wheaten (1977) and Liltved and Summerfelt (2007), where the authors found that a dose rate of 24,000-26,500 was adequate for bacterial disinfection. Most likely the up-flow filters collected and removed the dead and inactivated microorganism along with fine particulates from the treatment system thus lowering the COD over time. Although UV filtration is the direct cause of microorganism inactivity and or death this process is dependent on the up-flow particulate filtration for the

removal of suspended solids. Qualls et al., (1983) report that suspend solids can diminish the efficiency of UV disinfection by absorbing and scattering UV light. Additionally, the authors found that there was a correlation between COD and UV absorbance. A decrease in suspended solids and dissolved organic compound will allow for an improvement in UV disinfection. Due to this the efficiency of the UV disinfection in the up-flow and UV filtration treatment combination is influenced by the effectiveness of COD removal by the up-flow particulate filter.

2.3.2. Estimating the percent COD removal over time

The average percent COD removal for each filtration configuration described in table 2.2.1, was calculated at time (0, 4 and 8 hrs) (see table 2.3.3). The percent COD removal was calculated with the equation:

$$\text{Percent COD removal} = [1 - (COD_{(ti)}/COD_{(0)})] * 100 \qquad (2.6)$$

Where the $COD_{(ti)}$ is the COD concentration at time i and $COD_{(0)}$ is the initial COD concentration (time 0). For each treatment configuration the average percent removal approached equilibrium within four hours. Stated another way, the effect of the treatment system occurred in the first 4 hours of the treatment process and very little treatment occurred thereafter. The percent removed after 8 hrs for the up-flow filtration, up-flow and UV filtration, and up-flow, UV filtration with AOP, were not significantly different (12.6 ± 3.0%, 13.5 ± 5.0 %, and 14.9 ± 2.4% respectively). The up-flow, UV filtration and ozone contact had a removal percentage of 19.4 ± 3.4%, after 8 hrs, which was significantly higher than the up-flow filtration trial but not different from the other trial combinations mentioned above. The up-flow and ozone contact combination had the highest percent removal with a

removal percentage of 29.8 ± 2.8%. The lowest percent removal observed was the up-flow with no media trial with a removal percentage of 9.1 ± 2.9%. Both the up-flow and ozone contact and the up-flow with no media trials were significantly different after 8 hrs from each other and all the other trials.

As mentioned above we observed the highest percent COD removal (29.8 ± 2.8 %) with the up-flow filtration and ozone contact treatment configuration. Summerfelt et al., (1997) found a similar percent COD removal rate (36%) in a study using ozone delivered through an LHO and microscreen filter combination. This study was conducted over an 8 week period (comparer to our 8 hr) which may explain the slightly higher percent removal rates.

2.3.3 Estimating the COD removal rate from the trial results

The average $COD_{(0,4,8)}$ was measured and averaged for each trial configuration (described in table 2.2.1), and the results can be seen in table 2.3.3 and plotted in figures 2.3.2a, b, c, d, e, f, and g. All but two of the treatment trials, the up-flow no media and up-flow, UV filtration and ozone contact (1 of 2), had a significantly lower average COD after the first four hours of the experiment with a non significantly different average COD after the last four hours (see figures 2.3.2b, c, d, f, and g). The average COD was not statistically different in the up-flow no media trial over the eight hour trial period (see figure 2.3.2a). Whereas the average COD was significantly lower after the first fours and further lower after the last four hours of the up-flow, UV filtration and ozone contact (1 of 2) treatment (see figure 2.3.2e).

$COD_{(\Delta t)}$ removal rates were also calculated using the average $COD_{(0,4,8)}$ for the time intervals of time 0 to hour 4 and hour 4 to hour 8 can been seen in table 2.3.3. The $COD_{(\Delta t)}$ removal rate for up-flow with no media was not significantly different between first four hours and the last four hours. Additionally, the removal rate was not significantly different from zero in both the first four hours and the last four hours. In all the other trials we found that the $COD_{(\Delta t)}$ removal rate was significantly lower in the last four hours compared to the first four hours. Within these trials all $COD_{(\Delta t)}$ removal rates in the first four hours were significantly higher than zero and not significantly different than zero in the last four hours except for both of the up-flow, UV filtration, ozone contact configuration trials and the up-flow, UV filtration with AOP configuration. The $COD_{(\Delta t)}$ removal rates were significantly higher than zero in the first four hours and last four hours for these three trials.

Given that the removal rates were significantly higher than zero throughout the up-flow, UV filtration and ozone contact (2 of 2) and up-flow, UV filtration with AOP and the $COD_{(4)}$ and $COD_{(8)}$ for these trials were not significantly different it is thought that there is a COD generation term. It is possible that during these trials, waste from the biofilm and or waste from the up-flow filters and MBB could be shedding into the treatment water. On the other hand, the up-flow, UV filtration and ozone contact (1 of 2) had a removal rate significantly higher than zero and the $COD_{(8)}$ was significantly lower than $COD_{(4)}$. This indicates that the trial was not conducted for long enough duration to reach an equilibrium COD concentration in the treatment system.

As noted above the up-flow with no media unexpectedly had a higher COD removal rate than up-flow with media according to the model. A decrease of $COD_{(ti)}$ for the up-flow

no media can be observed in figure 2.3.2a for the first treatment on March 23, 2010 while the other two treatments demonstrate a fairly constant $COD_{(ti)}$ over the trial periods. This indicates COD removal rates were mostly likely due to settling solids as theorized above. The constant $COD_{(ti)}$ found in the latter two treatments were expected since there were no more readily settleable solids left in the system during these trial runs. Even though the model predicts that the up-flow no media has a higher $COD_{(\Delta t)}$ removal rate than the up-flow filter with media, the observed average $COD_{(\Delta t)}$ removal rate is significantly higher than zero in the first four hours for up-flow (media) whereas the observed average $COD_{(\Delta t)}$ removal rate is not significantly different than zero for up-flow no media (see table 2.3.3). This indicates that even with the observed COD removal rates found in the first run of the up-flow no media treatment the overall treatment did not show statistically that COD was removed from the system.

Interestingly, as the model predicts an increase of $COD_{(\Delta t)}$ removal rate with the addition of UV filtration to up-flow filtration the average observed $COD_{(\Delta t)}$ removal rate is lower for the up-flow and UV filtration when compared to up-flow filtration by itself in the first four hours (see table 2.3.3). This is most likely because the model uses $COD_{(0)}$ concentration as a covariate for prediction of the $COD_{(\Delta t)}$ removal rates where as table 2.3.3 shows the observed removal rates which are at various $COD_{(0)}$ concentrations in the different treatments. We theorize that as the $COD_{(0)}$ increases the observed $COD_{(\Delta t)}$ removal rates will increase within the different treatments until the $COD_{(ti)}$ reaches an equilibrium concentration. This makes it difficult to compare the observed $COD_{(\Delta t)}$ removal rates from the different trial against each other with each trial starting at a different $COD_{(0)}$. However,

the model used here accounts for the $COD_{(0)}$ and adjusts the $COD_{(\Delta t)}$ removal rates accordingly. Unfortunately with a commercial scale operation such as the LaPaz LLC it is very difficult to be able to replicate identical COD concentrations in the system which would be ideal for these filtration comparisons.

In an effort to normalize the removal rates between the different treatments, to compensate for the various $COD_{(0)}$, the percent COD removed was calculated. Similarly to the COD removal model we found that the up-flow and ozone contact had the best percent removal. As expected, the up-flow no media treatments had the lowest percent removal since the only method for fine organic removal would be through settling. The percent removal showed no significant difference between the other four trials except the up-flow, UV filtration and ozone contact was significantly better than up-flow filtration. This method used for normalizing the data was useful for pulling out the best and worst fine and dissolved organic removal treatments. However, determining an order of best to worst configurations was not determined through this method of normalizing the data or through utilizing the COD removal model.

Comparing the different combination of filters proved to be difficult due to the lack of control over standardizing the $COD_{(0)}$ between the different treatments. Had all the trials started at the same COD concentration it may have led to a more accurate comparison between the treatments. With the nature of commercial RAS there are many factors that make it difficult to reproduce similar water quality conditions over a six month period. Additionally the trial period of eight hours may not have been long enough to reach an equilibrium COD concentration in the treatment system for all of the trials.

2.5.0 Conclusions

The removal of fine and dissolved organic compounds through various filtration treatments was assessed in this research. We found that the use of ozone with up-flow particulate filtration and MBB significantly aided in the improvement of water quality conditions. This improvement may aid in biofilter efficiency, fish health and reduce oxygen demand in the culture tanks. Most importantly by enhancing these factors it may increase the growth rate of the fish and decrease the time for growout to harvest thus reducing the cost of production. AOP should be further investigated to determine if it is a practical technology for removal of fine and dissolved organic compounds in RAS. Utilizing these technologies and advancements for the improvement of cost and production of a quality product aids in the development of sustainable RAS for the farmer and consumer.

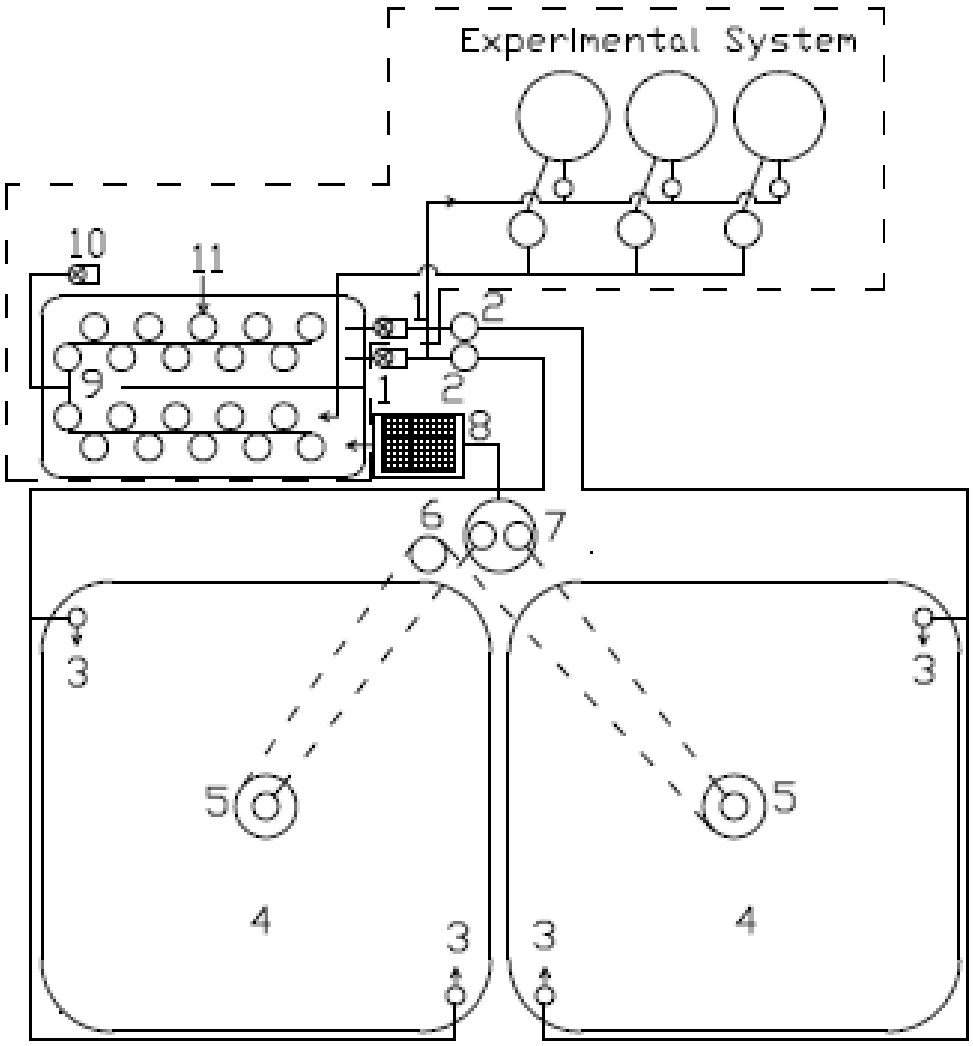

Figure 2.2.1. Design of the LaPaz RAS pod with an experimental filtration loop within the boxed in section; (1) pump, (2) oxygen saturator, (3) tank influent manifold, (4) culture tank, (5) double drain, (6) swirl separator, (7) overflow standpipes, (8) drum screen, (9) biofilter, (10) regenerator blower, (11) air diffusers.

