

Effects of a Bacterial Inoculum in Channel Catfish Ponds

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Abstract.—A commercial bacterial inoculum cultured on site called Biostart® was applied to three channel catfish *Ictalurus punctatus* ponds at Auburn, Alabama, USA, three times per week from May until October 1996. There were few significant differences ($P \leq 0.1$) in concentrations of water quality variables between ponds treated with bacteria and control ponds. In addition, bottom soil carbon and nitrogen did not differ between treated and control ponds. However, survival and net production of fish was significantly ($P \leq 0.1$) greater in ponds that received the bacterial inoculum than in controls. The mechanism by which the bacterial treatment influenced fish survival cannot be explained from data collected in this study. Further studies of probiotics are needed to define the potential benefits of these treatments to aquacultural production and to determine their mechanisms of action in pond ecosystems.

There has been considerable research in agriculture concerning the use of bacterial inocula to enhance the beneficial activities of bacteria in soils. Much of this work was done in the Soviet Union (Cooper 1959; Brown 1974), and positive responses were reported. Research in other nations has generally failed to show increase in crop yields following bacterial augmentation (Cooper 1959; Smith et al. 1961; Weaver et al. 1974), but research is continuing (Metting 1992; Rechcigl 1995). There also have been attempts to use bacterial inocula to augment wastewater treatment (Horsfall 1979; Schuetzle et al. 1982), but the results of these efforts have not resulted in the widespread use of bacterial augmentation of wastewater treatment systems.

Interest in bacterial inocula has spread to aquaculture, and ponds are sometimes inoculated with live microorganisms or with bacterial growth enhancers and extracellular enzyme preparations (Boyd 1995). Manufacturers and the vendors claim that augmentation of microbial activity with these products can improve water quality, en-

hance bottom soil conditions, reduce the incidence of diseases, and ultimately enhance aquacultural production. However, aside from advertisements and testimonials of satisfied users, it is difficult to find reliable documentation of positive benefits of bacterial inocula. Studies of bacterial inocula in channel catfish *Ictalurus punctatus* ponds failed to demonstrate differences in water quality and fish production between treated and control ponds (Boyd et al. 1984; Tucker and Lloyd 1985; Chiayvareesajja and Boyd 1993). Boyd and Pippopinyo (1994) applied a bacterial inoculum to fallow pond soils and were unable to demonstrate increases in soil respiration.

In studies mentioned above, inocula consisted of stabilized bacterial propagules packaged for direct application to ponds. These products were applied at intervals of one to several weeks. The present study was conducted to test one of the newer bacterial inocula that is cultured on site and applied to ponds at more frequent intervals.

Materials and Methods

Ponds

Six 400-m² earthen ponds with average depths of 0.9–1.0 m and maximum depths of 1.3–1.5 m on the Auburn University Fisheries Research Unit, Auburn, Alabama, USA, were used in this study. The ponds were rectangular (2:1) with earthen bottoms that gradually sloped, and the vertical edges were supported by wooden or concrete walls to maximum depths of 1.3 to 1.5 m at the drains. These ponds had been used for 25 yr in experiments in which fertilizers or feeds had been applied to promote fish production.

Ponds selected for use in this experiment were used in 1994 and 1995 in pond fertil-

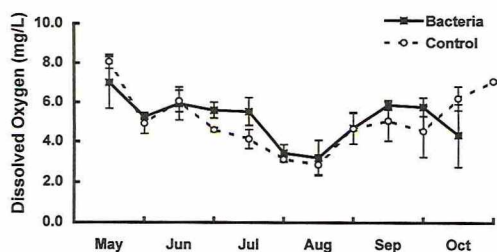


FIGURE 1. Dissolved oxygen concentrations measured in the early morning (0630–0730 h) in control ponds and in ponds treated with a bacterial inoculum at frequent intervals.

pond, and the three samples were combined to provide a composite sample for analysis. Samples were obtained between 0630 and 0700 h and immediately carried to the laboratory where analyses were conducted according to standard protocol (Eaton et al. 1995) for biochemical oxygen demand (standard 5-d test), soluble reactive phosphorus (ascorbic acid method), total ammonia-nitrogen (phenate method), nitrate-nitrogen by NAS reagent method (van Rijn 1993), chlorophyll *a* (acetone extraction and spectroscopy), and bacterial abundance (standard plate count medium). Twice each month, additional analyses were made for total alkalinity (acidimetry), total hardness (EDTA titration), pH (glass electrode), and specific conductance (conductivity meter). All samples were analyzed for chemical oxygen demand by the heat of dilution method (Boyd and Tucker 1992).

Soil samples (upper 5-cm layer) were collected from five places in each pond with

a core sampler before fish were stocked and on the day before ponds were drained. All cores from a pond were composited. Samples were dried at 60 C and pulverized to pass a 0.15-mm sieve. Soil samples were analyzed for total carbon and nitrogen with a Leco C-H-N Analyses (Leco Corporation, Saint Joseph, Michigan, USA).

At harvest, several fish were taken for detection of off-flavor in the flesh by a trained sensory panel according to a method described by van der Ploeg (1991).

