Promotion of Substrate Based Microbial Biofilm in Ponds - a Low Cost Technology to Boost Fish Production

K.M. Shankar, C.V. Mohan and M.C. Nandeesha

Abstract

Microbial biofilms have been found to increase fish production in ponds by increasing heterotrophic production through periphyton proliferation on available substrates. In this paper, the role of substrate based microbial biofilm in the production of *Cyprinus carpio* and *Labeo rohita* grown in ponds is investigated, using an easily available and biodegradable agricultural waste product (sugarcane bagasse) as substrate.

Introduction

In many parts of Asia, much of the aquaculture production comes from small and marginal farms. Improving existing farming systems becomes necessary to provide appropriate technologies to the large number of farmers. Development of viable, low cost technologies and their application to current farming practices will help increase the area under aquaculture as well as its yield. Fertilization and supplementary feeding are important in increasing production. This will add up to 40% to 70% of the production cost, depending on the intensity of feeding. However, endogenous food production can be increased through fertilization, thereby reducing the cost from the exogenous addition of artificial feed. Fertilization results in increased fish production through autotrophic and heterotrophic fish food production. The light dependent autotrophic food production is self limiting and hence has less potential for improvement. On the other hand, heterotrophic food production has no limit and hence has a large potential for improvement and exploitation. In addition, heterotrophic production can be promoted throughout a pond and even during the night.

Role of Biofilm Bacteria

The role of bacteria as food for both zooplankton and planktophagic fish needs no further emphasis (Kuznestov 1977; Moav et al. 1977; Shroeder 1987; Zhu et al.1990). Bacteria provide a significant source of carbon through extra-cellular polymer (Pearl 1978; Hobbie and Lee 1980), besides providing essential amino acids and vitamins (D'Agostino and Pravasoli 1970). However, the particle-bound sessile bacterial aggregates (biofilm) are preferred to their planktonic free cells by zooplankton and fish because of easy and economic exploitation (Ferguson and Rublee 1976). Bacteria are known to colonize on substrate and to develop into microcolonies with extra-cellular polymeric substance enrobing cells to form biofilm (Costerton 1984). Usually, bacterial biofilm cell density is 100 to 1 000 times greater than the planktonic population per unit weight. In aquatic environments, bacterial biofilm includes a complex community of other organisms such as algae, protozoa and fungi embedded in the extracellular matrix secreted by bacteria. However, current fertilization practices have little potential to promote sessile biofilm microorganisms. Hence, boosting fish production by promoting bacterial biofilm on suitable substrates in fertilized ponds is possible. A four-fold increase in the density and biomass of *Daphnia magna* through increased biofilm on a glass surface has been demonstrated by Langis et al. (1988). Agricultural wastes and aquatic weeds are easily available and biodegradable and seem to be more ideal for colonization of bacteria than inert glass and plastic surfaces.

Traditional Knowledge

Farmers in Africa have traditionally used tree branches and bamboo poles as fish aggregating devices. They also serve to promote the growth of fishes by increasing natural food availability through the production of periphyton on these substrates. Through this simple method of capture-cum-culture based fisheries, productions of 4-20 t/ha have been reported (Welcomme 1972; Hem et al. 1995). Similar systems are prevalent in Asia and are being used to exploit wild fisheries by artificially constructing reefs (brush parks) with tree branches. In Cambodia, these brush parks, locally known as *samarahs*, provide fish yields ranging from 1-4 t/ha/fishing season. Generally, these brush parks are rectangular in shape and more than 70 m^2 in area. Tree branches are used as substrate, coupled with floating aquatic weeds like water hyacinth (*Eichhornia crassipes*), which cover the entire surface area. The floating weeds are kept intact with ropes. Fish attracted to these sheltered areas are harvested 60 days after tree branches are laid in the water, by encircling the area with a net. Some farmers even provide feed like rice bran to attract/fatten the fish in the area. Though this system is officially banned, it is still practiced illegally. Even in ponds, tree branches and bamboo shoots that are used mainly to control poaching are slowly becoming popular as they also promote fish growth. Some farmers in Cambodia use paddy straw and palm leaves to clear turbidity in water and have noticed the benefits of these substrates not only in clearing the turbidity, but also in increasing plankton productivity.

Sugarcane bagasse tied to bamboo beams for suspension in experimental cisterns.

