Managing the accumulation of organic matter deposited on the bottom of shrimp ponds...Do chemical and biological probiotics really work?

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Accumulation of organic matter and appearance of black, anaerobic mud in pond bottom sediments is a concern to shrimp growers (Peterson and Daniels 1992). Accumulation of organic matter increases oxygen demand and the development of reducing and acidic conditions in bottom soils. These conditions favor several microbial-mediated biochemical processes including the production of reduced compounds, such as ammonia, nitrite, hydrogen sulfide, ferrous iron, manganous manganese and methane, that may adversely affect the growth of culture organisms (Avnimelech and Zohar 1986; Boyd 1995).

Therefore, any methods that reduce the accumulation of organic matter in pond sediments should enhance the quality of the pond ecosystem. Drying of pond bottoms between crops is effective in accelerating the decomposition of organic matter (Boyd and Teichert-Coddington 1994). Applications of nitrates may also increase the rate of decomposition of organic matter by oxidizing anaerobic sites within the pond and providing a readily available nitrogen source for microbial metabolism (Avnimelech and Zohar 1986). Liming materials are commonly broadcast over pond bottoms between crops to neutralize acidity and enhance microbial activity (Boyd 1995).

Deterioration of soil and water quality in aquaculture systems is often associated with decomposition of organic matter over time. Thus, shrimp farmers are eager to find a solution to this problem. A type of biotechnology called "bioremediation" or "bacterial augmentation" has received increasing attention among shrimp farmers in recent years. These products are available in a variety of presentations and consist mainly of: 1) bacterial inocula that contain live bacteria or spores in a medium that prevents their growth or germination until application, 2) inocula that contain extracellular enzymes, fruit or plant extracts, and 3) suspensions that combine enzymatic preparations and bacteria inocula. These products usually are marketed under the general term "probiotics". Advocates for the use of probiotics in aquaculture claim that these amendments enhance the rate of degradation of organic matter, increase levels of dissolved oxygen, eliminate undesirable waste products (nitrite, ammonia, carbon dioxide and sulfide), reduce the proportion of blue green algae, decrease feed conversion ratio, and increase production (Boyd 1995).

Bacterial inocula have proven to be effective in shrimp hatcheries by out-competing pathogenic bacteria for nutrients and other resources, thus reducing the risk of disease and improving larval growth and performance (Garriquez and Arevalo 1995; Rengpipat et al. 1998). Despite the encouraging results achieved in larviculture, improvement in soil conditions and water quality have not resulted after treatment with bacterial or enzymatic preparations (Boyd et al. 1984; Tucker and Lloyd 1985; Queiroz and Boyd 1998; Queiroz et al. 1998). In fact, in only one of these studies did production of the culture species actually improve (Queiroz and Boyd 1998). However, the factor actually responsible could not be identified.

We conducted a study to evaluate the ability of several commercial products currently used in shrimp ponds in Ecuador to accelerate decomposition of organic matter during the fallow period. Addition of calcium carbonate and sodium nitrate was also evaluated.

Ponds and type of amendments evaluated

Ponds for this study were located on two shrimp farms in the Guayas Province of Ecuador (Fig. 1). Farm A is located in the inner estuary of the Gulf of Guayaquil where soils are silty clays and salinities of pond water range from 10 to 20 ppt. Farm B is located on the Santa Elena peninsula. There, soils are sandy clay loams and salinities exceed 30 ppt. Three evaluations were performed for this study, and will be referred to as I (Farm A), II (Farm A), and III (Farm B). In evaluations I and III, the bottom soil respiration was measured *in situ* on 1-m^2 plots where the different amendments were applied. For II, soil respiration was measured in the laboratory. Soil samples were also removed at the beginning and end of each evaluation to measure the change in carbon concentration and pH. Immediately after ponds were drained and harvested, plots were established in pond bottoms, and treatments were randomly assigned to each plot. Biological amendments were diluted and sprayed over experimental units at doses recommended by vendors. Each amendment was then homogenized within each plot by mixing the upper 3-cm soil layer with a garden rake. For evaluation II, 400 g of soil from Farm A was added to 10-L plastic buckets that served as respiration chambers. All evaluations had durations of 7, 9 and 8 days, respectively. Each treatment was replicated four times. Evaluations were performed during the dry season, i.e., July - September of 1998.

