Evaluation of Bacterial Amendments for Improving Water Quality in Alabama Catfish Ponds

by

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Abstract

Four sets of experiments were conducted to evaluate the potential efficacy of bacterial amendments for improving water quality in channel catfish ponds. First, the effects of 12 bacterial amendments from eight companies on reducing total ammonia nitrogen, nitrite nitrogen and organic matter concentration in polluted pond water were evaluated in an environmentally controlled room. Then, a selected bacterial amendment (Waste & Sludge Reducer -WAS) was evaluated in channel catfish ponds at the E. W. Shell Fisheries Center. The third set of experiments was to evaluate selected bacterial amendments (Aqua PE and Oxyless) in channel catfish ponds with ammonium sulfate addition at the E. W. Shell Fisheries Center. The final set of experiments was to evaluate the efficacy of the bacterial amendment (Aqua PE) on water quality in Alabama commercial catfish ponds.

Concentrations of total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^-–N) and organic matter (as estimated from oxygen demand) in untreated water naturally decreased with time during 16 days. No large improvements were observed in accelerating nitrification or organic matter oxidation from the use of bacterial amendments in the laboratory study.

There were no differences ($P>0.05$) in total bacterial count after treatment with WAS on most of the days between May and September. Few differences ($P<0.05$) were observed in pH and concentrations of POM, Cha a, COD, SCOD, TAN and NO_2^-–N between ponds treated with WAS and control ponds. Channel catfish yield was not improved by treatment with WAS.

No larger improvements were observed in accelerating TAN removal rates in catfish ponds with ammonium sulfate addition from the use of the Aqua PE and Oxyless in combination. There were slightly beneficial effects on TAN removal from day 5 to day 8 in the ponds with high ammonium sulfate addition (225 kg/ha ammonium sulfate applied on day 4) after treatment with the bacterial amendments (Aqua PE and Oxyless).

There was less mean concentration of TAN ($P<0.05$) in commercial catfish ponds treated with the bacterial amendment (Aqua PE), which may because the dense algal bloom took up the ammonia. Compared to the untreated ponds (controls), Aqua PE gave beneficial effects on TAN removal ($P<0.05$) on 20 June (week 2) and 27 June (week 3). No differences ($P>0.05$) in mean concentrations of nitrite nitrogen was observed between ponds treated with Aqua PE and control ponds. However, there were less concentrations of nitrite nitrogen ($P<0.05$) in the ponds treated with Aqua PE on 27 June (week 3), 18 July (week 6), 1 August (week 8) and 22 August (week 11). There were higher ($P<0.05$) means of turbidity and Chl a concentration in Aqua PE treated pond. On 18 July (week 6), there were higher ($P<0.05$) turbidities in Aqua PE treated ponds. No improvements were observed in accelerating organic matter oxidation from the use of Aqua PE. No differences ($P>0.05$) was observed in pH, and concentrations of TN, TP after treatment with Aqua PE. Catfish yield was not improved by treatment with Aqua PE.

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Chapter 1 Introduction

Channel catfish (*Ictalurus punctatus*) ponds are being stocked at increasingly higher densities because of development of reliable mechanical aerators and other improvement in technology. High stocking densities result in the deterioration of water quality, including much greater concentrations of total ammonia nitrogen, nitrite nitrogen, and dissolved organic matter than in the past.

Pond waters receive large inputs of ammonia nitrogen, because ammonia is the nitrogenous waste product of fish and of bacteria that decompose organic wastes in ponds. Moreover, under certain conditions, nitrite nitrogen also accumulates in pond waters (Hargreaves 1998).

Ammonia nitrogen occurs in water as two forms, un-ionized ammonia (NH_3) and ammonium ion (NH_4^+) in a pH and temperature dependent equilibrium (Trussell, 1972):

$NH_3 + H_2O = NH_4^+ + OH^-$, $K_b = 10^{-4.74}$.

Although the ammonium ion is much less toxic to fish, high pH (8.5 to 9.5) that often occurs in Alabama catfish ponds after carbon dioxide is removed from water for phytoplankton photosynthesis during the daytime favors a high proportion ammonia (most toxic form).

Although ammonia diffuses from pond waters into the air (Gross et al., 1999) or is removed by phytoplankton (Tucker et al., 1984), a major control on ammonia concentration in ponds is microbial nitrification (Avnimelech et al., 1986; Gross et al., 2000; Hargreaves, 1998). In microbial nitrification, bacteria of the genera *Nitrosomonas* and *Nitrobacter* oxidize ammonia nitrogen to nitrate. There is no management practice proven effective for preventing a high concentration of total ammonia nitrogen in ponds other than limiting feed input, avoiding overfeeding, and providing aeration to maintain adequate dissolved oxygen for nitrifying bacteria (Boyd, 1998a).

Ammonia seldom kills fish directly, but it stresses fish causing them to eat less, grow slower, and be more susceptible to disease (Boyd, 1998a). A negative, linear relationship between channel catfish growth and increasing ammonia nitrogen concentration was reported over the concentration range of 0.07 to 1.2 mg/L during a month trial (Colt and Armstrong, 1981). Moreover, growth declined to 50% at 0.63 mg/L, and no weight gain was reported above 1.17 mg/L. Channel catfish ponds often have un-ionized ammonia nitrogen concentrations above 0.1 mg/L in the afternoon (Boyd, 1998a), and it is generally thought that high un-ionized ammonia concentration in catfish ponds negatively impacts growth and production.

Nitrite also can be toxic to fish, and this nitrogenous compound sometimes enters pond waters from anaerobic zones in sediment to reach concentrations of 5 to 20 mg/L. Nitrite also can enter water during the nitrification process if the rate of the first step on the oxidation that produces nitrite exceeds the rate of the second step that oxidizes nitrite to nitrate (Boyd, 1998a). Unlike ammonia nitrogen for which there is no effective means of counteracting its effect on fish at high concentrations, applications of common salt (NaCl) to ponds to maintain chloride concentration about 30 times greater than nitrite concentration can block nitrite entry across fish gills into the blood to prevent toxicity (Tucker and Hargreaves, 2004).

Relatively large amounts of organic matter enter catfish ponds in feed, and nutrients from feeding wastes encourage production of large amounts of organic matter by plankton photosynthesis. The organic matter load in ponds is mainly uneaten feed, fish feces, and living and dead plankton. This material decomposes naturally, but in some ponds considerable amounts of dissolved organic matter may accumulate in water and organic matter may build up in sediment (Boyd, 1995). Although the oxygen demand of organic matter in catfish ponds can be satisfied by mechanical aeration, the accumulation of organic matter in water or sediment is undesirable because it may harbor organisms – especially bacteria – capable of infecting fish (Avnimelech and Ritvo, 2003; Wakabayashi, 1991). Also, large amounts of organic matter in sediment favor anaerobic conditions at the soil-water interface (Boyd, 1995). Potential toxins such as nitrite and hydrogen sulfide may enter pond water from anaerobic sediment.

There has been considerable interest in applying living bacterial inocula to ponds in order to improve water and sediment quality (Boyd and Gross, 1998; Gräslund et al., 2003). Most bacterial amendments contain one or more species of *Bacillus*, *Lactobacillus*, *Nitrobacter*, *Nitrosomonas*, *Rhodobacter*, or *Rhodococcus* families. The bacterial amendments are advertised by vendors to enhance oxidation of organic matter, ammonia, nitrite, and reduced inorganic substances such as hydrogen sulfide. Most vendors also suggest that the products will lead to greater fish production. Although widely promoted commercially, there are no data to verify the benefits of these products. In fact several studies conducted mostly in research ponds have not revealed improvements in water quality following the application of bacterial amendments (Boyd et al., 1984; Chiayvareesajja and Boyd, 1993; Mischke, 2003; Queiroz and Boyd, 1998). However, most of these studies were conducted in ponds stocked at relatively low densities when compared to densities of catfish currently used in commercial ponds in Alabama. It is possible that water quality in ponds of previous studies did not deteriorate sufficiently to allow the effects of the bacterial amendments to be expressed (Boyd and Gross 1998). Moreover, the new generation bacterial amendments could possibly be more effective than the original ones used in aquaculture. Besides, the conditions under which benefits may be accrued from bacterial amendments use are still unknown. Therefore, the objectives of this study were to:

1. Test 12 bacterial amendments from eight companies to determine if they have potential for lessening total ammonia nitrogen, nitrite, and organic matter concentrations in water.
2. Evaluate the potential efficacy of a selected bacteria amendment (Waste & Sludge Reducer) for improving water quality in channel catfish ponds at the E. W. Shell Fisheries Center.
3. Evaluate the potential efficacy of the bacterial amendments (Aqua PE and Oxyless) in combination for lessening total ammonia nitrogen concentrations in catfish pond with ammonium sulfate addition at the E. W. Shell Fisheries Center.
4. Evaluate the potential efficacy of the bacterial amendment (Aqua PE) for improving water quality in commercial catfish ponds in Alabama.

Chapter 2 Literature Review

Channel Catfish (*Ictalurus punctatus*)

There are approximately 39 species of catfish in North America. However, less than twenty percent of them have been cultured for commercial production (Wellborn, 1988). The channel catfish (Fig 2.1) is a primary species which has been farm-raised for years, especially within the southeastern United States (Wolters and Johnson, 1994).

Fig 2.1-Channel catfish (*Ictalurus punctatus*).

Channel catfish, one of the most common freshwater catfish, belongs to the *Ictaluridae* family of *Siluriformes* order (Tucker and Hargreaves, 2004). It can be easily identified because of its distinctive forked tail and dark spots scattered around the body. Another special characteristic of channel catfish is several barbells, four of them located under the jaw and one on each tip of the maxilla, exist around its mouth. Of course, channel catfish has a characteristic anal fin with 24 to 29 rays, further distinguishing it from other catfish. The various color of channel catfish are dependent on location and environmental conditions. One common color of channel catfish is grayish-brown on top with dark brown dorsal fins. The dorsal area of the male may become completely black, dark blue, light blue, or silver in spawning season.

Fig 2.2-Pond used for culturing channel catfish (*Ictalurus punctatus*).

The channel catfish quickly became the standard species for commercial use because they are ideally suitable to a wide range of environmental conditions (Tucker and Robinson, 1990). Channel catfish are mainly mono-cultured in ponds (Fig 2.2), cages, raceways or circular tanks in the United States (FAO, 2013). Catfish appetite increases with increasing water temperatures; thus, they usually grow best in the summer at temperature around 30 ℃ (Wellborn, 1988). Channel catfish seem to have unlimited growth potential and the maximum age is nearly 40 years. They have been known to grow to more than 50 pounds, but they are typically cultured to plate size (about 1.5 lb) before reaching 2 years of age.

The channel catfish has been raised for more than a century in the Unites States, but the industry is a relatively new commercial aquaculture enterprise (Wellborn, 1987). Farm-raised catfish is an important aquaculture industry in the United States. The channel catfish is produced primarily in Alabama, Arkansas, Louisiana, and Mississippi. The catfish form these states comprised about 95 percent of the US total sales in 2012.

The United States catfish industry has grown rapidly since it began in the 1960s. The volume of processed catfish of the U.S. catfish industry increased from 225 million pounds in 1995 to 660 million pounds in 2003 (Hanson and Sites, 2012). The annual production value of catfish industry increased to $450 million in 2005, which is higher than the second highest annual production industry, trout industry, which is valued at $74 million.

Although the US channel catfish industry grew rapidly, the domestic production of catfish has significantly declined in recent years since the high mark in 2003 (Hanson and Sites, 2012). The catfish industry in the United States has been seriously influenced by many factors, such as high production cost, competition from imported catfish products, alternative species availability, and catfish quality.

Water Quality in Fish Ponds

Water quality in fish ponds includes all physical, chemical, and biological factors of water that affects the survival, reproduction, growth or management of fish or other aquatic creatures. There are various water quality variables in aquaculture ponds, but only a few of them play an essential role (Boyd and Tucker, 1992; Hepher and Pruginin, 1981).

Warmwater species grow best at 25 ℃ and 35 ℃. Temperature has an inevitable effects on chemical and biological processes. The rate of chemical and biological reactions generally double with 10 ℃ increase in temperature (Williams and Williams, 1967). Therefore, the dissolved oxygen requirements are generally quite critical for aquatic organisms.

The pH in freshwater ponds usually ranged between 6 and 9 with fluctuating daily by one or two units because of respiration and photosynthesis (Boyd, 1998a). In heavily stocked fish ponds, carbon dioxide concentrations can become high as a result of respiration. Then, the free carbon dioxide, which is released into water to produce carbonic acid, leads to low pH. Carbon dioxide seldom causes direct toxicity to fish, but fish will be stressed and even die if the pH drops lower than 5 or rises higher than 10. Catfish can tolerate carbon dioxide up to 30 mg/L as long as the dissolved oxygen concentration is above 5 mg/L (Wurts and Durborow, 1992).

Most of the dissolved oxygen in non-aerated fish ponds is generated from photosynthesis by phytoplankton (Isyagi et al., 2009). During the day, oxygen is generated.

After sunset, dissolved oxygen concentrations decline as photosynthesis stops and all plants and animals in the pond consume oxygen in a process called respiration. Thus, dissolved oxygen concentrations in the pond are normally highest in the late afternoon and lowest in the early morning. Normally, oxygen levels should be higher than 5 mg/L for good fish production (Boyd, 2003). Prolonged exposure to low dissolved oxygen levels will reduce fish growth and survival rates.

The turbidity of water is based on the amount of light scattered by particles in the water column (Kirk, 1985). There is a strong relationship between turbidity and total suspended solid because suspended solids can block the light from penetrating into the water. Thus, turbidity values are often used as an indicator of water clarity. There are serval sources of suspended solids such as silt or clay, inorganic materials, or organic matter such as algae, plankton and decaying material. Turbidity can also come from colored dissolved organic matter, fluorescent dissolved organic matter and other dyes. High turbidity has a negative effect on growth of phytoplankton and aquatic weeds, the primary producers in water ponds, by scattering and blocking sunlight for photosynthesis. The channel catfish, bottom dwellers, normally lives at the pond bottom to create a muddy water. Therefore, only superficial phytoplankton can survive in the pond because sunlight cannot penetrate deep.

Total alkalinity represents the quantity of base present and total hardness indicates the total concentration of divalent salts in water. Both influence the buffering capacity of the pond water. The recommended value of hardness is at least 20 mg/L and a range of 75 to 150 mg/L is ideal for fish culture (Bhatnagar and Devi, 2013; Swann and others, 1997).

The range of alkalinity between 75 and 200 mg/L, but not less than 20 mg/L is optimum in an aquaculture pond (Boyd and Bowman, 1997; Wurts and Durborow, 1992).

Ammonia nitrogen is the by-product from organic waste decomposition in water in two forms, un-ionized ammonia and ammonium ion (Trussell, 1972). Although the ammonium ion is much less toxic, a high proportion of ammonia (most toxic form) occurs in ponds with high pH and temperature after carbon dioxide is removed from water for phytoplankton photosynthesis during the a summer day. Ammonia seldom kills fish and shrimp directly, but it stressed them causing poor growth and low survival rate (Boyd, 1998a). High concentrations of ammonia in water impair the excretion of ammonia from the fish into the water across their gills, and thus cause gill damage and affect blood pH, enzyme systems efficiently. The damaged gills cannot extract oxygen from the water. Fish are able to tolerate 0.01 to 0.05 mg/L of un-ionized ammonia without a significant negative effect on production when dissolved oxygen and water temperature are within the recommended range (Boyd, 1998a). They can tolerate un-ionized ammonia up to 2 mg/L for only short times. There was a negative linear relationship between channel catfish growth and ammonia nitrogen concentration from 0.07 to 1.2 mg/L in a 1 month trial (Colt and Armstrong, 1981). Moreover, no weight gain was reported when the ammonia nitrogen concentration was greater than 1.17 mg/L. In aquatic ponds, ammonia can be directly absorbed by phytoplankton or broken down by nitrifying bacteria into toxic nitrite and then less toxic nitrate. Therefore, high concentrations of ammonia in water represent either a poor phytoplankton bloom or a nutrient overload at levels the normal bacteria in the pond cannot degrade.

Nitrite is an intermediate anion in an oxidation state between ammonium and nitrate in fresh and saline waters (Lewis and Morris, 1986). Total ammonia nitrogen is converted to nitrite, and then quickly turned to non-toxic nitrate by naturally-occurring bacteria. The concentrations of nitrite are typically less than 0.005 mg/L in oxygenated waters. High concentrations of nitrite also can be toxic to aquatic organisms by altering hemoglobin to methemoglobin that does not transport oxygen. When the water has high concentrations of nitrite, nitrite anion enters the bloodstream of fish through their gills and turns the blood to a brown color, which is called brown blood disease. The oxygen carrying capacity of the blood would be limited since brown blood could not carry enough oxygen despite adequate oxygen concentration in the water. High concentrations of nitrite in ponds frequently occur with the disruption of the nitrogen cycle due to decreased plankton or bacterial activity. A study by Russo and others (1981) showed that 96-h median lethal concentrations (LC_{50}) of rainbow trout (*Salmo gairdneri*) were less than 1 mg/L. Unlike ammonia nitrogen for which there is no effective way of counteracting its effect on aquatic organisms at a high level, application of chloride (in the form of common salt) at a ratio of chloride to nitrite of 30:1 prevents nitrite poisoning (Tucker and Hargreaves, 2004).

Uneaten feed, senescent phytoplankton and feces are the main sources of accumulated organic matter. More organic matter in ponds results in an increased demand for the available dissolved oxygen (Boyd, 1990). Therefore, excessive organic matter accumulation might cause severe oxygen depletion. Organic effluents also contain large quantities of suspended solids which increases turbidity and reduces the light available to photosynthetic organisms. In addition, organic matter accumulation provides a haven for certain disease organisms. Also, large accumulation of organic matter in sediment favors anaerobic conditions at the soil-water interface (Boyd, 1995). Under anaerobic conditions, organic matter often is decomposed with release of toxic substances such as NO_2^-, H_2S, NH_3 and CH_4 (Boyd and Bowman, 1997). The pollution caused by organic matter can be reduced if the organic matter is broken down into smaller less complex and toxic particles with the help of oxygen and bacteria (Bhatnagar and Devi, 2013).

The Use of Probiotics in Aquaculture

The term "probiotic" was initially introduced by Lilly and Stillwell (1965) for the organisms and substances to extend the growth of other species. The definition was revised by Fuller (1989) as "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". According to the FAO and WHO (2001), probiotic refers to the bacteria that contribute beneficial effects for both humans and animals.

The first application of probiotics in aquaculture was conducted by using spores of *Bacillus toyoi* to mix with feed to increase the growth rate of yellowtail (*Seriola quinqueradiata*) (Kozasa, 1986). The research on application of probiotics in aquaculture is increasing with the demand for environment friendly aquaculture (Gatesoupe, 1999). There were several reports on using probiotics as valuable environment-friendly alternative to antibiotic treatments to prevent infection from pathogenic bacteria in recent years (Boyd and Gross, 1998; FAO, 2001; Irianto and Austin, 2002; Kozasa, 1986). The benefits of the probiotics also include improving feed value, contribution to digestion and immune system, inhibition of pathogenic microorganisms and promoting growth (Kaushik et al., 2009; Sharma and Bhukar, 2000; Spanggaard et al., 2001; Verschuere et al., 2000).

Bacterial communities play an important role in maintaining water quality in aquaculture ponds, especially in those with high-density stocking rate (Boyd and Tucker, 1992; Mischke, 2003). Disruptions of bacterial communities in ponds may direct high-level accumulation of toxicity ammonia, nitrite and organic matter. There has been considerable interest worldwide in applying bacterial inocula to ponds in order to hasten ammonia nitrogen oxidation through bacterial nitrification (Boyd and Gross, 1998; Gräslund et al., 2003).

Many bacterial amendments are advertised and marketed by vendors to enhance oxidation of organic matter, ammonia, and nitrite and reduced inorganic substances as water quality conditioners to improve of growth and health of fish and shrimp in aquaculture. Most bacterial amendments contain one or more species of *Bacillus*, *Lactobacillus*, *Nitrobacter*, *Nitrosomonas*, *Rhodobacter*, or *Rhodococcus* families.

Although the beneficial effects of microbial inoculums on water quality have been revealed under controlled laboratory conditions (Barik et al., 2011), studies done 10 to 30 years ago in catfish ponds reported no benefit of bacterial amendments on water quality or production (Boyd et al., 1984; Boyd and Gross, 1998; Chiayvareesajja and Boyd, 1993; Gräslund et al., 2003; Queiroz and Boyd, 1998). However, most of these studies were conducted in ponds stocked at relatively low densities when compared to densities of catfish currently used in commercial ponds in Alabama. It is possible that water quality in ponds of previous studies did not deteriorate sufficiently to allow the effects of the bacterial amendments to be expressed (Boyd and Gross, 1998). Moreover, the new generation bacterial amendments could possibly be more effective than the original ones used in aquaculture. Research on bacterial amendments for improvement of water quality is needed, because these amendments are widely used throughout the world in aquaculture ponds, but the conditions under which benefits may be accrued from their use are unknown.

Rationale and Significance

Bacterial amendments have been used from time to time by a few catfish farmers in Alabama, but there has not been great interest in these products in the past. This may have been because total ammonia nitrogen concentrations were usually no more than 1 to 2 mg/L, due to relatively low stocking rates. Nitrite toxicity also usually could be controlled with salt at a ratio of chloride to nitrite of 30:1. Therefore, farmers did not see a need for bacterial amendments. Stocking rates in catfish ponds have increased greatly during the past two decades, and total ammonia nitrogen concentrations of 5 to 10 mg/L are not uncommon (Zhou and Boyd, 2015). It has been observed that when fish in ponds with high total ammonia concentrations become infected with a disease, they usually do not respond to treatment with medicated feed until ammonia concentrations decline.

Some catfish farms in west Alabama have begun to use a bacterial amendment (Aqua PE) from Europe that is sold by the company Eurovix, USA. It is likely that several other companies also will start an effort to sell bacterial amendments to catfish farmers. Therefore, studies should be conducted to ascertain if bacterial amendments actually reduce ammonia concentrations and improve other water quality conditions in ponds.

This research is needed because bacterial amendments are expensive; William Hemstreet also indicated that the cost of treating ponds apparently would be about $250 per hectare per year. This would be a significant increase in production cost, so it is important to determine if treatment with bacterial amendments provides benefits.

If a bacterial amendment that is effective in improving water quality – especially in reducing total ammonia nitrogen concentration – can be found, it would represent a major advancement in pond water quality management. On the other hand, if bacterial amendments are not effective for this purpose, the findings of this study would be valuable in preventing farmers from wasting money on an ineffective treatment.

Chapter 3 Bioremediation Treatments for Reducing Ammonia, Nitrite and Organic Matter in Polluted Water

ABSTRACT: The effects of 12 bacterial amendments from eight companies on reducing total ammonia nitrogen, nitrite nitrogen and organic matter concentration in polluted pond water were evaluated in an environmentally controlled room. Concentrations of total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^-–N) and organic matter (as estimated from oxygen demand) in untreated water naturally decreased with time during 16 days. No large improvements were observed in accelerating nitrification or organic matter oxidation from the use of these bacterial amendments in this study. However, some of the bacterial amendments showed minor effects on increasing the removal rates of TAN, nitrite nitrogen and organic matter on certain sampling dates. Compared to the untreated water, Aqua Prob EZ at suggestion dose gave the best results with less TAN on days 8 and 12, less nitrite nitrogen on day 8 and less organic matter on day 0 (initial measurement). At recommended doses, Aqua PE and WASTE & SLUDGE REDUCER (WSR) had a slightly beneficial effects on TAN removal on day 16 and day 0 (initial measurement), respectively. Aqua Bio-Trol at the recommended dose was successful at increasing nitrite nitrogen removal rate on day 12. Fritz Zyme 360 and Aqua PE with recommended doses promoted less organic matter on day 16. With higher doses or more frequent applications, there was less ($P<0.05$) TAN in water treated with Aqua Bio-Trol on day 0 (initial measurement) and day

2. Aqua PE at higher dose and Pond Protect (dry) with more frequent applications than recommended by manufacturers had little effect on decreasing the dissolved oxygen loss rates on days 1, 7, 9 and 13. Aqua Prob 4X, Lake & Pond Bacteria, Pond Protect (liquid), Sewper Rx, SHRIMPSHIELD and AQUA-TRON treatments were not successful ($P>0.05$) at increasing the removal rate of total ammonia nitrogen, nitrite nitrogen and organic matter.

Key Words: Polluted water, bacteria amendments, total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^-–N), organic matter.

Introduction

With ponds being stocked at increasingly higher densities, greater feed inputs result in much greater concentrations of total ammonia nitrogen, nitrite nitrogen and organic matter than in the past. Water quality deterioration, particularly nitrogenous wastes and excessive organic matter, in commercial ponds is considered as a major limiting factor for further intensification of aquaculture systems (Boyd, 1990; Colt and Armstrong, 1981; Colt and Tchobanoglous, 1978, 1976; Tucker and Robinson, 1990a).

Ammonia nitrogen from organic waste decomposition exists in water in two forms, un-ionized ammonia and ammonium ion (Trussell, 1972). Although the ammonium ion is much less toxic, a high proportion of ammonia (most toxic form) occurs in ponds with high pH and temperature after carbon dioxide is removed from water for phytoplankton photosynthesis during the summer daytime. Ammonia seldom kills fish and shrimp directly, but it stressed them causing poor growth and low survival rate (Boyd, 1998a). There was a negative linear relationship between channel catfish growth and ammonia nitrogen concentration from 0.07 to 1.2 mg/L in a month trial (Colt and Armstrong, 1981). Moreover, no weight gain was reported when the ammonia nitrogen concentration was greater than 1.17 mg/L.

High concentrations of nitrite also can be toxic to aquatic organisms by binding with hemoglobin to form methemoglobin that will not combine with molecular oxygen. A study by Russo and others (1981) showed 96-h median lethal concentrations (LC_{50}) of rainbow trout (*Salmo gairdneri*) were less than 1 mg/L. Unlike ammonia nitrogen for which there is no effective way of counteracting its effect on aquatic organisms, application of chloride (in the form of common salt) at a ratio of chloride to nitrite of 30:1 prevents nitrite poisoning (Tucker and Hargreaves, 2004).