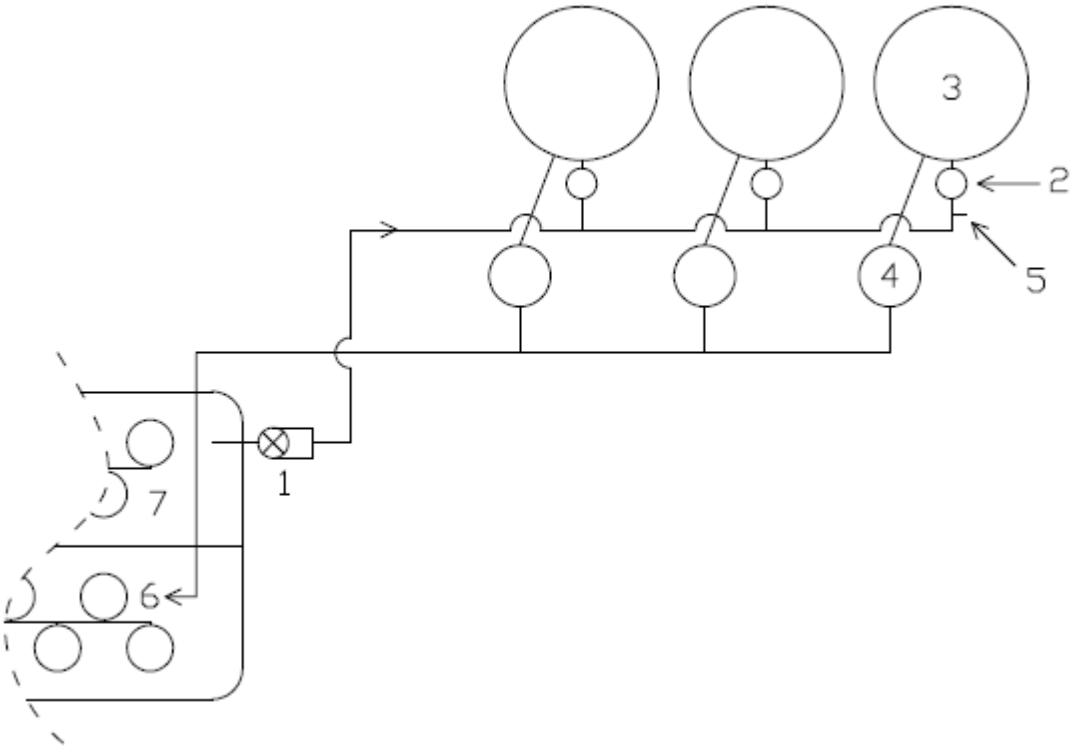

Figure 2.2.2a. Experimental loop; (1) pump, (2) UV sterilizer, (3) up-flow particulate, (4) LHO, (5) sampling port (in), (6) sampling port (out), (7) moving bed biofilter.

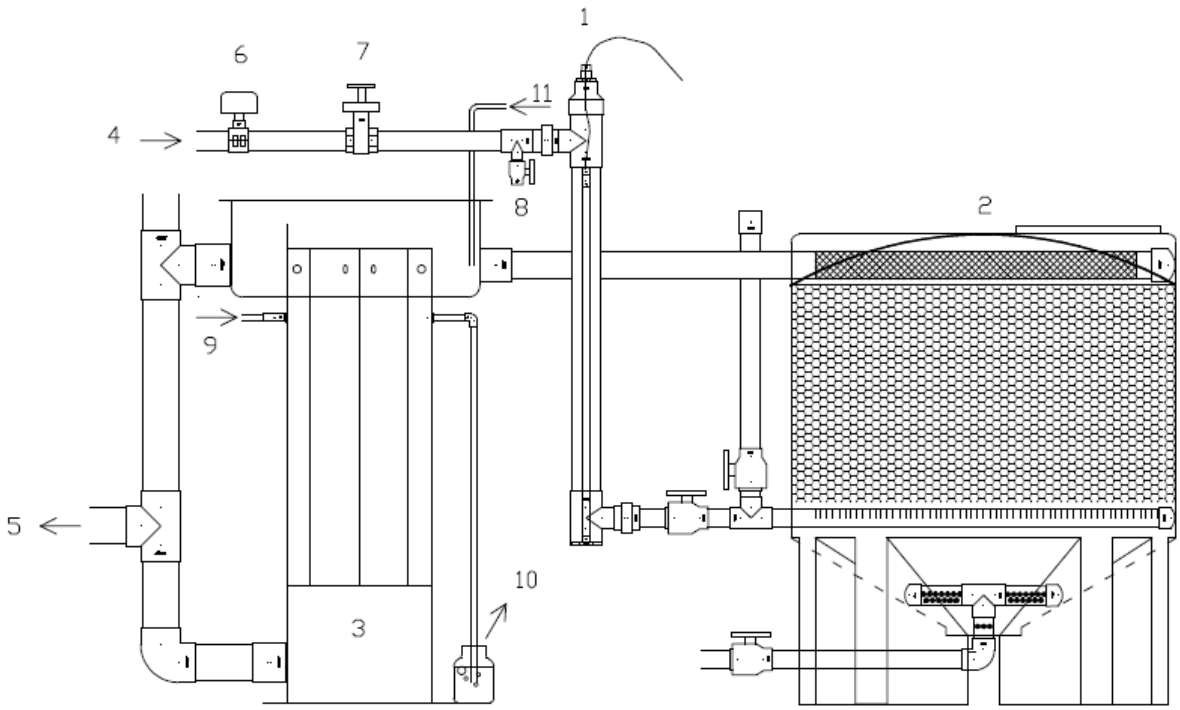

Figure 2.2.2b. Elevation view of the experimental system loop components without moving bed biofilter; (1) UV sterilizer, (2) up-flow particulate filter, (3) LHO, (4) influent to loop, (5) effluent from experimental loop/sample port out, (6) flow meter/totalizer, (7) gate valve to control flow rates, (8) sample port in, (9) ozone flow to LHO, (10) LHO off gas, (11) hydrogen peroxide addition site.

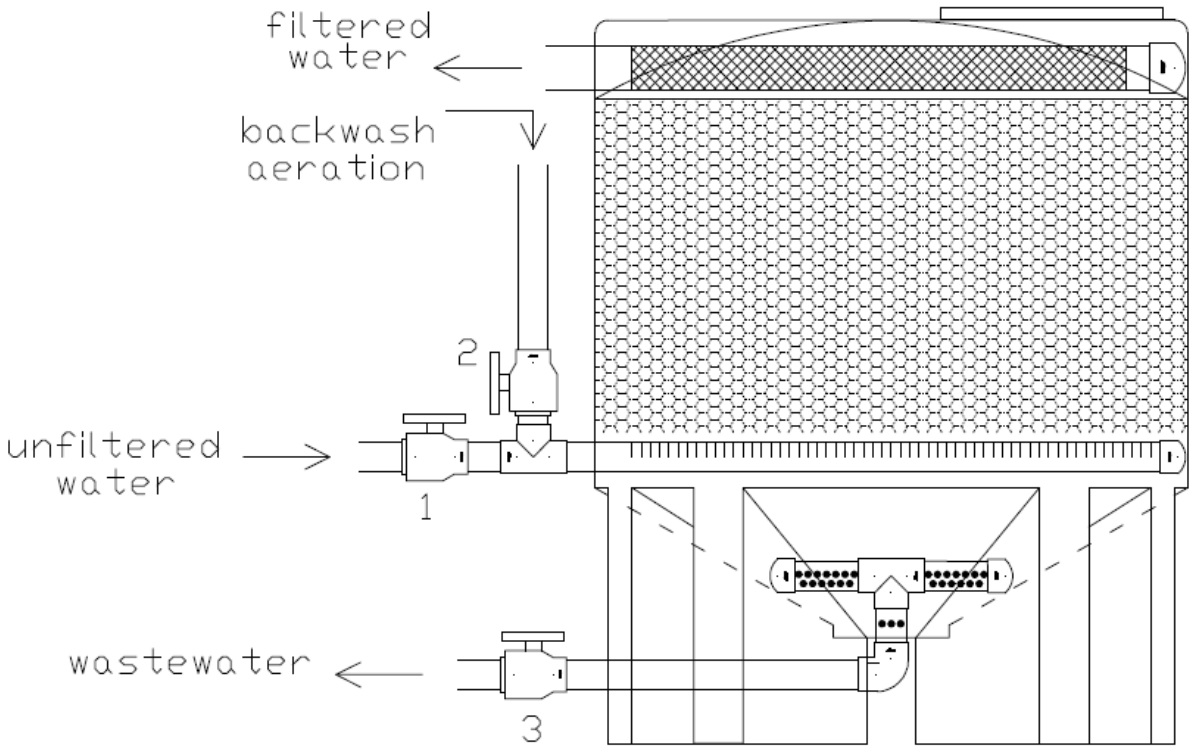

Figure 2.2.3. Up-flow particulate filter, 1135.5 liter conical tank with 0.75 m^3 of Kaldnes media. Steps in backwasing; 1) Close valve 1, to stop flow of water, open valve 2 to allow for aeration. 2) Aerate media for two minutes with low pressure air added to the pipe above valve 2. 3) Stop aeration and close valve 2. 4) Open valve 3 to drain tank of settled solids. 5) Once tank is drained close valve 3 and open valve 1 to continue normal operation.

