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Review

Biofilm in aquaculture production

P. K. Pandey*, Vivekanand Bharti and Kundan Kumar

Aquatic Environment and Health Management Division, Central Institute of Fisheries Education, Versova, Mumbai, 400061, India.

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A biofilm is an assemblage of microbial cells which is irreversibly associated with a surface and enclosed in a matrix of primarily polysaccharide material. It may form on a wide variety of surfaces, including living tissues, medical devices, industrial or potable water system pipe or natural aquatic systems. A well-diversified organism such as algae, bacteria, protozoa, arthropods, etc. may be observed in the biofilm assemblage. The biofilm structure depends on the nature of substratum, hydrodynamics of system, nutrient availability, light and grazing capacity of organism. It has been observed that the introduction of substrata for the development of biofilm in the aquaculture system play a significant role. Biofilm organisms are microscopic and highly nutritious. The organisms of biofilm may serve as single cell protein and are easily harvested by all size of cultured species in aquaculture as compared to planktonic organism in the water column. Biofilms are considered as good quality protein source (23-30%). Microalgae and heterotrophic bacteria are rich source of immune enhancers, growth promoters, bioactive compounds and dietary stimulants which can enhance growth performance of cultured organism. Substrata minimize the mortality by providing shelter and hiding places to cultured organisms. The attached nitrifying bacteria contained in biofilm improve the water quality by lowering ammonia waste from culture system through nitrification process. Biofilm based low cost technology will help resource poor farmers in generating protein rich nutrient in sustainable manner from aquaculture. An attempt has been made to review the role of biofilm in aquaculture.

Key words: Biofilm, fish growth, sustainable, water quality, survival, nutrient quality.

INTRODUCTION

Aquaculture has evolved on a large scale during the last two decades and has become a major source of protein for a large population of the world. The current worldwide growth rate of the aquaculture (8.9–9.1% per year since 1970s) is needed in order to cope with short supply of protein rich food, particularly in the developing countries (Gutierrez-Wing and Malone, 2006; Matos et al., 2006; Subasinghe, 2005). Aquaculture has emerged as one of the important branches of food production. Sustained productivity along with enhanced production is the major goal of aquaculture. Expansion of pond area and intensive farming in the pre-existing ponds has great potential for further development of aquaculture throughout the world. However, high stocking densities and frequent use of water, feeds and fertilizers in aquaculture intensification, leads to increased waste

*Corresponding author. E-mail: pkpandey@cife.edu.in. Tel: +91-9975112163. Fax: +91-22-26361573.

production (Beveridgeet al., 1997). On the other hand, operation of intensive aquaculture also demands huge investment and technical expertise, which are not affordable by resource-poor farmers. However, environmental considerations and economic conditions of poor farmers are the major limitations in the growth of aquaculture. Especially, intensive aquaculture coincides with the pollution of the water by an excess of organic materials and nutrients that are likely to cause acute toxic effects and long term environmental risks (Piedrahita, 2003) by increasing waste production (Beveridgeetal., 1997). To keep dissolved nutrients at low level, large amount of water must be exchanged, increasing the costs of production in aquaculture. An alternative way to maintain high water quality is biological treatment such as use of filters with a high surface-volume ratio, pre-colonized by microorganisms that absorb excess nutrients from the water (Wheaton, 1977). The relatively new alternatives to previous approaches are the application of bio-flocs and biofilm in aquaculture (Avnimelech, 2006). Biofilms - a microbial consortium associated with a matrix of extra-cellular polymeric substances bound to any sub-merged surfaces are responsible for many biogeochemical cycles in aquatic ecosystems, especially nitrogen cycling (Decho, 1990; Meyer-Reil, 1994). The major driving force is the intensive growth of heterotrophic bacteria to reduce the overload of unwanted components in the aquaculture. The presence of biofilms in aquaculture reduces the cost of shrimp production by minimizing water exchange (Thompson et al., 2002). It was also demonstrated in several studies that in the presence of biofilm, reduction, or even suppression of water exchange, did not cause any damage to the cultured organisms faced due to stress (Hopkins et al., 1995; McIntosh, 2000).