Uneaten feed, senescent phytoplankton and feces are the main sources of accumulated organic matter. Excessive organic matter accumulation increases turbidity, causes severe oxygen depletion and provides a haven for certain disease organisms (Boyd, 1990). Also, large accumulation of organic matter in sediment favors anaerobic conditions at the soil-water interface (Boyd, 1995). Under anaerobic conditions, organic matter often is decomposed with release of toxic substances such as NO_2^-, H_2S, NH_3 and CH_4 (Boyd and Bowman, 1997).

Bacterial communities in aquaculture ponds, especially those with high-density stocking rates, are essential for maintenance of optimum water quality (Boyd, 1998a; Mischke, 2003). Disruptions of bacterial communities in ponds may lead to accumulation of high concentrations of ammonia, nitrite and organic matter. There has been considerable interest worldwide in applying bacterial inocula to ponds to improve water quality through bioremediation (Boyd and Gross, 1998; Gräslund et al., 2003). Many bacterial amendments

– often incorrectly called probiotics – are advertised and marketed by vendors to enhance oxidation of organic matter, ammonia, and nitrite and reduced inorganic substances as water quality conditioners to improve growth and health of fish and shrimp in aquaculture. Most bacterial amendments contain one of more species of *Bacillus*, *Lactobacillus*, *Nitrobacter*, *Nitrosomonas*, *Rhodobacter*, or *Rhodococcus* families.

Although broadly promoted commercially, there are limited studies having been conducted to verify the efficiency of these amendments. Moreover, the conditions under which benefits may be accrued from their use are still unknown. Therefore, the objectives of this study were to evaluate the potential efficacy of bacteria amendments for lessening total ammonia nitrogen, nitrite and organic matter concentration in polluted pond water.

Materials and Methods

Twelve bacteria amendments (Table 3.1) obtained from eight companies were tested at regimens of original and two times (with recommended and double frequency of applications, respectively), and four, eight times doses and frequencies recommended by manufacturers. These tests were conducted in an environmentally-controlled room in the Aquatic Resource Laboratory at the E. W. Shell Fisheries Center (SFC), Auburn University, Auburn, Alabama.

For each amendment, water from an aquaculture pond on the SFC was used to fill 21, 20-L aquaria. The total alkalinity and total hardness concentrations of the water in each aquarium were adjusted by adding 100 mg/L $NaHCO_3$ and $CaSO_4 \cdot 2H_2O$, respectively. The aquaria each received air from a small carborundum diffuser to gently mix the water, and each was covered with a plastic lid to minimize evaporation. The temperature in the room was maintained at 25 ± 2 ℃, and the lights were set on a 12 hr on and off cycle.

Total ammonia nitrogen

The total ammonia nitrogen (TAN) concentration in each aquarium was artificially adjusted initially by adding 5 mg/L of TAN from NH_4Cl to each. Triplicate aquaria were treated with different regimens of a bacterial amendment, while three aquaria served as controls. These regimens included (1) dosage and frequency application recommended by manufacturers, (2) recommended dosage at 2x application frequency, (3) 2x dosage at recommended application frequency, (4) 2x dosage at 2x application frequency, (5) 4x dosage at recommended application frequency, (6) 8x dosage at recommended application frequency. Total ammonia nitrogen concentrations were measured by the salicylate method (Le and Boyd, 2012) on day 0 (a few hours after water was treated with bacteria amendment) and after 2, 4, 6, 8, 12, & 16 days. The pH and water temperature were measured on each sampling date, and total alkalinity and total hardness concentrations measured on days 0 (initial measurement), 8, and 16. This procedure was repeated for all bacterial amendments.

Nitrite nitrogen

The testing of bacterial amendments for removal of nitrite nitrogen from water was done similarly as described above for total ammonia nitrogen. The only difference was that the water in each aquarium was artificially polluted by adding 5 mg/L NO_2^-–N form $NaNO_2$ instead of NH_4Cl. Nitrite nitrogen concentrations were measured by the diazotization method (Eaton et al., 2005). Rates and frequencies of application were according the same arrangement used for ammonia nitrogen studies.

Organic matter

The source of organic matter for increasing the organic matter concentration to the water in the aquaria was from a standard 32% crude-protein-content, pelleted fish feed (Alabama Catfish Feed mill, Uniontown, AL, U.S.A). The fish feed was pulverized and placed in water to make an infusion. After 24-hr, the infusion was filtered to remove large particles, and aliquots of the filtered infusion were added to each aquarium to give an initial chemical oxygen demand concentration of about 50 mg/L. As described above for the ammonia trials, three replicates of aquaria were inoculated with bacterial amendment according to designed regimens and three aquaria for controls. On each sampling date (after few hours and 2, 4, 6, 8, 12, and 16 days), a sample of water from each aquarium was transferred to a standard biochemical oxygen demand bottle (300 mL), and the bottle was incubated at 20 ℃ for 1 day. During incubation, the dissolved oxygen concentration was determined and increased raised to near saturation twice at the beginning and after 16 hours by aid of a small air stone as described by Xinglong and Boyd (2005). The total decrease in dissolved oxygen concentration was measured during 24-hr incubations. The dissolved oxygen loss rate was used as an index of organic matter decomposition rate in each water sample.

Statistics

Correlation analyses were conducted and significant differences among mean ($P < 0.05$) were evaluated using oneway ANOVA with Tukey HSD (SPSS, IBM, New York, N.Y., U.S.A.).

Results and Discussion

Total Ammonia Nitrogen (TAN)

The concentration of TAN in untreated water of all controls for the study (n=36) decreased with time eventually decreasing to zero after 16 days (Fig. 3.1). The loss rate in TAN declined slightly with time, especially after 8 days. At this laboratory trial, the TAN values (n= 36) in untreated water are fit to a second order polynomial over time:

TAN (mg/L) = 0.0224* time (day) ^ 2 - 0.7773 * time (day) + 6.5434, $R^2 = 0.9769$.

The average changes in TAN are shown in Fig 3.2, for untreated water and water treated with bacterial amendments at the dose recommend by the manufacturers. As occurred with in TAN concentration in untreated water, the general trends of total TAN in water treated with various bacterial amendments was a gradual decrease during 16 days in the laboratory trial. The bacterial amendments, SHRIMPSHIELD, AQUA-TRON, Aqua Prob 4X (powder with nutrients), PondProtect (liquid), PondProtect (dry), FritzZyme 360, Lake & Pond Bacteria, Aqua Bio-Trol and Sewper Rx, at the manufacturers' suggested dose were ineffective ($P>0.05$) for increasing the TAN removal rate in ammonia polluted water during 16 days. After few hours, WAS at recommendation dose caused little effect ($P<0.05$) on lessening TAN concentrations when compared with TAN in untreated water. Aqua Prob EZ at the suggested dose had a beneficial effect ($P<0.05$) on increasing TAN removal rate at days 8 and 12. Aqua PE at the suggested dose appeared to offer some improvement ($P<0.05$) in ammonia nitrogen removal. The TAN concentration exhibited lower removal at day 16, but the TAN concentration had already decreased to small amount by this time.

Changes in TAN concentration of ammonia polluted water treated with amendments Aqua PE, Waste &Sludge Reducer (WSR) and Aqua Prob EZ at concentrations that range from the suggested dose to eight times that same dose, respectively, are shown in Table 3.2. The individual concentrations of TAN in water treated with Aqua PE, WSR and Aqua Prob EZ at the recommended dose were different ($P<0.05$) from controls on day 16, day 0 (initial measurement) and days 8 and12, respectively. No improvement was evident from these three amendments at greater concentrations or more frequent application than those recommended by the manufacturers on the day mentioned above. The TAN concentration was less ($P<0.05$) than that of the control at day 8 after water treated with Aqua PE at twice the suggested dose with two applications.

Concentrations of TAN measured in water treated with Aqua Bio-Trol at the recommended dose were not lower ($P>0.05$) than the control (Table 3.3). On sampling day 0 (initial measurement) and day 2, there was less ($P<0.05$) TAN in the water treated with Aqua Bio-Trol at higher doses or at more frequent application.

There was no decreases in TAN concentration ($P>0.05$) different from that observed in the controls for water following Fritz-Zyme 360, Aqua Prob 4X, Lake & Pond Bacteria, Pond Protect (dry), Pond Protect (liquid), Sewper Rx, SHRIMPSHIELD and AQUA-TRON treatments (Table 3.4 & Table 3.5).

Nitrite Nitrogen (NO_2^-–N)

The concentration of nitrite nitrogen in untreated water (controls) decreased from 5.22 mg/L to 0.22 mg/L in 16 days (Fig. 3.3). A strong linear relationship between the nitrite nitrogen contents and time was found with $R^2 = 0.9978$.

The average changes in nitrite nitrogen, for untreated water and those treated with bacterial amendments at the dose recommend by the manufacturers are found in Fig 3.4. Generally, all the amendments were not useful for increasing the rate of nitrite removal at the recommended dose, while some of them appeared to slow the natural nitrite decomposition process. The individual concentration of nitrite nitrogen in water treated Aqua Bio-Trol and Aqua Prob EZ at recommended doses were less ($P<0.05$) than concentration in the controls on day 12 and days 6 and 8.

However, no decrease in nitrite nitrogen concentration was evident on the sampling dates following treatment with these two amendments at greater concentrations or at more frequent application than recommended by manufacturers (Table 3.6).

No matter the application frequency or dose of bacterial amendments, concentrations of nitrite nitrogen were not different ($P >0.05$) or higher ($P <0.05$) in water treated with Fritz- Zyme 360, Aqua PE, Aqua Prob 4X, Lake & Pond Bacteria, Pond Protect (dry), Pond Protect (liquid), Sewper Rx, SHRIMPSHIELD, AQUA-TRON and Waste & Sludge Reducer (WSR) than in controls on any of the sampling dates (Table 3.7 and Table 3.8).

Organic Matter

The dissolved oxygen loss in 24 hours was measured as an index of organic matter decomposition. The rate of dissolved oxygen loss in untreated water (controls) (n=36) decreased quickly in the first 5 days, and then declined slowly until day 17 (Fig. 3.5). There was a strong second order polynomial relationship between dissolved oxygen loss in 24 hours (ΔDO) and time:

$$\Delta DO\ (mg/L) = 0.0847 * time\ (day)\ ^{\wedge}\ {}^{2} - 2.2256 * time\ (day) + 15.025,\ R^2 = 0.9486.$$

The average changes in dissolved oxygen loss, for untreated water and those treated with bacterial amendments at the recommendation dose recommend, as shown by Fig 3.6, also were discernible.

There was general trend of declining dissolved oxygen losses with time (days) in all polluted water samples. The bacterial amendments, SHRIMPSHIELD, AQUA-TRON, WAS, Aqua Prob 4X, PondProtect (liquid), PondProtect (dry), Lake & Pond Bacteria, Aqua PE, Aqua Bio-Trol and Sewper Rx, at the manufacturer's suggested doses were ineffectively ($P>0.05$) in increasing the rate of organic matter decomposition rates in organic matter polluted water during 16 days. FritzZyme 360 at the suggested dose appeared to offer some improvement on decreasing the rate of dissolved oxygen loss at day 17, but the organic matter has already almost completely decomposed at this time.

There was less ($P<0.05$) dissolved oxygen loss from water samples after treatment with Fritz Zyme 360 and Aqua Prob EZ at suggestion doses on day 17 and day 1 (Table 3.9). However, no improvement was evident from these two amendments at greater concentrations or more frequent application than recommended by manufactures.

There were not less ($P>0.05$) dissolved oxygen losses in water treated with Aqua PE and Pond Protect (dry) than in controls at recommended doses and applications frequencies (Table 3.10). Aqua PE has little effect ($P<0.05$) on decreasing the dissolved oxygen loss rate on day 1 when the dose reached 8 times the recommended. No improvement was observed by increasing the dose of Pond Protect (dry), but there was less ($P<0.05$) dissolved oxygen losses in water treated with Pond Protect (dry) at more frequent applications on days 7, 9 and 13.

There was no less organic matter on each sampling date was observed in polluted water treated with Aqua Prob 4X, Aqua Bio-Trol, Lake & Pond Bacteria, Pond Protect (liquid), Sewper Rx, SHRIMPSHIELD, AQUA-TRON and Waste & Sludge Reducer (WSR) (Table 3.11 and 3.12). This means, no matter whether higher dosage or more frequent applications are made, these eight amendments mentioned above have no effects ($P>0.05$) on increasing the removal rate of organic matter in organic matter polluted water.

Acknowledgements

Appreciation is extended to the Alabama Agricultural Experiment Station for partially funding this project. We also want to express our thanks to KEETON INDUSTRIES, AquaInTech,Inc, Novoymes, Fritz Industries, Inc., Outdoor Water Solutions, Eurovix USA, Inc., Brandt Consolidated, Inc. and Sludge Solutions Int'l for donating bacterial amendments.

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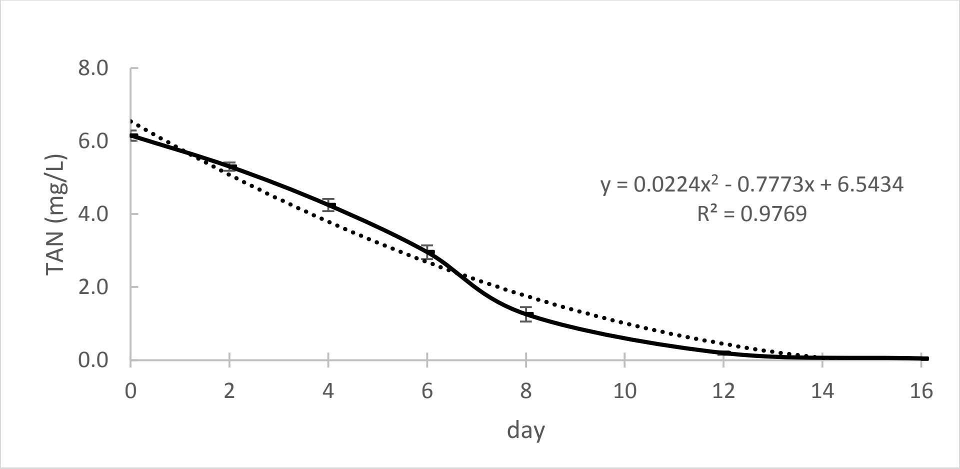

Fig 3.1- Relationship between total ammonia nitrogen (TAN) concentration and time (day) in ammonia polluted pond water (n=36). Standard errors are indicated by bars.

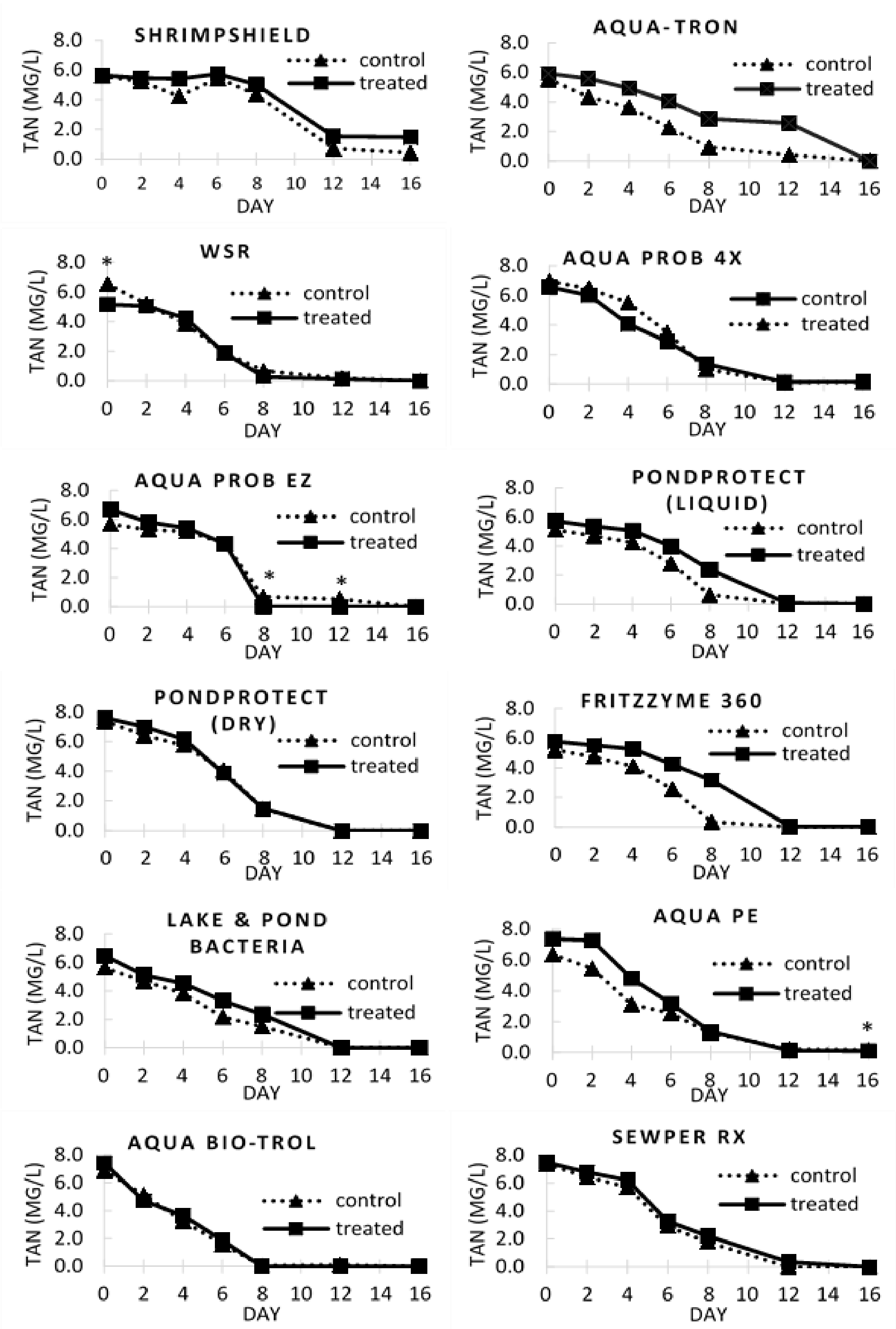

Fig 3.2- Average change in total ammonia nitrogen (TAN) concentration of ammonia polluted pond water with time after treatment by bacterial amendments. Results are presented as means (n=3). Stars indicate a significantly less concentration than control concentration ($P<0.05$).

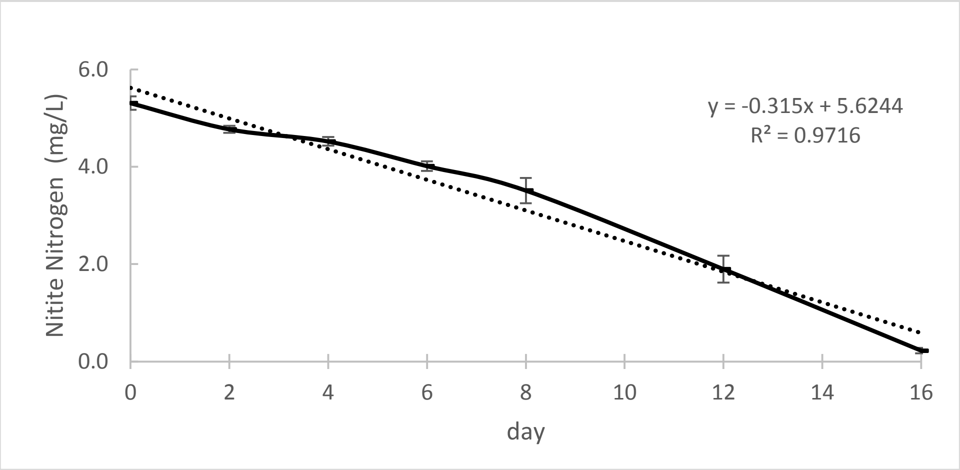

Fig 3.3- Relationship between the nitrite nitrogen (NO_2^-–N) and time (day) in nitrite polluted pond water (n= 36). Standard errors are indicated by bars.

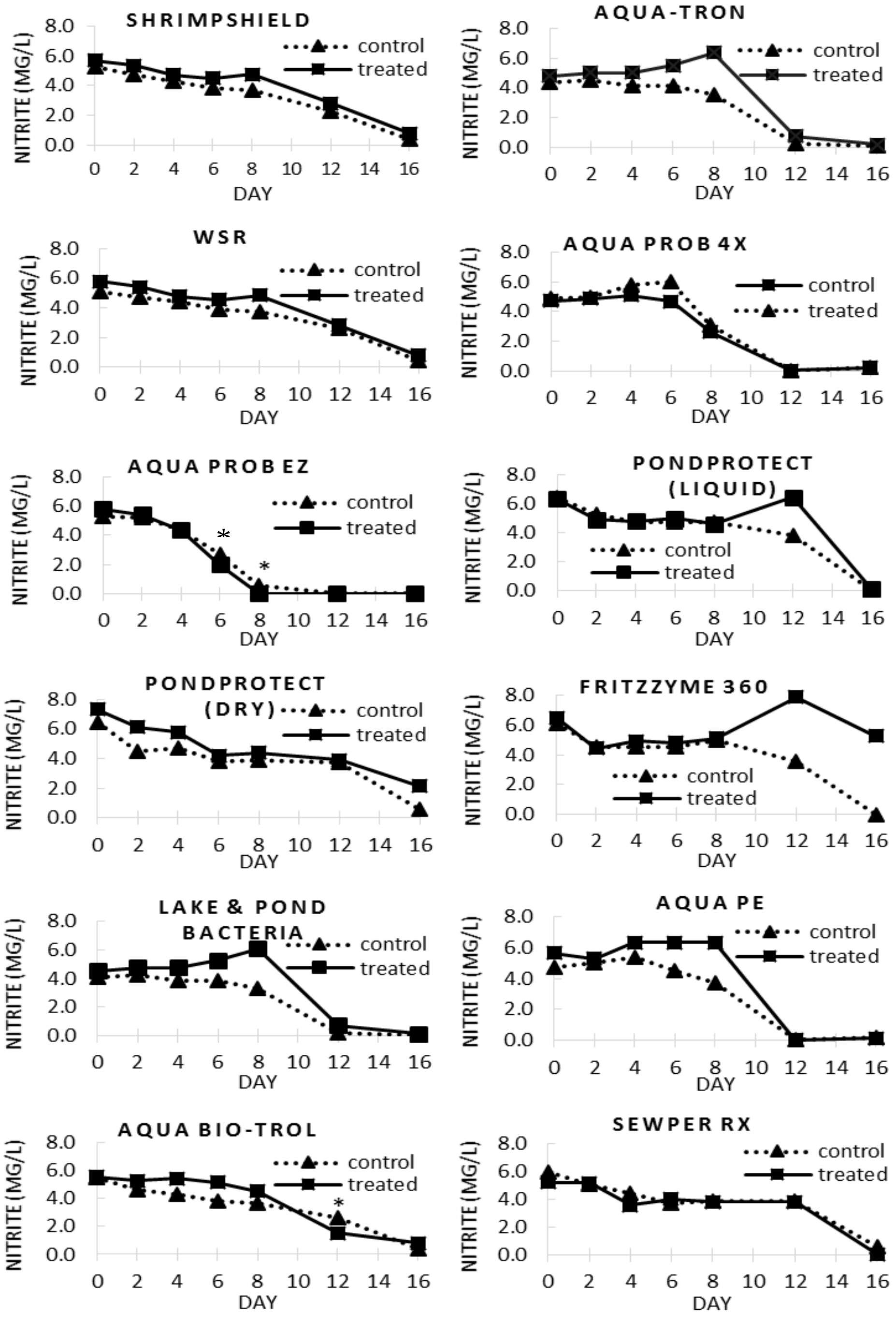

Fig 3.4- Average change in nitrite nitrogen (NO_2^- –N) concentration of nitrite polluted pond water with time following treatment by bacterial amendments. Results are presented as means (n=3). Stars indicate a significantly less concentration in the treatment than in the control ($P<0.05$).

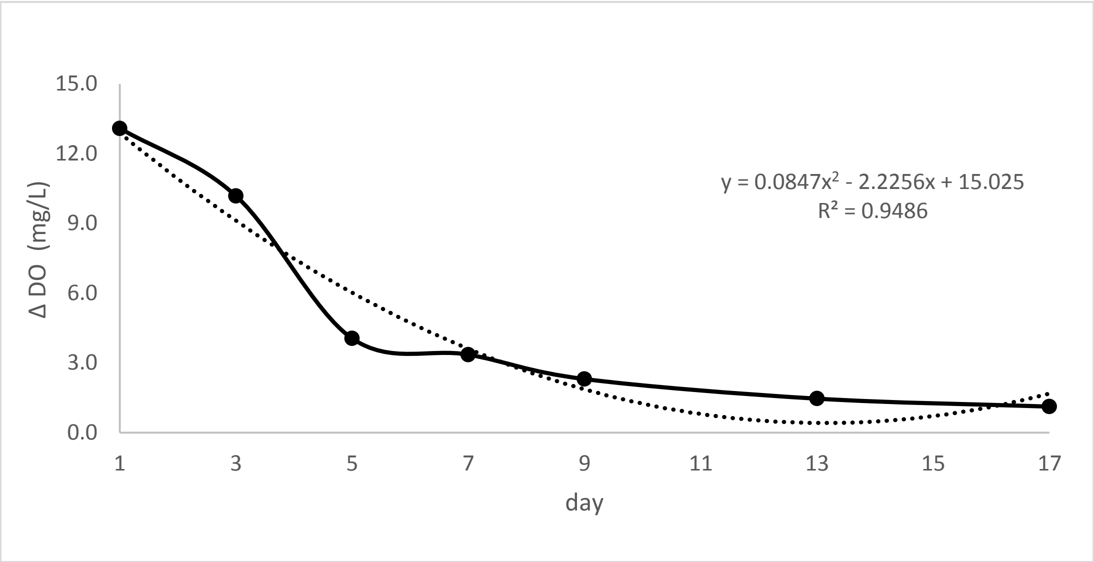

Fig 3.5- Relationship between the dissolve oxygen loss rate in 24 hours (Δ DO) and time (day) in organic matter polluted pond water (n= 36). Standard errors are indicated by bars.