Three biological amendments, termed A, B, and C, were purchased from local distributors. Amendment A is an enzymatic and bacterial suspension. According to the vendor, it contains the following ingredients: *Bacillus subtilis*, fermentation extracts from *Bacillus subtilis* and *Bacillus cereus*, protease, amylase, cellulase, fermented Yucca plant (*Yucca schigadera*) extract, peptides and water. Amendment B is advertised to contain a wide variety of enzymes, chelated micronutrients, organic complexes and vitamins, but it does not contain bacteria or other microorganisms. Both A and B are recommended for application during pond filling in doses that range from 0.5 to 1.0 L/ha. Amendment C is made from grapefruit (*Citrus paradassi*) and contains ascorbic acid (16.5 percent), amino acids, propylene glycol, glycerin, peptides, and others. The fourth commercial probiotic product, D, is a mixture of its active ingredient with calcium carbonate. The recommended application rate for soil preparation before pond filling is between 50 and 200 kg/ha. Calcium carbonate and a product consisting mainly of sodium nitrate were obtained from local distributors.

Soil samples were collected at the beginning and end of each evaluation. Samples from the first few millimeters of the soil surface from each plot were removed using a tablespoon. One composite sample was obtained from each treatment replicate by mixing several sub-samples. All samples were oven dried at 60°C and pulverized with a hammer mill-type soil crusher (Custom Laboratory Equipment Inc., Orange City, Florida, United States) to pass through a 20-mesh screen. Total carbon was determined with a LECO Induction Furnace Analyzer EC12. Dry soil pH was measured in 1:1 water - soil slurry with a glass electrode. Soil samples for evaluation I were lost because of a malfunction of the oven thermostat. Bulk density was determined according to the method described by Blake and Hartge (1986).

Measurement of soil respiration

Soil respiration *in situ* and in the laboratory was determined according to the technique described by Page et al. (1982). This technique consists of trapping the carbon dioxide evolved during soil respiration in an alkali solution placed inside an airtight chamber (Fig. 2). Commercially available, 10-L plastic buckets (24-cm tall and 23-cm diameter) were used as respiration chambers, while 8.7-cm diameter plastic Petri dishes were used as alkali containers. Once the treatments were applied to the soil surface, 5-cm PVC tubes were inserted vertically into the soil to support Petri dishes. Holes were drilled across the pipes to allow free movement of carbon dioxide into the respiration chamber. Plastic buckets were placed into position immediately after 20 mL of sodium hydroxide were pipetted into the Petri dish containing the alkali, by gently pressing the open end of the bucket into the soil to a depth of 2 to 4 cm. Four chambers without soil were carried through the procedure as blanks. These chambers were tightly capped with their respective bucket lids. To achieve an airtight seal, stopcock grease was placed around the border of the lid in contact with bucket opening.

The amount of carbon dioxide evolved in soil respiration was estimated by the following equation:

$$CO_2\ (\mathrm{mg/m^2}) = \frac{(B-V)\ N\ 22}{A}$$

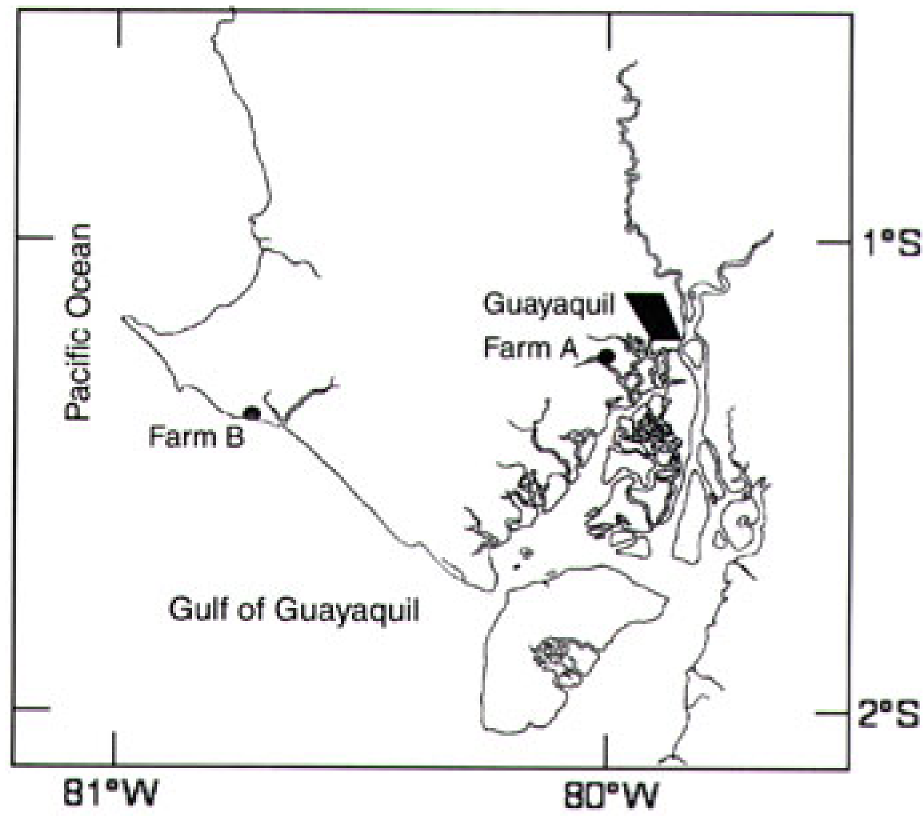

Fig. 1. Gulf of Guayaquil showing location of shrimp farms where evaluations were conducted.

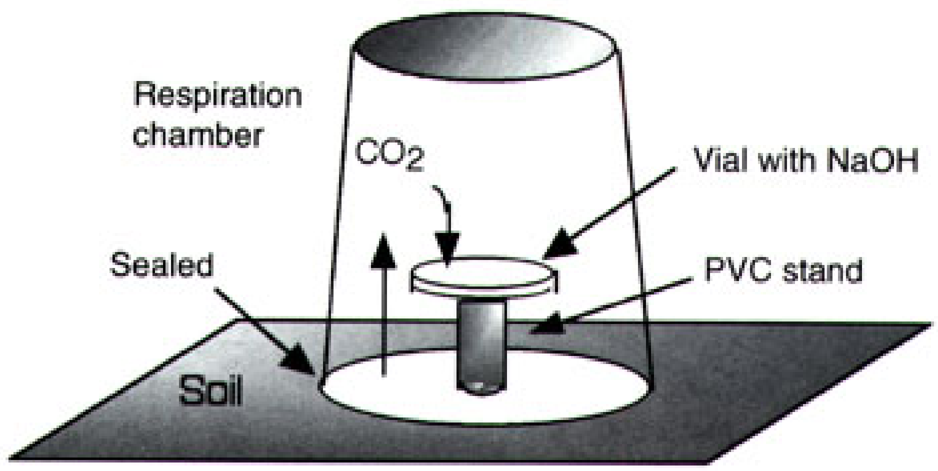

Fig. 2. In situ technique used to measure soil respiration.

where

B = acid used to titrate NaOH in the blank (mL)

V = acid used to titrate NaOH in treatment (mL)

N = Normality of hydrochloric acid (1.00 N)

22 = Equivalent weight for CO_2

A = Area of soil confined in respiration chamber (m^2)

Data Analysis

Analysis of variance and assumptions on equality of variance and normality of population means were computed with the statistical package JMP, SAS Institute Inc. All means were tested for statistical differences with Duncan's multiple range test at a probability level of 0.05.

Results

Soil variables measured during the evaluations are presented in Table 1. All experiments had similar soil pH, although differences in soil moisture content occurred for the different evaluations. The moisture content of soils confined under the respiration chambers did not change throughout the experiments.