Samarahs *(brushparks) suspended in a river in Cambodia.*

Role of Bacterial Biofilm in Fish Culture

In view of the potential of biofilm bacteria to increase fish production, several studies have been conducted at the College of Fisheries, Mangalore, India since 1992. Important among them are: i) promoting and quantifying microbial biofilm to increase fish production in ponds (Umesh 1993); ii) developing bacterial biofilm in ponds for possible application in the oral vaccination of carps (Shankar et al.1993); and iii) studying the influence of different substrates on heterotrophic food production and fish growth (Ramesh 1994). Additionally, the use of bacterial pathogen biofilm as a better alternative to its free cell form for oral vaccination of fish has been investigated. Biofilm of *Aeromonas hydrophila* has been successfully developed on chitin particles *in vitro* for use in oral vaccination with promising results (Azad et al. 1997). This article presents some salient findings on the potential of substrate based biofilm production in ponds to increase fish production.

SUGARCANE BAGASSE AS SUBSTRATE

A preliminary study was conducted to understand the effect of sugarcane bagasse on fish growth and plankton production in small cement tanks. Sugarcane bagasse (0.5 kg) was suspended in 1 m^2 cement tanks and fertilized with 0.3 kg cattle dung (3 000 kg/ha) and 5 g urea (50 kg/ha) in three replicates. Tanks fertilized with 0.6 kg cattle dung (6 000 kg/ha) and 10 g urea (100 kg/ha) served as control. Ten tilapia *(Oreochromis mossambicus*) and common carp *(Cyprinus carpio)* fry were stocked in each of these tanks in a 1:1 ratio. In the 91-day culture period, growth of *O. mossambicus* and *C. carpio* was higher by 47.6% and 49.7%, respectively, than the control treatment. Zooplankton production was higher in the bagasse treatment but, interestingly, lower phytoplankton production was observed. These findings provide indirect evidence that the substrate enhanced fish growth through heterotrophic food production consisting most likely of bacterial biofilm. Based on the results obtained in these preliminary studies, a further investigation was carried out in three 25 m^2 cement cisterns over a period of 133 days. Sugarcane bagasse was suspended in cisterns fertilized with cattle dung and urea at the dosages mentioned. Cisterns fertilized with urea and cattle dung only served as control. *C. carpio* and rohu *(Labeo rohita)* were stocked at 10 000/ha for growth studies. Water quality, bacteria in water (planktonic) and on bagasse (biofilm), zooplankton in water and on bagasse, and fish growth were studied in detail (Fig. 1). At the end of culture period, growth of *C. carpio* and *L. rohita* was higher by 47.4% and 47.5%, respectively, as compared to the control. The species of fish used in the studies are known to feed on particle bound organisms (Das and Moitra 1955; Schroeder 1983). Tilapia *(O. niloticus)* feeding on substrates has been documented by Shresta and Knud-Hansen (1994) and the findings of Hem et al. (1995) clearly indicate that an acadja system could enhance the yield of tilapia, owing to their preference for periphyton.

Analysis of the data revealed that the increase in growth of fish in tanks with bagasse was largely due to the increased zooplankton density in the water (Fig. 2). This in turn was influenced by a phenomenal increase in biofilm on the substrate (Fig. 3) on which both zooplankton and fish grazed. Interestingly, the production of phytoplankton and planktonic bacterial density in the water in the control and the bagasse tanks was similar. The biofilm bacterial density on substrate per unit weight was 100 times more than that in the water (Fig. 3). The actual density could be much higher since the complete separation of active and quiescent cells in biofilm for enumeration was not possible. Biodegradable substrates therefore can support an enormous density of biofilm bacteria. Schroeder's (1978) observation that more than half of the observed fish yields in intensively manured ponds results from the direct consumption of heterotrophic production and the remaining from the consumption of conventional food by fish was verified. In this study, the quantity of substrate and fertilizer was fixed arbitrarily, assuming that 8 000 kg/ha/yr of organic matter (5 000 kg sugarcane bagasse and 3 000 kg cattle dung) would not affect water quality. The dose and frequency of application needs to be standardized considering the effect of organic matter on dissolved oxygen and bacterial and zooplankton biomass. Average dissolved oxygen in bagasse treated tanks was 3.94 ppm, while in control tanks, it was 6.61 ppm. A slight decrease in dissolved oxygen levels was noted in the bagasse tanks during the first week, which is characteristic of water with predominant heterotrophic food production (Moriarty 1987). However, this will not have affected the two species of carps and tilapia, which are capable of tolerating a low oxygen level, although about 6 ppm of dissolved oxygen is required for better growth (Huet 1972). Average total ammonia was relatively lower in tanks with bagasse (7.14 µg at.N/l) than in the control tanks (13.17 µg at.N/l). The high density of bacterial biofilm that developed on the substrate may have brought down ammonia concentrations. Langis et al. (1988) recorded lower ammonia levels in aquaria suspended with glass panels for biofilm formation.