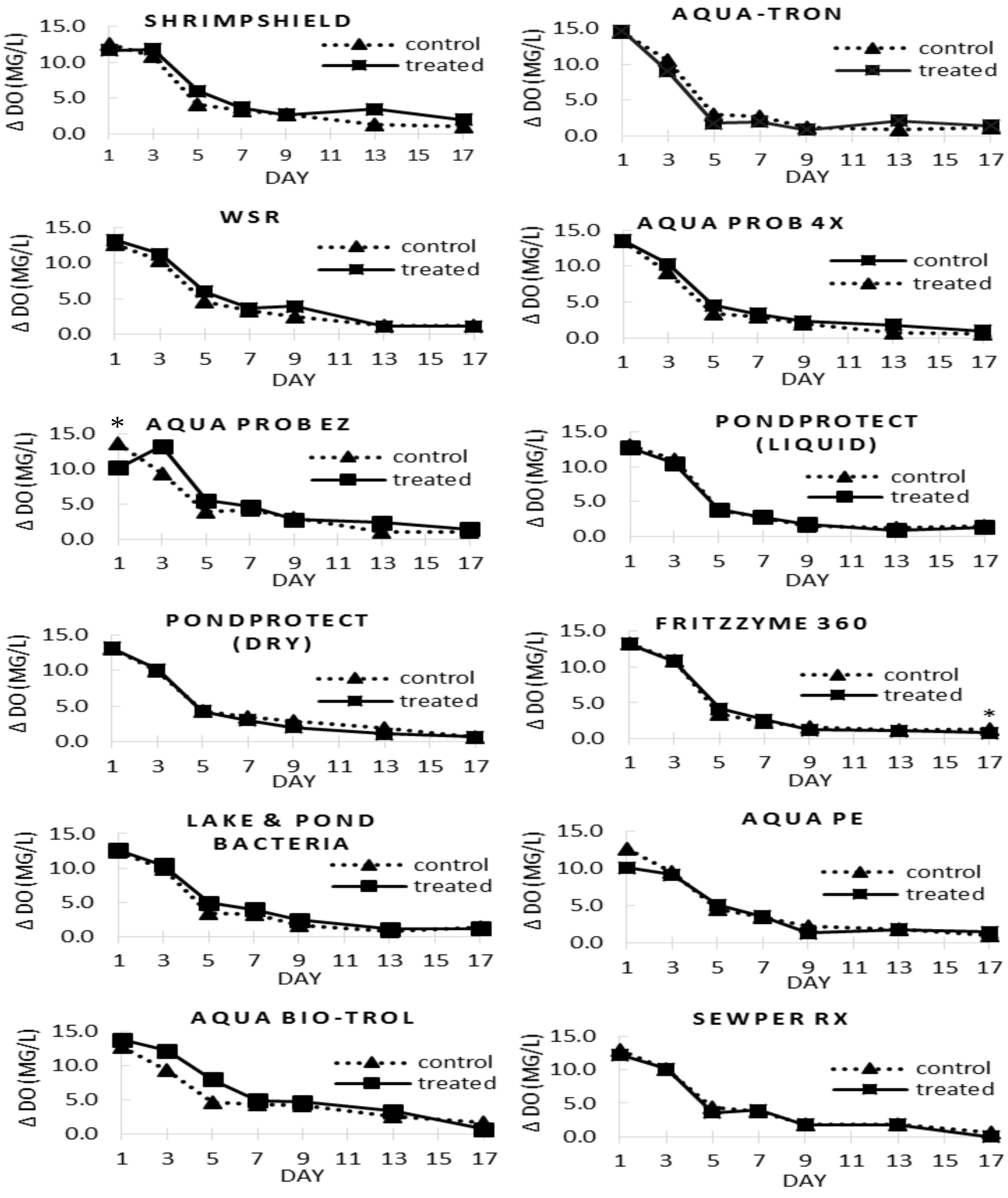

Fig 3.6- Average change in the dissolved oxygen loss (Δ DO) in 24 hour in organic matter polluted pond water with time after treatment by bacterial amendments. Results are presented as means (n=3). Stars indicate significant difference concentration in the treatments as compared to the control (P<0.05).

Table 3.1- Company, location and brand names used in this study

Company	**Location**	**Product Name**	**Recommended Regimen**
KEETON INDUSTRIES	Wellington, CO	SHRIMPSHIELD	0.015 g/L/every 3 day
		AQUA-TRON	0.0125 g/L/every week
		WASTE &SLUDGE REDUCER	0.075 g/L
AquaInTech,Inc	Lynnwood, WA	Aqua Prob 4X (powder w/ nutrients)	0.025 g/L/every week
		Aqua Prob EZ	0.0125 g/L/every 3 day
Novoymes	Portland, ME	PondProtect (liquid)	0.003 mL/L/every 5 day
		PondProtect (dry)	0.0101 g/L/every 5 day
Fritz Industries, Inc.	Mesquite, TX	FritzZyme 360	0.0005 g/L
Outdoor Water Solutions	Springdale, AR	Lake & Pond Bacteria	0.0005 g/L
Eurovix USA, Inc.	Portage, MI	Aqua PE	0.00125 g/L
Brandt Consolidated, Inc.	Springfield, IL	Aqua Bio-Trol	0.0025 g/L
Sludge Solutions Int'l	Tallahassee, FL	Sewper Rx	0.0015 g/L/every week

Table 3.2- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Aqua PE, Waste &Sludge Reducer (WSR) and Aqua Prob EZ.

Treatment				Total ammonia nitrogen (TAN) concentration (mg/L)						
				days						
Product name	Application frequency	Dose		0	2	4	6	8	12	16
Aqua PE	1	control		6.37 a	5.46 a	3.14 a	2.55 ad	1.28 a	0.20 a	0.16 a
		0.00125 g/L	1X	7.37 a	7.27 b	4.80 b	3.17 ae	1.37 ab	0.12 a	**0.11 bc**
		0.0025 g/L	2X	6.57 a	5.73 ab	4.10 ab	3.03 ac	1.64 b	0.12 a	**0.11 bc**
		0.005 g/L	4X	7.22 a	6.33 ab	5.84 b	3.98 be	1.22 a	0.22 a	**0.12 bc**
		0.01 g/L	8X	7.03 a	5.45 a	2.97 a	2.23 bcd	1.09 a	0.09 a	0.13 ac
	2	0.00125 g/L/every week	1X	6.83 a	5.90 a	4.59 a	3.27 a	1.60 a	0.11 a	**0.11 b**
		0.0025 g/L/every week	2X	5.12 a	5.72 a	3.89 a	2.76 a	**0.82 b**	0.40 a	**0.11 b**
WSR	1	control		6.55 a	5.17 a	3.85 a	1.91 a	0.67 a	0.18 a	ND a
		0.075 g/L	1X	**5.16 b**	5.04 a	4.22 a	1.88 a	0.28 a	0.12 a	ND a
		0.15 g/L	2X	**5.27 b**	5.09 a	4.32 a	2.39 a	1.07 a	0.10 a	ND a
		0.3 g/L	4X	**5.32 b**	4.92 a	4.24 a	1.76 a	ND a	0.13 a	ND a
		0.6 g/L	8X	**5.20 b**	4.95 a	4.00 a	2.06 a	1.19 a	0.14 a	ND a
	3	0.075 g/L/ every 5 days	1X	**5.28 b**	4.85 ab	3.68 a	2.13 a	1.44 a	0.09 a	0.03 a
		0.15 g/L/every 5 days	2X	**5.18 b**	4.59 ab	2.91 a	1.54 a	ND a	0.13 a	ND a
Aqua Prob EZ	6	control		5.69 ab	5.30 a	5.19 a	4.34 a	0.69 a	0.53 a	ND a
		0.0125 g/L/every 3 day	1X	6.68 a	5.83 b	5.43 a	4.34 a	**ND b**	**ND b**	ND a
		0.025 g/L/every 3 day	2X	5.28 b	5.13 a	5.00 a	3.68 a	0.17 ab	**ND b**	ND a
		0.05 g/L/every 3 day	4X	5.60 b	5.46 ab	5.04 a	4.04 a	**ND b**	**ND b**	ND a
		0.1 g/L/every 3 day	8X	5.91 ab	5.10 a	4.71 a	3.71 a	**ND b**	**ND b**	ND a
	17	0.0125 g/L/every day	1X	5.41 a	5.36 a	5.35 a	3.77 a	**ND b**	**ND b**	NA a
		0.025 g/L/every day	2X	5.62 a	5.37 a	5.29 a	4.79 a	**ND b**	**ND b**	NA a

Results are presented as mean (n=3);
Values with different letters with in a column are significantly different (P<0.05);
Bold means significantly lower (P<0.05) than the control

Table 3.3- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Aqua Bio-Trol.

Treatment				Total ammonia nitrogen (TAN) concentration (mg/L)						
				days						
Product name	Application frequency	Dose		0	2	4	6	8	12	16
Aqua Bio-Trol	1	control		6.86 a	5.13 a	3.25 a	1.58 a	0.07 a	0.11 a	ND a
		0.025 g/L	1X	7.42 a	4.72 a	3.63 a	1.86 a	ND a	0.01 a	ND a
		0.05 g/L	2X	**5.11 b**	4.37 ab	3.63 a	1.90 a	ND a	ND a	ND a
		0.1 g/L	4X	**5.52 b**	4.66 a	3.75 a	1.80 a	0.02 a	0.12 a	ND a
		0.2 g/L	8X	**5.35 b**	**3.61 b**	2.49 a	1.98 a	ND a	0.07 a	ND a
	2	0.025 g/L/ every week	1X	**4.99 b**	**4.32 b**	1.84 a	1.38 a	ND a	0.08 a	0.08 a
		0.05 g/L/ every week	2X	**5.13 b**	4.39 ab	2.63 a	1.50 a	ND a	0.11 a	0.02 a

Results are presented as mean (n=3);
Values with different letters with in a column are significantly different ($P<0.05$);
Bold means significantly lower ($P<0.05$) than the control

Table 3.4- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Fritz- Zyme 360, Aqua Prob 4X, Lake & Pond Bacteria, Pond Protect (dry), Pond Protect (liquid), Sewper Rx, SHRIMPSHIELD and AQUA-TRON at recommended application frequency.

Treatment				Total ammonia nitrogen (TAN) concentration (mg/L)						
				days						
Product name	Recommended application frequency	Dose		0	2	4	6	8	12	16
Fritz- Zyme 360	1	control		5.20 a	4.73 a	4.11 a	2.56 a	0.35 a	0.02 a	0.03 a
		0.0005 g/L	1X	5.76 ab	5.50 b	5.25 b	4.24 b	3.16 bd	0.04 a	0.02 a
		0.001 g/L	2X	6.04 b	5.12 ab	4.64 c	3.69 b	2.62 b	0.02 a	0.02 a
		0.002 g/L	4X	5.42 ab	5.33 bc	4.57 ac	3.90 b	2.48 bc	0.02 a	0.02 a
		0.004g/L	8X	5.33 ab	4.98 ac	4.75 bc	3.97 b	3.42 d	0.23 b	0.02 a
Aqua Prob 4X	2	control		6.58 a	6.02 a	4.07 a	2.90 a	1.37 a	0.16 a	0.18 a
		0.0025 g/L/every week	1X	6.96 a	6.48 a	5.52 a	3.52 a	1.01 a	0.12 a	0.13 a
		0.005 g/L/every week	2X	6.75 a	5.94 a	5.71 a	4.32 a	1.17 a	0.10 a	0.12 a
		0.01 g/L/every week	4X	6.55 a	5.71 a	3.77 a	2.34 a	0.85 a	0.14 a	0.10 a
		0.02 g/L/every week	8X	6.65 a	5.46 a	3.59 a	2.55 a	1.00 a	0.11a	0.44 a
Lake & Pond Bacteria	1	control		5.63 a	4.67 ab	3.82 a	2.18 a	1.51 a	0.01 a	0.01 a
		0.0005 g/L	1X	6.49 b	5.11 ab	4.52 bc	3.32 a	2.36 a	0.01 a	0.01 a
		0.001 g/L	2X	6.12 ab	4.59 a	3.67 a	1.43 a	0.11 a	ND a	0.00 a
		0.002 g/L	4X	6.19 ab	5.23 b	4.11 ac	2.53 a	1.13 a	ND a	0.02 a
		0.004 g/L	8X	5.73 ab	5.08 ab	4.17 ac	2.13 a	1.40 a	0.03 a	0.01 a
Pond Protect (dry)	3	control		7.38 a	6.42 a	5.74 ab	4.08 ac	1.47 a	ND a	ND a
		0.001 g/L/every 5 days	1X	7.61 a	7.01 b	6.17 a	3.86 ad	1.48 a	ND a	ND a
		0.002 g/L/every 5 days	2X	7.35 a	6.54 ab	5.68 ab	4.20 ac	0.85 a	ND a	ND a
		0.004 g/L/every 5 days	4X	7.55 a	6.87 ab	5.89 ab	4.72 bc	0.71 a	ND a	ND a
		0.008 g/L/every 5 days	8X	7.36 a	6.60 ab	5.51 b	4.66 bcd	1.72 a	0.03 a	ND a
Pond Protect (liquid)	3	control		5.11 a	4.69 a	4.22 a	2.78 a	0.63 a	0.02 a	0.02 a
		0.003 mL/L/every 5 days	1X	5.71 a	5.37 b	5.07 b	3.97 a	2.37 a	0.07 a	0.02 a
		0.006 mL/L/every 5 days	2X	5.50 a	5.22 ab	4.90 b	3.71 a	1.63 a	0.01 a	0.02 a
		0.012mL/L/every 5 days	4X	5.48 a	5.20 ab	4.65 ab	3.36 a	0.96 a	0.04 a	0.02 a
		0.024 mL/L/every 5 days	8X	5.58 a	5.04 ab	4.39 a	2.71 a	0.62 a	0.01 a	0.02 a
Sewper Rx	1	control		7.37 a	6.45 a	5.73 a	2.90 a	1.77 a	ND a	ND a

		0.0015 g/L	1X	7.48 a	6.80 a	6.22 a	3.26 ac	2.21 a	0.35 a	ND a
		0.003 g/L	2X	7.42 a	6.83 a	5.94 a	3.86 bc	2.50 a	0.24 a	ND a
		0.006 g/L	4X	7.66 a	6.68 a	6.05 a	3.99 bc	2.43 a	0.23 a	ND a
		0.012 g/L	8X	7.44 a	6.64 a	5.86 a	2.74 ac	1.64 a	0.00 a	ND a
SHRIMPSHIELD	6	control		5.58 a	5.24 a	4.23 a	5.45 a	4.33 a	0.70 a	0.42 a
		0.015 g/L/every 3 day	1X	5.64 a	5.46 a	5.41 a	5.73 ab	5.02 a	1.53 a	1.46 a
		0.03 g/L/every 3 day	2X	5.64 a	5.29 a	5.14 a	5.28 b	4.89 a	1.45 a	1.25 a
		0.06 g/L/every 3 day	4X	5.86 a	5.45 a	4.99 a	4.92 b	5.94 a	3.30 a	1.10 a
		0.12 g/L/every 3 day	8X	5.68 a	5.08 a	4.60 a	4.31 a	6.01 a	7.71 a	0.32 a
AQUA-TRON	1	control		5.51 a	4.33 a	3.64 a	2.26 a	0.94 ab	0.42 a	0.01 a
		0.001 g/L	1X	5.90 a	5.57 b	4.91 b	4.03 b	2.85 b	2.56 b	0.01 a
		0.002 g/L	2X	6.21 a	4.87 ab	4.04 a	2.97 a	0.69 ab	0.10 a	0.02 a
		0.004 g/L	4X	5.91 a	5.04 ab	4.01 a	2.65 a	0.45 a	0.20 a	0.01 a
		0.008 g/L	8X	5.85 a	4.90 ab	3.71 a	2.41 a	0.26 a	0.14 a	0.01 a

Results are presented as mean (n=3).
Values with different letters with in a column are significantly different (P<0.05)

Table 3.5- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Fritz- Zyme 360, Aqua Prob 4X, Lake & Pond Bacteria, Pond Protect (dry), Pond Protect (liquid), Sewper Rx, SHRIMPSHIELD and AQUA-TRON at doses and more application frequencies greater than recommended by the manufacturers.

Treatment				Total ammonia nitrogen (TAN) concentration (mg/L)						
				days						
Product name	Application frequency		Dose	0	2	4	6	8	12	16
Fritz Zyme 360	2		control	5.20 a	4.73 a	4.11 a	2.56 a	0.35 a	0.02 a	0.03 a
		1X	0.0005g/L/every week	5.30 a	5.16 b	5.09 b	3.97 a	3.27 b	0.22 b	0.03 a
		2X	0.001 g/L/every week	5.58 a	5.20 b	4.76 b	3.85 b	2.79 b	0.09 a	0.02 a
Aqua Prob 4X	4		control	6.58 a	6.02 a	4.07 a	2.90 a	1.37 a	0.16 a	0.18 a
		1X	0.0025 g/L/ every 4 days	6.43 a	5.28 a	3.40 a	2.74 a	1.25 a	0.09 a	0.08 a
		2X	0.005 g/L/every 4 days	6.39 a	5.31 a	5.18 a	3.55 a	1.60 a	0.12 a	0.11 a
Lake & Pond Bacteria	2		control	5.63 a	4.67 a	3.82 a	2.18 a	1.51 a	0.01 a	0.01 a
		1X	0.0005 g/L/ every week	5.70 a	4.67 a	3.91 a	3.06 a	2.57 a	0.08 b	0.01 a
		2X	0.001 g/L/ every week	6.25 a	4.92 a	4.16 a	2.15 a	1.45 a	0.01 a	0.01 a
Pond Protect (dry)	5		control	7.38 a	6.42 a	5.74 a	4.08 a	1.47 ac	ND a	ND a
		1X	0.001 g/L/every 3 days	7.58 a	6.58 a	6.14 a	4.41 a	2.22 a	0.12 b	ND a
		2X	0.002 g/L/every 3 days	7.48 a	6.66 a	5.81 a	4.41 a	0.79 bc	ND a	ND a
Pond Protect (liquid)	5		control	5.11 a	4.69 a	4.22 a	2.78 a	0.63 a	0.02 a	0.02 a
		1X	0.003mL/L/ every 3 days	5.25 a	4.29 a	3.92 a	2.40 a	0.82 a	0.04 a	0.02 a
		2X	0.006mL/L/ every 3 days	5.60 a	4.72 a	3.98 a	2.29 a	0.28 a	0.01 a	0.02 a
Sewper Rx	2		control	7.37 a	6.45 a	5.73 a	2.90 a	1.77 a	ND a	ND a
		1X	0.0015 g/L/ every week	7.54 a	6.51 a	5.45 a	2.25 a	1.48 a	0.35 a	ND a
		2X	0.003 g/L/ every week	7.36 a	6.63 a	5.80 a	2.65 a	1.43 a	0.13 a	ND a
SHRIMP SHIELD	17		control	5.58 a	5.24 a	4.23 a	5.45 a	4.33 a	0.70 a	0.42 a
		1X	0.015 g/L/every day	5.79 a	5.60 a	5.24 a	5.40 a	5.59 b	1.29 a	0.42 a
		2X	0.03 g/L/every day	5.69 a	5.41 a	5.04 a	4.96 a	5.93 b	6.03 b	0.91 a
AQUA-TRON	2		control	5.51 a	4.33 a	3.64 a	2.26 a	0.94 a	0.42 a	0.01 a
		1X	0.001 g/L/ every week	5.55 a	4.69 a	3.75 a	2.89 b	1.51 a	0.25 a	0.01 a
		2X	0.002 g/L/ every week	5.84 a	4.68 a	3.88 a	3.41 ab	1.42 a	0.17 a	0.01 a

Results are presented as mean (n=3);
Values with different letters with in a column are significantly different ($P<0.05$);

Table 3.6- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with Aqua Bio-Trol and Aqua Prob EZ.

Treatment				Nitrite nitrogen (NO_2^-–N) concentration (mg/L)						
				days						
Product name	Application frequency	Dose		0	2	4	6	8	12	16
Aqua Bio-Trol	1	control		5.47 a	4.62 a	4.34 a	3.80 a	3.66 a	2.59 a	0.40 a
		0.025 g/L	1X	5.58 a	5.27 b	5.43 b	5.15 b	4.53 a	**1.53 b**	0.83 a
		0.05 g/L	2X	5.36 a	4.67 ad	5.46 b	4.41 c	4.01 a	**1.41 b**	0.80 a
		0.1 g/L	4X	5.50 a	5.33 b	5.73 b	4.99 bc	4.28 a	**1.42 b**	0.50 a
		0.2 g/L	8X	5.21 a	5.15 bd	5.32 b	4.62 c	3.82 a	**1.77 b**	0.72 a
	2	0.025 g/L/ every week	1X	5.04 a	5.01 a	4.99 ab	4.44 b	3.46 a	**1.52 b**	1.26 b
		0.05 g/L/ every week	2X	5.16 a	4.98 a	5.63 b	4.77 b	4.10 a	**1.67 b**	0.64 ab
Aqua Prob EZ	6	control		5.30 a	5.19 ab	4.34 a	2.69 a	0.53 a	ND a	ND a
		0.0125 g/L/every 3 day	1X	5.83 b	5.43 a	4.34 a	**2.00 b**	**ND b**	ND a	ND a
		0.025 g/L/every 3 day	2X	5.13 a	5.00 ab	3.68 a	2.17 ab	**ND b**	ND a	ND a
		0.05 g/L/every 3 day	4X	5.46 ab	5.04 ab	4.04 a	**1.85 b**	**ND b**	ND a	ND a
		0.1 g/L/every 3 day	8X	5.10 a	4.71 b	3.71 a	**1.86 b**	**ND b**	ND a	ND a
	17	0.0125 g/L/every day	1X	5.36 a	5.35 a	3.77 a	**1.87 b**	**ND b**	ND a	ND a
		0.025 g/L/every day	2X	5.37 a	5.29 a	4.79 a	**2.03 b**	**ND b**	ND a	ND a

Results are presented as mean (n=3);
Values with different letters with in a column are significantly different ($P<0.05$);
Bold means significantly lower ($P<0.05$) than the control

Table 3.7 -Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with Fritz- Zyme 360, Aqua PE, Aqua Prob 4X, Lake & Pond Bacteria, Pond Protect (dry), Pond Protect (liquid), Sewper Rx, SHRIMPSHIELD, AQUA-TRON and Waste & Sludge Reducer (WSR) at doses and application frequencies recommended by the manufacturers.