Respiration in evaluation I decreased as levels of calcium carbonate increased (Fig. 3). Differences in soil respiration re-

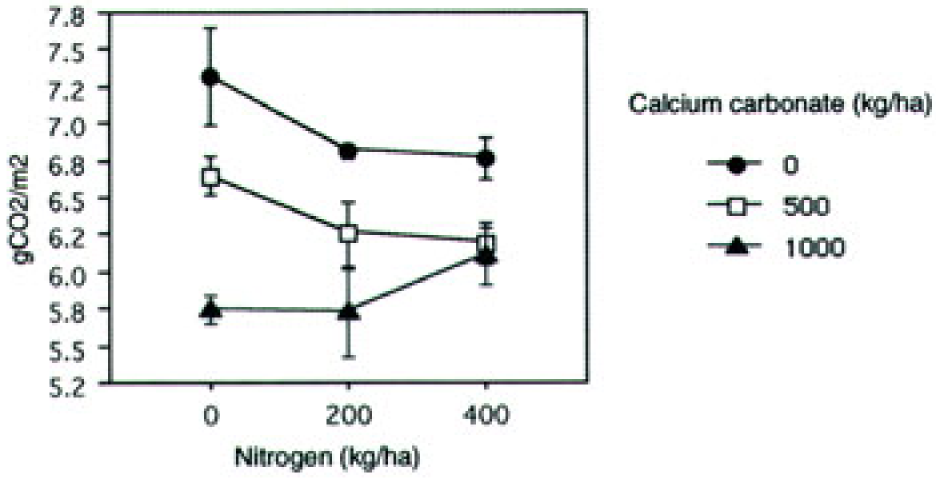

Fig. 3. Soil respiration for different combinations of calcium carbonate and nitrogen applied to a shrimp pond during the fallow period on Farm A. Vertical bars represent standard error of means.

Table 1. Summary of soil properties at the beginning of each evaluation.

	Evaluation I	Evaluation II	Evaluation III
Total carbon (%)	1.56	1.30	1.38
pH	7.45	7.75	7.50
Moisture (%)	61.7	44.1	50.6
Soil Type	silty clay	silty clay	sandy clay loam

Table 2. Cumulative respiration rate (g CO_2/m^2) of pond soil treated with biological amendments during fallow period in Farm A after 7 days. There were no significant differences among amendments.

Amendment	Application rate	Respiration g CO_2/m^2 Mean	SD
None (Control)	———	7.5	0.2
BIO	0.5 L/ha	7.2	1.1
BIO	1.0 L/ha	7.3	1.4
DG	0.5 L/ha	7.8	0.6
DG	1.0 L/ha	7.2	0.6
KLD	100 kg/ha	7.3	0.5
KLD	200 kg/ha	7.6	0. 7

sulting from the addition of calcium carbonate were highly significant. No statistically significant interaction among factors was found. Respiration was not affected by the addition of biological amendments (Tables 2 to 4). No difference was found between controls and different treatments for any of the biological amendments. Soil respiration values at Farm B were higher than those obtained at Farm A. The average soil respiration for trials I through III were 7.36, 6.58 and 14.31 g CO_2/m^2, respectively.

The difference in total carbon for the upper 0.5 cm soil layer as measured in samples collected at the beginning and end of evaluations II and III did not differ significantly among treatments. A net decrease in total carbon concentration was observed at the end of evaluations II and III in all treatments except for B and D in evaluation II (Table 3). Average decreases in soil carbon concentration for evaluations II and III were 0.01 and 0.10 percent, respectively.

The amount of carbon decomposed (lost) from soils in Farm B was estimated from respiration and bulk density data, and compared to actual carbon measurements. The average soil respiration of 14.25 g CO_2/m^2 was equivalent to the release of 3.89 g C/m^2. The bulk density for this particular pond was 1.15 g/cm^3 and a release of 3.89 g c/m^2 is equivalent to 0.08% C [3.89 g C ∏ (4,000 cm^3 x 1.15 g/cm^3)]. This result agrees well with the measured carbon loss of 0.10 percent.