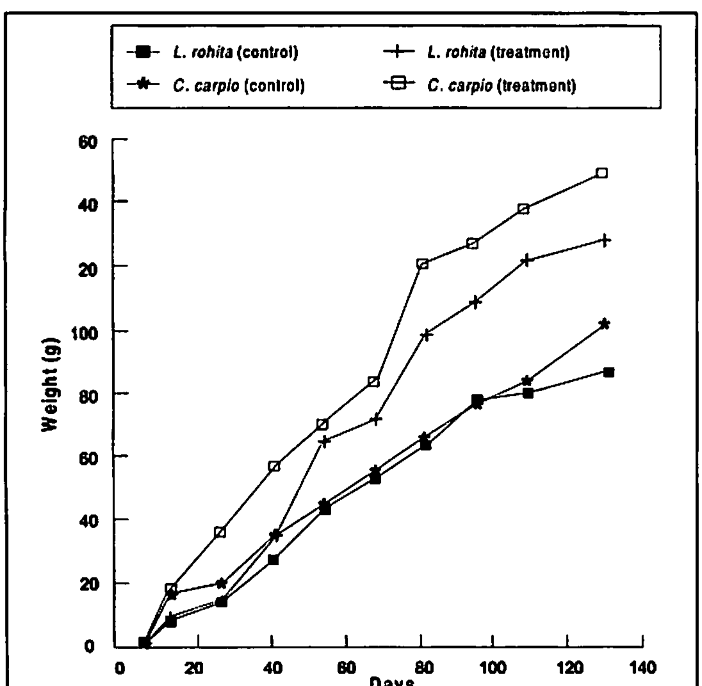

Fig. 1. Growth of C. carpio and L. rohita *in control and bagasse treated tanks.*

In a subsequent study, a comparative analysis of the different substrates such as sugarcane bagasse, paddy straw and water hyacinth were evaluated for their efficacy in increasing fish production. Among the three substrates evaluated, growth of both *L. rohita* and *C. carpio* was significantly higher in all the three different substrate treated ponds as compared to control treatment. The weight attained by *C. carpio* was 47%, 32% and 21% higher and that of *L. rohita* was 47%, 29% and 18% higher in sugarcane bagasse, paddy straw and dried water hyacinth, respectively, than the control treatment. The difference in growth between the three different substrates is likely to result from the varying C:N ratios of the substrates.

Conclusion

The results obtained indicate the potential for increasing fish production at low cost by adding easily available and biodegradable agricultural substrates into ponds. In addition to the substrates used in this study, there are a variety of other biodegradable substrates which need to be tested for their suitability. Further studies such as standardization of dosage, frequency of application of substrates, practical applicability of the technique in larger water bodies, growth of different fish species, etc. on substrate based technology are in progress.

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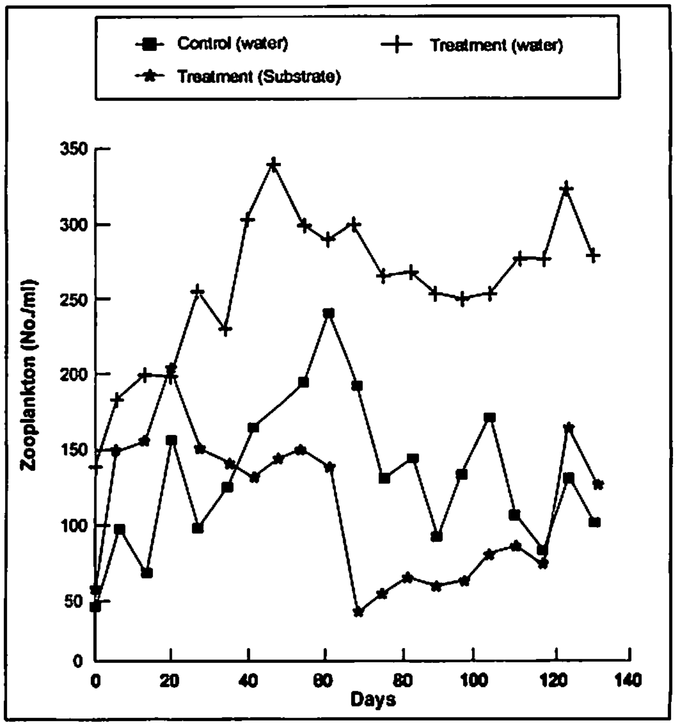