Treatment				Nitrite nitrogen (NO_2^-–N) concentration (mg/L)						
				days						
Product name	Application	Dose		0	2	4	6	8	12	16
Fritz- Zyme 360	1	control		6.08 a	4.55 ab	4.54 a	4.51 a	4.96 a	3.56 a	ND a
		0.0005 g/L	1X	6.51 a	4.43 a	4.91 a	4.80 a	5.10 a	7.89 b	5.27 b
		0.001 g/L	2X	6.18 a	4.54 ab	4.65 a	4.39 a	4.80 a	7.09 b	5.01 b
		0.002 g/L	4X	6.51 a	4.83 b	4.83 a	4.29 a	4.92 a	7.48 b	1.41 c
		0.004g/L	8X	5.77 a	4.51 ab	4.73 a	4.38 a	4.59 a	7.37 b	5.22 b
Aqua PE	1	control		4.73 a	5.00 ac	5.39 a	4.52 a	3.70 a	0.02 a	0.18 a
		0.00125 g/L	1X	5.66 b	5.30 ad	6.33 bc	6.34 bcd	6.31 bde	0.02 a	0.15 a
		0.0025 g/L	2X	4.88 a	4.60 bc	5.44 ac	5.19 ad	4.83 acf	0.02 a	0.19 a
		0.005 g/L	4X	5.22 ab	5.57 d	6.96 b	6.82 bc	7.03 bdf	0.02 a	0.27 a
		0.01 g/L	8X	5.02 ab	5.27 ad	6.91 b	6.34 bd	5.26 ce	0.02 a	0.23 a
Aqua Prob 4X	2	control		4.70 a	4.85 a	5.07 a	4.66 a	2.61 a	0.02 a	0.19 a
		0.0025 g/L/every week	1X	4.88 a	4.94 a	5.76 a	5.99 a	3.11 a	0.01 a	0.23 a
		0.005 g/L/every week	2X	4.77 a	4.81 a	5.43 a	4.75 a	1.98 a	0.01 a	0.17 a
		0.01 g/L/every week	4X	4.75 a	5.20 a	6.05 a	5.40 a	2.44 a	0.01 a	0.22 a
		0.02 g/L/every week	8X	5.04 a	5.07 a	5.97 a	5.33 a	2.24 a	0.01 a	0.21 a
Lake & Pond Bacteria	1	control		4.09 a	4.27 ab	3.87 a	3.86 a	3.29 a	0.18 a	0.04 a
		0.0005 g/L	1X	4.55 a	4.78 ab	4.78 b	5.27 ab	6.12 a	0.70 a	0.14 a
		0.001 g/L	2X	4.17 a	3.97 a	4.55 ab	4.53 ab	6.20 a	0.50 a	0.44 a
		0.002 g/L	4X	4.17 a	5.10 b	4.70 ab	5.40 b	6.14 a	0.46 a	0.07 a
		0.004 g/L	8X	4.14 a	4.78 ab	4.43 ab	4.72 ab	5.38 a	0.25 a	0.06 a
Pond Protect (dry)	3	control		6.41 a	4.49 a	4.74 a	3.81 a	3.88 ac	3.74 a	0.56 a
		0.001 g/L/every 5 days	1X	7.30 a	6.10 b	5.76 b	4.18 b	4.37 b	3.88 a	2.14 a
		0.002 g/L/every 5 days	2X	7.14 a	5.25 ab	6.40 b	3.94 ab	3.86 a	3.73 a	1.78 a
		0.004 g/L/every 5 days	4X	7.59 a	5.70 ab	6.43 b	4.17 b	4.25 bc	3.78 a	1.20 a
		0.08 g/L/every 5 days	8X	6.44 a	5.60 ab	5.55 ab	3.82 a	3.85 a	5.06 a	1.90 a
Pond Protect (liquid)	3	control		6.37 a	5.24 a	4.76 a	4.76 a	4.74 a	3.83 a	0.03 a
		0.003 mL/L/every 5 days	1X	6.32 a	4.89 a	4.79 a	4.99 ab	4.59 a	6.44 a	0.11 a
		0.006 mL/L/every 5 days	2X	6.39 a	5.07 a	4.88 a	5.00 ab	4.74 a	5.64 a	0.34 a

		0.012mL/L/every 5 days	4X	6.49 a	4.94 a	4.97 a	5.15 ab	4.71 a	5.25 a	0.35 a
		0.024 mL/L/every 5 days	8X	7.20 a	5.66 a	4.95 a	5.34 b	5.46 a	5.48 a	0.41 a
Sewper Rx	1	control		5.91 a	5.09 a	4.40 a	3.73 a	3.82 a	3.86 a	0.61 a
		0.0015 g/L	1X	5.22 a	5.14 a	3.58 a	3.99 a	3.82 a	3.81 a	0.03 ab
		0.003 g/L	2X	5.98 a	5.13 a	4.80 a	3.81 a	3.86 a	3.90 a	0.76 ab
		0.006 g/L	4X	6.52 a	5.52 a	5.42 a	3.82 a	3.88 a	4.00 a	1.56 ab
		0.012 g/L	8X	6.67 a	6.01 a	5.27 a	3.79 a	3.91 a	4.12 a	3.07 b
SHRIMPSHIELD	6	control		5.24 a	4.74 a	4.28 a	3.88 a	3.68 a	2.23 a	0.37 a
		0.015 g/L/every 3 day	1X	5.69 a	5.34 ab	4.70 ab	4.48 b	4.77 a	2.80 ab	0.77 ab
		0.03 g/L/every 3 day	2X	5.76 a	4.78 a	5.25 b	4.09 b	4.64 a	2.71 ab	0.99 ab
		0.06 g/L/every 3 day	4X	6.09 a	5.44 b	5.52 b	4.46 b	4.76 a	2.75 ab	0.95 ab
		0.12 g/L/every 3 day	8X	5.75 a	5.47 b	5.09 ab	4.11 ab	4.38 a	3.14 b	1.68 b
AQUA-TRON	1	control		4.33 a	4.51 ab	4.11 a	4.10 a	3.54 a	0.23 a	0.09 a
		0.001 g/L	1X	4.79 a	5.02 ab	5.02 b	5.51 ab	6.36 a	0.75 a	0.19 a
		0.002 g/L	2X	4.41 a	4.21 a	4.80 ab	4.78 ab	6.44 a	0.55 a	0.49 a
		0.004 g/L	4X	4.41 a	5.34 b	4.94 ab	5.64 b	6.38 a	0.51 a	0.12 a
		0.008 g/L	8X	4.39 a	5.02 ab	4.68 ab	4.96 ab	5.62 a	0.30 a	0.11 a
WSR	1	control		5.09 a	4.70 a	4.42 a	3.88 a	3.75 a	2.61 a	0.42 a
		0.075 g/L	1X	5.77 ab	5.42 ab	4.78 ab	4.56 b	4.85 a	2.81 a	0.79 ab
		0.15 g/L	2X	5.84 ab	4.86 ab	5.33 ab	4.17 ab	4.72 a	2.73 a	1.01 ab
		0.3 g/L	4X	6.17 b	5.52 b	5.60 b	4.54 b	4.84 a	2.76 a	0.96 ab
		0.6 g/L	8X	5.83 ab	5.55 b	5.17 ab	4.19 ab	4.46 a	3.16 a	1.69 b

Results are presented as mean (n=3);

Values with different letters with in a column are significantly different (P<0.05);

Table 3.8- Changes in nitrite nitrogen (NO_2^-N) concentration at various days after treated with Fritz-Zyme 360, Aqua PE, Aqua Prob 4X, Lake & Pond Bacteria, Pond Protect (dry), Pond Protect (liquid), Sewper Rx, SHRIMPSHIELD, AQUA-TRON and Waste & Sludge Reducer (WSR) at higher doses and more frequent applications that recommended by manufacturers.

Treatment				Nitrite nitrogen (NO_2^- –N) concentration (mg/L)						
				days						
Product name	Application frequency	Dose		0	2	4	6	8	12	16
Fritz Zyme 360	2	control		6.08 a	4.55 a	4.54 a	4.51 a	4.96 a	3.56 a	ND a
		0.0005g/L/every week	1X	5.17 a	5.09 b	4.46 a	4.03 a	4.39 a	7.67 b	2.07 b
		0.001 g/L/every week	2X	5.95 a	4.82 ab	4.65 a	4.24 a	4.71 a	7.41 b	2.83 b
Aqua Prob PE	2	control		4.73 a	5.00 a	5.39 a	4.52 a	3.70 a	0.02 a	0.18 a
		0.00125 g/L/every week	1X	5.19 a	5.12 a	5.91 a	6.22 b	5.41 b	0.02 a	0.24 a
		0.0025 g/L/every week	2X	4.90 a	4.95 a	5.64 a	5.48 ab	5.47 b	0.01 a	0.16 a
Aqua Prob 4X	4	control		4.70 a	4.85 a	5.07 a	4.66 a	2.61 a	0.02 a	0.19 a
		0.0025 g/L/ every 4 days	1X	4.73 a	4.63 a	5.55 a	5.27 a	1.68 a	0.01 a	0.20 a
		0.005 g/L/every 4 days	2X	4.56 a	4.71 a	5.62 a	4.92 a	1.82 a	0.01 a	0.18 a
Lake & Pond Bacteria	2	control		4.09 a	4.27 a	3.87 a	3.86 a	3.29 a	0.18 a	0.04 a
		0.0005 g/L/ every week	1X	4.15 a	4.34 a	4.09 a	4.24 ab	4.71 a	0.79 a	0.35 a
		0.001 g/L/ every week	2X	3.89 a	4.68 a	5.00 b	5.39 b	6.41 a	0.60 a	0.17 a
Pond Protect (dry)	5	control		6.41 a	4.49 a	4.74 a	3.81 a	3.88 a	3.74 a	0.56 a
		0.001 g/L/ every 3 days	1X	6.23 a	6.06 b	5.33 a	3.81 a	4.00 a	3.78 a	3.23 b
		0.002 g/L/every 3 days	2X	7.03 a	5.55 b	6.26 b	4.01 a	4.06 a	4.39 a	1.56 ab
Pond Protect (liquid)	5	control		6.37 a	5.24 a	4.76 a	4.76 a	4.74 a	3.83 a	0.03 a
		0.003mL/L/ every 3 days	1X	6.63 a	5.30 a	4.85 a	4.82 a	4.98 a	5.99 a	2.25 a
		0.006mL/L/ every 3 days	2X	7.14 a	5.55 a	4.97 a	5.01 a	5.18 a	5.57 a	0.98 a
Sewper Rx	2	control		5.91 a	5.09 a	4.40 a	3.73 a	3.82 a	3.86 a	0.61 a
		0.0015 g/L/ every week	1X	6.58 a	5.34 a	5.45 a	3.93 a	3.97 a	4.10 a	0.48 a
		0.003 g/L/ every week	2X	6.64 a	5.64 a	4.34 a	4.03 a	3.93 a	4.08 a	0.60 a
SHRIMP SHIELD	17	control		5.24 a	4.74 a	4.28 a	3.88 a	3.68 a	2.23 a	0.37 a
		0.015 g/L/every day	1X	5.65 a	5.25 a	4.96 a	3.99 ab	4.23 a	2.89 a	1.36 a
		0.03 g/L/every day	2X	5.86 a	5.29 a	5.20 a	4.48 b	4.80 a	3.02 a	0.78 a
AQUA-TRON	2	control		4.33 a	4.51 a	4.11 a	4.10 a	3.54 a	0.23 a	0.09 a
		0.001 g/L/ every week	1X	4.39 a	4.58 a	4.34 a	4.48 ab	4.95 a	0.84 a	0.40 a
		0.002 g/L/ every week	2X	4.14 a	4.92 a	5.25 b	5.64 b	6.66 a	0.64 a	0.22 a

WSR	3	control		5.09 a	4.70 a	4.42 a	3.88 a	3.75 a	2.61 a	0.42 a
		0.075 g/L/ every 5 days	1X	5.73 a	5.33 a	5.04 a	4.08 ab	4.31 a	2.91 a	1.37 a
		0.15 g/L/ every 5 days	2X	5.94 a	5.37 a	5.29 a	4.56 b	4.88 a	3.04 a	0.79 a

Results are presented as mean (n=3);

Values with different letters with in a column are significantly different ($P<0.05$);

Table 3.9- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with Fritz Zyme 360 and Aqua Prob EZ.

Treatment				Dissolved oxygen loss (Δ DO, mg/L) in 24 hours						
				days						
Product name	Application frequency	Dose		1	3	5	7	9	13	17
Fritz Zyme 360	1	control		13.24 a	10.96 ab	3.47 a	2.32 a	1.57 a	1.17 a	1.38 a
		0.0005 g/L	1X	13.26 a	10.84 ab	4.16 a	2.63 ab	1.24 a	1.06 a	**0.81 b**
		0.001 g/L	2X	12.80 a	9.21 a	3.02 a	2.39 a	1.22 a	0.81 a	**0.62 b**
		0.002 g/L	4X	12.76 a	10.57 ab	4.35 a	3.00 ab	1.70 a	1.08 a	**0.86 ab**
		0.004g/L	8X	12.73 a	11.86 b	4.09 a	3.62 b	3.60 b	0.99 a	**0.74 b**
	2	0.0005g/L/every week	1X	12.43 a	11.21 a	4.38 a	2.78 a	1.95 a	0.95 a	**0.63 b**
		0.001 g/L/every week	2X	12.85 a	9.28 a	2.70 a	2.14 a	1.37 a	0.75 a	**0.51 b**
Aqua Prob EZ	6	control		13.69 a	9.40 ab	3.86 a	4.12 a	3.08 ab	1.06 a	0.97 a
		0.0125 g/L/every 3 day	1X	**10.18 bc**	13.25 ac	5.51 a	4.66 a	2.81 a	2.36 ac	1.42 a
		0.025 g/L/every 3 day	2X	12.03 acd	8.11 b	4.10 a	2.66 a	2.66 a	1.89 ac	0.92 a
		0.05 g/L/every 3 day	4X	12.45 ac	14.20 c	5.44 a	3.98 a	4.62 b	3.66 bc	2.25 b
		0.1 g/L/every 3 day	8X	**9.72 bd**	12.19 abc	6.16 a	4.06 a	4.19 ab	5.44 b	3.15 c
	17	0.0125 g/L/every day	1X	12.06 a	13.54 a	6.12 a	3.98 a	4.02 a	1.68 a	1.07 a
		0.025 g/L/every day	2X	12.20 a	12.62 a	5.51 a	3.89 a	3.98 a	2.36 a	1.43 a

Results are presented as mean (n=3);
Values with different letters with in a column are significantly different (P<0.05);
Bold means significantly lower (P<0.05) than the control

Table 3.10- Changes in dissolved oxygen loss in 24 hours (Δ DO, mg/L) at various days after treatment with Aqua PE and Pond Protect (dry).

Treatment				Dissolved oxygen loss (Δ DO, mg/L) in 24 hours						
				days						
Product name	Application frequency	Dose		1	3	5	7	9	13	17
Aqua PE	1	control		12.67 a	9.51 a	4.55 a	3.52 a	2.27 a	1.87 a	0.96 a
		0.00125 g/L	1X	10.09 ab	9.15 a	5.13 a	3.57 a	1.30 a	1.83 a	1.47 a
		0.0025 g/L	2X	10.54 ab	9.27 a	4.60 a	3.92 ab	1.59 a	1.80 a	0.76 a
		0.005 g/L	4X	10.54 ab	11.37 a	4.35 a	5.11 b	2.50 a	1.30 a	0.77 a
		0.01 g/L	8X	**9.70 b**	9.33 a	4.45 a	3.60 a	1.77 a	1.01 a	0.82 a
	2	0.00125 g/L/every week	1X	11.14 a	11.35 a	4.65 a	5.14 b	2.22 a	1.74 a	0.83 a
		0.0025 g/L/every week	2X	14.15 a	10.65 a	4.55 a	3.76 ab	1.46 a	1.07 a	0.52 a
Pond Protect (dry)	3	control		13.10 a	10.01 a	4.32 a	3.53 ab	2.84 a	1.86 a	0.63 a
		0.001 g/L/every 5 days	1X	13.12 a	10.11 a	4.27 a	3.03 b	2.06 b	1.10 b	0.67 a
		0.002 g/L/every 5 days	2X	13.94 b	11.92 ab	5.74 a	4.56 c	2.20 ab	0.92 b	0.60 a
		0.004 g/L/every 5 days	4X	14.10 b	12.59 ab	3.84 b	3.16 ab	1.97 b	0.90 b	0.50 a
		0.08 g/L/every 5 days	8X	13.95 b	13.12 b	4.86 ab	3.44 ab	2.65 ab	1.63 a	1.29 b
	5	0.001 g/L/every 3 days	1X	13.61 b	12.23 a	4.91 a	3.71 b	2.17 ab	**1.03 b**	0.53 a
		0.002 g/L/every 3 days	2X	14.02 b	12.30 a	4.06 a	**3.01 c**	**2.03 b**	**1.00 b**	0.57 a

Results are presented as mean (n=3);
Values with different letters with in a column are significantly different (P<0.05);
Bold means significantly lower (P<0.05) than the control

Table 3.11- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with Aqua Prob 4X, Aqua Bio-Trol, Lake & Pond Bacteria, Pond Protect (liquid), Sewper Rx, SHRIMPSHIELD, AQUA-TRON and Waste & Sludge Reducer (WSR) at application rates recommended by the manufacturers.

Treatment				Dissolved oxygen loss (Δ DO, mg/L) in 24 hours						
				days						
Product name	Application	Dose		1	3	5	7	9	13	17
Aqua Prob 4X	2	control		13.61 a	10.29 a	4.55 a	3.37 a	2.27 a	1.82 a	0.95 a
		0.0025 g/L/every week	1X	13.57 a	9.10 a	3.42 a	2.90 a	1.98 a	0.73 ab	0.53 a
		0.005 g/L/every week	2X	11.99 a	9.05 a	3.73 a	3.17 a	2.18 a	0.43 b	0.71 a
		0.01 g/L/every week	4X	11.77 a	9.70 a	4.29 a	3.88 a	3.59 a	0.89 ab	1.36 a
		0.02 g/L/every week	8X	13.43 a	11.18 a	4.12 a	3.54 a	2.88 a	1.15 ab	1.14 a
Aqua Bio-Trol	1	control		12.71 a	9.35 a	4.62 a	4.30 a	4.17 a	2.52 a	1.69 a
		0.025 g/L	1X	13.74 b	12.11 b	7.94 b	4.93 a	4.68 a	3.33 a	0.73 a
		0.05 g/L	2X	14.26 b	12.03 b	7.07 b	5.31 a	4.28 a	2.17 a	0.96 a
		0.1 g/L	4X	13.16 ab	11.80 b	7.09 b	5.84 a	5.44 a	2.91 a	0.85 a
		0.2 g/L	8X	13.63 ab	11.71 b	8.09 b	5.78 a	5.26 a	3.51 a	2.54 a
Lake & Pond Bacteria	1	control		12.30 a	9.84 a	3.46 a	3.23 a	1.73 a	0.80 a	1.46 a
		0.0005 g/L	1X	12.59 a	10.41 a	4.94 b	3.96 ab	2.42 a	1.15 a	1.21 a
		0.001 g/L	2X	12.19 a	9.15 a	4.84 b	4.12 ab	1.79 a	1.65 a	1.47 a
		0.002 g/L	4X	14.05 a	8.84 a	4.73 ab	4.21 ab	2.24 a	1.26 a	1.44 a
		0.004 g/L	8X	12.90 a	9.14 a	5.03 b	4.46 b	2.41 a	1.56 a	1.17 a
Pond Protect (liquid)	3	control		13.02 a	10.96 a	3.83 a	2.71 a	1.60 a	1.25 a	1.55 a
		0.003 mL/L/every 5 days	1X	12.72 a	10.45 a	3.86 a	2.83 a	1.80 a	0.96 a	1.40 a
		0.006 mL/L/every 5 days	2X	12.65 a	10.81 a	3.66 a	2.71 a	1.61 a	0.99 a	0.59 a
		0.012mL/L/every 5 days	4X	12.32 a	10.06 a	3.83 a	2.78 a	1.75 a	0.94 a	0.73 a
		0.024 mL/L/every 5 days	8X	12.37 a	11.44 a	3.94 a	2.79 a	1.65 a	1.25 a	1.15 a
Sewper Rx	1	control		12.91 a	10.09 a	4.40 a	3.73 a	1.83 a	1.86 a	0.61 ab
		0.0015 g/L	1X	12.22 a	10.14 a	3.58 a	3.99 a	1.82 a	1.81 a	0.03 a
		0.003 g/L	2X	12.98 a	10.13 a	4.79 a	3.81 a	1.86 a	1.90 a	0.76 ab
		0.006 g/L	4X	13.52 a	10.53 a	5.42 a	3.82 a	1.88 a	2.00 a	1.56 ab
		0.012 g/L	8X	13.67 a	11.00 a	5.27 a	3.79 a	1.91 a	2.12 a	3.07 b
SHRIMPSHIELD	6	control		12.57 a	10.80 a	4.04 a	3.32 a	2.65 a	1.32 a	0.98 a
		0.015 g/L/every 3 day	1X	11.65 a	11.80 ab	5.97 a	3.63 a	2.65 a	3.44 b	1.96 ab
		0.03 g/L/every 3 day	2X	12.17 a	11.27 a	5.27 a	4.04 a	3.57 a	4.67 b	3.88 ab

		0.06 g/L/every 3 day	4X	11.40 a	13.45 b	5.84 a	4.37 a	3.51 a	7.69 c	3.30 ab
		0.12 g/L/every 3 day	8X	11.25 a	13.39 b	9.49 b	6.80 b	5.15 a	7.84 c	6.34 b
AQUA-TRON	1	control		14.52 a	10.63 a	2.93 a	2.73 a	1.14 a	0.82 a	1.09 a
		0.001 g/L	1X	14.70 a	8.96 a	1.73 a	1.96 a	0.85 a	2.06 a	1.38 ab
		0.002 g/L	2X	14.42 a	9.42 a	2.44 a	2.19 a	0.48 a	1.57 a	2.18 b
		0.004 g/L	4X	13.76 a	8.65 a	1.55 a	1.63 a	0.31 a	1.62 a	1.87 ab
		0.008 g/L	8X	14.10 a	10.21 a	2.32 a	1.91 a	0.47 a	1.56 a	1.46 ab
WSR	1	control		12.71 a	10.35 a	4.62 a	3.37 a	2.52 a	1.19 a	1.19 a
		0.075 g/L	1X	13.23 a	11.30 a	5.98 a	3.71 a	3.95 a	1.15 a	1.15 a
		0.15 g/L	2X	11.57 a	9.87 a	4.68 a	3.50 a	3.63 a	2.26 a	2.26 a
		0.3 g/L	4X	12.96 a	9.96 a	4.56 a	3.56 a	2.53 a	3.07 a	3.07 a
		0.6 g/L	8X	13.90 a	10.83 a	6.63 a	5.00 a	1.97 a	1.83 a	2.03 a

Results are presented as mean (n=3);

Values with different letters with in a column are significantly different (P<0.05);

Table 3.12- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with Aqua Prob 4X, Aqua Bio-Trol, Lake & Pond Bacteria, Pond Protect (liquid), Sewper Rx, SHRIMPSHIELD, AQUA-TRON and Waste & Sludge Reducer (WSR) at higher doses and greater application frequencies that recommended by manufacturers.

Treatment				Dissolved oxygen loss in 24 hours (Δ DO, mg/L)						
				days						
Product name	Application frequency	Dose		1	3	5	7	9	13	17
Aqua Prob 4X	4	control		13.61 a	10.29 a	4.55 a	3.37 a	2.27 a	1.82 a	0.95 a
		0.0025 g/L/ every 4 days	1X	12.92 a	10.12 a	3.58 a	3.22 a	2.30 a	0.61 b	0.14 a
		0.005 g/L/every 4 days	2X	13.08 a	10.42 a	4.06 a	3.32 a	2.71 a	1.04 ab	0.53 a
Aqua Bio-Trol	2	control		12.71 a	9.35 a	4.62 a	4.30 a	4.17 a	2.52 a	1.69 a
		0.025 g/L/ every week	1X	13.44 ab	11.56 b	6.27 ab	5.77 a	5.24 a	3.06 a	1.08 a
		0.05 g/L/ every week	2X	13.92 b	11.53 b	6.91 b	5.83 a	5.04 a	1.48 a	0.63 a
Lake & Pond Bacteria	2	control		12.30 a	9.84 a	3.46 a	3.23 a	1.73 a	0.80 a	1.46 a
		0.0005 g/L/ every week	1X	13.03 a	9.49 a	4.82 ab	5.39 b	4.60 b	1.69 a	1.41 a
		0.001 g/L/ every week	2X	13.03 a	8.81 a	5.05 b	3.99 a	1.78 ab	1.54 a	1.37 a
Pond Protect (liquid)	5	control		13.02 a	10.96 a	3.83 a	2.71 a	1.60 a	1.25 a	1.55 a
		0.003mL/L/ every 3 days	1X	12.86 a	10.94 a	3.96 a	2.64 a	1.33 a	1.46 a	1.45 a
		0.006mL/L/ every 3 days	2X	12.96 a	11.31 a	3.36 a	2.61 a	1.86 a	1.47 a	1.14 a
Sewper Rx	2	control		12.91 a	10.09 a	4.40 a	3.73 a	1.83 a	1.86 a	0.61 a
		0.0015 g/L/ every week	1X	13.58 a	10.34 a	5.44 a	3.92 a	1.98 a	2.10 a	0.48 a
		0.003 g/L/ every week	2X	13.64 a	10.64 a	4.34 a	4.03 a	1.93 a	2.08 a	0.60 a
SHRIMP SHIELD	17	control		12.57 a	10.80 a	4.04 a	3.32 a	2.65 a	1.32 a	0.98 a
		0.015 g/L/every day	1X	11.28 a	13.11 b	7.15 b	4.74 a	2.89 a	3.66 b	2.75 a
		0.03 g/L/every day	2X	11.80 a	13.91 b	10.46 c	7.68 b	6.56 b	7.39 c	4.34 a
AQUA-TRON	2	control		14.52 a	10.63 a	2.93 a	2.73 a	1.14 a	0.82 a	1.09 a
		0.001 g/L/ every week	1X	13.18 a	12.71 a	2.89 a	2.24 a	0.60 a	0.95 a	1.52 a
		0.002 g/L/ every week	2X	14.09 a	10.64 a	4.11 a	2.84 a	1.39 a	1.76 a	1.65 a
WSR	3	control		12.71 a	10.35 a	4.62 a	3.37 a	2.52 a	1.19 a	1.19 a
		0.075 g/L/ every 5 days	1X	12.56 a	10.68 a	5.79 a	4.13 a	4.71 a	3.35 a	3.35 a
		0.15 g/L/every 5 days	2X	14.03 a	10.82 a	5.78 a	4.34 a	4.27 a	3.17 a	3.17 a

Results are presented as mean (n=3).
Values with different letters with in a column are significantly different ($P<0.05$).

Chapter 4 The Influence of the Bacterial Amendment (Waste & Sludge Reducer) on Water Quality in Channel Catfish (*Ictalurus punctatus*) Ponds

ABSTRACT: The effects of a selected bacterial amendment (Waste & Sludge Reducer - WAS) in channel catfish ponds were evaluated at the E. W. Shell Fisheries Center, Auburn University, Alabama. Three ponds were treated with the bacterial amendment WSR at 3 times the dose recommended by the manufacturer every 2 weeks, and three served as controls. There were no differences ($P>0.05$) in total bacterial count after treatment with WAS on most of the days between May and September. Few differences ($P<0.05$) were observed in pH and concentrations of POM, Cha A, COD, SCOD, TAN and NO_2^-–N, between ponds treated with WAS and control ponds. Channel catfish yield was not improved by treatment with WAS. The potential benefits of this bacterial amendment and the exact mechanism of the bacterial amendment's action requires further study.

Key Words: Channel catfish ponds, bacterial amendment, water quality

Introduction

Channel catfish (*Ictalurus punctatus*) have been cultured for many years as the dominant farm-raised aquaculture species in the United States, especially in the southeastern states of Alabama, Arkansas, Mississippi, and Texas (FAO, 2013; Hanson and Sites, 2013). In 2003, the volume of catfish processed increased to 660 million pounds from 225 million pounds in 1995. However, in recent years, catfish consumption has declined with only 334 million pounds of catfish being processed in 2013.

Channel catfish ponds are being stocked at increasingly higher densities because of development of reliable mechanical aerator and other improvement in technology High stocking densities result in the deterioration of water quality, including much greater concentrations of total ammonia nitrogen, nitrite nitrogen, and dissolved organic matter as well as more sediment organic matter accumulation than in the past. Fish stressed by un-ionized ammonia and nitrite eat less, grow slower, and are more susceptible to disease, while organic matter accumulation provides havens for certain disease organisms. Therefore, water quality has been recognized as a limiting factor in the catfish industry (Boyd, 1998a; Colt et al., 1981; Craig S. Tucker, 2009; Tucker and Robinson, 1990b).

Although studies done 10 to 30 years ago in catfish ponds reported no benefit of bacterial augmentation on water and sediment quality (Boyd et al., 1984; Boyd and Gross, 1998; Chiayvareesajja and Boyd, 1993; Gräslund et al., 2003; Queiroz and Boyd, 1998), the beneficial effects of microbial inoculums on water quality have been revealed under controlled laboratory conditions (Barik et al., 2011). In spite of the conflicting record of success, there is interest worldwide in using bacterial amendments for the maintenance of optimum water quality in aquaculture ponds.