Table 2.2.1. Trial configurations

Test	Number of Trials	Duration (hrs)	Number of samples
Up-flow	3	8	42 Samples per trial
Up-flow and UV	4	8	42 Samples per trial
Up-flow and ozone	3	8	42 Samples per trial
Up-flow, UV and ozone	6	8	42 Samples per trial
Up-flow, UV, ozone with *H_2O_2	3	8	42 Samples per trial
Up-flow (no media)	3	8	42 Samples per trial

All configurations include the moving bed biofilter as a sump.
*0.354 mg H_2O_2 : 1.0 mg ozone

Table 2.3.2. Difference of least squares means for $COD_{(\Delta t)}$ removal rates between treatments.

Treatments	**Up-flow UV AOP**	**Up-flow UV Ozone**	**Up-flow Ozone**	**Up-flow UV**	**Up-flow**
Up-flow no media Diff. LSM (g/hr)	Not Significant	Not Significant	Significant at $\alpha = 0.05$ 19.23 ± 7.98	Not Significant	Significant at $\alpha = 0.05$ -22.01 ± 8.78
Up-flow Diff. LSM (g/hr)	Significant at $\alpha = 0.05$ 31.47 ± 9.48	Significant at $\alpha = 0.05$ 20.36 ± 7.42	Significant at $\alpha = 0.05$ 41.24 ± 10.14	Significant at $\alpha = 0.05$ 30.17 ± 9.06	
Up-flow UV	Not Significant	Not Significant	Not Significant		Significant at $\alpha = 0.05$ -30.17 ± 9.06
Up-flow Ozone Diff. LSM (g/hr)	Not Significant	Significant at $\alpha = 0.05$ -20.88 ± 7.39		Not Significant	Significant at $\alpha = 0.05$ -41.24 ± 10.14
Up-flow UV Ozone Diff. LSM (g/hr)	Not Significant		Significant at $\alpha = 0.05$ 20.88 ± 7.39	Not Significant	Significant at $\alpha = 0.05$ -20.36 ± 7.42

Table 2.3.3. Average COD Removal Fraction, average $COD_{(ti)}$ and average $COD_{(\Delta t)}$ removal rate during the 8 hour trial periods at time 0, 4 hours and 8 hours for the different treatments.

Treatment	Percent COD Removal	$CODin_{(ti)}$ (mg/l)	$COD_{(\Delta in)}$ removal rate (g/hr)	Time (hr)
Up-flow	0.0 ± 0.0 %	25.38 ± 2.52		0
no media	10.0 ± 3.0 %	22.70 ± 1.46	10.14 ± 11.02	4
	9.1 ± 2.9 %	22.93 ± 1.49	-0.85 ± 7.88	8
Up-flow	0.0 ± 0.0 %	35.57 ± 1.88		0
	14.6 ± 2.4 %	30.28 ± 0.88	20.02 ± 7.86	4
	12.6 ± 3.0 %	30.98 ± 0.58	-2.67 ± 3.99	8
Up-flow UV	0.0 ± 0.0 %	23.80 ± 1.49		0
	13.4 ± 4.5 %	21.32 ± 0.36	9.38 ± 1.53	4
	13.5 ± 5.0 %	21.44 ± 0.35	-0.45 ± 0.50	8
Up-flow	0.0 ± 0.0 %	25.41 ± 2.58		0
Ozone	26.6 ± 3.1 %	18.48 ± 1.06	26.20 ± 10.55	4
	29.8 ± 2.8 %	17.71 ± 1.27	2.93 ± 6.26	8
Up-flow UV		35.87 ± 2.99		0
Ozone		29.31 ± 0.97	24.82 ± 3.14	4
(1 of 2)	* 0.0 ± 0.0 %	26.70 ± 1.15	9.87 ± 1.50	8
	*14.4 ± 3.0 %			
Up-flow UV	*19.4 ± 3.4 %	22.89 ± 0.86		0
Ozone		20.28 ± 0.78	9.90 ± 1.16	4
(2 of 2)		19.68 ± 0.56	2.26 ± 0.96	8
Up-flow UV	0.0 ± 0.0 %	23.06 ± 0.43		0
AOP	11.3 ± 2.3 %	20.45 ± 0.52	9.87 ± 2.54	4
	14.9 ± 2.4 %	19.62 ± 0.57	3.13 ± 2.93	8

* By analyzing the percent fractional removal rates the data was normalized for the two Up-flow, UV filtration and ozone contact trials and reported as one trial.

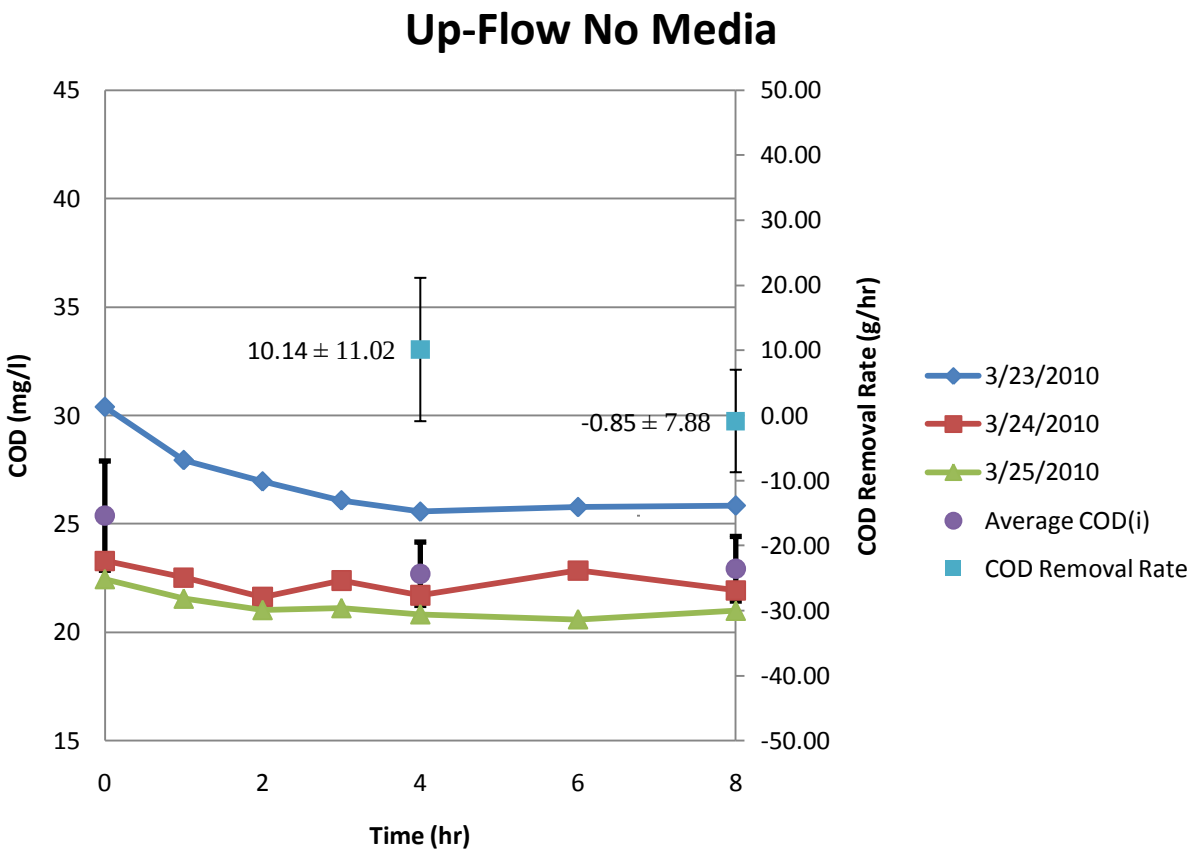

Figure 2.3.2a. Change in COD and $COD_{(\Delta t)}$ removal rate over time for Up-flow no media trials.

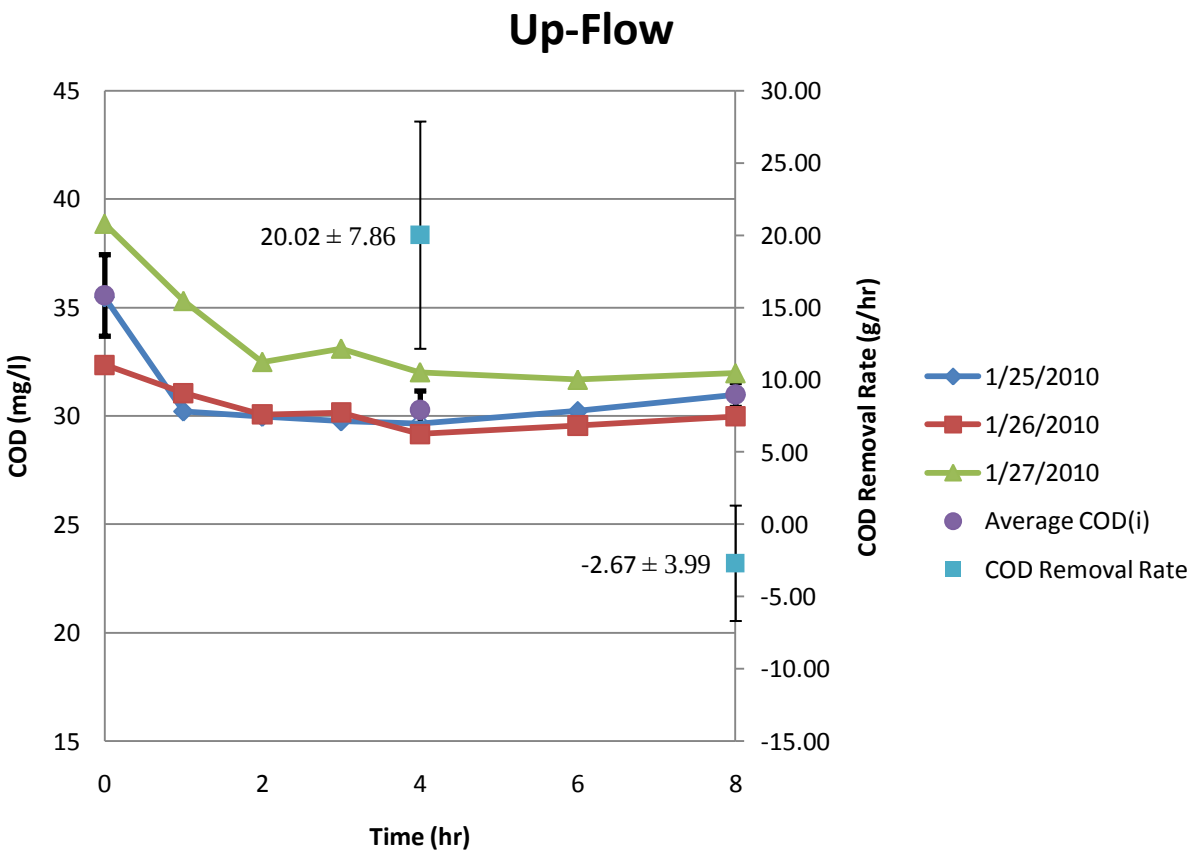

Figure 2.3.2b. Change in COD and $COD_{(\Delta t)}$ removal rate over time for Up-flow filtration trials.