Fig. 2. Zooplankton production in control and bagasse treated tanks.

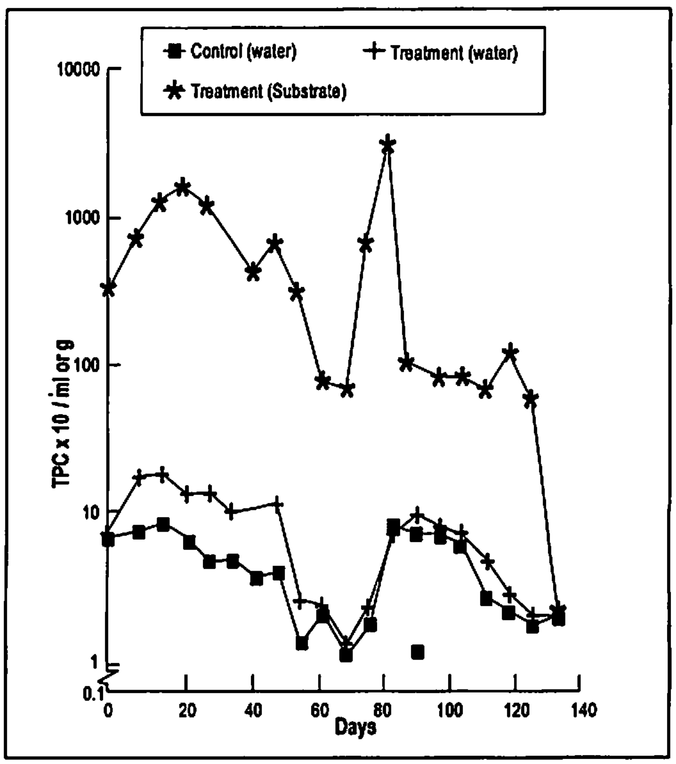

Fig. 3. Total plate count (TPC) of bacteria in control and bagasse treated tanks.

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K.M. SHANKAR, C.V. MOHAN *and* M.C. NANDEESHA *are from the Department of Aquaculture, College of Fisheries, Mangalore-575002, India.*

The Potential for Crop Rotation in Controlling Diseases in Shrimp Culture

Jose O. Paclibare, Marc C.J. Verdegem, Willem B. van Muiswinkel and Bram E.A. Huisman

Abstract

The use of antibiotics and other chemicals in controlling shrimp pathogens becomes ineffective as the strains grow more resistant to these chemicals. Moreover, the bacterial pathogen (*Vibrio harveyi*) produces biofilm coating that protects it from drying and disinfection procedures that are followed during pond preparation. Biological control is being considered as an alternative means of preventing shrimp disease outbreak. The main principle behind biological control is to enhance the growth of beneficial microorganisms which serve as antagonists of target pathogens. The paper discusses shrimp and tilapia crop rotation as a form of effective biological control, a technique which is already being practiced in Indonesia and the Philippines.

The farming of tiger shrimp *(Penaeus monodon)* contributes significantly to the economies of the Philippines and other countries in the Asia-Pacific region. However, in recent years its production in many of these countries has declined due to persistent disease problems. For example, in the Philippines, shrimp exports dropped from 30 462 t in 1991 to 18 275 t in 1995. In Negros Occidental, the major shrimp-farming area of the Philippines, only 10 out of 200 intensive shrimp farms are currently operating. The rest of the farms ceased operation because of disease problems. The luminous bacterium *Vibrio harveyi* has been associated with many of these disease outbreaks.