It is possible that the water quality in the ponds of previous studies did not deteriorate sufficiently to allow the effects of the bacterial amendments to be expressed (Boyd and Gross, 1998). Moreover, the new generation bacterial amendments could possibly be more effective than the original ones used in aquaculture. Therefore, the objectives of this study were to evaluate the potential efficacy of a selected bacteria amendment (Waste & Sludge Reducer) in catfish ponds.

Materials and Methods

Six ponds (0.04-ha) at the E. W. Shell Fisheries Center, Auburn University, Alabama were stocked on 3 March, 2014 with 15,000 fingerling channel catfish (*Ictalurus punctatus*) per hectare. The fingerlings had an average weight of 30.8 g/fish. Three ponds were treated with the bacterial amendment WASTE & SLUDGE REDUCER (WSR) purchased from Keeton Industries (Wellington, CO, USA) at three times the dose recommended by the manufacturer (initial dose = 0.68 kg/0.04-ha; maintenance = 0.23 kg/ 0.04-ha/2 weeks), and three ponds served as controls. Five fingerling grass carp (*Ctenopharyngodon idellus*) were stocked into each pond on 3 March, 2014 for aquatic macrophyte control. Fish were fed daily with a 32% crude protein feed (Alabama Catfish Feed Mill, Uniontown, AL, U.S.A). Feeding rate was adjusted weekly based on estimated weight gain, a food conversion of 1.5, and observed rate of feed consumption. Water was added routinely to replace evaporation and seepage from ponds, but water exchange was not applied. Dissolved oxygen concentration was measured daily at dawn and ponds were aerated, particularly at night, when necessary.

Water samples were collected weekly between 5 May and 29 Sep 2014, with a 90-cm water column sampler (Boyd and Tucker, 1992). Samples were transported to the laboratory and analyzed immediately for pH, water temperature, nitrite nitrogen, particulate organic matter, chlorophyll a, and soluble and total chemical oxygen demand according to standard protocol (Eaton et al. 2005). Total ammonia nitrogen was measured weekly by the salicylate method (Le and Boyd, 2012).

Ponds were drained and fish were harvested between 9 Oct and 11 Oct, 2014. The fish from each pond were counted and weighed.

Statistics

Differences among means were assessed for statistical significance ($P<0.05$) using two-tailed t-tests (Microsoft Excel, San Diego, CA, USA).

Results and Discussion

There were no differences ($P>0.05$) in mean of pH, total bacterial count, and concentrations of particle organic matter (POM), chlorophyll a (Chl a), total ammonia nitrogen (TAN) and nitrite nitrogen (NO_2^-–N), chemical oxygen demand (COD) and soluble chemical oxygen demand (SCOD) after treatment with the bacterial amendment Waste & Sludge Reducer (Table 4.1).

Averages for pH, total bacterial count, and concentrations of POM and Chl a, for untreated ponds and ponds treated with WAS are presented in Fig 4.1. The pH of pond waters ranged from 7.51 and 9.82. There were no differences ($P>0.05$) in pH, total bacterial count, and concentrations of POM and Chl a after treatment with the bacterial amendment (WAS) on most of sampling dates between May and September. The total bacterial count in all six pond waters remained stable in the first 3 months and increased after late-August. On five sampling dates, there were higher ($P<0.05$) total bacterial count in ponds treated with the bacterial amendment (WAS). The general trend of POM concentration in treated and untreated water was a gradual increase between May and September. There were higher concentrations of POM ($P<0.05$) in pond water treatment with WAS on 12 May, 18 Aug and 1 Sep. Concentrations of Cha a in pond water could not be detected until mid-June, and then generally increased over time. Higher Cha a ($P<0.05$) were detected in control ponds in early-May and late-June.

There were few differences ($P<0.05$) between ponds treated with WAS and the control ponds in concentrations of TAN and nitrite nitrogen, COD and SCOD (Fig 4.2). The TAN concentrations in all ponds were less than 0.2 mg/L until August. On one sampling date in August, there was a higher ($P<0.05$) TAN concentration in the control, but on another sampling data in September, there was a greater concentration ($P<0.05$) of TAN in the ponds treated with WAS. The nitrite nitrogen of all pond waters for the study increased with time, especially after mid-August. Concentrations of nitrite nitrogen were higher ($P<0.05$) in WAS treated ponds than in control ponds on one sampling date (14 July). The COD and SCOD were similar between treated and control ponds between May and September. Similarly as described for TAN, on one sampling date in July there was more ($P<0.05$) COD in the control, but on another sampling data in September, there was a larger ($P<0.05$) COD in the ponds treated with WAS. Only on the first sampling date, May 5, there was significantly less ($P<0.05$) SCOD in ponds treated with WAS.

Fish production data are summarized in Table 4.2. Harvest weights of catfish averaged 3,023 kg/ha in ponds treated with WAS, and 2,955 kg/ha for control ponds. No significant difference ($P>0.05$) in fish surviving, net production, average weight per fish and feed conversion rate (FCR) were observed in this study. Catfish yield was not improved by treatment with WAS.

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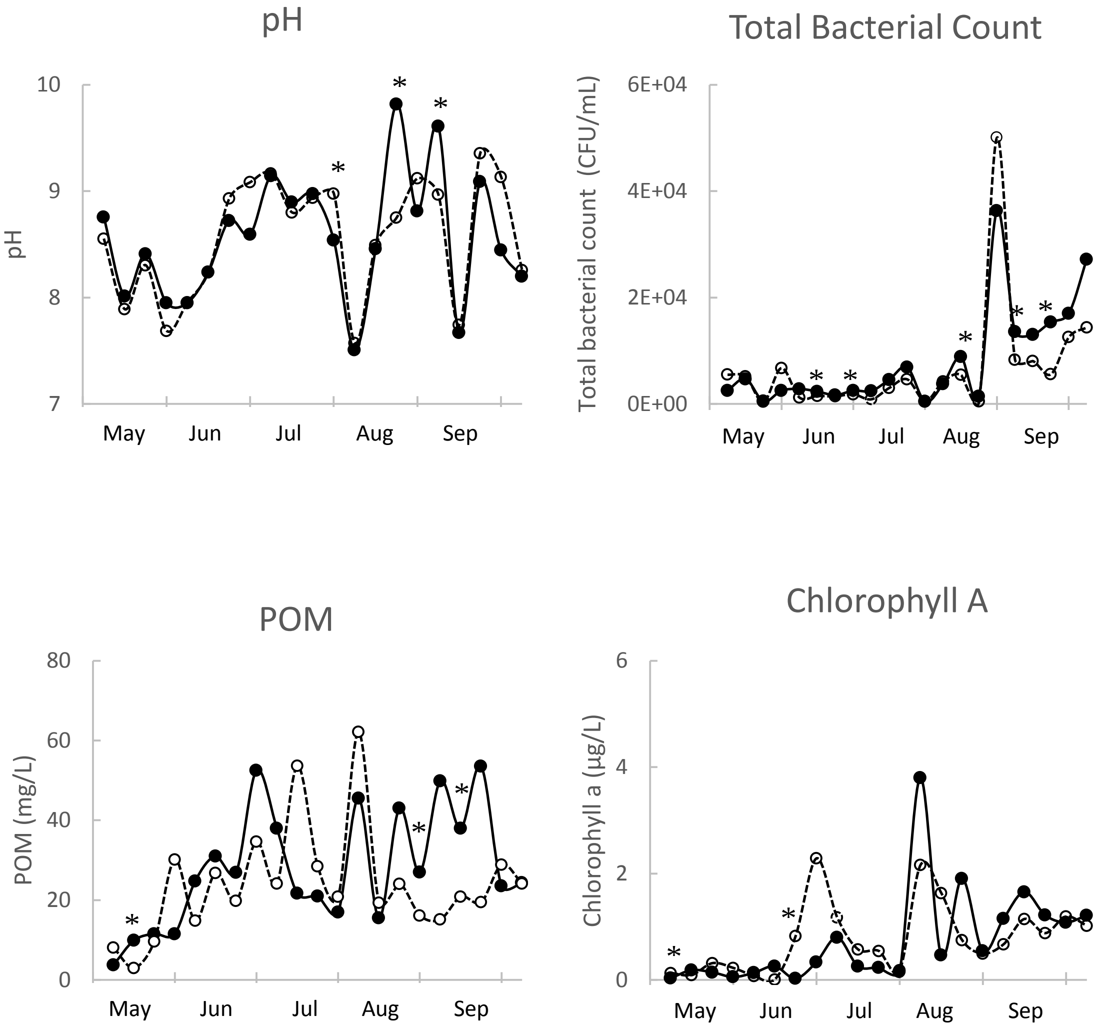

Fig 4.1- The pH, total bacterial count, and concentrations of particulate organic matter (POM), chlorophyll a (Chl a) in three channel catfish ponds treaded with a bacterial amendment , Waste & Sludge Reducer (dots and solid lines) and in three control ponds (open circles and dashed lines). Stars indicate a different ($P<0.05$) in concentrations of variables between treatment and control ponds.

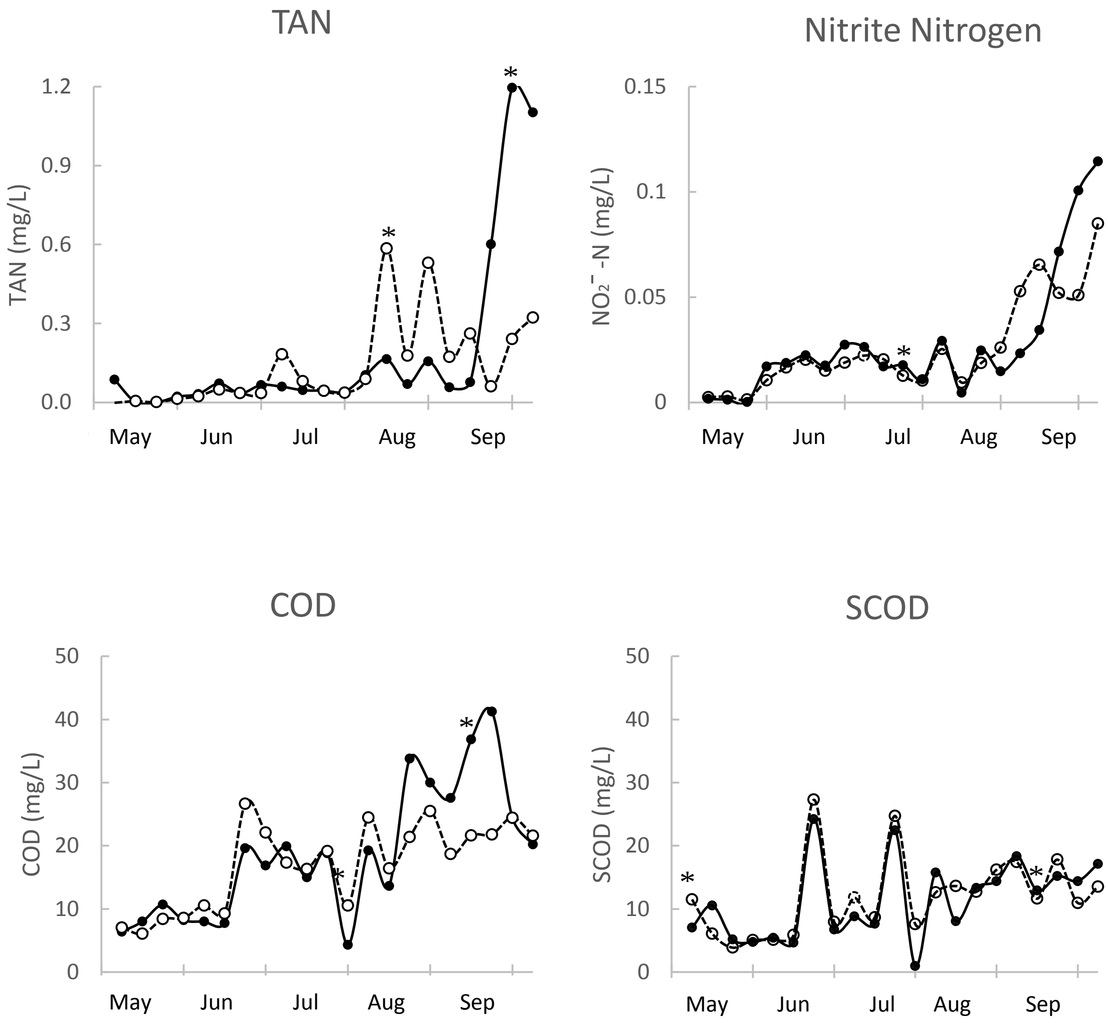

Fig 4.2- Concentrations of total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^-–N), chemical oxygen demand (COD) and soluble chemical oxygen demand (SCOD) in three channel catfish ponds treaded with a bacterial amendment , WAS (dots and solid lines) and in three control ponds (open circles and dashed lines). Stars indicate a different ($P<0.05$) in concentrations of variables between treatment and control ponds.

Table 4.1- Mean of pH, total bacterial count, and concentrations of particulate organic matter (POM), chlorophyll a (Chl a), total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^-–N), chemical oxygen demand (COD) and soluble chemical oxygen demand (SCOD) in three channel catfish ponds receiving a bacterial amendment (WAS) and in three control ponds

Variable	**Treated**	**Control**
pH	8.49 a	8.57 a
Total bacterial count	8.12E+03 a	6.77E+03 a
Particulate organic matter (mg/L)	28.09 a	24.03 a
Chlorophyll a (µg/L)	0.75 a	0.78 a
Total ammonia nitrogen (mg/L)	0.191 a	0.140 a
Nitrite nitrogen (mg/L)	0.028 a	0.026 a
Chemical oxygen demand (mg/L)	18.60 a	17.05 a
Soluble chemical oxygen demand (mg/L)	11.34 a	12.01 a

Means within a row with a letter in common are not different ($P>0.05$)

Table 4.2- Average production data for channel catfish ponds receiving a bacterial amendment (WAS) and in control ponds

Variable	**Treated**	**Control**
Stocking rate (per hectare)	15,000	15,000
Fish surviving until harvest (per hectare)	11,625 a	11,817 a
Net production (kg/ha)	3023 a	2955 a
Average harvest weight per fish (kg)	0.26 a	0.25 a
FCR (feed convertion rate)	1.11 a	1.14 a

Values with same letter with in a column are not different (P>0.05)

Chapter 5 Effects of Bacterial Amendments (Aqua PE and Oxyless) on Reducing Total Ammonia Nitrogen in Polluted Channel Catfish Ponds

ABSTRACT: The effects of selected bacterial amendments (Aqua PE and Oxyless) were evaluated in polluted channel catfish ponds at the E. W. Shell Fisheries Center, Auburn University, Alabama. Three ponds were treated with bacterial amendments (Aqua PE and Oxyless) at the dose recommended by the manufacturer and three served as controls. No improvements were observed in accelerating the rates of TAN removal in ponds with ammonium sulfate addition from the use of the Aqua PE and Oxyless in combination. There were minor beneficial effects on TAN removal from day 5 to day 8 in ponds with high ammonium sulfate addition (225 kg/ha ammonium sulfate applied on day 4) after treatment with the bacterial amendments (Aqua PE and Oxyless).

***Key Words*:** Channel catfish ponds, bacterial amendment, total ammonia nitrogen, nitrate nitrogen

Introduction

Catfish ponds can be produced at increasingly higher densities because of better mechanical aeration techniques and other technological advances. In the southern United States, average yield of catfish ponds has increased from less than 2,000 kg/ha in the 1960s to approximately 5,000 kg/ha in recent years (Hanson and Sites, 2012). With higher stocking densities, pond waters receive much greater inputs of ammonia nitrogen, which is the major nitrogenous waste product of fish and of bacteria that decompose organic wastes in ponds (Boyd and Tucker, 2014). Ammonia nitrogen occurs in water as two forms, un-ionized ammonia (NH_3) and ammonium ion (NH_4^+) in a pH and temperature dependent equilibrium:

$NH_3 + H_2O = NH_4^+ + OH^-$, $K_b = 10^{-4.74}$.

As can be seen form the equation above, the proportion of ammonia nitrogen in NH_3 form increases with greater pH. This form is considered toxic to fish, while NH_4^+ is comparatively non-toxic (Warren, 1962). The pH in catfish ponds trend to increase during daytime because of carbon dioxide removal from water for phytoplankton photosynthesis. Thus many catfish ponds in Alabama have high NH_3 concentration especially during the afternoon in warm months (Zhou and Boyd, 2015).

A negative, linear relationship between channel catfish growth and increasing ammonia nitrogen concentration was reported over the concentration range of 0.07 to 1.2 mg/L during a month trial (Colt and Armstrong, 1981). Moreover, no weight gain was reported when the ammonia nitrogen concentration was above 1.17 mg/L. It was also observed that when fish in ponds with high concentration of total ammonia nitrogen become infected with disease, they usually do not respond to treatment with medicated feed until ammonia concentrations are decreased to an acceptable level (William Hemstreet, Alabama fish farming center, personal communication). Ammonia seldom kills fish and shrimp directly, but it can stress them causing them to eat less, grow slower and be more susceptible to disease (Boyd and Tucker, 1998).

Although some ammonia diffuses from pond waters into the air (Gross et al., 1999) or is removed by phytoplankton (Tucker et al., 1984), a major process of that lessen ammonia concentration by transforming it to nitrate (a non-toxic form of nitrogen) in ponds is microbial nitrification (Avnimelech et al., 1986; Gross et al., 2000; Hargreaves, 1998). Microbial nitrification is the sequential, two-step oxidation of ammonia nitrogen to nitrate by bacteria of the genera *Nitrosomonas* and *Nitrobacter*. Disruptions of bacterial communities in ponds may high-levels of accumulation of ammonia and fish toxicity (Boyd, 1998b; Mischke, 2003). There has been considerable interest worldwide in applying bacterial inocula to ponds in order to hasten ammonia nitrogen oxidation through bacterial nitrification (Boyd and Gross, 1998; Gräslund et al., 2003). Although commercial bacterial amendments (Aqua PE and Oxyless) are promoted for use in Alabama ponds to lessen high ammonia concentration, studies have not been conducted to verify the efficiency of these two amendments. Thus, there is no knowleage of the conditions under which benefits may be accrued on scientific justification for trail use. Therefore, the objectives of this study were to evaluate the potential efficacy of the bacterial amendment combination (Aqua PE and Oxyless) for lessening total ammonia nitrogen concentration in catfish ponds.

Materials and Methods

The bacterial amendment (Aqua PE and Oxyless) were purchased from Eurovix USA, Inc. and tested at doses recommended by manufacturer (Aqua PE: 62.5 tablets/ha; Oxyless: 25 tablets/ha) in six ponds (0.04-ha) stocked with channel catfish at 15000/ha at the E. W. Shell Fisheries Center, Auburn University, Alabama.

Three ponds were treated with the bacterial amendments Aqua PE and Oxyless, and three served as controls. The water in each pond was artificially polluted by adding different amounts of ammonium sulfate fertilizer (21% N). Six ponds were treated as follows:

Pond 1 = B_0+A_1

Pond 2 = B_1+A_1

Pond 3 = B_0+A_2

Pond 4 = B_2+A_2

Pond 5 = B_0+A_3

Pond 6 = B_3+A_3

A_1 = low ammonia concentration – 112.5 kg/ha ammonium sulfate applied on 30 September, 2014 (day 0);

A_2 = medium ammonia concentration – 150 kg/ha ammonium sulfate applied on 2 October, 2014 (day 2);

A_3 = high ammonia concentration – 225 kg/ha ammonium sulfate applied on 4 October, 2014 (day 4);

B_0= without bacterial amendment applied;

B_1= bacterial amendment combination (Aqua PE and Oxyless) applied on 30 September, 2014 (day 0);

B_2= bacterial amendment combination (Aqua PE and Oxyless) applied on 2 October, 2014 (day 2);

B_3= bacterial amendment combination (Aqua PE and Oxyless) applied on 4 October, 2014 (day 4);

Water samples were collected daily between day 0 and day 8 for Pond 1 and Pond 2, between day 2 and day 8 for Pond 3 and Pond 4, and between day 4 and day 8 for Pond 5 and Pond 6. Samples were transported to the laboratory and analyzed immediately for pH, total ammonia nitrogen and nitrate nitrogen. Water pH was measured by the electrometric method (Franson and Eaton, 2005) with an Orion pH meter Model 230 and glass electrode. Total ammonia nitrogen was measured by the salicylate method (Le and Boyd, 2012) and nitrate nitrogen was analyzed by Szechrome NAS reagent method (Polysciences, Inc., Technical Data Sheet 239).

Results and Discussion

The pH of pond water ranged from 7.75 and 9.54 (Fig 5.1). The concentrations of total ammonia nitrogen (TAN) in all ammonia polluted water generally increased until day 5, remained stable on days 6 and 7, and then decreased quickly on day 8 (Fig 5.2). No clearly improvements were observed in accelerating the rate of TAN removal from polluted water resulting from the use of the Aqua PE and Oxyless in combination. Compared to the untreated water, the bacterial amendments in combination (Aqua PE and Oxyless) showed slightly beneficial effects on TAN removal from day 5 to day 8 in pond with the high ammonia addition (225 kg/ha ammonium sulfate on day 4).

Most of the nitrate, the least toxic form of combined inorganic nitrogen, found in natural waters is the end product of nitrification (Boyd, 1998a). The change in concentrations of TAN and nitrate nitrogen in ponds receiving low ammonium sulfate addition in response to application of bacterial amendments is shown in Fig 5.3. The concentrations of TAN in the pond with low ammonium sulfate additions generally increased from 4.5 m/L to 6.5 mg/L during the first 5 days, remained stable on days 5, 6 and 7, and then rapidly dropped from about 6.5 mg/L to 5 mg/L on day 8. Nitrate behaved similarly to TAN, no matter whether treated or untreated with bacterial amendments, the concentration of nitrate nitrogen slightly increased during the first 3 days. Conversely, in the ponds with low ammonium sulfate addition, the concentrations of nitrate nitrogen sharply increased from 1.9 mg/L to 4.2 mg/L in the control and from 1.0 mg/L to 1.6 mg/L after treatment with bacterial amendments on the last day of the trail.

Changes in concentrations of TAN and nitrate nitrogen in ponds receiving medium ammonium sulfate addition in response to application of bacterial amendments are shown in Fig 5.4. The concentrations of TAN and nitrate nitrogen in these ponds generally increased until day 7. On day 8, as the TAN in treated and untreated ponds, the concentrations of nitrate nitrogen in the control pond decreased, while nitrate nitrogen concentration increased on the last sampling day (day 8) in the medium ammonia addition pond that received bacterial amendments.

Concentrations of TAN in ponds of the high ammonium sulfate addition increased to about 7.35 mg/L on day 5, remained stable on days 6 and 7, and then decreased form around 6.85 mg/L to 5.25 mg/L on day 8 (Fig 5.5). Concentrations of nitrate nitrogen remained stable until day 7, but sharply increased form 0.5mg/L to 2.3 mg/L on day 8. It appears that much ammonia nitrogen was transformed into nitrate nitrogen through biological oxidation by bacteria of the genera *Nitrosomonas* and *Nitrobacter*. However, there were not obvious differences in concentrations of ammonia nitrogen and nitrate nitrogen in the treated and untreated ponds. Thus, the oxidation of TAN by nitrifying bacterial would have occurred with or without the addition of the bacterial amendments.

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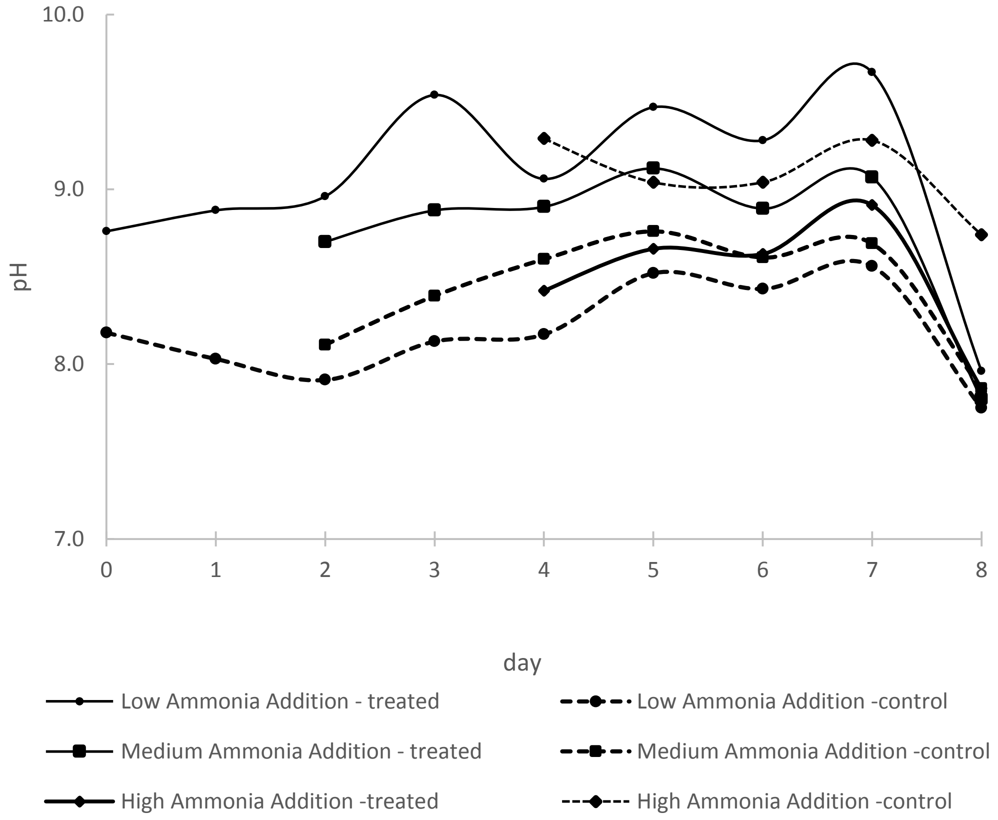

Fig 5.1- The pH of water in ponds over time following treatment by the bacterial amendments, Aqua PE and Oxyless.