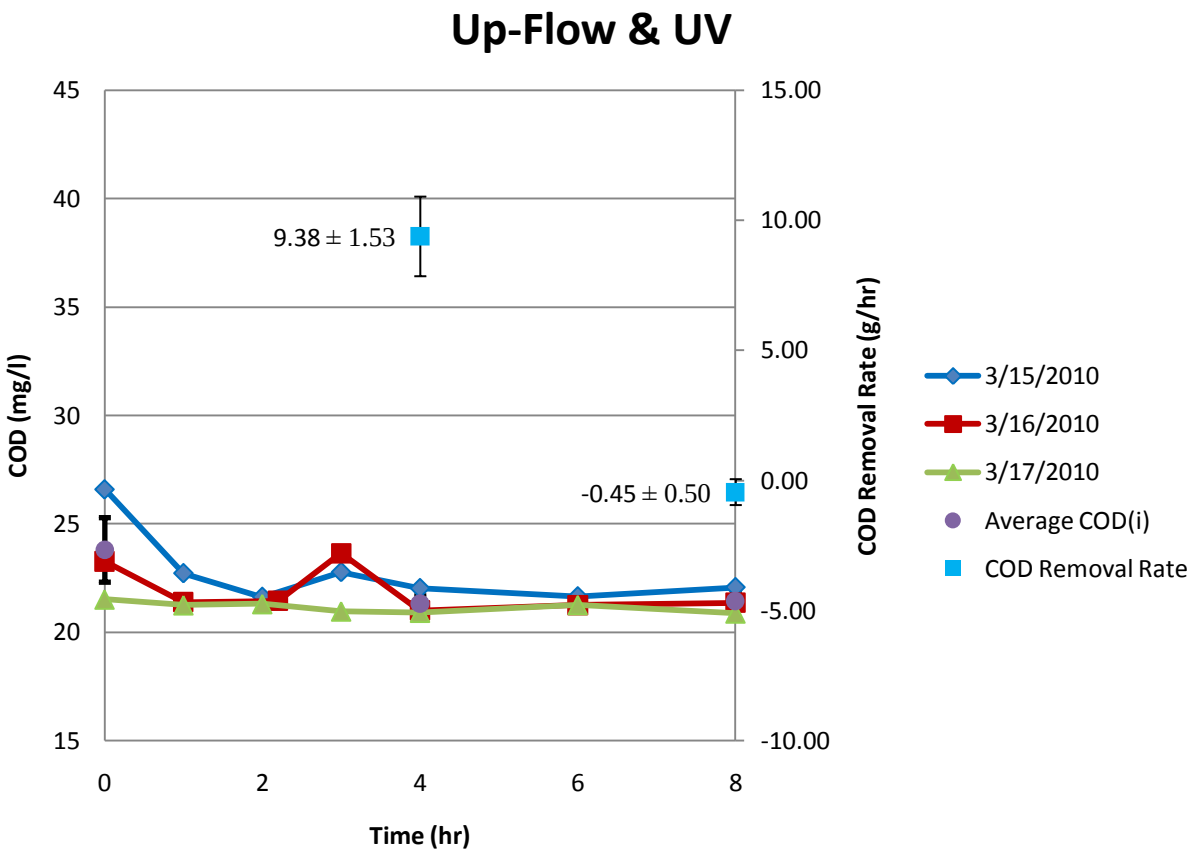

Figure 2.3.2c. Change in COD and $COD_{(\Delta t)}$ removal rate over time for Up-flow and UV filtration trials.

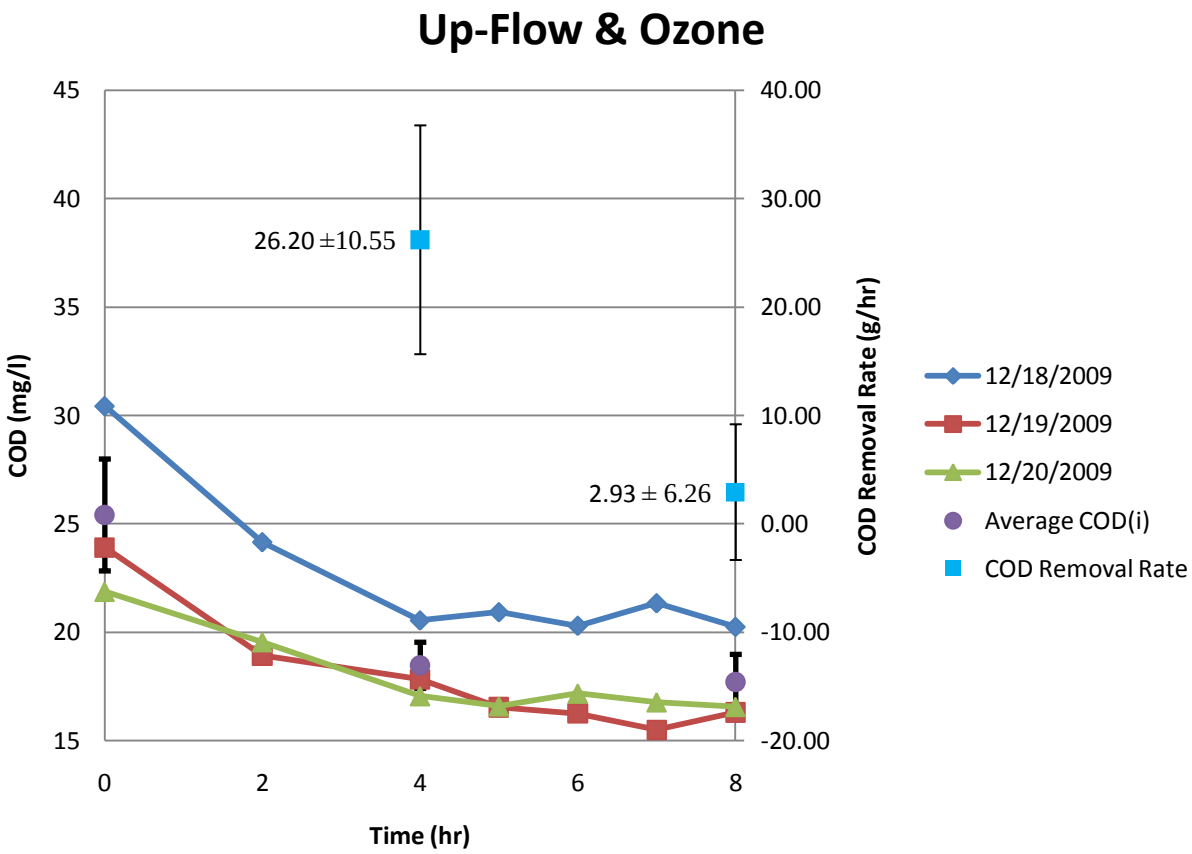

Figure 2.3.2d. Change in COD and $COD_{(\Delta t)}$ removal rate over time for Up-flow and ozone trials.

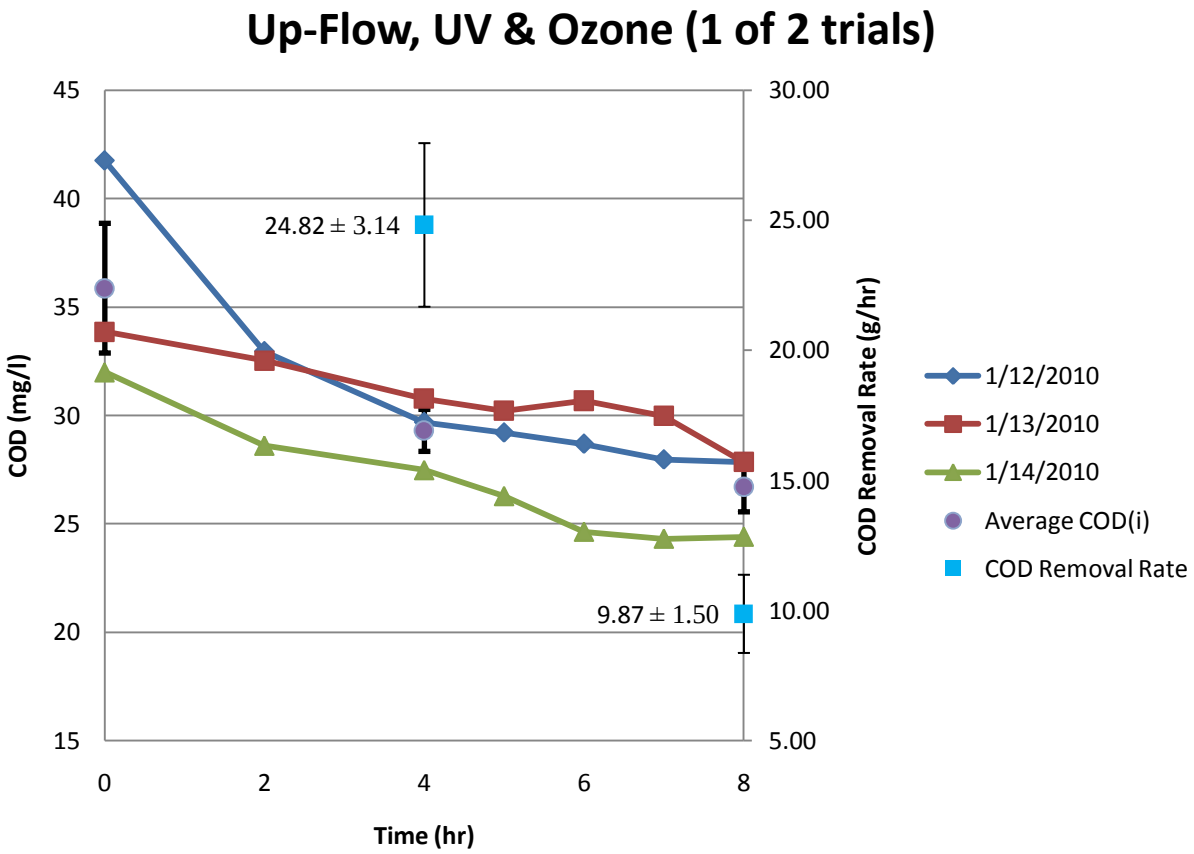

Figure 2.3.2e. Change in COD and $COD_{(\Delta t)}$ removal rate over time for Up-flow, UV filtration and ozone contact for high range CODin trials.

