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[Communication]

Bacterial Bioaugmentation of Channel Catfish Ponds

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Abstract.—Twelve, 0.1-ha earthen ponds at Stoneville, Mississippi were used in a 2-year, double-blind study of the effects of a *Bacillus*-based bacterial bioaugmentation product on water quality and production of channel catfish *Ictalurus punctatus*. Each year, six ponds were treated weekly with the microbial product from late May or early June through October, and six ponds were designated as untreated controls. Mean concentrations of chlorophyll *a*, nitrite-N, and total ammonia-N did not significantly differ ($P > 0.05$) between bacteria-treated and untreated ponds in the first year; however, in the second year, mean chlorophyll-*a* concentrations were higher ($P \leq 0.05$) and nitrite-N and total ammonia-N concentrations were lower ($P \leq 0.05$) in bacteria-treated ponds than in untreated ponds. Reductions in dissolved inorganic nitrogen concentrations were more likely due to increased phytoplankton growth (i.e., increased algal assimilation) than to a direct effect of bacterial inoculation. The mechanism by which bioaugmentation may have enhanced phytoplankton growth is unknown and contradicts several past studies. Net fish production and feed conversion ratios were not affected by bacterial treatment in either year ($P > 0.05$); accordingly, there is no economic incentive to use *Bacillus*-bacterial bioaugmentation products in channel catfish ponds.

Aquaculture production in ponds that are operated with long hydraulic residence times is ultimately limited by the capacity of the pond microbial community to treat wastes produced during culture (Hargreaves and Tucker 2003). To intensify aquaculture production beyond that achievable in static-water ponds, the production system must be designed or managed to increase waste-treatment capacity. One approach to aquaculture intensification is to export wastes produced during culture and treat them outside the culture unit. Waste treatment may be accomplished in separate units of the overall culture system (as in recirculating aquaculture systems) or by discharging wastes to public waters (as in raceways and net-pen culture).

An alternative approach to intensifying aquaculture production is to increase the capacity of the within-pond microbial community to remove or transform metabolic wastes. This is analogous to the goal of increasing the capacity of soil or aquatic microbial communities to biodegrade pollutants through "engineered bioremediation." Engineered bioremediation (in contrast to intrinsic bioremediation, which is the ability of the native microflora to treat wastes) can be accomplished either by improving environmental conditions to stimulate the growth and activity of naturally occurring microorganisms (biostimulation) or by supplementing naturally occurring microflora with organisms produced in culture (bioaugmentation).

Although the term "biostimulation" is not used in aquaculture, the use of mechanical aeration in ponds is a good example of the concept. In addition to the primary goal of providing dissolved oxygen for cultured animals, aeration also improves conditions for organic matter decomposition (Ayub et al. 1993) and nitrification (Hargreaves and Tucker 1996).

Bioaugmentation is based on the concept that the rate of a particular microbially mediated process may be limited by the abundance of microorganisms carrying out that process. As such, process rates can be increased by adding an allochthonous source of microorganisms to the system. Typically, microorganisms used in bioaugmentation are isolated from nature by means of enrichment techniques and then grown in mass laboratory cultures. Bioaugmentation is most commonly used to remediate soils contaminated with hazardous organic pollutants such as crude oil, petroleum products, or pesticides (Vogel and Walter 2001; Wagner-Döbler 2003; Thompson et al. 2005). Bioaugmentation has also been used in wastewater treatment (van Limbergen et al. 1998) and in lake and pond management (Boyd et al. 1984; Chiayuvareesajja and Boyd 1993; Quieroz and Boyd 1998; Duvall and Anderson 2001; Duvall et al. 2001). Treatment success has been variable regardless of the specific application.

Despite the uneven record of success, microbial products are aggressively marketed for aquaculture use. Specific claims for bacterial supplements sold for use in aquaculture vary among products, but general claims include improved water quality, reduced algal growth, enhanced sediment conditions, and improved aquaculture production. The study described below evaluates the effect of a *Bacillus*-based bioaugmentation product on water quality and production of channel catfish *Ictalurus punctatus* in a 2-year, double-blind study.