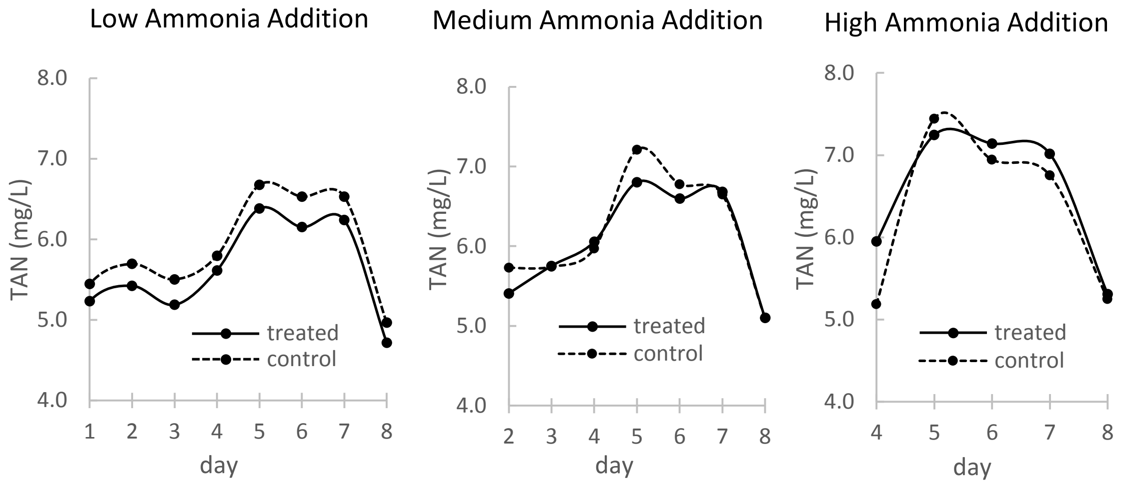

Fig 5.2- Changes in total ammonia nitrogen (TAN) concentration in ponds treated with bacterial amendments (Aqua PE and Oxyless).

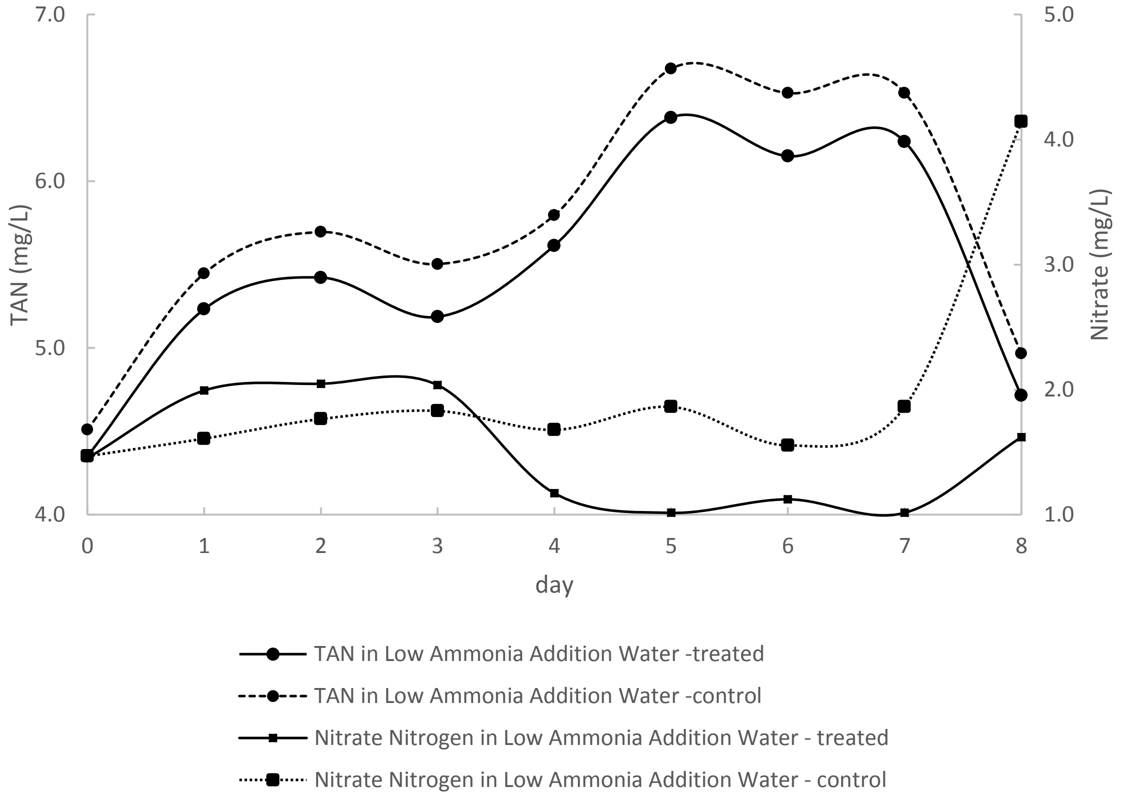

Fig 5.3- Concentrations of total ammonia nitrogen (TAN) and nitrate nitrogen in the ponds treated with 112.5 kg/ha ammonium sulfate either received bacterial amendments (Aqua PE and Oxyless) or served as controls.

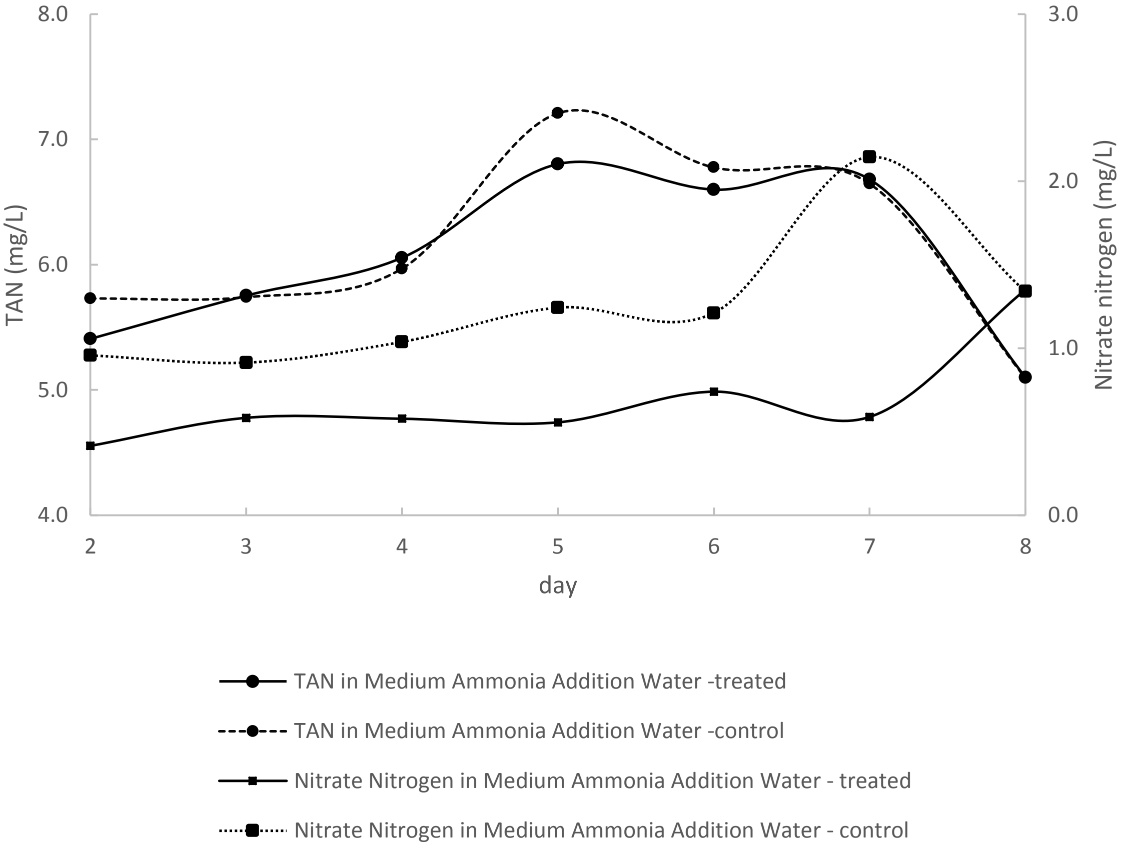

Fig 5.4- Concentrations of total ammonia nitrogen (TAN) and nitrate nitrogen in the ponds treated with 150 kg/ha ammonium sulfate either received bacterial amendments (Aqua PE and Oxyless) or served as controls.

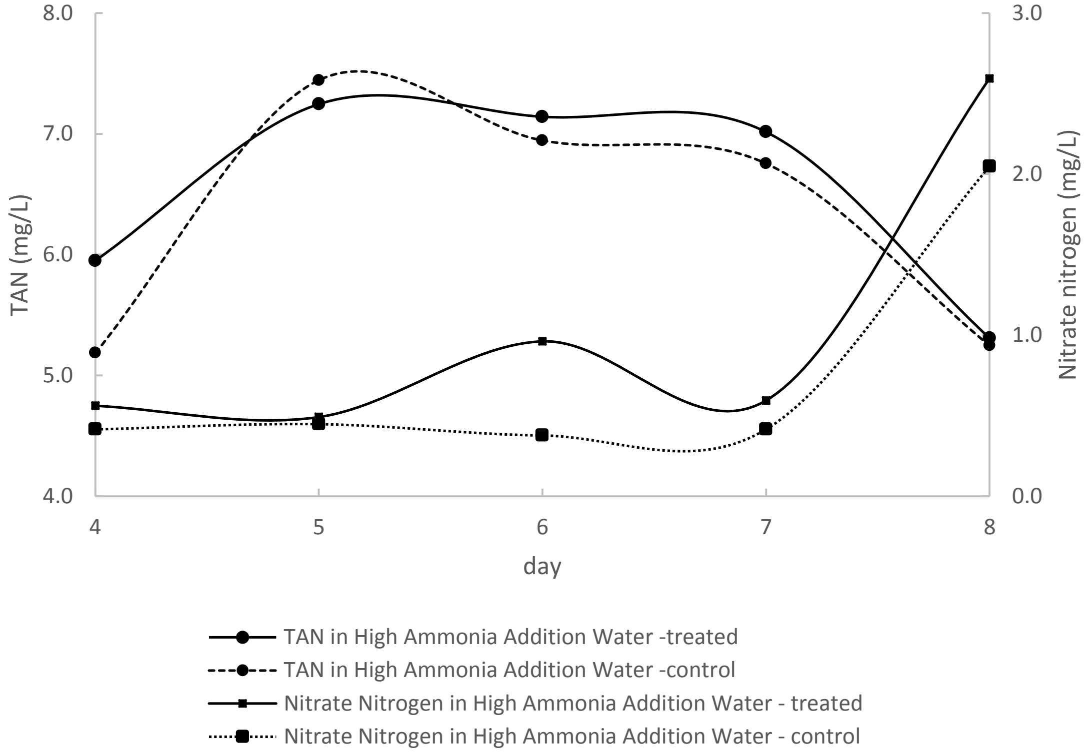

Fig 5.5- Concentrations of total ammonia nitrogen (TAN) and nitrate nitrogen in the ponds treated with 225 kg/ha ammonium sulfate either received bacterial amendments (Aqua PE and Oxyless) or served as controls.

Chapter 6 The Influence of the Bacterial Amendment (Aqua PE) on Water Quality in Alabama Commercial Catfish (*Ictalurus punctatus*) Ponds

ABSTRACT: The effects on water quality of treatment of commercial catfish ponds with a bacterial amendment (Aqua PE) were evaluated in Alabama Catfish, Inc. in west central Alabama. There was less mean concentration of TAN ($P<0.05$) in ponds treated with the bacterial amendment (Aqua PE). Compared to the untreated ponds (controls), Aqua PE gave beneficial effects on TAN removal ($P<0.05$) on 20 June (week 2) and 27 June (week 3). No differences ($P>0.05$) in mean concentrations of nitrite nitrogen was observed between ponds treated with Aqua PE and control ponds. However, there were less concentrations of nitrite nitrogen ($P<0.05$) in the ponds treated with Aqua PE on 27 June (week 3), 18 July (week 6), 1 August (week 8) and 22 August (week 11). There were higher ($P<0.05$) means of turbidity and Chl a concentration in Aqua PE treated pond. On 18 July (week 6), there were higher ($P<0.05$) turbidities in Aqua PE treated ponds. No improvements were observed in accelerating organic matter oxidation from the use of Aqua PE. No differences ($P>0.05$) was observed in pH, and concentrations of TN, TP after treatment with Aqua PE. Catfish yield was not improved by treatment with Aqua PE.

Key Words: Channel catfish ponds, bacterial amendment (Aqua PE), water quality

Introduction

With increased stocking density, water quality in ponds for production of channel catfish (*Ictalurus punctatus*) often deteriorates during the growing season as the result of accumulation of wastes from feed inputs. Greater feed inputs are resulting in higher concentrations of total ammonia nitrogen, nitrite nitrogen, and dissolved organic matter than were observed in the past (Boyd, 1995; Hargreaves, 1998). Pond waters usually develop dense phytoplankton blooms in response to nutrients added in feed and not converted into fish biomass (Boyd, 1998). There was higher turbidity and greater concentrations of total nitrogen and total phosphorus in aquaculture ponds with greater stocking densities (Luz et al., 2012; Van Khoi and Fotedar, 2010).

High concentration of ammonia in aquaculture ponds can cause poor growth and low survival rate (Boyd and Tucker, 1998). The total ammonia nitrogen (TAN) concentrations in ponds often are monitored by farmers, and when the concentrations exceed 2 or 3 ppm, various techniques for reducing TAN concentrations may be implemented. The most common management practices are water exchange, limiting feed input and providing aeration to maintain adequate dissolved oxygen for nitrifying bacteria (Boyd, 1998). High concentrations of nitrite also can be toxic to aquatic organisms by altering hemoglobin. Studies showed that 96-h median lethal concentrations (LC50) for fishes ranged from 0.7 mg/L for rainbow trout (*Salmo gairdneri*) to 27 mg/L for channel catfish (*Ictalurus punctatus*) (Konikoff, 1975; Russo et al., 1981). Excessive organic matter accumulation increases turbidity which inhibits plant growth, decreases oxygen production, limits availability of natural food, and provides a haven for certain disease organisms (Boyd, 1990). Also, large accumulation of organic matter in sediment favors anaerobic conditions at the soil-water interface (Boyd, 1995). Under anaerobic conditions, decomposition of organic matter leads to microbial production of toxic substances such as NO_2^-, H_2S, NH_3 and CH_4 (Boyd and Bowman, 1997).

Bacterial communities play an important role in maintaining water quality in aquaculture ponds, especially in those with high-density stocking rate (Boyd and Tucker, 1992; Mischke, 2003). Disruptions of bacterial communities in ponds may cause accumulation of potentially toxic levels of ammonia and nitrite or lead to dissolved oxygen depletion. There has been considerable interest worldwide in applying biological products including live bacterial inocula to aquaculture ponds for use as water quality conditioners (Boyd and Gross, 1998; Gräslund et al., 2003). Although the commercial bacterial product, Aqua PE, is broadly promoted in Alabama ponds for reducing high ammonia concentration, studies have not been conducted to verify the efficiency of this practice. The objectives of this study were to evaluate the potential efficacy of the bacteria product (Aqua PE) for improving water quality in commercial catfish ponds in Alabama.

Materials and Methods

Six commercial catfish ponds (average 2.83-ha) with high stocking densities of catfish at the Alabama Catfish, Inc. in west central Alabama were chosen. Four ponds were treated with the bacterial amendment Aqua PE from Eurovix USA, Inc., and two served as controls. Catfish ponds in Alabama are typically about 1.5 m in average depth (Boyd et al., 2000). The ponds were managed as multiple-batch system in order to conserve water and allow year around harvest. Marketable-size fish were harvested at intervals, and fingerlings were stocked as replacements. Fish was fed daily with a 32% crude protein feed by truckmounted feeders and fish were always offered more feed than they could consume. Water was added routinely to replace evaporation and seepage from ponds, but water exchange was not applied. Dissolved oxygen was measured routinely and ponds were aerated (6 kW/ha), at night in particular, between May and October.

Water samples were collected from ponds weekly between 13 Jun and 11 Sep, 2013, by dipping surface water with a dipper attached at the end of a 3-m plastic rod (Boyd and Tucker, 1992). Samples were placed in 1-L plastic bottles and held on ice in insulated chests during transported to the laboratory at Auburn University and analyzed immediately for pH, nitrite nitrogen, chlorophyll a, and total chemical oxygen demand according to standard protocol (Eaton et al. 2005). Turbidity of the sample was measured with a Orbeco-Hellige Model 965-10 A Direct Reading Turbidimeter (Orbeco Analytical Systems, Inc, New Jersey, USA). Total nitrogen and total phosphorus analyses were made according to Gross and Boyd (1998). Total ammonia nitrogen was measured by the salicylate method (Le and Boyd, 2012). Data about water surface area, total feed input, and total harvested fish weights were provided by the farm owner.

Statistics

Differences ($P<0.05$) among means were assessed using two-tailed t-tests (Microsoft Excel, San Diego, CA, USA).

Results and Discussion

There were no differences ($P>0.05$) in means of pH, and concentrations of total nitrogen (TN), total phosphorus (TP) and nitrite nitrogen (NO_2^-–N) after treatment with the bacterial amendment – Aqua PE (Table 6.1). Means of turbidity, concentrations of chlorophyll a (Chl a) and chemical oxygen demand (COD) in ponds after treatment with Aqua PE were higher than in control ponds ($P>0.05$). There was less mean concentration of total ammonia nitrogen (TAN) in four catfish ponds treated with Aqua PE, which may not because of Aqua PE, but because the dense algal bloom took up the ammonia.

Average changes in turbidity and concentrations of TN, TP and Chl a, for untreated water and water treated with Aqua PE are shown in Fig 6.1. There were no differences ($p>0.05$) in concentrations of TN, TP, and Chl a after treatment with the bacterial amendment (Aqua PE) on any the sampling days between June and September. Concentrations of TN in all ponds were less than 2 mg/L, excepting on 1 August (week 8). The concentrations of TP remained stable and under 0.3 mg/L between 13 June (week1) and 20 June (week 2) and between 18 July (week 6) and 12 September (week 14). From 27 June (week 3) to 11 July (week 5), the concentrations of TP in treated and control ponds ranged from 0.58 mg/L to 1.45 mg/L. Only on 18 July (week 6), there was higher ($P<0.05$) turbidity in Aqua PE treated pond. Variation in Cha a concentration among catfish ponds usually is high (Boyd, 1979), and this study was no exception.

The pH of pond waters ranged from 6.88 and 9.23. On two sampling dates in June, there was a higher ($p<0.05$) TAN concentration in the ponds treated with Aqua PE (Fig 6.2). No improvements was observed in accelerating organic matter oxidation from the use of the bacterial amendment (Aqua PE) during this study. The COD was similar in treated and untreated (control) ponds between June and September. However, compared to the untreated (control) water, the bacterial amendment (Aqua PE) showed some effects (P

<0.05) on decreasing nitrite nitrogen on 27 June (week 3), 18 July (week 6), 1 August (week 8) and 22 August (week 11).

Fish production data are summarized in Table 6.2. Consistent with previous studies done 18 years ago in catfish ponds, bacterial amendments did not affect production of catfish (Boyd and Gross, 1998; Queiroz and Boyd, 1998). There were no differences ($P>0.05$) in harvest weights of catfish after treatment with Aqua PE (7,255 kg/ha in ponds treated with Aqua PE and 9,540 kg/ha for control ponds). No difference ($P>0.05$) in feed conversion rate (FCR) were observed in this study. Catfish yield was not improved by treatment with Aqua PE.

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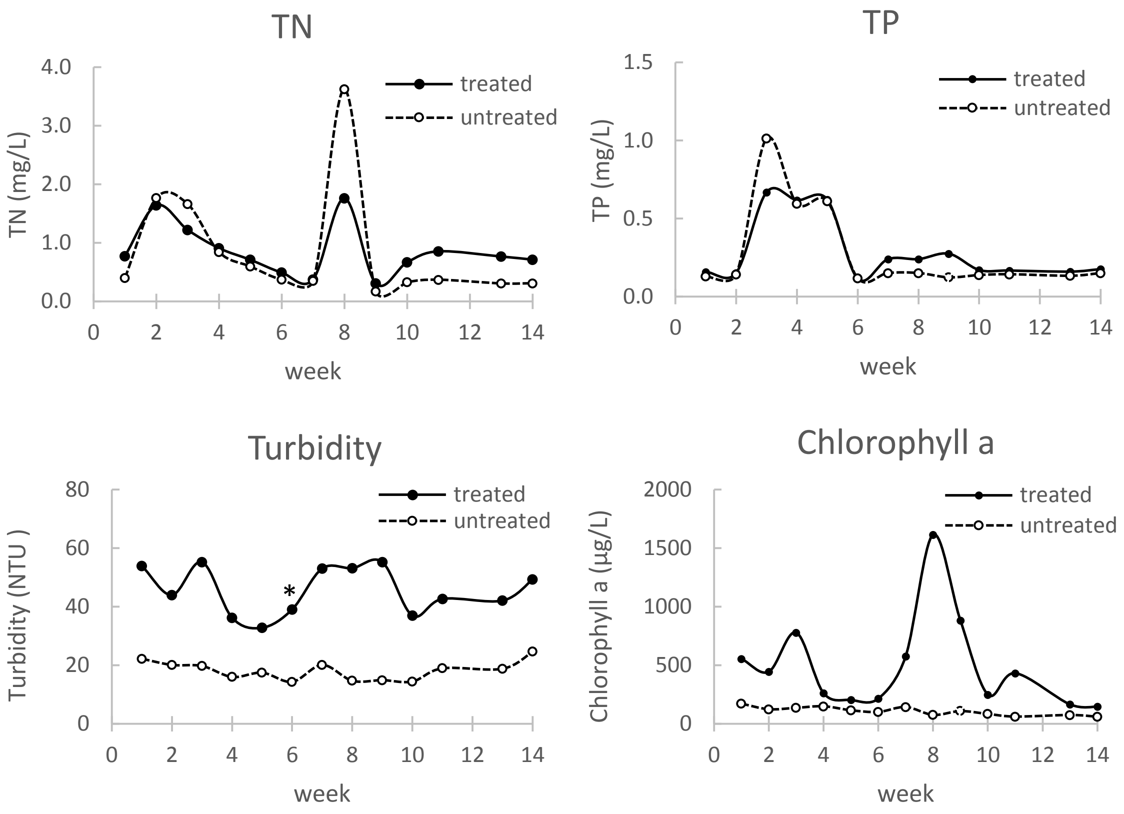

Fig 6.1- Turbidity and concentrations of total nitrogen (TN), total phosphorus (TP), and chlorophyll a (Chl a) in four channel catfish ponds treaded with a bacterial amendment, Aqua PE, (dots and solid lines) and in two untreated (control) ponds (open circles and dashed lines). Stars indicate difference ($P<0.05$) in concentrations of variable between treatment and control.

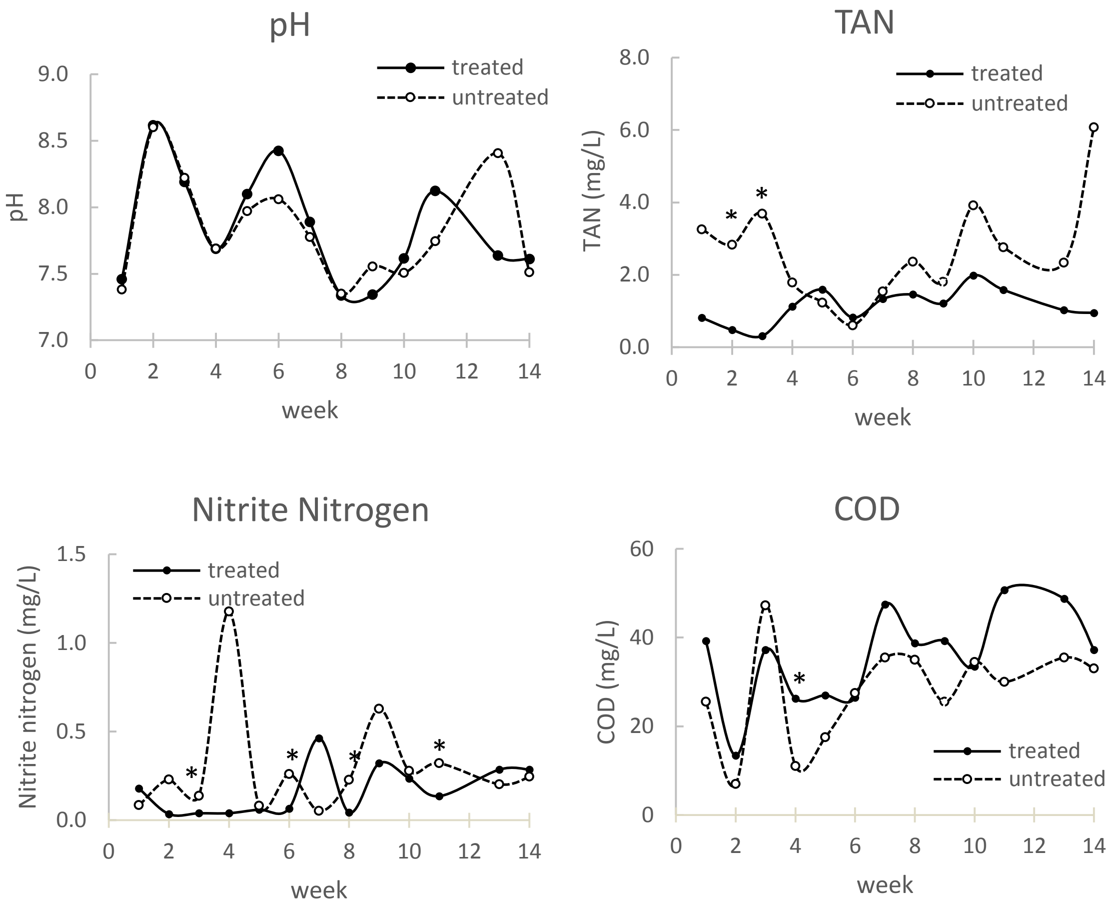

Fig 6.2- The pH and concentrations of total ammonia nitrogen (TAN), nitrite nitrogen, chemical oxygen demand (COD) in four channel catfish ponds treaded with a bacterial amendment, Aqua PE, (dots and solid lines) and in two untreated (control) ponds (open circles and dashed lines). Stars indicate difference ($P<0.05$) in concentrations of variable between treatment and control.