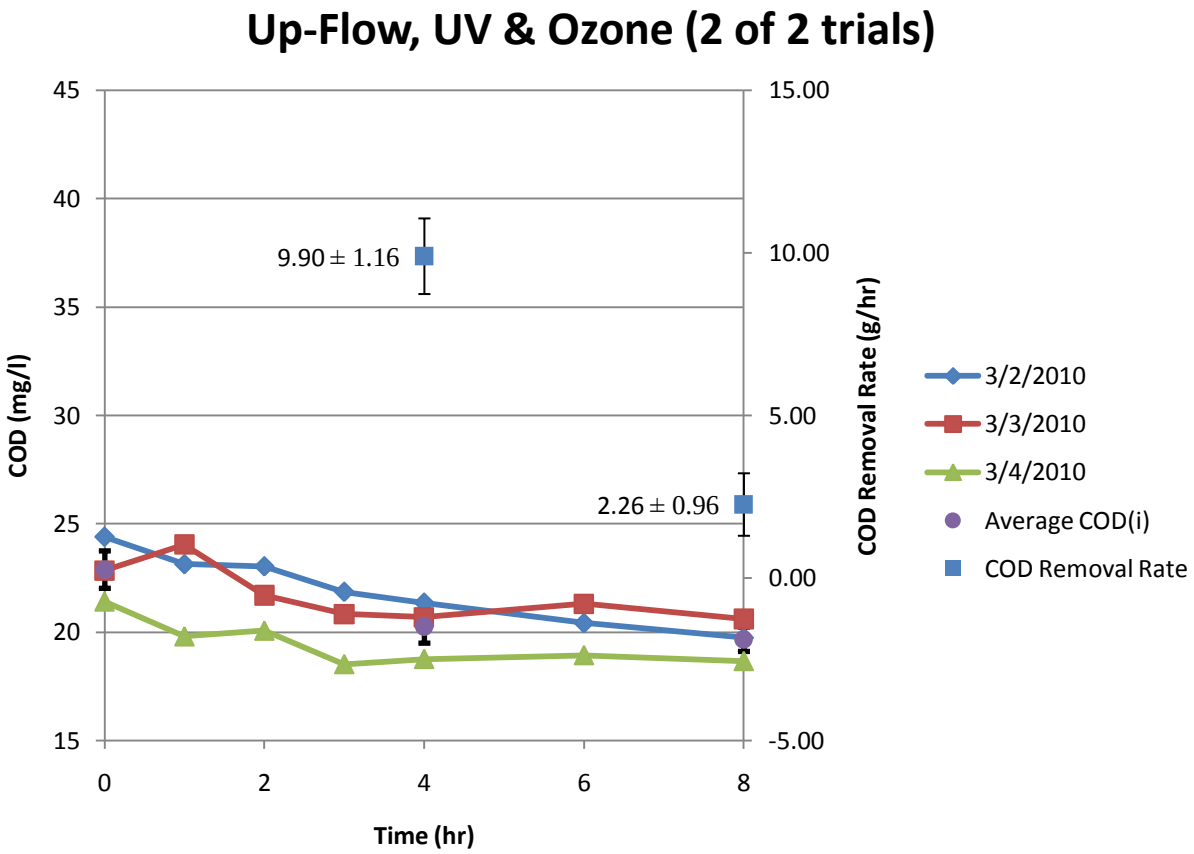

Figure 2.3.2f. Change in COD and $COD_{(\Delta t)}$ removal rate over time for Up-flow, UV filtration and ozone contact for low range CODin trials.

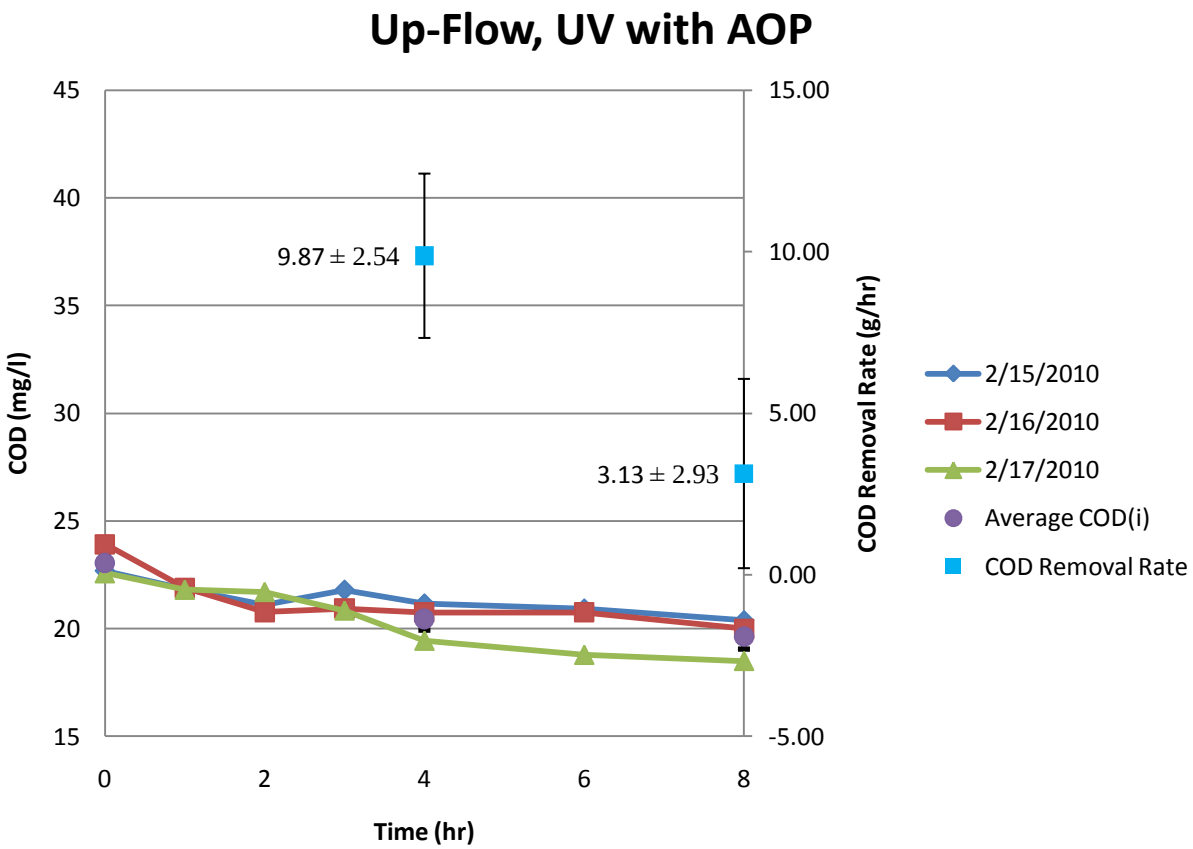

Figure 2.3.2g. Change in COD and $COD_{(\Delta t)}$ removal rate over time for Up-flow, UV filtration with AOP trials.

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CHAPTER 3

Removal of the Off-Flavor Compounds Geosmin and 2-Methylisoborneol in Recirculating Aquaculture Systems

3.1.0. Introduction

The demand for quality food is ever-increasing as the world population rapidly expands. Aquaculture helps offset the pressures placed on wild stocks of fishes and aquatic invertebrates. One of the major problems with the culture of farmed raised fish is producing unmarketable fish due to earthy and musty off-flavors. Fish farmers lose millions of dollars every year from off-flavor episodes (Tucker, 2000). Additionally, the aquaculture industry receives a negative perception when subpar farm raised fish are sold in the seafood market. If a consumer intentionally buys a farmed raised fish and finds that it has a bad flavor they are less likely to buy farmed raised fish again. The earthy musty flavors that are found in farmed raised fish and drinking water are caused by the semivolatile compounds geosmin (Gerber and Lechevalier, 1965) and 2-Methylisoborneol (2-MIB) (Gerber, 1969). These naturally occurring compounds are produced as secondary metabolites from a variety of microorganisms, most notably actinomycetes and cyanobacteria (Jüttner and Watson, 2007). Geosmin and 2-MIB are detectable by human taste and smell at low concentrations down to 10 ηg/l. Although these compounds have an undesirable flavor they have not been found to cause any health problems to humans.

These off-flavor compounds are biosynthesized inside the microorganism from three different pathways: the 2-methylerythritol-4-phosphate pathway, the mevalonate pathway or the leucine pathway (Jüttner and Watson, 2007). Once the compounds are synthesized they

are released into the aquatic environment and or maintained within the cell (Jüttner and Watson, 2007). Once in the water, geosmin and 2-MIB are passively transported into the flesh of the fish. They most readily enter the fish through the gills. Although some studies have shown that geosmin and 2-MIB can enter through the gut and skin, the magnitudes of these transport mechanisms are much less than that of the gills (Howgate, 2004). Once these compounds pass through the gills they enter the blood stream and are deposited into the fatty tissues of the fish. The driving force for bioaccumulation of these compounds is due to the hydrophobic and lypophilic nature of the compounds (Schüürmann and Klein, 1988). Fish associated with off-flavors caused by geosmin and 2-MIB have been recognized worldwide. Interestingly, the concentration of the detectable level of off-flavor varies between different fish species. This has been explained by the natural flavor of the fish masking the off-flavor such that a stronger flavored fish with a certain concentration of geosmin would have a normal taste whereas a milder fish with the same concentration of geosmin could have a strong earthy flavor (Persson, 1980; Schrader, 2005).

Depuration of these compounds from fish is possible through purging in water free of geosmin and 2-MIB. A correlation has been found with both the absorption and depuration of the off-flavor compounds depending on water temperature and fat content of the fish. Johnsen et al., (1996), observed the absorption and depuration rates increase with an increase in temperature, although absorption rates are much higher than depuration rates at similar temperatures. Additionally, a fish with a higher fat content has a higher bioaccumulation of 2-MIB than a leaner fish (Johnsen and Lloyd, 1992). The initial depuration rates of 2-MIB in

a lean fish compared to a fattier fish are the same; however, the fatter fish retains a concentration of 2-MIB for a longer duration (Johnsen and Lloyd, 1992).

Currently fish grown in recirculating aquaculture systems (RAS) are typically purged in clean water for off-flavor depuration prior to harvest. As mentioned earlier depuration rates depend on the fishes' fat content and water temperature. A large cool water fish such a sturgeon could take better than a month of purging before it reaches a marketable flavor. There are additive costs associated with purging such as: capital cost for purging systems, electricity (pumps, blowers etc.), water, oxygen, space and the risk of mortalities. The delay in harvest caused by purging also has a downstream affect of delaying the progress of moving the next batch of fish up into the growout tanks until the tank space is open after purging and harvesting has been completed.

Although there has been little work on the remediation of geosmin or 2-MIB from the water in an RAS, off flavor remediation has been extensively studied by the drinking water industry. These compounds are typically treated by oxidation and / or adsorption and with limited success with biological processes (McGuire, 1999). Although adsorption with the use of activated carbon is an effective treatment of off-flavor removal, it can be very expensive. Ozone treatment has been shown to be an effective oxidant in the removal of geosmin and 2-MIB. Typically ozone is produced on site with an ozone generator from a stream of pure oxygen gas. Ozone is dissolved into the water where it reacts and oxidizes the off-flavor compounds. Additionally, ozone combined with hydrogen peroxide (Park et al., 2006) or UV radiation (Meunier et al., 2006) enhances the degradation of the geosmin and 2-MIB. Combining ozone with hydrogen peroxide, ozone with UV radiation or hydrogen

peroxide with UV radiation facilitates the creation of hydroxyl radicals which are much stronger oxidants than ozone alone. The Advanced Oxidative Process (AOP) is the utilization of these hydroxyl radicals for oxidation. A very recent study using AOP (UV with hydrogen peroxide and ozone with hydrogen peroxide) for the degradation of geosmin and 2-MIB in the water from a RAS water showed some promise for the treatment of these compounds (Klausen and Grønborg, 2010). The authors found degradation rates for these compounds however; they were significantly lower than observed in similar tap water and deionized water studies. They attribute the lower degradation rates to higher concentration of dissolved and fine particulate organic compounds found in RAS water. The organic compounds act as hydroxyl radical scavenger thus competing for oxidation with the targeted off-flavor compounds. Even though the degradation rates were lower in RAS water, this study shows potential for the use of the AOP in RAS for the removal of off-flavor compounds. Since many RAS use pure oxygen to maintain dissolved oxygen levels for fish culture it would not be difficult to add an ozone generator and a low head oxygenator (LHO) for ozone / water contact at a RAS.

