Evaluation of Bio-organic Catalyst in Channel Catfish Ponds

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ABSTRACT

A Bio-organic catalyst (AC+, Neozyme International) was tested in ponds at Auburn, Alabama, for its effect on water quality, soil organic carbon, and channel catfish production. Ponds treated with AC+ had higher concentrations of dissolved oxygen (DO) than control ponds during summer months even though all ponds were aerated mechanically. Data on water quality and soil organic carbon suggested that the primary influence of AC+ was to inhibit phytoplankton productivity which in turn lessened the nighttime oxygen demand. Although fish production did not increase as the result of greater DO availability, fish harvest and survival were higher in the treated ponds (P=0.1). At the maximum daily feeding rate of 75kg/ha, water quality was not severely impaired in any of the ponds. The bio-organic catalyst product might have greater benefits in ponds with higher stocking and feeding rates. Information on the mechanism of action of bio-catalyst additions in pond ecosystems would be useful in determining their potential benefits to pond aquaculture.

INTRODUCTION

Amendments high in enzymatic activity such as bio-organic catalysts have been developed to accelerate natural microbial process in soils, water or other media. These bio-organic catalysts products have been used in agriculture, wastewater treatment, clean up of oil spills, remediation of sites contaminated with hazardous wastes. Enzymes are catalysts that accelerate biochemical reactions, and microorganisms excrete extracellular enzymes break large molecules into smaller fragments that can be absorbed by microbial cells (Anderson 1987), and they can cause transformations of various compounds to accelerate microbial processes (Dick and Tabatabai 1992). Enough success has been obtained with some of these amendments to encourage companies to produce and market them for a variety of applications (Glass 1992).

Some of these companies recommend the use of their products in pond aquaculture. According to information from one manufacturer (Neozymes International, Inc, Aliso Viejo, California), their product can improve water quality through increasing the solubility of hydrophobic material, improving gas diffusion by increasing dissolved oxygen levels, and accelerating breakdown of organic matter. Therefore, the present study was initiated to evaluate the use of this bio-organic catalyst (BOC), which contains derived catalysts, surface modifying synthetic compounds, and other proprietary ingredients, in channel catfish (Ictalurus punctatus) ponds.

MATERIALS AND METHODS

Ponds

Nine 400-m^2 levee ponds on the Auburn University Fisheries Research Unit (FRU) were used in this study. Ponds were square with earthen bottoms that gradually sloped from depths of about 20-40cm at the vertical edges supported by wooden or concrete walls to 130-150cm at the drains. Ponds volumes ranged from 450 to 480m^3. These ponds have been used annually for experiments on water quality and pond fertilization for 25 years.

Soils used for construction of these ponds were typic, Kandiudults (clayey, kaolinitic, and thermic). They are acidic, reddish brown soils of low cation exchange capacity with base saturation less than 35%, and in their native state, these soils have low concentrations of phosphorus and organic matter (McNutt 1981). Auburn, Alabama, is located at 32.5 N latitude.

The normal annual temperature is 17.2^{o}C (normal minimum = 11.1^{o}C; normal maximum = 23.2^{o}C); normal annual rainfall is 1,434mm.

Ponds are supplied by water from a reservoir filled by runoff from woodland (Boyd 1990). This water has total alkalinity and total hardness values below 20mg/L as $CaCO_3$ and soluble reactive phosphorus concentrations less than 0.005mg/L. These ponds are treated most years with 500 to 1,000kg/ha of agricultural limestone to maintain adequate pH, alkalinity, and hardness.

Management

The ponds were stocked at a rate equal to 15,000 channel catfish fingerlings per hectare in April 1996. The average fish weight at stocking was 10.7kg per thousand fingerlings. A 32% crude protein, pelleted feed was offered 7 days per week at 3% of body weight per day. Feeding rates were increased weekly according to an assumed feed conversion (weight of feed applied/weight gained by the fish) of 1.6. Ponds were inspected daily, and any dead fish were removed. Daily feeding rate did not exceed 75kg/ha. When this rate was reached, it was continued until fish harvest. All ponds had a 0.25-kW vertical pump aerator (Air-O-Lator Corporation, Kansas City, Missouri) connected to a timer. Aerators were operated from dusk to dawn from the end of June until harvest in October. Water levels in ponds were maintained 10-20cm below the tops of standing drain pipes to prevent overflow after rains. Water was added from a pipeline when necessary to replace evaporation and seepage losses.

Ponds were drained and fish were harvested and weighed on 15 October.