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Methods

This study was conducted over 2 years in 12, 0.1-ha earthen ponds at the Delta Research and Extension Center, Stoneville, Mississippi. Ponds were constructed on the Sharkey soil series of very fine, smectitic, thermic Chromic Epiaquerts of the Yazoo–Mississippi River floodplain in northwest Mississippi. Water was supplied from a well pumping from the Mississippi River Alluvial Aquifer, which is the water source for all catfish aquaculture in northwest Mississippi. Pond water levels were maintained below the tops of standpipes so that no water was discharged from ponds during the sampling period. Total alkalinity and total hardness of pond waters varied between 100 and 200 mg/L as $CaCO_3$, with about 70% of the hardness contributed by calcium. Each pond was equipped with a 0.75-kW surface spray-type aerator that was operated from 2200 to 0800 each night throughout the study. Pond construction, soil type, water supply, and water management were typical of those used in channel catfish aquaculture in northwest Mississippi (Tucker 1996).

In Year 1 of the study, all ponds were stocked on 30 March 1997 with 1,500 fingerling channel catfish (15,000 fish/ha), with an average fish weight of 30 g/fish. Fish were fed a 32% crude protein, floating catfish feed on a maintenance ration (45 kg/ha 3 d per week) until mid-May, after which fish were fed daily to satiation until the end of the study on 8 November 1997. In Year 2, ponds were stocked on 16 April 1998 with 2,300 channel catfish fingerlings (23,000 fish/ha), with an average fish weight of 45 g/fish. Fish were fed a 32% crude protein, floating catfish feed at 45 kg/ha 3 d per week until mid-May, after which fish were fed daily to satiation until the end of the study on 28 October 1998. Fish were fed in both years by personnel with no knowledge of the study's nature or assignment of treatments to particular ponds.

The bacterial bioaugmentation product (Impact A; Environmental Dynamics, Inc., Natural Bridge Station, Virginia) and a "placebo" consisting only of the dry inert carrier were delivered to the research site in the spring of each year in containers marked only as "A" and "B." The identity of the two sets of containers was known only to the manufacturer. Containers were reassigned "X" and "Y" labels by an uninvolved person at Stoneville so that neither the manufacturer nor the on-site investigators knew the identity of the treated and untreated ponds during the studies. Treatments (X or Y) were assigned to the 12 ponds by ballot procedure each year. Coding of product identity and pond treatment assignment were sent each year to Claude Boyd at Auburn University, Alabama, and held until fish were harvested and data were summarized.

Product and placebo were applied weekly from 11 June to 15 October in Year 1 (19 total applications) and from 26 May to 23 October in Year 2 (20 total applications). Each year, the initial treatment rate was 1 kg of material per pond (approximately 1 g/m^3), followed weekly with 0.35 kg of material per pond (approximately 0.35 g/m^3). The materials were mixed with pond water in a plastic container at 20 L/kg of product and allowed to stand for 2 h before application to the pond.

Water samples for measurement of chlorophyll *a*, total ammonia, and nitrite were collected at biweekly intervals during the treatment period each year. Single samples were collected 20 cm beneath the surface, about 5 m from the bank at the deepest point in each pond, with a 2-L Kemmerer bottle. Analyses were initiated within 30 min of collection by using the following procedures: chlorophyll *a* by extraction into acetone–methanol followed by spectrophotometry (Pechar 1987), nitrite by diazotization (APHA et al. 1992), and total ammonia by the phenate method (APHA et al. 1992).

Chlorophyll-*a*, nitrite, and total ammonia data were compared statistically between treatments for sampling dates during the entire treatment period and for sampling dates after 1 August of each year. Comparisons made late in the growing season are often instructive in studies of water quality management in channel catfish ponds because treatment effects may be cumulative and appear only in the later stages of the study. Also, water quality in channel catfish ponds tends to deteriorate over time as nutrient loading increases in response to greater feed additions as fish grow. Positive effects of treatment are, therefore, most important at that time. Analyses were conducted for a completely randomized design with repeated measures taken biweekly. Data were analyzed with the MIXED Procedure in SAS version 9.1 software (SAS Institute, Cary, North Carolina) by using covariance structure autoregressive order 1. Net channel catfish production (harvested biomass minus stocking biomass) and feed conversion ratios (weight feed offered divided by net fish production) were analyzed by using Student's *t*-test. A probability level of 0.05 was accepted as significant for all comparisons.