An experimental side stream treatment loop was added to a commercial RAS and tested for geosmin and 2-MIB removal rates. The experimental loop consisted of UV irradiation, up-flow particulate filtration and ozone contact with and without hydrogen peroxide dosing. The objective of this study was to determine if the experimental loop, in various configurations, could produce an effective process for the removal of off-flavor compounds in a RAS. Additionally, we wanted to determine the effect each filtration combination (see table 3.2.1) had on the removal geosmin and 2-MIB in order to find the best

treatment combination for the removal of these off-flavor compounds. We hypothesized that the highest removal rates would be observed using up-flow filtration, UV sterilization with AOP.

Table 3.2.1. Trial configurations

Test	Number of Trials	Duration (hrs)	Number of samples
Up-flow	3	8	6 Samples per trial
Up-flow and UV	4	8	6 Samples per trial
Up-flow and ozone	3	8	6 Samples per trial
Up-flow, UV and ozone	6	8	6 Samples per trial
Up-flow, UV, ozone with *H_2O_2	3	8	6 Samples per trial
Up-flow (no media)	3	8	**6 Samples per trial

All configurations include the moving bed biofilter as a sump.
*0.354 mg H_2O_2 : 1.0 mg ozone, **4 sample bottles were broken during shipment and one was lost

3.2.0. Materials and methods

The RAS, off-flavor remediation loop and test methodology is discussed in detail in the Chapter 2 material and methods, sections: 2.2.0, 2.2.1, 2.2.2, 2.2.3, and 2.2.4. Note the off-flavor remediation loop is referred to as the fine and dissolved organic remediation loop in Chapter 2.

3.2.1 Trials

The trials for testing the off-flavor remediation loop began in October, 2009. From data collected in Chemical Oxygen Demand (COD) ranging experiments we decided to carry on the trials for a period of 8 hours. See Table 3.2.1 for a description of the trial configurations.

During the 8 hr time period there were 3 sampling test times; time 0, 4 and 8 hrs. Two samples were collected at each test time, one from the inlet port just prior to the UV sterilizer furthest downstream and the other from the effluent flowing out of the LHOs (see figure 2.2.2a). At each sample time the water flow rate and total flow was recorded. For trials using ozone, ozone flow rate, total flow, ozone concentration g/m^3 and ozone concentration in the aqueous phase was recorded. The sample water was collected in 125 ml serum glass bottles (ACE Glass Inc, Vineland, NY) which were filled to the top to prevent volatization and sealed with a crimped cap. Each water sample was immediately placed in a cooler with ice for preservation and transportation in the dark. The samples were sent by overnight courier to the David H. Murdock Research Institute (DHMRI) in Kannapolis, NC for geosmin and 2-MIB analysis.

3.2.2 Geosmin and 2-MIB analysis

The methods for geosmin and 2-MIB analysis are as follows (DHMRI, personal communication):

Preparation of Standards & Samples – Geosmin and 2-MIB standards were purchased from Sigma Aldrich (47525-U, St. Louis, MO). For quantitative analysis, a seven point calibration curve 1, 5, 10, 30, 50, 100, 500 ppt in water. An internal standard of 2-IMP, was purchased from Sigma Aldrich (47527-U, St. Louis, MO) and prepared at a concentration of 500ppt in water. For standard analysis, 1mL of standard was mixed with 1mL of internal standard solution in a 20mL headspace vial with a stir bar. For sample analysis, 1mL of test water sample was mixed with 1mL of internal standard solution in a 20mL headspace vial with a stir bar.

SPME Gas Chromatography Mass Spectrometry Parameters – SPME extraction was performed on a single magnet mixer (Chromsys, Alexandria, VA). Samples were incubated at 60°C for 1 minute and then the SPME fiber (DVB/CAR/PDMS) was exposed for 45 minutes during the extraction. After extraction the fiber was desorbed onto the column at 230°C for 5 minutes. Volatile components were separated on a Varian VF-5MS column (20Mx0.25umx0.25um df). The oven was initially held at 70°C for 5 minutes and ramped at 10°C/min to a maximum temperature of 250°C. The mass spectrometry was set to acquire 60 – 600 amu.

Data Analysis – Calibration curve, samples and blanks were processed on the AnalyzerPro software. Raw peak areas for geosmin, 2-MIB, and 2-IMP were exported to Microsoft Excel for further processing and data calculations.

3.2.3 Calculating removal rates

Geosmin and 2-MIB removal rates were calculated in order to compare removal efficiencies for the different treatments conducted as noted in table 3.2.1. Geosmin and 2-MIB removal rates will be referred to as GEO removal rate and MIB removal rate respectively. We calculated the removal rates using two different methods. One method we call "instantaneous removal" which is calculated by using an inlet and outlet samples at time t to determine the removal rate between sample periods. Instantaneous removal rates were calculated as follows:

$$GEO_{(in\text{-}out)}\ \text{Removal Rate} = [GEO_{in(ti)} - GEO_{out(ti)}] \times \text{flow rate} \qquad (3.1)$$

(ηg/hr) (ηg/l) (ηg/l) (l/hr)

$$MIB_{(in\text{-}out)}\ \text{Removal Rate} = [MIB_{in(ti)} - MIB_{out(ti)}] \times \text{flow rate} \quad (3.2)$$

(ηg/hr) (ηg/l) (ηg/l) (l/hr)

Additionally, removal rates were calculated according to the change in geosmin and 2-MIB concentrations over a period of time during the experimental trials. These removal rates were calculated as follows:

$$GEO_{(\Delta t)}\ \text{Removal Rate} = [(GEO_{in(ti)} - GEO_{in(ti+1)}) \times V] / \Delta t \quad (3.3)$$

(ηg/hr) (ηg/l) (ηg/l) (l) (hr)

$$MIB_{(\Delta t)}\ \text{Removal Rate} = [(MIB_{in(ti)} - MIB_{in(ti+1)}) \times V] / \Delta t \quad (3.4)$$

(ηg/hr) (ηg/l) (ηg/l) (l) (hr)

Where: V = volume of the treatment system (liters)

3.3.0 Results and discussion

The data was analyzed in three ways: Using statistical software to determine if the treatments yielded significantly different removal rates, analyzing the percent removal over time between the treatments, and lastly comparing removal rates over time between the trials.

In an effort to determine the affects of the different treatments on the removal of geosmin and 2-MIB SAS (SAS version 9.1.3, SAS Institute Inc, North Carolina) was used for statistical analysis. The mixed procedure in SAS was used to first determine if there was a significance between removal rates of geosmin or 2-MIB between the treatments and secondly to determine which treatments were significantly different. We analyzed the data using both techniques mentioned above for calculated removal rates (see equations 3.1, 3.2, 3.3 and 3.4).

The statistical analysis showed no significance at the $\alpha = 0.05$ for a difference between the treatments and the removal rates calculated using $MIB_{(in-out)}$, $GEO_{(\Delta t)}$ and $MIB_{(\Delta t)}$. In other words the removal rates observed for 2-MIB, calculated either way (equations 3.2 and 3.4), was not statistically different between any of the treatments. Additionally, the observed removal rates for $GEO_{(\Delta t)}$ (equation 3.3) were not statistically different between any of the treatments. Although, $GEO_{(in-out)}$ removal rates showed, with significance at the $\alpha = 0.05$ level, that at least one of the treatments had a different removal rate for the others. Comparing the difference of least squares means between the treatments showed that the treatment utilizing up-flow filtration, UV sterilization and AOP had a significantly ($\alpha = 0.05$) different $GEO_{(in-out)}$ removal rate than that of all the other trials. Interestingly, this treatment demonstrated lower removal rates than all of the other treatments. These results were unexpected and counter to our hypothesis. The AOP process has been shown to be an effective treatment for the degradation of geosmin and 2-MIB (Gunton, 2003; Klausen and Grønborg, 2010; Koch et al, 1992; Meunier et al., 2006; Park et al., 2006) which nonetheless should have yielded higher removal rates than up-flow no media or at least similar removal rates to up-flow filtration, since up-flow filtration was also used in the AOP trials. The up-flow no media trials more or less have no process for the removal of off-flavor compounds whereas the up-flow filters (with media) could possibly act as biofilters in removal these off-flavor compounds.

We believe there were errors either with sampling techniques, sample storage, sample preservation, sample mix up, contamination, and or sample analysis. A few noteworthy sampling problems we ran into were:

- Four of the 18 up-flow and UV filtration with AOP samples were found to be out of range (higher than 500 η/l) for the analysis. This was not brought to the authors attention until 4 months after analysis. The samples were rerun and it is possible there was biological activity in the stored samples over the four months.
- Additionally 2 of the 18 samples for the up-flow trials were found out of range for analysis (higher than 500 ηg/l). This was not brought to our attention until close to 5 months after analysis. Again the samples were rerun, and it is possible there was biological activity in the sample over the five months.
- Four of the 18 samples from the up-flow no media trials were broken during shipment and another one of the samples (total of 5 from this treatment) was misplaced and never analyzed.

The comparative analysis for the percent removal and removal rates between the different treatments were inconclusive. We observed removal and generation of geosmin and 2-MIB within each treatment (See Figure 3.3.1 and 3.3.2). In several cases there was a large increase in concentration of off-flavor compounds in the first four hours followed by 100% removal in the following four hours. Within the trials there were discrepancies where one day we would observe removal rates, the next day we would observe generation rates and the next day could be a combination of generation and removal rates within the same treatment. Off-flavor generation could have occurred through off-flavor producing organisms found in the biofilm (Skjevrak et al., 2005) of the pipes, moving bed biofilter and or the up-flow particulate filter. However, it is unlikely to observe a change in geosmin concentration from

52.92 ηg/l at time 0, 77.03 ηg/l after 4 hours and then 1005.23 ηg/l after another 4 hrs. Additionally, using the same treatment we observed a change in geosmin concentration starting at 1066.76 ηg/l at time 0, 29.80 ηg/l after 4 hours and finally 25.72 ηg/l after another 4 hours.

Over the six months of conducting these experiments there were two treatments, up-flow filtration with ozone contact and up-flow filtration, UV sterilization with ozone contact, where 2-MIB was never detected in the system water. Off-flavor compounds have been found to change seasonably (Westerhoff et al., 2005) which could explain these findings since these two treatments were conducted in mid December and mid January. All the other treatments were carried out either before or after these two treatments and they all had detectable levels of geosmin and 2-MIB in system water.