Bio-organic Catalyst Treatment

A bio-organic catalyst product marketed under the trade name AC+ was obtained from Neozymes International, Inc, Aliso Viejo, California. The experiment was designed so that the treatment applied to any given pond was not revealed until after the ponds were harvested. The researchers assigned each pond a letter (A-I) by ballot. The manufacturer bottle 960ml aliquots of AC+ to provide 2mg/L in ponds (based on 480m^3 volume) and equal volumes of a placebo consisting of herbal tea that looked like AC+. Bottles were identified by letters A to I to correspond to letters assigned to ponds and the application date and shipped to Auburn University. This provided three treatments of three replications each as follows: high AC+ treatment – 2mg/L AC+ weekly (8mg/L per month); low AC+ treatment – 2mg/L AC+ one week followed by applications of placebo for three weeks (2mg/L per month); control – placebo at weekly intervals. The manufacturer sent the code for identifying bottle contents to Dr Craig, S Tucker, Delta Research and Extension Center, Stoneville, Mississippi, and he revealed it to the researchers at the end of the study. These treatments were applied between 13 May 1996 and 11 October 1996. Placebo and AC+ were splashed over pond surfaces, and the aerator was then operated for 1 hour to mix the materials with the pond waters.

Measurements

Dissolved oxygen and temperature were measured one or more times per day with a polarographic oxygen meter (Yellow Springs Instrument Company, Yellow Springs, Ohio) to verify that fish were not subjected to low oxygen stress. Water samples were collected between 0630 and 0700hr at 2 weeks intervals with a 90cm water column sampler (Boyd and Tucker 1992). Samples were taken from three places in each pond and combined to give a composite for analysis. Analyses were conducted for pH (glass electrode), total alkalinity (acidimetry), total hardness (EDTA titration), specific conductance (conductivity meter), chemical oxygen demand (potassium dichromate – sulfuric acid oxidation), biochemical oxygen demand (standard 5 day test), soluble reactive phosphorus (ascorbic acid method), total ammonia nitrogen (phenate method), chlorophy11 **a** (membrane filtration, acetone extraction, and spectroscopy) and bacterial abundance (standard plate count). Standard protocol was followed for these analyses (Eaton et al 1989). Nitrate was determined by the NAS reagent method (van Rijn 1993). Before stocking and 1 week before harvest, five soil samples (upper 5cm layers) were collected from each pond with a 5cm diameter core sampler. On each sampling, the samples from a given pond were combined to make a composite sample. Soil samples were dried at 60^{o}C and pulverized in a hammer mill. They were analysed for total carbon by an induction furnace carbon analyser (Leco Corporation, St Joseph Michigan). At the harvest time, the following fish production data were collected: number and total weight of fish, percentage survival, average weight of individual fish and food conversion ratio.

Data Analysis

Means were tested for statistical differences with Duncan's multiple range test. A large degree of variation is typically encountere3d in water quality and fish production variables in experiments conducted in earthen, aquaculture ponds (Boy et al 1994). Because of this large variation, the amount of replication necessary to declare significant differences at the 0.05 probability level in pond aquaculture experiments is often prohibitive. Therefore, in this study, we declared means to be different if the probability was 0.1 or less.

RESULTS

Total alkalinity and total hardness concentrations in pond water were between 25 and 30mg/L in May, and they increased to 35 to 40mg/L in October. Specific conductance of pond waters was within the range 75 to 105 umhos/cm and morning pH values were between 7.7 and 8.2. Morning water temperature in ponds increased from 23°C on 16 May to 29°C on 8 August and declined to 18°C by 14 October. No influence of AC+ treatment could be detected on pH, specific conductance, water temperature, alkalinity and hardness.

Because ponds were aerated nightly, dissolved oxygen (DO) concentrations were seldom less than 3mg/L. From July to September, when water temperature and feeding rates were high, DO concentrations often were 1 or 2mg/L greater in ponds treated with AC+ than in control ponds (Figure 1), but the high AC+ and low AC+ treatments were similar in DO concentration. Concentrations of soluble reactive phosphorus (SRP) were quite similar in all ponds during most of the study, but there were large increases in SRP concentration in the high AC+ treatment on two dates and in the low AC+ treatment on one date (Figure 1). On a few dates, nitrate-nitrogen (NO_3-N) concentration also tended to be higher in ponds treated with AC than in control ponds. During the summer, there was a trend of greater concentrations of total ammonia nitrogen (TAN) in AC+ ponds than in control ponds (Figure 1). Also, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) concentrations often were slightly lower during the summer in ponds treated with AC+ as compared to controls (Figure 1).Chlorophy11 **a** concentration (an index of phytoplankton abundance) tended to be higher during August and September in control ponds than in ponds of the AC+ treatments. From June through August, there was a trend of greater bacteria abundance in control ponds than in AC+ ponds (Figure 1). Even though trends of difference in water quality between control and AC+ ponds were detectable at certain times, grand means for treatments were similar (Table1). They only significant difference was for SRP concentration, and the difference resulted from high concentrations of SRP in AC+ ponds on a few dates.