Table 6.1- Mean of pH, turbidity, and concentrations of particulate organic matter (POM), total nitrogen (TN), total phosphorus (TP), chlorophyll a (Chl a), total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^-–N) and chemical oxygen demand (COD) in catfish ponds receiving a bacterial amendment (Aqua PE) and in control ponds.

Variable	**Treated**	**Control**
pH	7.85 a	7.83 a
Turbidity	45.67 a	18.19 b
Total nitrogen (mg/L)	0.86 a	0.85 a
Total phosphorus (mg/L)	0.29 a	0.28 a
Chlorophyll a (μg/L)	501.76 a	107.02 b
Total ammonia nitrogen (mg/L)	1.13 a	2.63 b
Nitrite nitrogen (mg/L)	0.17 a	0.30 a
Chemical oxygen demand (mg/L)	35.80 a	28.06 b

Means within a row with a letter in common are not different ($P>0.05$)

Table 6.2- Average production for catfish ponds receiving a bacterial amendment (Aqua PE) and in control ponds

Variable	Treated	Control
Average production (kg/ha)	7255 a	9540 a
FCR (feed conversion rate)	1.84 a	2.27 a

Values with same letter with in a column are not different (P> 0.05)

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Appendix A- Lab Study

Table A.1.1- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with FritzZyme 360.

day	0	2	4	6	8	12	16
control	5.20 a	4.73 a	4.11 a	2.56 a	0.35 a	0.02 a	0.03 a
0.0005 g/L	5.76 ab	5.50 b	5.25 b	4.24 b	3.16 bd	0.04 a	0.02 a
0.001 g/L	6.04 b	5.12 ab	4.64 c	3.69 b	2.62 b	0.02 a	0.02 a
0.002 g/L	5.42 ab	5.33 bc	4.57 ac	3.90 b	2.48 bc	0.02 a	0.02 a
0.004g/L	5.33 ab	4.98 ac	4.75 bc	3.97 b	3.42 d	0.23 b	0.02 a
0.0005g/L/every week	5.30 a	5.16 b	5.09 b	3.97 a	3.27 b	0.22 b	0.03 a
0.001 g/L/every week	5.58 a	5.20 b	4.76 b	3.85 b	2.79 b	0.09 a	0.02 a

Values with same letter with in a row are not different (P>0.05)

Table A.1.2- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Aqua PE.

day	0	2	4	6	8	12	16
control	6.37 a	5.46 a	3.14 a	2.55 ad	1.28 ab	0.20 a	0.16 a
0.00125 g/L	7.37 a	7.27 b	4.80 b	3.17 ae	1.37 ab	0.12 a	0.11 bc
0.0025 g/L	6.57 a	5.73 ab	4.10 ab	3.03 ac	1.64 b	0.12 a	0.11 bc
0.005 g/L	7.22 a	6.33 ab	5.84 b	3.98 be	1.22 a	0.22 a	0.12 bc
0.01 g/L	7.03 a	5.45 a	2.97 a	2.23 bcd	1.09 a	0.09 a	0.13 ac
0.00125 g/L/every week	6.83 a	5.90 a	4.59 a	3.27 a	1.60 a	0.11 a	0.11 b
0.0025 g/L/every week	5.12 a	5.72 a	3.89 a	2.76 a	0.82 b	0.40 a	0.11 b

Values with same letter with in a row are not different (P>0.05)

Table A.1.3- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Aqua Prob 4X (powder w/ nutrients).

day	0	2	4	6	8	12	16
control	6.58 a	6.02 a	4.07 a	2.90 a	1.37 a	0.16 a	0.18 a
0.0025 g/L/every week	6.96 a	6.48 a	5.52 a	3.52 a	1.01 a	0.12 a	0.13 a
0.005 g/L/every week	6.75 a	5.94 a	5.71 a	4.32 a	1.17 a	0.10 a	0.12 a
0.01 g/L/every week	6.55 a	5.71 a	3.77 a	2.34 a	0.85 a	0.14 a	0.10 a
0.02 g/L/every week	6.65 a	5.46 a	3.59 a	2.55 a	1.00 a	0.11a	0.44 a
0.0025 g/L/ every 4 days	6.43 a	5.28 a	3.40 a	2.74 a	1.25 a	0.09 a	0.08 a
0.005 g/L/every 4 days	6.39 a	5.31 a	5.18 a	3.55 a	1.60 a	0.12 a	0.11 a

Values with same letter with in a row are not different (P>0.05)

Table A.1.4- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Aqua Bio-Trol.

day	0	2	4	6	8	12	16
control	6.86 a	5.13 a	3.25 a	1.58 a	0.07 a	0.11 a	ND a
0.025 g/L	7.42 a	4.72 a	3.63 a	1.86 a	ND a	0.01 a	ND a
0.05 g/L	5.11 b	4.37 ab	3.63 a	1.90 a	ND a	ND a	ND a
0.1 g/L	5.52 b	4.66 a	3.75 a	1.80 a	0.02 a	0.12 a	ND a
0.2 g/L	5.35 b	3.61 b	2.49 a	1.98 a	ND a	0.07 a	ND a
0.025 g/L/ every week	4.99 b	4.32 b	1.84 a	1.38 a	ND a	0.08 a	0.08 a
0.05 g/L/ every week	5.13 b	4.39 ab	2.63 a	1.50 a	ND a	0.11 a	0.02 a

Values with same letter with in a row are not different (P>0.05)

Table A.1.5- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Aqua Prob EZ.

day	0	2	4	6	8	12	16
control	5.69 ab	5.30 a	5.19 a	4.34 a	0.69 a	0.53 a	ND a
0.0125 g/L/every 3 day	6.68 a	5.83 b	5.43 a	4.34 a	ND b	ND b	ND a
0.025 g/L/every 3 day	5.28 b	5.13 a	5.00 a	3.68 a	0.17 ab	ND b	ND a
0.05 g/L/every 3 day	5.60 b	5.46 ab	5.04 a	4.04 a	ND b	ND b	ND a
0.1 g/L/every 3 day	5.91 ab	5.10 a	4.71 a	3.71 a	ND b	ND b	ND a
0.0125 g/L/every day	5.41 a	5.36 a	5.35 a	3.77 a	ND b	ND b	ND a
0.025 g/L/every day	5.62 a	5.37 a	5.29 a	4.79 a	ND b	ND b	ND a

Values with same letter with in a row are not different (P>0.05)

Table A.1.6- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Lake & Pond Bacteria.

day	0	2	4	6	8	12	16
control	5.63 a	4.67 ab	3.82 a	2.18 a	1.51 a	0.01 a	0.01 a
0.0005 g/L	6.49 b	5.11 ab	4.52 bc	3.32 a	2.36 a	0.01 a	0.01 a
0.001 g/L	6.12 ab	4.59 a	3.67 a	1.43 a	0.11 a	ND a	0.00 a
0.002 g/L	6.19 ab	5.23 b	4.11 ac	2.53 a	1.13 a	ND a	0.02 a
0.004 g/L	5.73 ab	5.08 ab	4.17 ac	2.13 a	1.40 a	0.03 a	0.01 a
0.0005 g/L/ every week	5.70 a	4.67 a	3.91 a	3.06 a	2.57 a	0.08 b	0.01 a
0.001 g/L/ every week	6.25 a	4.92 a	4.16 a	2.15 a	1.45 a	0.01 a	0.01 a

Values with same letter with in a row are not different (P>0.05)

Table A.1.7- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Pond protect (dry).

day	0	2	4	6	8	12	16
control	7.38 a	6.42 a	5.74 ab	4.08 ac	1.47 a	ND a	ND a
0.001 g/L/every 5 days	7.61 a	7.01 b	6.17 a	3.86 ad	1.48 a	ND a	ND a
0.002 g/L/every 5 days	7.35 a	6.54 ab	5.68 ab	4.20 ac	0.85 a	ND a	ND a
0.004 g/L/every 5 days	7.55 a	6.87 ab	5.89 ab	4.72 bc	0.71 a	ND a	ND a
0.08 g/L/every 5 days	7.36 a	6.60 ab	5.51 b	4.66 bcd	1.72 a	0.03 a	ND a
0.001 g/L/every 3 days	7.58 a	6.58 a	6.14 a	4.41 a	2.22 a	0.12 b	ND a
0.002 g/L/every 3 days	7.48 a	6.66 a	5.81 a	4.41 a	0.79 bc	ND a	ND a

Values with same letter with in a row are not different (P>0.05)

Table A.1.8- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Pond protect (liquid).

day	0	2	4	6	8	12	16
control	5.11 a	4.69 a	4.22 a	2.78 a	0.63 a	0.02 a	0.02 a
0.003 mL/L/every 5 days	5.71 a	5.37 b	5.07 b	3.97 a	2.37 a	0.07 a	0.02 a
0.006 mL/L/every 5 days	5.50 a	5.22 ab	4.90 b	3.71 a	1.63 a	0.01 a	0.02 a
0.012mL/L/every 5 days	5.48 a	5.20 ab	4.65 ab	3.36 a	0.96 a	0.04 a	0.02 a
0.024 mL/L/every 5 days	5.58 a	5.04 ab	4.39 a	2.71 a	0.62 a	0.01 a	0.02 a
0.003mL/L/ every 3 days	5.25 a	4.29 a	3.92 a	2.40 a	0.82 a	0.04 a	0.02 a
0.006mL/L/ every 3 days	5.60 a	4.72 a	3.98 a	2.29 a	0.28 a	0.01 a	0.02 a

Values with same letter with in a row are not different (P>0.05)

Table A.1.9- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with Sewper Rx.

day	0	2	4	6	8	12	16
control	7.37 a	6.45 a	5.73 a	2.90 a	1.77 a	ND a	ND a
0.0015 g/L	7.48 a	6.80 a	6.22 a	3.26 ac	2.21 a	0.35 a	ND a
0.003 g/L	7.42 a	6.83 a	5.94 a	3.86 bc	2.50 a	0.24 a	ND a
0.006 g/L	7.66 a	6.68 a	6.05 a	3.99 bc	2.43 a	0.23 a	ND a
0.012 g/L	7.44 a	6.64 a	5.86 a	2.74 ac	1.64 a	0.00 a	ND a
0.0015 g/L/ every week	7.54 a	6.51 a	5.45 a	2.25 a	1.48 a	0.35 a	ND a
0.003 g/L/ every week	7.36 a	6.63 a	5.80 a	2.65 a	1.43 a	0.13 a	ND a

Values with same letter with in a row are not different (P>0.05)

Table A.1.10- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with SHRIMPSHIELD.

day	0	2	4	6	8	12	16
control	5.58 a	5.24 a	4.23 a	5.45 a	4.33 a	0.70 a	0.42 a
0.015 g/L/every 3 day	5.64 a	5.46 a	5.41 a	5.73 ab	5.02 a	1.53 a	1.46 a
0.03 g/L/every 3 day	5.64 a	5.29 a	5.14 a	5.28 b	4.89 a	1.45 a	1.25 a
0.06 g/L/every 3 day	5.86 a	5.45 a	4.99 a	4.92 b	5.94 a	3.30 a	1.10 a
0.12 g/L/every 3 day	5.68 a	5.08 a	4.60 a	4.31 a	6.01 a	7.71 a	0.32 a
0.015 g/L/every day	5.79 a	5.60 a	5.24 a	5.40 a	5.59 b	1.29 a	0.42 a
0.03 g/L/every day	5.69 a	5.41 a	5.04 a	4.96 a	5.93 b	6.03 b	0.91 a

Values with same letter with in a row are not different (P>0.05)

Table A.1.11- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with AQUA-TRON.

day	0	2	4	6	8	12	16
control	5.51 a	4.33 a	3.64 a	2.26 a	0.94 ab	0.42 a	0.01 a
0.001 g/L	5.90 a	5.57 b	4.91 b	4.03 b	2.85 b	2.56 b	0.01 a
0.002 g/L	6.21 a	4.87 ab	4.04 a	2.97 a	0.69 ab	0.10 a	0.02 a
0.004 g/L	5.91 a	5.04 ab	4.01 a	2.65 a	0.45 a	0.20 a	0.01 a
0.008 g/L	5.85 a	4.90 ab	3.71 a	2.41 a	0.26 a	0.14 a	0.01 a
0.001 g/L/ every week	5.55 a	4.69 a	3.75 a	2.89 b	1.51 a	0.25 a	0.01 a
0.002 g/L/ every week	5.84 a	4.68 a	3.88 a	3.41 ab	1.42 a	0.17 a	0.01 a

Values with same letter with in a row are not different (P>0.05)

Table A.1.12- Changes in total ammonia nitrogen (TAN) concentration at various days after treatment with WASTE &SLUDGE REDUCER (WAS).

day	0	2	4	6	8	12	16
control	6.55 a	5.17 a	3.85 a	1.91 a	0.67 a	0.18 a	ND a
0.075 g/L	5.16 b	5.04 a	4.22 a	1.88 a	0.28 a	0.12 a	ND a
0.15 g/L	5.27 b	5.09 a	4.32 a	2.39 a	1.07 a	0.10 a	ND a
0.3 g/L	5.32 b	4.92 a	4.24 a	1.76 a	ND a	0.13 a	ND a
0.6 g/L	5.20 b	4.95 a	4.00 a	2.06 a	1.19 a	0.14 a	ND a
0.075 g/L/ every 5 days	5.28 b	4.85 ab	3.68 a	2.13 a	1.44 a	0.09 a	0.03 a
0.15 g/L/every 5 days	5.18 b	4.59 b	2.91 a	1.54 a	ND a	0.13 a	ND a

Values with same letter with in a row are not different (P>0.05)

Table A.2.1- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with FritzZyme 360.

day	0	2	4	6	8	12	16
control	6.08 a	4.55 ab	4.54 a	4.51 a	4.96 a	3.56 a	ND a
0.0005 g/L	6.51 a	4.43 a	4.91 a	4.80 a	5.10 a	7.89 b	5.27 b
0.001 g/L	6.18 a	4.54 ab	4.65 a	4.39 a	4.80 a	7.09 b	5.01 b
0.002 g/L	6.51 a	4.83 b	4.83 a	4.29 a	4.92 a	7.48 b	1.41 c
0.004g/L	5.77 a	4.51 ab	4.73 a	4.38 a	4.59 a	7.37 b	5.22 b
0.0005g/L/every week	5.17 a	5.09 b	4.46 a	4.03 a	4.39 a	7.67 b	2.07 b
0.001 g/L/every week	5.95 a	4.82 ab	4.65 a	4.24 a	4.71 a	7.41 b	2.83 b

Values with same letter with in a row are not different ($P>0.05$)

Table A.2.2- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with Aqua PE.

day	0	2	4	6	8	12	16
control	4.73 a	5.00 ac	5.39 a	4.52 a	3.70 a	0.02 a	0.18 a
0.00125 g/L	5.66 b	5.30 ad	6.33 bc	6.34 bcd	6.31 bde	0.02 a	0.15 a
0.0025 g/L	4.88 a	4.60 bc	5.44 ac	5.19 ad	4.83 acf	0.02 a	0.19 a
0.005 g/L	5.22 ab	5.57 d	6.96 b	6.82 bc	7.03 bdf	0.02 a	0.27 a
0.01 g/L	5.02 ab	5.27 ad	6.91 b	6.34 bd	5.26 ce	0.02 a	0.23 a
0.00125 g/L/every week	5.19 a	5.12 a	5.91 a	6.22 b	5.41 b	0.02 a	0.24 a
0.0025 g/L/every week	4.90 a	4.95 a	5.64 a	5.48 ab	5.47 b	0.01 a	0.16 a

Values with same letter with in a row are not different ($P>0.05$)

Table A.2.3- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with Aqua Prob 4X (powder w/ nutrients).

day	0	2	4	6	8	12	16
control	4.70 a	4.85 a	5.07 a	4.66 a	2.61 a	0.02 a	0.19 a
0.0025 g/L/every week	4.88 a	4.94 a	5.76 a	5.99 a	3.11 a	0.01 a	0.23 a
0.005 g/L/every week	4.77 a	4.81 a	5.43 a	4.75 a	1.98 a	0.01 a	0.17 a
0.01 g/L/every week	4.75 a	5.20 a	6.05 a	5.40 a	2.44 a	0.01 a	0.22 a
0.02 g/L/every week	5.04 a	5.07 a	5.97 a	5.33 a	2.24 a	0.01 a	0.21 a
0.0025 g/L/ every 4 days	4.73 a	4.63 a	5.55 a	5.27 a	1.68 a	0.01 a	0.20 a
0.005 g/L/every 4 days	4.56 a	4.71 a	5.62 a	4.92 a	1.82 a	0.01 a	0.18 a

Values with same letter with in a row are not different (P>0.05)

Table A.2.4- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with Aqua Bio-Trol.

day	0	2	4	6	8	12	16
control	5.47 a	4.62 a	4.34 a	3.80 a	3.66 a	2.59 a	0.40 a
0.025 g/L	5.58 a	5.27 b	5.43 b	5.15 b	4.53 a	1.53 b	0.83 a
0.05 g/L	5.36 a	4.67 ad	5.46 b	4.41 c	4.01 a	1.41 b	0.80 a
0.1 g/L	5.50 a	5.33 b	5.73 b	4.99 bc	4.28 a	1.42 b	0.50 a
0.2 g/L	5.21 a	5.15 bd	5.32 b	4.62 c	3.82 a	1.77 b	0.72 a
control	5.47 a	4.62 a	4.34 a	3.80 a	3.66 a	2.59 a	0.40 a
0.025 g/L/ every week	5.04 a	5.01 a	4.99 ab	4.44 b	3.46 a	1.52 b	1.26 b

Values with same letter with in a row are not different (P>0.05)

Table A.2.5- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with Aqua Prob EZ.

day	0	2	4	6	8	12	16
control	5.30 a	5.19 ab	4.34 a	2.69 a	0.53 a	NA a	NA a
0.0125 g/L/every 3 day	5.83 b	5.43 a	4.34 a	2.00 b	NA b	NA a	NA a
0.025 g/L/every 3 day	5.13 a	5.00 ab	3.68 a	2.17 ab	NA b	NA a	NA a
0.05 g/L/every 3 day	5.46 ab	5.04 ab	4.04 a	1.85 b	NA b	NA a	NA a
0.1 g/L/every 3 day	5.10 a	4.71 b	3.71 a	1.86 b	NA b	NA a	NA a
0.0125 g/L/every day	5.36 a	5.35 a	3.77 a	1.87 b	NA b	NA a	NA a
0.025 g/L/every day	5.37 a	5.29 a	4.79 a	2.03 b	NA b	NA a	NA a

Values with same letter with in a row are not different ($P>0.05$)

Table A.2.6- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with Lake & Pond Bacteria.

day	0	2	4	6	8	12	16
control	4.09 a	4.27 ab	3.87 a	3.86 a	3.29 a	0.18 a	0.04 a
0.0005 g/L	4.55 a	4.78 ab	4.78 b	5.27 ab	6.12 a	0.70 a	0.14 a
0.001 g/L	4.17 a	3.97 a	4.55 ab	4.53 ab	6.20 a	0.50 a	0.44 a
0.002 g/L	4.17 a	5.10 b	4.70 ab	5.40 b	6.14 a	0.46 a	0.07 a
0.004 g/L	4.14 a	4.78 ab	4.43 ab	4.72 ab	5.38 a	0.25 a	0.06 a
0.0005 g/L/ every week	4.15 a	4.34 a	4.09 a	4.24 ab	4.71 a	0.79 a	0.35 a
0.001 g/L/ every week	3.89 a	4.68 a	5.00 b	5.39 b	6.41 a	0.60 a	0.17 a

Values with same letter with in a row are not different ($P>0.05$)

Table A.2.7- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with Pond protect (dry).

day	0	2	4	6	8	12	16
control	6.41 a	4.49 a	4.74 a	3.81 a	3.88 ac	3.74 a	0.56 a
0.001 g/L/every 5 days	7.30 a	6.10 b	5.76 b	4.18 b	4.37 b	3.88 a	2.14 a
0.002 g/L/every 5 days	7.14 a	5.25 ab	6.40 b	3.94 ab	3.86 a	3.73 a	1.78 a
0.004 g/L/every 5 days	7.59 a	5.70 ab	6.43 b	4.17 b	4.25 bc	3.78 a	1.20 a
0.08 g/L/every 5 days	6.44 a	5.60 ab	5.55 ab	3.82 a	3.85 a	5.06 a	1.90 a
0.001 g/L/ every 3 days	6.23 a	6.06 b	5.33 a	3.81 a	4.00 a	3.78 a	3.23 b
0.002 g/L/every 3 days	7.03 a	5.55 b	6.26 b	4.01 a	4.06 a	4.39 a	1.56 ab

Values with same letter with in a row are not different ($P>0.05$)

Table A.2.8- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with Pond protect (liquid).

day	0	2	4	6	8	12	16
control	6.37 a	5.24 a	4.76 a	4.76 a	4.74 a	3.83 a	0.03 a
0.003 mL/L/every 5 days	6.32 a	4.89 a	4.79 a	4.99 ab	4.59 a	6.44 a	0.11 a
0.006 mL/L/every 5 days	6.39 a	5.07 a	4.88 a	5.00 ab	4.74 a	5.64 a	0.34 a
0.012mL/L/every 5 days	6.49 a	4.94 a	4.97 a	5.15 ab	4.71 a	5.25 a	0.35 a
0.024 mL/L/every 5 days	7.20 a	5.66 a	4.95 a	5.34 b	5.46 a	5.48 a	0.41 a
0.003mL/L/ every 3 days	6.63 a	5.30 a	4.85 a	4.82 a	4.98 a	5.99 a	2.25 a
0.006mL/L/ every 3 days	7.14 a	5.55 a	4.97 a	5.01 a	5.18 a	5.57 a	0.98 a

Values with same letter with in a row are not different ($P>0.05$)

Table A.2.9- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with Sewper Rx.

day	0	2	4	6	8	12	16
control	5.91 a	5.09 a	4.40 a	3.73 a	3.82 a	3.86 a	0.61 a
0.0015 g/L	5.22 a	5.14 a	3.58 a	3.99 a	3.82 a	3.81 a	0.03 ab
0.003 g/L	5.98 a	5.13 a	4.80 a	3.81 a	3.86 a	3.90 a	0.76 ab
0.006 g/L	6.52 a	5.52 a	5.42 a	3.82 a	3.88 a	4.00 a	1.56 ab
0.012 g/L	6.67 a	6.01 a	5.27 a	3.79 a	3.91 a	4.12 a	3.07 b
0.0015 g/L/ every week	6.58 a	5.34 a	5.45 a	3.93 a	3.97 a	4.10 a	0.48 a
0.003 g/L/ every week	6.64 a	5.64 a	4.34 a	4.03 a	3.93 a	4.08 a	0.60 a

Values with same letter with in a row are not different (P>0.05)

Table A.2.10- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with SHRIMPSHIELD.

day	0	2	4	6	8	12	16
control	5.24 a	4.74 a	4.28 a	3.88 a	3.68 a	2.23 a	0.37 a
0.015 g/L/every 3 day	5.69 a	5.34 ab	4.70 ab	4.48 b	4.77 a	2.80 ab	0.77 ab
0.03 g/L/every 3 day	5.76 a	4.78 a	5.25 b	4.09 b	4.64 a	2.71 ab	0.99 ab
0.06 g/L/every 3 day	6.09 a	5.44 b	5.52 b	4.46 b	4.76 a	2.75 ab	0.95 ab
0.12 g/L/every 3 day	5.75 a	5.47 b	5.09 ab	4.11 ab	4.38 a	3.14 b	1.68 b
0.015 g/L/every day	5.65 a	5.25 a	4.96 a	3.99 ab	4.23 a	2.89 a	1.36 a
0.03 g/L/every day	5.86 a	5.29 a	5.20 a	4.48 b	4.80 a	3.02 a	0.78 a

Values with same letter with in a row are not different (P>0.05)

Table A.2.11- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with AQUA-TRON.

day	0	2	4	6	8	12	16
control	4.33 a	4.51 ab	4.11 a	4.10 a	3.54 a	0.23 a	0.09 a
0.001 g/L	4.79 a	5.02 ab	5.02 b	5.51 ab	6.36 a	0.75 a	0.19 a
0.002 g/L	4.41 a	4.21 a	4.80 ab	4.78 ab	6.44 a	0.55 a	0.49 a
0.004 g/L	4.41 a	5.34 b	4.94 ab	5.64 b	6.38 a	0.51 a	0.12 a
0.008 g/L	4.39 a	5.02 ab	4.68 ab	4.96 ab	5.62 a	0.30 a	0.11 a
0.001 g/L/ every week	4.39 a	4.58 a	4.34 a	4.48 ab	4.95 a	0.84 a	0.40 a
0.002 g/L/ every week	4.14 a	4.92 a	5.25 b	5.64 b	6.66 a	0.64 a	0.22 a

Values with same letter with in a row are not different ($P>0.05$)

Table A.2.12- Changes in nitrite nitrogen (NO_2^-–N) concentration at various days after treatment with WASTE &SLUDGE REDUCER (WAS).

day	0	2	4	6	8	12	16
control	5.09 a	4.70 a	4.42 a	3.88 a	3.75 a	2.61 a	0.42 a
0.075 g/L	5.77 ab	5.42 ab	4.78 ab	4.56 b	4.85 a	2.81 a	0.79 ab
0.15 g/L	5.84 ab	4.86 ab	5.33 ab	4.17 ab	4.72 a	2.73 a	1.01 ab
0.3 g/L	6.17 b	5.52 b	5.60 b	4.54 b	4.84 a	2.76 a	0.96 ab
0.6 g/L	5.83 ab	5.55 b	5.17 ab	4.19 ab	4.46 a	3.16 a	1.69 b
0.075 g/L/ every 5 days	5.73 a	5.33 a	5.04 a	4.08 ab	4.31 a	2.91 a	1.37 a
0.15 g/L/every 5 days	5.94 a	5.37 a	5.29 a	4.56 b	4.88 a	3.04 a	0.79 a