3.4.0. Conclusions

In this study the results of testing the capability of a multiple component filtration loop for the removal of geosmin and 2-MIB in an RAS proved to be inconclusive. The off-flavor analysis data was inconsistent throughout the different treatments. The lack of consistency may be from sampling error, improper storage/preservation, sample mix up, contamination or bad analysis. Unfortunately, no statistically significant conclusions can be developed from the results of these tests.

This research would have fit in nicely into the "story" of off-flavor remediation in aquaculture. To our knowledge this was the first study utilizing these technologies in a commercial scale RAS production system for the removal of off-flavor. Additionally, this was the first study, to our knowledge, utilizing the AOP (an ozone and peroxide mix) for the

removal of geosmin and 2-MIB in aquaculture system water. This study should be repeated given the potential for the remediation of off-flavors in RAS. If an off-flavor removal process is found to be a successful alternative to purging in RAS, there could be a large economic benefit for the farmer.

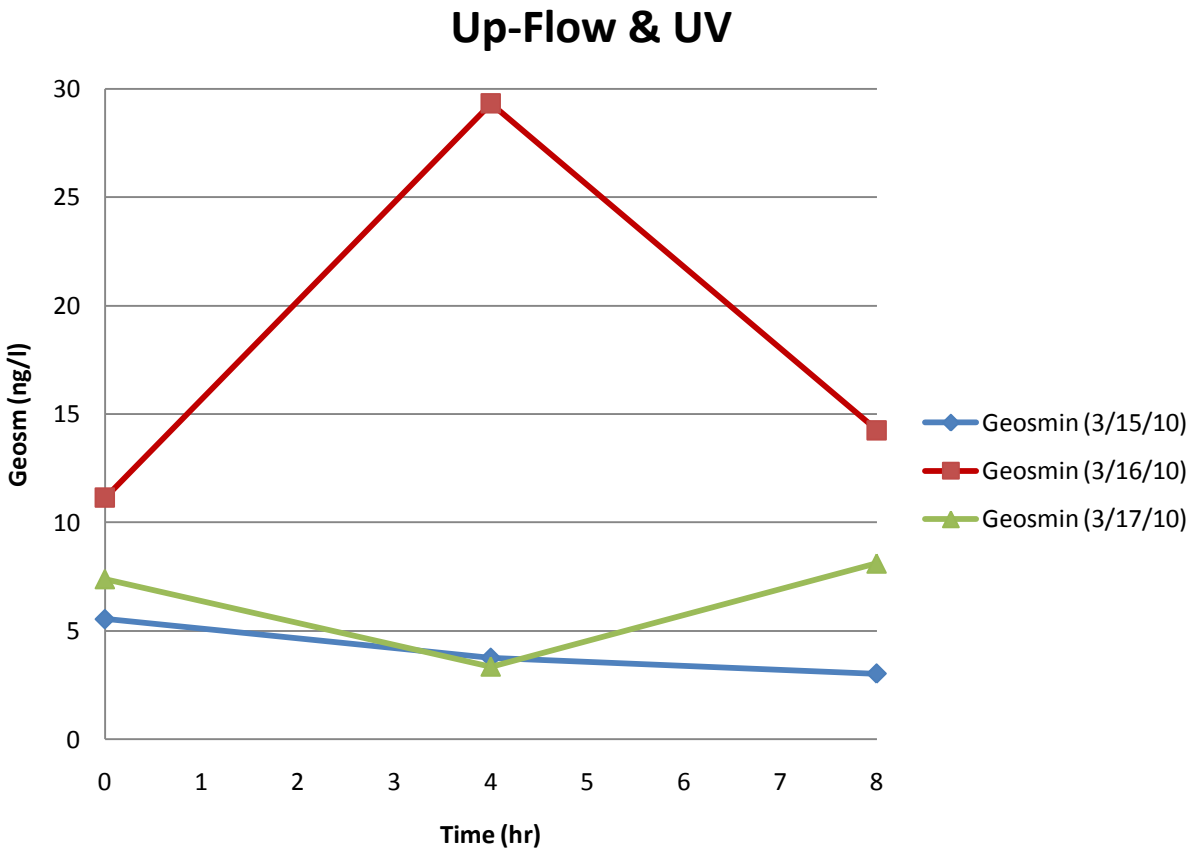

Figure 3.3.1: Change in geomin concentration over time for up-flow and UV filtration trials.

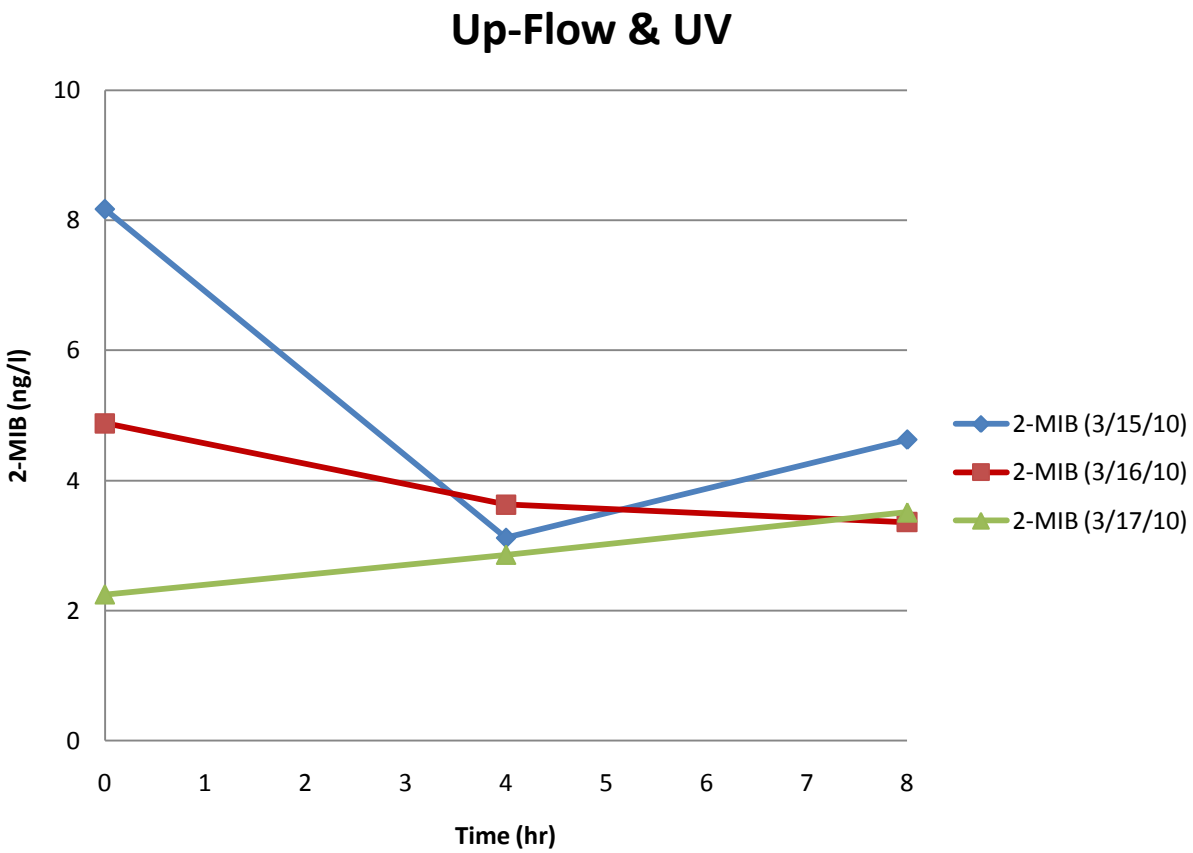

Figure 3.3.2: Change in 2-MIB concentration over time for up-flow and UV filtration trials.

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APPENDICES

APPENDIX A: Ranging Experiments for Chapter 2

Ranging Experiments

The study was begun by conducting a number of ranging experiments. That is, we ran short studies to determine the rate at which the experimental loop could change the quality of the water within the RAS biofilter and experimental treatment loop. The first two ranging experiments were carried out using the up-flow particulate filters, UV sterilizers and LHOs (see Table A.1a and A.1b). The first ranging trial lasted 5 hours while the second ranging trial lasted for 4 hours due to low Dissolved Oxygen (D.O.) conditions in the fish culture tanks. Four samples were taken every hour starting with an initial sample taken at time zero. Two of the samples (duplicate inlet samples) were collected at the sampling port prior to the UV sterilizer furthest downstream and the other two (duplicate outlet samples) were collected from the effluent flow out from the experimental treatment loop. One sample from each of the duplicate inlet and outlet samples were analyzed at the Environmental Analysis Laboratory for Chemical Oxygen Demand (COD) and ammonia. The remaining inlet and outlet samples were analyzed on-site for pH, D.O., ORP, suspended solids, turbidity (FAU) and color (Pt-Co).

The COD analysis was conducted with low range, 0 to 150 mg/l, COD test vials (Hach Company, Loveland, CO). After reviewing the results of these initial ranging trials, test results indicated that that future COD tests should be analyzed using the ultra low range, 0 to 40 mg/l, test vials in order to get more accurate results. From the initial ranging experiments, the results suggested that COD removal rates were highest during the first 2 hours of the experimental trial. From the COD data gathered in these ranging trials we

concluded that we should obtain more samples during the first hour of the next ranging trial to capture more data points of the ΔCOD over time in an effort to find the time frame in which the system has an steep decrease in COD concentration before reaching an equilibrium COD concentration.

TAN Analysis

Analysis of Total Ammonia Nitrogen (TAN) was conducted in early ranging experiments to determine if the up-flow particulate filters provided a significant nitrification within the experimental loop. However, the effectiveness of the remediation loop in nitrification was suspended because the TAN concentrations within the experimental treatment loop were below the range of accuracy for the lab equipment. We assumed that the TAN concentration was low because of the main system's biofilter, which supplied the water for the remediation loop, was continuously removing TAN causing the concentration to be below accurate limits of detection. As a result TAN analysis was discontinued in future trials.

Other chemical analysis

Dissolved oxygen, ORP, turbidity, suspended solids and color analysis were analyzed on-site concurrently with the sampling period. These parameters were analyzed to determine if there was a correlation with changes in COD concentrations. From the early ranging trials we determined that changes in D.O., ORP, turbidity, suspended solids and color analysis were not reliable indicators of changes in COD concentration. Therefore, we decided to suspend analysis of these parameters for subsequent trials.

Further experimental ranging trials

The next 9 experimental trials were conducted over three days, with 3 trials per day. Between same day trials the growout tanks were placed back on the RAS for one hour with the total flow through the remediation loop reduced from 738 lpm (195 gpm) to 341 lpm (90 gpm). Additionally, when ozone or UV sterilization was used during the trials, they were turned off during the hour between the trails. This was done to introduce the untreated growout tank water with the previously treated trial water in an effort to replicate similar conditions for the three trial runs on the same day. One inlet (prior to the UV sterilizer furthest downstream) and one outlet (effluent from the LHOs) sample was taken at time 0, 20 min, 40 min, 1 hr and 2 hrs. On the first day of sampling the remediation loop included the up-flow particulate filters only. The trials on the second and third day utilized the up-flow particulate filter combined with UV sterilizers and the up-flow particulate filters with ozone contactors respectively (Table A.2a, b and c).