For all treatments the pH dropped, most likely as a result of the decomposition of organic matters (Tables 3 and 4). Acidification of soil by aerobic decomposition is caused by mineralization of CO_2 and nitrification. Hydrogen ion reacts with Al $(OH)_3$ in soil to release AL^{3+} that replaces basic cations on soil colloids, and causes soil pH to decrease. Reaction of CO_2, either with calcium carbonate or water, results in an underestimation of microbial respiration.

No statistical differences were found among treatments. The decrease in pH at the end of the experiment averaged higher in evaluation II than in trial III, despite lower carbon mineralization in evaluation II.

Accumulation of organic matter – can it be managed?

This study revealed no benefits in the application of bacterial inocula or enzymatic suspension to enhance the decomposition of organic matter during fallow periods under the conditions tested. Boyd and Pippopinyo (1994) also did not observe an increase in respiration rates of pond bottom soils treated with bacterial suspensions. The factor(s) that contributed to the decrease in soil respiration following calcium carbonate treatment could not be defined, but we suspect that calcium carbonate was added in excess of the amount needed to neutralize soil acidity. Excess calcium carbonate would react with CO_2 released in soil respiration and cause erroneously low soil respiration measurements. Liming is beneficial for acidic conditions because it reacts with hydrogen ions produced during displacement of Al^{3+} from soil colloid by Ca^{2+}, thus increasing soil pH and lowering the base unsaturation. Boyd and Pippopinyo (1994) showed that lime applications to an acidic soil favored soil respiration. Disruption of the ionic equilibria of soil colloids by calcium ions from liming is another possible explanation. Cations such as Mg^{2+}, K^+, Fe^{+3} are essential for microbial growth (Millis 1988). Microbial

Table 3. Cumulative respiration rate (g CO_2/m^2), carbon reduction (mg/kg) and pH change of soil treated with biological amendments and incubated in respiration chambers for 9 days. Negative values indicate an increase; N* = nitrogen added at 100kg/ha. There were no significant differences among amendments.

Amendment	Application rate	Respiration (g CO_2/m^2)		Carbon reduction (mg/kg)		pH Change	
		Mean	SD	Mean	SD	Mean	SD
None (Control)	—	7.5	0.2	33	802	0.2	0.1
B	1.0 L/ha	7.2	1.1	-167	321	0.4	0.2
A	1.0 L/ha	7.8	0.6	433	650	0.3	0.1
C	0.8 kg/ha	7.2	0.6	300	624	0.3	0.3
D	200 kg/ha	7.3	0.5	-133	305	0.2	0.0
D + N*	200 kg/ha	7.6	0.7	133	586	0.2	0.1

Table 4. Cumulative respiration rate (g CO_2/m^2), carbon reduction (mg/kg) and pH change of pond soil treated with biological amendments during fallow period in Farm B after 8 days. Negative values indicate an increase. N* = nitrogen added at 100 kg/ha. There were no significant differences among amendments.

Amendment	Application rate	Respiration (g CO_2/m^2)		Carbon reduction (mg/kg)		pH Change	
		Mean	SD	Mean	SD	Mean	SD
None (Control)	—	14.0	0.4	225	1,443	0.0	0.2
B	1.0 L/ha	14.7	0.3	600	1,820	-0.1	0.2
A	1.0 kg/ha	14.6	0.4	200	1,494	-0.2	0.4
C	10 kg/ha	14.3	0.7	800	2,208	0.3	0.1
D	500 kg/ha	14.2	0.6	1,025	1,480	0.3	0.1
D+N*	500 kg/ha	14.8	0.9	2,150	592	0.0	0.1

cells are also in close equilibrium with the ionic composition of soil colloids and their attachment to clay particles is strongly affected by electrostatic forces (Millis 1988, Vancura and Kunk 1988). The accumulation of salts and ions increases the ionic strength and alters the ionic composition of the soil solution. The ionic composition of the soil may have reached a new equilibrium due to dissolution of calcium carbonate, possibly hindering microbial activity.