Results

Pond water quality was typical of channel catfish ponds managed as "single-crop" systems wherein fish are stocked in spring, grown through the summer, and harvested in late autumn or winter. That is, phytoplankton abundance (as measured by chlorophyll-*a*

TABLE 1.—Least-squares means of water quality variables in untreated control ponds and in ponds treated weekly with a commercial bacterial bioaugmentation product. Means were compared in each of the two study years for biweekly water samples taken throughout the treatment period (11 June to 15 October in Year 1 and 26 May to 23 October in Year 2) and for samples collected only after 1 August of each year. Means in a row followed by different letters differ significantly ($\alpha = 0.05$). Means in a row not followed by letters are not significantly different.

Variable	Least-squares mean		Pooled standard error
	Untreated	Treated	
Year 1, all samples			
Chlorophyll a (μg/L)	118	103	9
Nitrite-N (mg/L)	0.08	0.09	0.01
Total ammonia-N (mg/L)	0.35	0.47	0.06
Year 1, samples after 1 August			
Chlorophyll a (μg/L)	134	115	11
Nitrite-N (mg/L)	0.12	0.13	0.02
Total ammonia-N (mg/L)	0.48	0.64	0.09
Year 2, all samples			
Chlorophyll a (μg/L)	141 z	174 y	12
Nitrite-N (mg/L)	0.10 z	0.04 y	0.01
Total ammonia-N (mg/L)	0.47 z	0.30 y	0.04
Year 2, samples after 1 August			
Chlorophyll a (μg/L)	145 z	187 y	16
Nitrite-N (mg/L)	0.12 z	0.05 y	0.02
Total ammonia-N (mg/L)	0.52 z	0.30 y	0.06

concentrations), nitrite-N, and total ammonia-N increased through the growing season as fish grew and feeding rates increased (data not shown). Mean values for all water quality variables were higher late in the growing season compared with the season-long average in both years (Table 1). Mean chlorophyll-*a* concentrations in both years were lower than that reported in other studies in channel catfish ponds with the same general range of nutrient loading rates (Tucker 1996, 2006) but are nevertheless typical of hypertrophic ecosystems.

In Year 1, mean concentrations of chlorophyll *a*, nitrite-N, and total ammonia-N did not significantly differ ($P > 0.05$) between untreated and treated ponds for comparisons made with all samples and comparisons made with samples collected late in the growing season (Table 1). Likewise, net channel catfish production and feed conversion ratios did not significantly differ ($P > 0.05$) between treatments in Year 1 (Table 2).

In Year 2, channel catfish stocking rates were increased to assess treatment effects when feeding rates and nutrient loading were higher. Mean chlorophyll-*a* concentrations were higher ($P \leq 0.05$) and nitrite-N and total ammonia-N concentrations lower ($P \leq 0.05$) in bacteria-treated ponds than in untreated ponds across all sampling dates and for late-season samples (Table 1). Net channel catfish production and feed conversion ratios were not affected ($P > 0.05$) by bacterial bioaugmentation (Table 2).

Discussion

The product tested in this study is a mixture of three species of the spore-forming bacterium *Bacillus* (Logan and Bartlett 1998). The product is formulated as a dry powder with an inert carrier and "micronutrients." Based on patent claims, the treatment rate used in this study provided weekly inocula of about 10^3 bacteria/mL, which is within the same general range of inoculation rates used in studies of other *Bacillus*-based lake and pond bioaugmentation products (Chiayuvareesajja and Boyd 1993; Quieroz and Boyd 1998; Duvall and Anderson 2001; Duvall et al. 2001).

Modes of action for *Bacillus*-based bioaugmentation products vary, but typical claims are that introduced bacteria compete with phytoplankton for dissolved inorganic nitrogen and phosphorus, thereby reducing waterborne concentrations of those nutrients. Because microbial products are not registered as pesticides, label claims cannot include specific reference to reduced algal growth, although this is an implied outcome of reduced nutrient availability. In fact, microbial bioaugmentation products are commonly

TABLE 2.—Channel catfish production variables (means and 95% confidence intervals) in untreated ponds and in ponds treated weekly with a commercial bacterial bioaugmentation product. Feed conversion ratio is the weight of feed offered divided by net catfish production.