Pond soils on the FRU do not naturally contain free calcium carbonate (McNutt 1981) and only small amounts of residual calcium carbonate could have resulted from agricultural limestone applications. Therefore, the total carbon concentrations in pond soil were considered to result primarily from organic carbon. In May, averages and standard deviations for percentages of soil carbon were as follows: control, 0.81$\pm$ 0.15; low AC+, 1.00 $\pm$ 0.30; high AC+, 0.70 $\pm$ 0.18. Soil carbon increased in all ponds during the study, and percentages found in October were: control, 1.26 $\pm$ 0.14; low AC+, 1.40 $\pm$ 0.91; high AC+, 1.21 $\pm$ 0.59. These data suggest that AC+ treatment did not influence the rate of increase of soil organic carbon in pond bottoms.

Fish production data are summarized in Table 2. A portion of the fish in all ponds were infected by proliferative gill disease and enteric septicemia of catfish (Edwardsiela ictaluri) and survival ranged from 56.1% in controls to 75.7% in high AC+ ponds. Nevertheless, similar amounts of feed were applied in all ponds, and fish tended to be larger at harvest in ponds that had the lowest survival. Feed conversion ration and net fish production did not differ among treatments (P<0.1).

DISCUSSION

Even though ponds were aerated during the night, there was a trend of higher DO concentrations in the ponds treated with AC+ as compared to control ponds. Differences in DO concentration were most obvious in August and September. This difference resulted because higher concentrations of COD and BOD and more phytoplankton in control ponds than in AC+ ponds caused a greater nighttime demand for oxygen in control ponds.

One explanation for the lower oxygen demand in AC+ ponds is that additions of bio-organic catalysts accelerated the decomposition of organic matter to lower BOD and COD. This explanation is consistent with the observation that SRP, TAN and NO_3-N concentration were greater in AC+ ponds, because greater decomposition would have caused more nutrient recycling. However, greater nutrient recycling should have stimulated phytoplankton productivity in the AC+ ponds, but this effect was not observed. Also, similar rates of organic carbon accumulation in pond soils of the three treatments is not consistent with enhanced decomposition of organic matter in AC+ ponds.

A more feasible explanation for the high concentration of chlorophy11 **a** BOD, COD at times in the control ponds is that AC+ have reduced the phytoplankton growth rate. Boyd (1973) and Boyd et al. (1978) demonstrated that BOD and COD concentrations in pond waters had a strong positive correlation with chlorophy11 **a** concentrations (phytoplankton abundance). An increase in chlorophy11 **a** concentration should cause an increase in BOD and COD concentrations. Nutrient inputs to all treatments in feed were similar, and the tendency of greater nutrient concentration in AC+ ponds relative to controls could have resulted from less uptake of nutrients by smaller phytoplankton biomass in AC+ ponds instead of greater rates of nutrient recycling in AC+ ponds. Boyd and Musi (1981) showed that the rate of decline in spikes of SRP made to pond waters increased with increasing phytoplankton abundance. Tucker et al. (1984) demonstrated that TAN concentrations in fish pond waters decreased as phytoplankton abundance increase. Thus, lower nutrient concentrations and higher BOD and COD values in control ponds are consistent with the explanation that AC+ have reduced the phytoplankton abundance. Further support of the effects of AC+ on phytoplankton growth rates comes from the claim by the manufacturer that AC+ can be used to reduce the abundance of nuisance algal growth in water. Because of the proprietary name of AC+, its composition was not divulged by Neozymes International. Without this information, one cannot speculate on the mechanism by which AC+ might have influenced phytoplankton growth.

Differences on fish harvest and survival were observed (Table 2). These results could be related to the action of the AC+, however the high variation between the ponds due to the few replicates used and the disease that has occurred requires further investigations.

The improvement in DO concentration in AC+ ponds did not cause greater fish production. This is not surprising because the stocking rate was conservative and the feeding rate did not exceed 75kg/ha per day. When mechanical aeration is used, dangerously low DO concentration, excessive TAN concentration, and other water quality impairment seldom occurs unless feeding rates exceed 100 to 120kg/ha per day (Boyd 1990; Tucker and Boyd 1995). The likelihood of water quality improvement and enhanced fish production through the use of bio-organic catalysts supplementation would be greater in ponds where stocking and feeding rates are high enough to cause more severe water quality deterioration than observed in the present study. Although there were not marked differences in water quality or fish production data between the low and high AC+ treatments, it seems better to use weekly treatments. The bio-catalyst molecules probably slowly degrade to non-functional form and more frequent application would provide a more constant concentration of bio-catalysts.