After analyzing the ΔCOD over time the results suggested that more than 2 hours of time was needed to reach an equilibrium COD concentration for the system. For future sampling a three hour experimental trial periods was used.

Three additional ranging trials were conducted all during the same day. Sampling occurred every hour for 3 hours starting at time zero. The method of "dirtying" the water for an hour between trials on the same day was followed as described above. A sampling regime of one inlet and one outlet sample was followed. The configuration of the remediation loop for these trials consisted of up-flow particulate filters, UV sterilizers and ozone contactors with H_2O_2 injection. Hydrogen Peroxide was added at a rate of 0.15:1 (grams of peroxide

per g of ozone), 0.354:1 (peroxide g:ozone g) and 1:1 (peroxide g:ozone g) in that order for the three trials (See Table A.3). An ozone concentration of 0.5 mg/l was estimated by a mass balance calculation. In hindsight Henry's law should have been used to calculate the ozone concentration in the water inside the LHOs. During the first trial, 0.15:1 peroxide g/ozone g, the D.O. in the growout tanks dropped and the trial was stopped after the second hour for consideration of the fishes' health (Table A.3). The following two trials continued for the three hour duration with no D.O. problems (Table A.3).

The results from the ranging trials indicated that ΔCOD with respect to time did not reach an equilibrium COD concentration during the 3 hour periods allowed. From these ranging tests we determined that a sample period of 8 hrs was required to reach an equilibrium COD concentration in the treated water.

Experimental trials

The final experimental trials commenced in October, 2009. As noted above, from data collected in the ranging experiments we determined that the experimental trial periods of 8 hours was appropriate.

Table A.1a Preliminary Test, up-flow particulate filtration, UV sterilization and ozone contact

Sample	pH	D.O. (mg/l)	ORP (mV)	Suspended Solids (mg/l)	Color (Pt-Co)	COD (mg/l)	NH_3 (mg/l)	$COD_{(in-out)}$ Removal Rate (g/hr)	$COD_{(\Delta t)}$ Removal Rate (g/hr)
1 in	7.46	7.2	178	3	49	27	0.3	527.35	-1014.38
1 out	7.50	8.9	182	4	55	16	0.13		
2 in	7.76	7.3	171	3	2	94	0.03	3706.89	1150.64
2 out	7.64	10.8	176	2	5	17	0.03		
3 in	7.79	7.5	170	0	34	18	0.05	181.47	0
3 out	7.68	11.1	175	0	0	14	0.04		
4 in	7.78	7.9	167	0	21	18	0.04	95.77	30.28
4 out	7.69	12.0	168	0	0	16	0.03		
5 in	7.77	8.0	167	0	29	16	0.03	0	-30.28
5 out	7.66	12.1	167	0	0	16	0.02		
6 in	7.78	8.0	164	0	25	18	0.03	95.19	.
6 out	7.67	12.2	165	0	0	16	0.02		

Table A.1b Preliminary Test, up-flow particulate filtration, UV sterilization and ozone contact

Sample	D.O. (mg/l)	ORP (mV)	Turbidity (FAU)	Color (Pt-Co)	COD (mg/l)	NH_3 (mg/l)	$COD_{(in-out)}$ Removal Rate (g/hr)	$COD_{(\Delta t)}$ Removal Rate (g/hr)
1 in	8.1	178	1	9	20	0.02	142.32	45.42
1 out	12.0	180	2	32	17	0.03		
2 in	8.2	178	4	41	17	0.01	-46.71	15.14
2 out	12.6	180	0	0	18	0.01		
3 in	8.3	175	1	40	16	0.02	-48.42	-15.14
3 out	12.8	192	0	6	17	0.02		
4 in	8.3	192	0	34	17	0.02	0	30.28
4 out	12.8	195	0	6	17	0.02		
5 in	8.3	187	3	23	15	0.01	-48.11	.
5 out	12.8	190	0	0	16	0.01		

Table A.2a. Preliminary Tests, up-flow particulate filtration

	Sample	COD (mg/l)	$COD_{(in-out)}$ Removal Rate (g/hr)	$COD_{(\Delta t)}$ Removal Rate (g/hr)
Trial 1	1 in	21.75	209.90	90.84
	1 out	17.32		
	2 in	19.75	167.26	13.17
	2 out	16.46		
	3 in	19.46	111.01	38.61
	3 out	17.18		
	4 in	18.61	46.70	13.02
	4 out	17.61		
	5 in	17.75	67.82	.
	5out	16.32		
Trial 2	1 in	22.61	279.47	71.31
	1 out	16.75		
	2 in	21.04	98.02	71.76
	2 out	19.04		
	3 in	19.46	59.43	-136.26
	3 out	18.18		
	4 in	22.46	138.14	32.39
	4 out	19.61		
	5 in	20.32	167.56	.
	5out	16.89		
Trial 3	1 in	21.75	116.76	13.17
	1 out	19.32		
	2 in	21.46	0	84.03
	2 out	21.46		
	3 in	19.61	-95.90	-97.20
	3 out	21.61		
	4 in	21.75	87.37	67.07
	4 out	19.89		
	5 in	17.32	48.59	.
	5out	16.32		

Table A.2b. Preliminary Tests, up-flow particulate filtration with UV sterilization

	Sample	COD (mg/l)	$COD_{(in-out)}$ Removal Rate (g/hr)	$COD_{(\Delta t)}$ Removal Rate (g/hr)
Trial 1	1 in	20.18	171.87	64.95
	1 out	16.61		
	2 in	18.75	121.90	71.31
	2 out	16.18		
	3 in	17.18	-13.72	6.36
	3 out	17.46		
	4 in	17.04	27.92	4.39
	4 out	16.46		
	5 in	16.75	6.80	.
	5out	16.61		
Trial 2	1 in	19.89	157.97	12.72
	1 out	16.61		
	2 in	19.61	84.04	78.12
	2 out	17.75		
	3 in	17.89	20.44	38.61
	3 out	17.46		
	4 in	17.04	28.32	0
	4 out	16.46		
	5 in	17.04	41.09	.
	5out	16.18		
Trial 3	1 in	20.04	164.76	64.95
	1 out	16.61		
	2 in	18.61	55.18	64.95
	2 out	17.46		
	3 in	17.18	-26.74	0
	3 out	17.75		
	4 in	17.18	-46.76	13.02
	4 out	18.18		
	5 in	16.32	-13.79	.
	5out	16.61		

Table A.2c. Preliminary Tests, up-flow particulate filtration with ozone contact

	Sample	COD (mg/l)	$COD_{(in-out)}$ Removal Rate (g/hr)	$COD_{(\Delta t)}$ Removal Rate (g/hr)
Trial 1	1 in	20.18	155.34	84.48
	1 out	16.75		
	2 in	18.32	73.52	64.95
	2 out	16.61		
	3 in	16.89	6.39	38.61
	3 out	16.75		
	4 in	16.04	-6.43	-10.75
	4 out	16.18		
	5 in	16.75	19.44	.
	5out	16.32		
Trial 2	1 in	18.61	45.33	32.70
	1 out	17.61		
	2 in	17.89	43.94	12.72
	2 out	16.89		
	3 in	17.61	70.70	39.06
	3 out	16.04		
	4 in	16.75	0	6.51
	4 out	16.75		
	5 in	16.32	0	.
	5out	16.32		
Trial 3	1 in	18.46	45.24	-45.42
	1 out	17.46		
	2 in	19.46	94.90	58.14
	2 out	17.32		
	3 in	18.18	105.79	104.01
	3 out	15.89		
	4 in	15.89	-19.31	-10.90
	4 out	16.32		
	5 in	16.61	38.84	.
	5out	15.75		

Table A.3. Preliminary Tests, up-flow particulate filtration, UV sterilization with AOP

	Sample	COD (mg/l)	$COD_{(in-out)}$ Removal Rate (g/hr)	$COD_{(\Delta t)}$ Removal Rate (g/hr)
Trial 1	1 in	33.56	186.72	34.37
	1 out	29.36		
0.15 mg	2 in	31.29	111.62	14.08
peroxide/	2 out	28.77		
1.0 mg	3 in	30.36	163.38	.
ozone	3 out	26.71		
	4 in	.	.	.
	4 out	.		
Trial 2	1 in	35.27	248.22	46.03
	1 out	29.73		
0.354 mg	2 in	32.23	155.52	17.41
peroxide/	2 out	28.76		
1.0 mg	3 in	31.08	108.88	28.46
ozone	3 out	28.61		
	4 in	29.20	10.82	.
	4 out	28.95		
Trial 3	1 in	34.58	61.41	32.70
	1 out	33.21		
1.0 mg	2 in	32.42	87.01	20.14
peroxide/	2 out	30.48		
1.0 mg	3 in	31.09	47.05	-31.95
ozone	3 out	30.05		
	4 in	33.20	192.16	.
	4 out	28.95		

Appendix B: Ultralow range COD analysis for Chapter 2

Spectrophotometer and COD analysis protocol

Prior to analysis the Spectronic 401 was turned on for 2 hours to allow the spectrophotometer to warm up and stabilize. The spectrophotometer absorbance wave length was set at 345 nm and zeroed with a vial containing 5 ml deionized water. According to Boyles (1997), the Hach ultra low range test vials have the highest sensitivity at the wave length of 345 nm. As described in the Hach COD protocol, 2.0 ml of each sample, using a micro pipette, was added to the ultra low range test vials. The vials were then mixed and digested in the Hach DRB200 Reactor at 150 ºC for two hours. The vials were then mixed again and cooled to room temperature for two hours. Each vial was analyzed three times and averaged to reduce error.

A standard curve of absorbance vs. COD concentration was made for each lot number of COD test vials. COD standards were made at concentrations of 0, 5, 10, 15, 20, 30, 40 mg/l and a Quality Control (QC) of either 2.07 or 20.7 mg/l. It was found that between the lot numbers the slope of the standard curve was nearly constant throughout and the y-intercept varied between the different lot numbers. Due to this, the slope was averaged and the follow equation for COD was determined:

$$COD = [Abs_{(COD[0])} - Abs_{(sample)}] / 0.204$$

Where 0.0204 is the slope, $Abs_{(COD[0])}$ is the absorbance reading for the digested vial containing deionized water (y-intercept, adjusts standard curve up or down according to lot number) and $Abs_{(sample)}$ is the absorbance reading of the sample.

All COD samples from the same trial were digested and analyzed (42 samples) at the same time. An additional 4 samples, $COD_{[0]}$, $COD_{[10]}$, $QC_{[2.07 \text{ or } 20.7]}$ and a sample

spiked with 10 mg/l for percent recovery, were run concurrently, for a total of 46 samples per trial. The $COD_{[0]}$ sample absorbance was used as one of the parameters in the equation for determining COD. The $COD_{[10]}$, $QC_{[2.07 \text{ or } 20.7]}$ and the spiked sample (for percent recovery) was used to check the accuracy of the sample analysis.

REFERENCE

Boyles, W., 1997. The science of chemical oxygen demand, technical information series, booklet no. 9. Method 7053, Hach Company, USA.