Variable	Mean (95% confidence interval)		*t*-value	*P*-value
	Untreated	Treated		
Year 1				
Net production (kg/ha)	4,901 (4,428–5,374)	4,930 (4,457–5,403)	0.09	0.93
Feed conversion ratio	1.81 (1.71–1.92)	1.78 (1.68–1.89)	0.52	0.62
Year 2				
Net production (kg/ha)	5,634 (5,297–5,971)	5,690 (5,353–6,028)	0.26	0.80
Feed conversion ratio	2.04 (1.94–2.13)	2.03 (1.93–2.13)	0.08	0.94

used by lake and pond managers as alternatives to traditional algicides (Duvall and Anderson 2001; Duvall et al. 2001).

Effects of bioaugmentation on channel catfish pond water quality were inconsistent and difficult to interpret. In Year 1, treatment had no effect on water quality or fish production. We therefore decided to increase fish stocking and feeding rates to evaluate treatment effects under more intensive culture conditions. In Year 2, bacterial bioaugmentation of ponds was associated with higher phytoplankton standing crops and lower nitrite-N and total ammonia-N concentrations relative to untreated ponds.

Decreased dissolved inorganic nitrogen concentrations in Year 2 were probably not the direct result of bioaugmentation. Although water column bacteria are responsible for a significant fraction of orthophosphate and dissolved inorganic nitrogen uptake in oligotrophic waters, their role is much reduced in ecosystems with higher nutrient loading rates, such as aquaculture ponds, where phytoplankton uptake dominates inorganic nutrient budgets (Hargreaves 1997; Biddanda et al. 2001; Cotner and Biddanda 2002; Danger et al. 2007). This is probably the basis for observations in past research, where total ammonia levels were not reduced in aquaculture ponds treated with bacterial supplements (Tucker and Lloyd 1985; Chiayuvareesajja and Boyd 1993; Quieroz and Boyd 1998). Moreover, if the *Bacillus*-based product used in the present study acted according to claims for similar products, the outcome of reduced nitrogen availability should have been lower—rather than higher—chlorophyll-*a* concentrations.

The inverse relationship between phytoplankton biomass and dissolved inorganic nitrogen in Year 2 is consistent with typical nitrogen dynamics in channel catfish ponds (Tucker et al. 1983; Tucker and van der Ploeg 1993; Hargreaves and Tucker 1996). As explained above, algal uptake is the primary warm-weather sink for dissolved inorganic nitrogen (Hargreaves and Tucker 1996; Hargreaves 1997, 1998), and factors that increase or decrease phytoplankton growth rates have the opposite effect on dissolved inorganic nitrogen concentrations. The water quality trends seen in Year 2 could therefore be explained if bacterial bioaugmentation enhanced phytoplankton growth, thereby increasing algal uptake of dissolved inorganic nitrogen. However, the mechanism by which addition of exogenous bacteria may have increased phytoplankton biomass in the second year of this study is unknown. The results in Year 2 differ from those seen in the first year and also differ from results of other studies showing that bacterial bioaugmentation has no effect on lake or pond phytoplankton abundance (Boyd et al. 1984; Tucker and Lloyd 1985; Quieroz and Boyd 1998; Duvall and Anderson 2001; Duvall et al. 2001). In addition, the implied claim for similar bioaugmentation products is that their use will reduce, rather than increase, phytoplankton standing crops (Duvall and Anderson 2001; Duvall et al. 2001). Based on the lack of a tenable mechanistic explanation for the trends seen in Year 2, as well as contradictions with past studies, it is possible that a confounding factor gave rise to a Type 1 statistical error, and the apparent relationship between bacterial bioaugmentation and water quality in Year 2 may be spurious.

Regardless of the impacts on water quality in the second year, bacterial bioaugmentation had no effect on fish growth or feed conversion in either year. Therefore, within the limits of the study conditions, there is no economic incentive to use *Bacillus*-based microbial products in catfish aquaculture. This conclusion is supported by other studies in channel catfish ponds that have failed to demonstrate positive outcomes from bacterial bioaugmentation (Boyd et al. 1984; Tucker and Lloyd 1985; Quieroz and Boyd 1998).

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