This study shows that bio-organic catalysts supplementation can effect improvement in water quality even in aquaculture ponds stocked and fed at conservative rates. Further studies are needed to show if larger benefit can be achieved in ponds where stocking and feeding rates are higher and water quality conditions are more critical. Information on the mechanisms of action of extracellular enzyme additions in ponds would also be useful in assessing the potential benefits of these products and defining the conditions under which they can be effective.

REFERENCES

Anderson JM 1987. Production and decomposition in aquatic ecosystems and implications for aquaculture. Pages 123-147 in DJW Moriarty and RSV Pullin eds. Detritus and Microbial Ecology in Aquaculture. ICLARM Conference Proceedings 14, International Centre for Living Aquatic Resources Management, Manila, Philippines.

Boyd CE 1973. The chemical oxygen demand of waters and biological materials from ponds. Transactions of the American Fisheries Society 102:606-611.

Boyd CE 1990. Water Quality in Ponds for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama.

Boyd CE and Y Musig 1980. Orthophosphate update by phytoplankton and sediment. Aquaculture 22:165-173

Boyd CE and CS Tucker 1992. Water Quality and Pond Soil Analyses for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama.

Boyd CE and CS Tucker 1995. Sustainability of channel catfish farming. World Aquaculture 26:45-53.

Boyd CE, RP Romaire and E Johnston 1978. Predicting early morning dissolved oxygen concentrations in channel catfish ponds. Transactions of the American Fisheries Society 107:484-492.

Boyd CE, E Hernandez, JC Williams and RP Romaire 1994. Effects of sampling technique on precision estimates for water quality variables in fish culture ponds. Journal of Applied Aquaculture 4:1-18.

Dick WA and MA Tabatabai 1992. Significance and potential uses of soil enzymes. Pages 95-127 in FV Meting, ed.. Soil Microbial Ecology. Marcel Dekker, Inc, New York, New York.

Eaton AD, LS Clesceri and AE Greenburg 1995. Standard Methods for the Examination of Water and Wastewater, 19ed. American Public Health Association, Washington DC.

Glass DJ 1992. Commercialisation of soil microbial technologies. Pages 595-618 in FB. Meeting ed. Soil Microbial Ecology. Marcel Dekker, Inc, New York, New York.

McNutt RB 1981. Soil Survey of Lee County, Alabama. National Cooperative Soil Survey, Soil Conservation Service, United States Department of Agriculture, Washington DC.

Tucker CS, SW Lloyd and RL Busch 1984. Relationships between phytoplankton periodicity and the concentrations of total and un-ionized ammonia in channel catfish ponds. Hydrobiologia 111:75-79.

Van Rijn J 1993. Methods to Evaluate Water Quality in Aquaculture. Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel.

TABLE 1

Grand means of water quality data collected from channel catfish ponds treated with extracellular enzymes at 4 week intervals (low AC+), weekly intervals (high AC+) and controls. There were three replications of each treatment.

	Treatment		
Variables	**Low AC+**	**High AC+**	**Control**
Dissolved oxygen (mg/L)	5.25 a	5.59 a	52.26 a
Soluable reactive phosphorus (mg/L)	0.039a	0.038 a	0.020 b
Total ammonia nitrogen (mg/L0	0.45 a	0.35 a	0.25 a
Nitrate nitrogen (mg/L	0.18 a	0.13 a	0.09 a
Chemical oxygen demand (mg/L)	23.5 a	19.0 a	25.1 a
Biochemical oxygen demand (mg/L)	7.8 a	7.8 a	8.12 a
Chlorophy11 **a** (ug/L)	64.5 a	54.5 a	62.2 a
Bacterial abundance (cells/mL x 10^2)	10.5 a	11.7 a	13.0 a

[a,b]Means indicated by the same letter did not differ significantly at P = 0.1. Horizontal comparisons only.

TABLE 2

Average channel catfish production data for ponds treated with extracellular enzymes at 4 week intervals (low AC+), weekly intervals (high AC+) and controls. There were three replications of each treatment.

	Treatment		
Variables	**Low AC+**	**High AC+**	**Control**
Stocking rate (number/ha)	15,000 a	15,000 a	15,000 a
Feed input (kg,ha)	6,128 a	6,298 a	6,008 a
Fish harvested (number/ha)	10,225 a	11,350 a	8,425 b
Survival (%)	68.2 ab	75.7 a	56.1 b
Average harvest weight per fish(g)	342 a	328 a	400 a
Net production (kg/ha)	3,502 a	3,728 a	3,301 a
Fee conversion ratio	1.75 a	1.77 a	1.82 a

[a,b]Means indicated by the same letter did not differ significantly at P = 0.1. Horizontal comparisons only.

FIGURE 1

Average water quality parameters that were measured biweekly in earthen catfish ponds in the fisheries research unit, Auburn Alabama between May to October 1996.