Vendors recommend application of products A and B once during pond refilling at a dose of 0.5 to 1.0 L per hectare. This application rate is equivalent to a dilution factor of 1 in 10^7 (1 liter in 10 million liters). This concentration may be too small to influence native bacterial populations in ponds. Product C is advertised as a bactericide and is recommended for use to control infectious diseases caused by different pathogenic bacteria. Yet, application of this product did not affect respiration adversely.

Despite the belief of most shrimp producers that organic carbon is being deposited at high rates in their ponds over the years, recent data show otherwise. Sonnenholzner and Boyd (2000) found an average organic carbon concentration of 1.4 percent in bottom soils of shrimp ponds constructed on non-mangrove land in Ecuador. Total carbon in pond soils constructed on former mangrove land ranged between 2.5 and 14 percent. Shrimp farming in Ecuador is conducted mainly under semi-extensive and semi-intensive conditions. Yields range from 300 to 1,800 kg/ha/yr, with an average of 722 kg/ha/yr (Jory 1998). Feed inputs range from 300 to 2300 kg/ha/yr, and reported feed conversion ratios (FCR) range from 0.8 to 1.3 (Laniado 1997). Boyd and Teichert-Coddington (1995) reported that at a FCR of 2:1, approximately 11.5 percent of carbon in feed is recovered in shrimp. A carbon recovery of 15 percent is obtained with a FCR of 1.5, a value similar to those reported in shrimp production systems in Ecuador. Therefore, the average yearly carbon load per hectare from feed for a FCR of 1.5 is estimated to be 490 kg [(1,083 kg feed x 0.92 dry matter x 0.52 carbon) – (722 kg shrimp x 0.25 dry matter x 0.15 carbon)]. If we assume that this amount of carbon is incorporated in the first 5-cm layer, we would expect an increase of 0.08% C per hectare per year [(490kg ∏ (500 m^3 x 1,200 kg/m^3)]. However, this calculated amount deposited on the pond bottom may be increased several times by remains of dead phytoplankton. Boyd (1985) determined that for each kilogram of fish produced, 2.59 kg of COD was added by phytoplankton production. Munsiri et al. (1996) found an increase of 1 percent organic carbon in the soil of the bottom of semi-intensive shrimp ponds with 8 to 10 consecutive years of production. Studies have demonstrated that most of the organic carbon accumulated during the previous crops is decomposed during pond drying between crops and eroded from the soil surface during harvest (Ayub et al. 1993; Boyd et al. 1994). The average decrease in organic carbon during the fallow period measured in farm B was 0.11 percent, which represents a reduction of 8 percent of the total carbon initially present. Ayub et al. (1993) found that organic carbon decreased 0.19 percent, from 1.65 percent to 1.46 percent during a 5-week fallow period.

Excessive deposition of organic matter may become a problem in draining channels and other deeper parts of ponds where smaller colloidal particles tend to settle. In addition, greater rates of deposition occur in the corners of ponds where suspended particles are carried by prevailing winds. Localized, oxygen-depleted sediments can be treated with sodium nitrate to prevent low redox potential and formation of hydrogen sulfide and other toxic metabolites

Failure of probiotics to enhance organic matter decomposition probably resulted because soils were not extremely high in organic matter content or deficient in microorganisms or extracellular enzymes. There may be conditions in aquaculture ponds where there are inadequate populations of microorganisms or concentrations of extracellular enzymes to efficiently decompose organic matter. In such conditions, probiotics might provide benefits, but at present, conditions requiring probiotics to enhance organic matter decomposition cannot be identified in ponds.

Soil conditions influencing organic matter decomposition that can be easily managed are pH and moisture. Brackishwater soils that are neutral or slightly alkaline probably do not need to be limed to enhance microbial activity. Therefore, farm managers should verify the soil pH before applying lime.

Drying of soil allows better aeration and enhances decomposition, but if soils become excessively dry, the rate of decomposition declines. The goal of management practices for pond bottoms should be maintenance of those conditions favorable for microbial activity.

Notes and References

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