Data Analysis

Means were tested for statistical differences with *t*-tests. A large degree of variation is typically encountered in water quality and fish production variables in experiments conducted in earthen, aquaculture ponds (Boyd 1990; Boyd 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 and Discussion

Total alkalinity and total hardness were similar in all ponds and increased from 25–30 mg/L in May to 35–40 mg/L in October. There was a corresponding increase in specific conductance from 45–50 μ mhos/cm to 85–95 μ mhos/cm. The pH of pond waters ranged between 7.2 and 8.6. No dif-

TABLE 1. Grand means and standard deviations for water quality variables in channel catfish ponds receiving a bacterial inoculum (treated) and in control ponds for the period 8 May to 14 October 1996. None of the means of treated and control ponds differed at a probability level of 0.1.

Variable	Treated	Control
Dissolved oxygen (mg/L)	5.29 $\pm$ 1.74	5.26 $\pm$ 1.76
Soluble reactive phosphorus (mg/L)	0.037 $\pm$ 0.062	0.020 $\pm$ 0.027
Total ammonia nitrogen (mg/L)	0.45 $\pm$ 0.79	0.28 $\pm$ 0.56
Nitrate nitrogen (mg/L)	0.13 $\pm$ 0.21	0.09 $\pm$ 0.14
Chemical oxygen demand (mg/L)	21.7 $\pm$ 13.8	25.1 $\pm$ 16.2
Biochemical oxygen demand (mg/L)	9.1 $\pm$ 5.2	8.1 $\pm$ 4.6
Chlorophyll <i>a</i> (μ g/L)	76.7 $\pm$ 83.9	62.1 $\pm$ 59.2
Bacterial abundance (cells/mL $\times 10^3$)	13.6 $\pm$ 19.3	13.0 $\pm$ 11.9

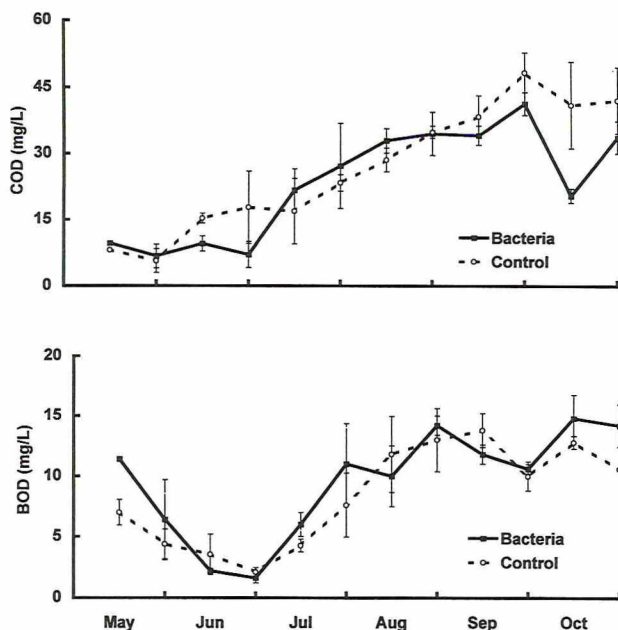


FIGURE 4. Concentrations of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in control ponds and in ponds treated with a bacterial inoculum at frequent intervals.

on 16 May to 29 C on 8 August and then decreased to 18 C by 14 October. The apparent difference in DO concentration between treated and control ponds (Fig. 1) were not significant ($P > 0.1$). Grand means for DO concentrations for treated and control ponds (Table 1) were not different ($P > 0.1$).

There were individual dates when concentrations of soluble reactive phosphorus, total ammonia-nitrogen, and nitrate-nitrogen were greater ($P \leq 0.1$) in ponds treated with the inoculum than in control ponds

(Fig. 2). However, grand means for these three variables did not differ between the treated and control ponds ($P > 0.1$). Chlorophyll *a* concentrations increased in both treatments from May until August and remained high until harvest in October (Fig. 3). No differences ($P > 0.1$) could be detected between chlorophyll *a* concentrations in treated and control ponds on individual dates or for grand means (Table 1).

TABLE 2. Average concentrations and standard deviations of total carbon and nitrogen in bottom soils of channel catfish ponds receiving a bacterial inoculum (treated) and in control ponds. Means for treated and control ponds were not different at the beginning of the study in May or at the end in October at a probability level of 0.1.

Time	Control	Treated
Carbon (%)		
May	0.81 $\pm$ 0.15	0.76 $\pm$ 0.26
October	1.34 $\pm$ 0.69	1.90 $\pm$ 0.15
Nitrogen (%)		
May	0.04 $\pm$ 0.02	0.04 $\pm$ 0.02
October	0.11 $\pm$ 0.08	0.16 $\pm$ 0.02

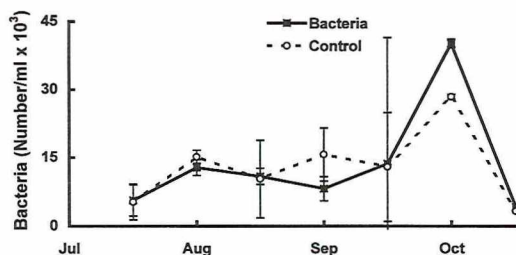


FIGURE 5. Bacterial abundance in control ponds and in ponds treated with a bacterial inoculum at frequent intervals.

determine their mechanisms of action in pond ecosystems.

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