Values with same letter with in a row are not different ($P>0.05$)

Table A.3.1- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with FritzZyme 360.

day	1	3	5	7	9	13	17
control	13.24 a	10.96 ab	3.47 a	2.32 a	1.57 a	1.17 a	1.38 a
0.0005 g/L	13.26 a	10.84 ab	4.16 a	2.63 ab	1.24 a	1.06 a	0.81 b
0.001 g/L	12.80 a	9.21 a	3.02 a	2.39 a	1.22 a	0.81 a	0.62 b
0.002 g/L	12.76 a	10.57 ab	4.35 a	3.00 ab	1.70 a	1.08 a	0.86 ab
0.004g/L	12.73 a	11.86 b	4.09 a	3.62 b	3.60 b	0.99 a	0.74 b
0.0005g/L/every week	12.43 a	11.21 a	4.38 a	2.78 a	1.95 a	0.95 a	0.63 b
0.001 g/L/every week	12.85 a	9.28 a	2.70 a	2.14 a	1.37 a	0.75 a	0.51 b

Values with same letter with in a row are not different ($P>0.05$)

Table A.3.2- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with Aqua PE.

day	1	3	5	7	9	13	17
control	12.67 a	9.51 a	4.55 a	3.52 a	2.27 a	1.87 a	0.96 a
0.00125 g/L	10.09 ab	9.15 a	5.13 a	3.57 a	1.30 a	1.83 a	1.47 a
0.0025 g/L	10.54 ab	9.27 a	4.60 a	3.92 ab	1.59 a	1.80 a	0.76 a
0.005 g/L	10.54 ab	11.37 a	4.35 a	5.11 b	2.50 a	1.30 a	0.77 a
0.01 g/L	9.70 b	9.33 a	4.45 a	3.60 a	1.77 a	1.01 a	0.82 a
0.00125 g/L/every week	11.14 a	11.35 a	4.65 a	5.14 b	2.22 a	1.74 a	0.83 a
0.0025 g/L/every week	14.15 a	10.65 a	4.55 a	3.76 ab	1.46 a	1.07 a	0.52 a

Values with same letter with in a row are not different ($P>0.05$)

Table A.3.3- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with Aqua Prob 4X (powder w/ nutrients).

day	1	3	5	7	9	13	17
control	13.61 a	10.29 a	4.55 a	3.37 a	2.27 a	1.82 a	0.95 a
0.0025 g/L/every week	13.57 a	9.10 a	3.42 a	2.90 a	1.98 a	0.73 ab	0.53 a
0.005 g/L/every week	11.99 a	9.05 a	3.73 a	3.17 a	2.18 a	0.43 b	0.71 a
0.01 g/L/every week	11.77 a	9.70 a	4.29 a	3.88 a	3.59 a	0.89 ab	1.36 a
0.02 g/L/every week	13.43 a	11.18 a	4.12 a	3.54 a	2.88 a	1.15 ab	1.14 a
0.0025 g/L/ every 4 days	12.92 a	10.12 a	3.58 a	3.22 a	2.30 a	0.61 b	0.14 a
0.005 g/L/every 4 days	13.08 a	10.42 a	4.06 a	3.32 a	2.71 a	1.04 ab	0.53 a

Values with same letter with in a row are not different (P>0.05)

Table A.3.4- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with Aqua Bio-Trol.

day	1	3	5	7	9	13	17
control	12.71 a	9.35 a	4.62 a	4.30 a	4.17 a	2.52 a	1.69 a
0.025 g/L	13.74 b	12.11 b	7.94 b	4.93 a	4.68 a	3.33 a	0.73 a
0.05 g/L	14.26 b	12.03 b	7.07 b	5.31 a	4.28 a	2.17 a	0.96 a
0.1 g/L	13.16 ab	11.80 b	7.09 b	5.84 a	5.44 a	2.91 a	0.85 a
0.2 g/L	13.63 ab	11.71 b	8.09 b	5.78 a	5.26 a	3.51 a	2.54 a
0.025 g/L/ every week	13.44 ab	11.56 b	6.27 ab	5.77 a	5.24 a	3.06 a	1.08 a
0.05 g/L/ every week	13.92 b	11.53 b	6.91 b	5.83 a	5.04 a	1.48 a	0.63 a

Values with same letter with in a row are not different (P>0.05)

Table A.3.5- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with Aqua Prob EZ.

day	1	3	5	7	9	13	17
control	13.69 a	9.40 ab	3.86 a	4.12 a	3.08 ab	1.06 a	0.97 a
0.0125 g/L/every 3 day	10.18 bc	13.25 ac	5.51 a	4.66 a	2.81 a	2.36 ac	1.42 a
0.025 g/L/every 3 day	12.03 acd	8.11 b	4.10 a	2.66 a	2.66 a	1.89 ac	0.92 a
0.05 g/L/every 3 day	12.45 ac	14.20 c	5.44 a	3.98 a	4.62 b	3.66 bc	2.25 b
0.1 g/L/every 3 day	9.72 bd	12.19 abc	6.16 a	4.06 a	4.19 ab	5.44 b	3.15 c
0.0125 g/L/every day	12.06 a	13.54 a	6.12 a	3.98 a	4.02 a	1.68 a	1.07 a
0.025 g/L/every day	12.20 a	12.62 a	5.51 a	3.89 a	3.98 a	2.36 a	1.43 a

Values with same letter with in a row are not different (P>0.05)

Table A.3.6- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with Lake & Pond Bacteria.

day	1	3	5	7	9	13	17
control	12.30 a	9.84 a	3.46 a	3.23 a	1.73 a	0.80 a	1.46 a
0.0005 g/L	12.59 a	10.41 a	4.94 b	3.96 ab	2.42 a	1.15 a	1.21 a
0.001 g/L	12.19 a	9.15 a	4.84 b	4.12 ab	1.79 a	1.65 a	1.47 a
0.002 g/L	14.05 a	8.84 a	4.73 ab	4.21 ab	2.24 a	1.26 a	1.44 a
0.004 g/L	12.90 a	9.14 a	5.03 b	4.46 b	2.41 a	1.56 a	1.17 a
0.0005 g/L/ every week	13.03 a	9.49 a	4.82 ab	5.39 b	4.60 b	1.69 a	1.41 a
0.001 g/L/ every week	13.03 a	8.81 a	5.05 b	3.99 a	1.78 ab	1.54 a	1.37 a

Values with same letter with in a row are not different (P>0.05)

Table A.3.7- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with Pond protect (dry).

day	1	3	5	7	9	13	17
control	13.10 a	10.01 a	4.32 a	3.53 ab	2.84 a	1.86 a	0.63 a
0.001 g/L/every 5 days	13.12 a	10.11 a	4.27 a	3.03 b	2.06 b	1.10 b	0.67 a
0.002 g/L/every 5 days	13.94 b	11.92 ab	5.74 a	4.56 c	2.20 ab	0.92 b	0.60 a
0.004 g/L/every 5 days	14.10 b	12.59 ab	3.84 b	3.16 ab	1.97 b	0.90 b	0.50 a
0.08 g/L/every 5 days	13.95 b	13.12 b	4.86 ab	3.44 ab	2.65 ab	1.63 a	1.29 b
0.001 g/L/every 3 days	13.61 b	12.23 a	4.91 a	3.71 b	2.17 ab	1.03 b	0.53 a
0.002 g/L/every 3 days	14.02 b	12.30 a	4.06 a	3.01 c	2.03 b	1.00 b	0.57 a

Values with same letter with in a row are not different (P>0.05)

Table A.3.8- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with Pond protect (liquid).

day	1	3	5	7	9	13	17
control	13.02 a	10.96 a	3.83 a	2.71 a	1.60 a	1.25 a	1.55 a
0.003 mL/L/every 5 days	12.72 a	10.45 a	3.86 a	2.83 a	1.80 a	0.96 a	1.40 a
0.006 mL/L/every 5 days	12.65 a	10.81 a	3.66 a	2.71 a	1.61 a	0.99 a	0.59 a
0.012mL/L/every 5 days	12.32 a	10.06 a	3.83 a	2.78 a	1.75 a	0.94 a	0.73 a
0.024 mL/L/every 5 days	12.37 a	11.44 a	3.94 a	2.79 a	1.65 a	1.25 a	1.15 a
0.003mL/L/ every 3 days	12.86 a	10.94 a	3.96 a	2.64 a	1.33 a	1.46 a	1.45 a
0.006mL/L/ every 3 days	12.96 a	11.31 a	3.36 a	2.61 a	1.86 a	1.47 a	1.14 a

Values with same letter with in a row are not different (P>0.05)

Table A.3.9- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with Sewper Rx.

day	1	3	5	7	9	13	17
control	12.91 a	10.09 a	4.40 a	3.73 a	1.83 a	1.86 a	0.61 ab
0.0015 g/L	12.22 a	10.14 a	3.58 a	3.99 a	1.82 a	1.81 a	0.03 a
0.003 g/L	12.98 a	10.13 a	4.79 a	3.81 a	1.86 a	1.90 a	0.76 ab
0.006 g/L	13.52 a	10.53 a	5.42 a	3.82 a	1.88 a	2.00 a	1.56 ab
0.012 g/L	13.67 a	11.00 a	5.27 a	3.79 a	1.91 a	2.12 a	3.07 b
0.0015 g/L/ every week	13.58 a	10.34 a	5.44 a	3.92 a	1.98 a	2.10 a	0.48 a
0.003 g/L/ every week	13.64 a	10.64 a	4.34 a	4.03 a	1.93 a	2.08 a	0.60 a

Values with same letter with in a row are not different (P>0.05)

Table A.3.10- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with SHRIMPSHIELD.

day	1	3	5	7	9	13	17
control	12.57 a	10.80 a	4.04 a	3.32 a	2.65 a	1.32 a	0.98 a
0.015 g/L/every 3 day	11.65 a	11.80 ab	5.97 a	3.63 a	2.65 a	3.44 b	1.96 ab
0.03 g/L/every 3 day	12.17 a	11.27 a	5.27 a	4.04 a	3.57 a	4.67 b	3.88 ab
0.06 g/L/every 3 day	11.40 a	13.45 b	5.84 a	4.37 a	3.51 a	7.69 c	3.30 ab
0.12 g/L/every 3 day	11.25 a	13.39 b	9.49 b	6.80 b	5.15 a	7.84 c	6.34 b
0.015 g/L/every day	11.28 a	13.11 b	7.15 b	4.74 a	2.89 a	3.66 b	2.75 a
0.03 g/L/every day	11.80 a	13.91 b	10.46 c	7.68 b	6.56 b	7.39 c	4.34 a

Values with same letter with in a row are not different (P>0.05)

Table A.3.11- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with AQUA-TRON.

day	1	3	5	7	9	13	17
control	14.52 a	10.63 a	2.93 a	2.73 a	1.14 a	0.82 a	1.09 a
0.001 g/L	14.70 a	8.96 a	1.73 a	1.96 a	0.85 a	2.06 a	1.38 ab
0.002 g/L	14.42 a	9.42 a	2.44 a	2.19 a	0.48 a	1.57 a	2.18 b
0.004 g/L	13.76 a	8.65 a	1.55 a	1.63 a	0.31 a	1.62 a	1.87 ab
0.008 g/L	14.10 a	10.21 a	2.32 a	1.91 a	0.47 a	1.56 a	1.46 ab
0.001 g/L/ every week	13.18 a	12.71 a	2.89 a	2.24 a	0.60 a	0.95 a	1.52 a
0.002 g/L/ every week	14.09 a	10.64 a	4.11 a	2.84 a	1.39 a	1.76 a	1.65 a

Values with same letter with in a row are not different (P>0.05)

Table A.3.12- Changes in dissolved oxygen loss (Δ DO, mg/L) in 24 hours at various days after treatment with WASTE &SLUDGE REDUCER (WAS).

day	1	3	5	7	9	13	17
control	12.71 a	10.35 a	4.62 a	3.37 a	2.52 a	1.19 a	1.19 a
0.075 g/L	13.23 a	11.30 a	5.98 a	3.71 a	3.95 a	1.15 a	1.15 a
0.15 g/L	11.57 a	9.87 a	4.68 a	3.50 a	3.63 a	2.26 a	2.26 a
0.3 g/L	12.96 a	9.96 a	4.56 a	3.56 a	2.53 a	3.07 a	3.07 a
0.6 g/L	13.90 a	10.83 a	6.63 a	5.00 a	1.97 a	1.83 a	2.03 a
0.075 g/L/ every 5 days	12.56 a	10.68 a	5.79 a	4.13 a	4.71 a	3.35 a	3.35 a
0.15 g/L/every 5 days	14.03 a	10.82 a	5.78 a	4.34 a	4.27 a	3.17 a	3.17 a

Values with same letter with in a row are not different (P>0.05)

Appendix B- E.W. Shell Fisheries Study

Table B.1- The pH in three channel catfish ponds treaded with a bacterial amendment (Waste & Sludge Reducer) and in three untreated pond (controls).

	pH		
week	treated	untreated	p-value
1	8.76	8.55	0.59
2	8.01	7.89	0.73
3	8.41	8.3	0.86
4	7.95	7.69	0.63
5	7.95	7.95	1.00
6	8.24	8.24	1.00
7	8.72	8.93	0.63
8	8.59	9.08	0.46
9	9.14	9.17	0.92
10	8.9	8.8	0.71
11	8.97	8.94	0.88
12	8.54	8.98	0.03
13	7.51	7.57	0.61
14	8.46	8.49	0.96
15	9.82	8.75	0.09
16	8.81	9.12	0.54
17	9.61	8.97	0.08
18	7.67	7.74	0.60
19	9.09	9.36	0.51
20	8.45	9.13	0.23
21	8.20	8.26	0.91

Table B.2- Total bacterial count in three channel catfish ponds treaded with a bacterial amendment (Waste & Sludge Reducer) and in three untreated pond (controls).

Total bacterial count			
week	treated	untreated	p-value
1	2.53E+03	5.53E+03	0.11
2	4.70E+03	5.17E+03	0.71
3	5.00E+02	4.03E+02	0.12
4	2.49E+03	6.75E+03	0.19
5	2.82E+03	1.23E+03	0.13
6	2.35E+03	1.51E+03	0.01
7	1.66E+03	1.46E+03	0.79
8	2.50E+03	1.84E+03	0.08
9	2.45E+03	8.93E+02	0.19
10	4.54E+03	3.03E+03	0.28
11	6.90E+03	4.65E+03	0.30
12	4.37E+02	5.47E+02	0.49
13	3.77E+03	4.23E+03	0.60
14	8.87E+03	5.47E+03	0.02
15	1.45E+03	4.87E+02	0.11
16	3.63E+04	5.01E+04	0.64
17	1.36E+04	8.33E+03	0.09
18	1.30E+04	8.07E+03	0.12
19	1.54E+04	5.60E+03	0.02
20	1.70E+04	1.26E+04	0.53
21	2.72E+04	1.44E+04	0.10

Table B.3- Concentrations of chlorophyll a (Chl a) in three channel catfish ponds treaded with a bacterial amendment, Waste & Sludge Reducer and in three untreated pond (controls).

Chlorophyll a (µg/L)			
week	treated	untreated	p-value
1	0.03	0.13	0.03
2	0.18	0.09	0.40
3	0.14	0.31	0.12
4	0.06	0.22	0.20
5	0.13	0.07	0.23
6	0.26	0.01	0.18
7	0.03	0.83	0.05
8	0.33	2.29	0.39
9	0.80	1.17	0.40
10	0.26	0.57	0.49
11	0.23	0.54	0.25
12	0.16	0.18	0.82
13	3.80	2.16	0.40
14	0.47	1.63	0.08
15	1.91	0.75	0.37
16	0.55	0.50	0.83
17	1.15	0.67	0.44
18	1.65	1.15	0.11
19	1.22	0.88	0.64
20	1.08	1.19	0.88
21	1.21	1.02	0.77

Table B.4- Concentrations of particulate organic matter (POM) in three channel catfish ponds treaded with a bacterial amendment, Waste & Sludge Reducer and in three untreated pond (controls).

Particulate organic matter (mg/L)			
week	treated	untreated	p-value
1	3.78	8.11	0.27
2	9.89	3.00	0.05
3	11.56	9.67	0.67
4	11.54	30.15	0.26
5	24.79	14.89	0.54
6	31.01	26.77	0.79
7	26.89	19.81	0.12
8	52.50	34.67	0.36
9	38.00	24.17	0.27
10	21.67	53.67	0.29
11	21.00	28.50	0.51
12	17.00	20.83	0.36
13	45.50	62.17	0.64
14	15.50	19.33	0.40
15	43.00	24.08	0.22
16	27.00	16.17	0.00
17	49.85	15.17	0.23
18	37.97	20.83	0.07
19	53.56	19.50	0.21
20	23.52	28.88	0.37
21	24.33	24.17	0.98

Table B.5- Concentrations of chemical oxygen demand (COD) in three channel catfish ponds treaded with a bacterial amendment, Waste & Sludge Reducer and in three untreated pond (controls).

Chemical oxygen demand (mg/L)			
week	treated	untreated	p-value
1	6.40	7.04	0.52
2	8.00	6.08	0.10
3	10.67	8.41	0.65
4	8.23	8.55	0.89
5	8.00	10.56	0.32
6	7.75	9.30	0.55
7	19.63	26.68	0.13
8	16.87	22.08	0.32
9	19.93	17.31	0.36
10	15.00	16.33	0.53
11	18.81	19.14	0.87
12	4.29	10.56	0.06
13	19.25	24.50	0.16
14	13.64	16.43	0.45
15	33.79	21.39	0.13
16	30.00	25.50	0.27
17	27.55	18.68	0.20
18	36.86	21.66	0.08
19	41.25	21.78	0.27
20	24.44	24.44	1.00
21	20.20	21.58	0.74

Table B.6- Concentrations of soluble chemical oxygen demand (SCOD) in three channel catfish ponds treaded with a bacterial amendment, Waste & Sludge Reducer and in three untreated pond (controls).

Soluble chemical oxygen demand (mg/L)			
week	treated	untreated	p-value
1	7.04	11.52	0.06
2	10.56	6.08	0.37
3	5.17	3.88	0.53
4	4.75	5.07	0.72
5	5.44	5.12	0.52
6	4.65	5.89	0.21
7	24.23	27.29	0.61
8	6.75	7.97	0.35
9	8.82	11.76	0.42
10	7.67	8.67	0.42
11	22.44	24.75	0.47
12	0.99	7.59	0.24
13	15.75	12.60	0.51
14	8.06	13.64	0.17
15	13.33	12.71	0.73
16	14.40	16.20	0.57
17	18.37	17.42	0.88
18	12.93	11.64	0.66
19	15.18	17.82	0.73
20	14.41	10.97	0.12
21	17.15	13.56	0.34

Table B.7- Concentrations of total ammonia nitrogen (TAN) in three channel catfish ponds treaded with a bacterial amendment, Waste & Sludge Reducer and in three untreated pond (controls).

Total ammonia nitrogen (mg/L)			
week	treated	untreated	p-value
1	0.086	-0.002	0.29
2	0.001	0.004	0.55
3	-0.010	0.000	0.45
4	0.022	0.013	0.24
5	0.032	0.023	0.65
6	0.072	0.049	0.26
7	0.034	0.035	0.89
8	0.066	0.035	0.16
9	0.059	0.182	0.40
10	0.046	0.080	0.31
11	0.045	0.043	0.88
12	0.041	0.036	0.65
13	0.103	0.088	0.62
14	0.163	0.584	0.09
15	0.069	0.177	0.48
16	0.155	0.530	0.41
17	0.057	0.172	0.44
18	0.077	0.261	0.31
19	0.601	0.060	0.36
20	1.195	0.240	0.06
21	1.101	0.322	0.12

Table B.8- Concentrations of nitrite nitrogen (NO_2^-–N) in three channel catfish ponds treaded with a bacterial amendment, Waste & Sludge Reducer and in three untreated pond (controls).

Nitrite nitrogen (mg/L)			
week	treated	untreated	p-value
1	0.002	0.002	0.49
2	0.001	0.003	0.17
3	0.000	0.001	0.33
4	0.017	0.011	0.13
5	0.019	0.016	0.83
6	0.022	0.020	0.84
7	0.017	0.015	0.67
8	0.027	0.019	0.26
9	0.026	0.022	0.44
10	0.017	0.020	0.57
11	0.018	0.013	0.09
12	0.011	0.010	0.87
13	0.029	0.025	0.40
14	0.005	0.009	0.40
15	0.025	0.019	0.31
16	0.015	0.026	0.56
17	0.023	0.053	0.49
18	0.034	0.065	0.34
19	0.072	0.052	0.62
20	0.101	0.051	0.54
21	0.114	0.085	0.65

Appendix C- Alabama Commercial Catfish Ponds Study

Table C.1- The pH in four channel catfish ponds treaded with a bacterial amendment (Aqua PE) and in two untreated pond (controls).

	pH		
week	treated	untreated	p-value
1	7.46	7.38	0.60
2	8.62	8.60	0.97
3	8.19	8.22	0.94
4	7.69	7.69	0.98
5	8.10	7.97	0.56
6	8.43	8.06	0.43
7	7.89	7.78	0.55
8	7.34	7.35	0.98
9	7.34	7.56	0.48
10	7.62	7.51	0.49
11	8.12	7.75	0.50
13	7.64	8.41	0.23
14	7.61	7.51	0.41

Table C.2- Turbidity in four channel catfish ponds treaded with a bacterial amendment (Aqua PE) and in two untreated pond (controls).

Turbidity			
week	treated	untreated	p-value
1	53.93	22.20	0.35
2	43.98	20.10	0.27
3	55.25	19.75	0.29
4	36.25	16.00	0.14
5	32.80	17.45	0.23
6	39.05	14.35	0.00
7	53.05	20.10	0.34
8	53.18	14.70	0.26
9	55.23	14.90	0.31
10	36.95	14.45	0.14
11	42.65	18.95	0.25
13	42.08	18.80	0.18
14	49.35	24.70	0.34

Table C.3- Total Nitrogen (TN) in four channel catfish ponds treaded with a bacterial amendment (Aqua PE) and in two untreated pond (controls).

Total Nitrogen (mg/L)			
week	treated	untreated	p-value
1	0.77	0.40	0.48
2	1.64	1.77	0.90
3	1.22	1.66	0.57
4	0.91	0.84	0.85
5	0.71	0.59	0.62
6	0.49	0.37	0.71
7	0.38	0.35	0.91
8	1.76	3.62	0.54
9	0.31	0.17	0.49
10	0.67	0.33	0.36
11	0.85	0.37	0.27
13	0.77	0.30	0.30
14	0.71	0.31	0.41

Table C.4- Total Phosphorus (TP) in four channel catfish ponds treaded with a bacterial amendment (Aqua PE) and in two untreated pond (controls).

Total Phosphorus (mg/L)			
week	treated	untreated	p-value
1	0.16	0.13	0.26
2	0.15	0.14	0.26
3	0.67	1.01	0.29
4	0.62	0.59	0.29
5	0.62	0.61	0.65
6	0.11	0.12	0.76
7	0.24	0.15	0.43
8	0.24	0.15	0.43
9	0.27	0.12	0.48
10	0.17	0.14	0.29
11	0.17	0.14	0.35
13	0.16	0.13	0.23
14	0.18	0.15	0.48

Table C.5- Concentrations of chlorophyll a (Chl a) in four channel catfish ponds treaded with a bacterial amendment (Aqua PE) and in two untreated pond (controls).

Chlorophyll a (μg/L)			
week	treated	untreated	p-value
1	555.14	170.47	0.47
2	444.02	123.17	0.41
3	779.15	134.77	0.41
4	261.95	148.16	0.38
5	203.04	114.24	0.48
6	214.65	99.96	0.14
7	575.89	141.91	0.54
8	1614.98	74.97	0.52
9	881.34	107.10	0.48
10	249.45	82.85	0.33
11	431.97	60.69	0.27
13	165.11	74.08	0.24
14	146.15	58.91	0.35

Table C.6- Concentrations of total ammonia nitrogen (TAN) in four channel catfish ponds treaded with a bacterial amendment (Aqua PE) and in two untreated pond (controls).

Total ammonia nitrogen (mg/L)			
week	treated	untreated	p-value
1	0.82	3.26	0.14
2	0.48	2.83	0.01
3	0.31	3.69	0.01
4	1.12	1.79	0.43
5	1.59	1.24	0.71
6	0.82	0.61	0.70
7	1.34	1.54	0.84
8	1.46	2.36	0.24
9	1.22	1.80	0.53
10	1.99	3.91	0.13
11	1.59	2.76	0.55
13	1.03	2.33	0.24
14	0.95	6.07	0.16

Table C.7- Concentrations of nitrite nitrogen (NO_2^-–N) in four channel catfish ponds treaded with a bacterial amendment (Aqua PE) and in two untreated pond (controls).

Nitrite nitrogen (mg/L)			
week	treated	untreated	p-value
1	0.18	0.08	0.66
2	0.04	0.23	0.20
3	0.04	0.14	0.03
4	0.04	1.18	0.16
5	0.06	0.08	0.53
6	0.07	0.26	0.02
7	0.46	0.05	0.35
8	0.04	0.23	0.01
9	0.32	0.63	0.30
10	0.24	0.28	0.86
11	0.14	0.32	0.09
13	0.29	0.20	0.81
14	0.29	0.25	0.78

Table C.8- Concentrations of chemical oxygen demand (COD) in four channel catfish ponds treaded with a bacterial amendment (Aqua PE) and in two untreated pond (controls).

Chemical oxygen demand (mg/L)			
week	treated	untreated	p-value
1	39.25	25.50	0.22
2	13.38	7.00	0.20
3	37.25	47.25	0.74
4	26.25	11.00	0.09
5	27.00	17.50	0.39
6	26.50	27.50	0.91
7	47.50	35.50	0.32
8	38.75	35.00	0.78
9	39.25	25.50	0.22
10	33.50	34.50	0.88
11	50.75	30.00	0.25
13	48.75	35.50	0.13
14	37.25	33.00	0.61