Thompson et al. (2002) indicated that nitrogen uptake by a biofilm may help to reduce the occurrence of pathogenic bacteria in culture system, as these pathogenic microorganisms normally occur in situations where nitrogenous compounds reach extremely high values (Austin and Austin, 1999; Brock and Main, 1994). Moreover, many microalgae present in biofilms are able to produce antibiotics that prevent pathogenic bacterial growth (Austin and Day, 1990; Alabiet al., 1999). Inhabiting protozoa in biofilms can also control the abundance of pathogenic bacteria through grazing (Thompson et al., 1999). Thus, it is possible that biofilm removal can increase the risk of developing pathogenic bacteria (Thompson et al., 2002).

The manipulation of C:N ratio by use of biofilm on substrata in freshwater finfish and prawn production in extensive pond enhances aquaculture production (van Dam et al., 2002; Azim and Little, 2006; Hargreaves, 2006). The development of biofilm requires installation of hard substrata or application of cheap carbohydrates resources which could potentially be produced within the farmer's traditional agricultural systems (Asaduzzaman et al., 2008). It has been observed that both survival and growth of freshwater prawn were significantly higher due to provision of substratum as compared to traditional production system without substratum (Cohen et al., 1983; Tidwell and Bratvold, 2005; Uddin et al., 2006). The biofilm acts as additional natural food, shelter to minimize territorial effects, improves water quality through trapping of suspended solids, organic matter breakdown, enhances nitrification through the microbial activity and heterotrophic production of single cell protein (McIntosh, 2000; Hari et al., 2004; Crab et al., 2007; Avnimelech, 2007). The main objective of this review is to assess the effect of biofilm in enhancing aquaculture production in a sustainable manner.

BIOFILM FORMATION IN AQUATIC ENVIRONMENT

Biofilms may form on a wide variety of surfaces, including living tissues, medical devices, industrial or potable water system pipe or natural aquatic systems (Donlan, 2002; Kordmahaleh and Shalke, 2013). Biofilm formation begins with the accumulation of organic molecules on any submerged surface. Few hours after the establishment of the macromolecular film, bacterial colonization begins (Whal, 1989). The main advantage of biofilm formation is the protection of the organisms from the effects of an adverse environment. A multispecies microbial culture can provide and maintain appropriate physical and chemical environments for further growth and survival (King et al., 2008).

Biofilm formation is a dynamic process, capable of responding to environmental stimuli. Initially, cells organize themselves into micro-colonies, followed by division and cell recruitment, they grow and encase themselves in an extracellular matrix. Within this matrix, complex and differentiated associations can be formed, which facilitate nutrient uptake (Hall-Stoodley et al., 2004; Toutain et al., 2004). The redistribution of attached cells by surface motility is one of the most important mechanisms of biofilm formation (Hall-Stoodley and Stoodley, 2002). The development of biofilm depends on constituents of the organism(s), the properties of the surface being colonized and the physico-chemical conditions of the aqueous environment. Detachment from biofilms can be caused by a number of factors including external perturbations or internal processes, though many species appear to use dispersal as an active means of colonizing new niches (Sauer et al., 2002; Hall-Stoodley et al., 2004). Biofilm formation is generally thought to proceed as follows: (1) individuals colonize a surface, (2) individuals form micro-colonies and (3) microcolonies form biofilms (Johnson, 2008).

Nutrient availability in aquaculture ecosystem

Nutrients supply energy for the growth and development of heterotrophic bacterial population. Pradeep et al. (2004) reported increase in aerobic heterotrophic bacterial populations in water over due course of time and reached at peak during the 4th week, followed by subsequent decrease, attributing to the role of nutrients availability. Initially, higher levels of nutrients lead to bacterial multiplication and with reduction in nutrient levels, the population starts declining. The change in the diversity of bacterial community strongly depends on tem-poral change in the quality and quantity of organic sub-strata available (Gatune et al., 2012). Inorganic nutrients have a strong effect on periphyton biomass (Aizaki and Sakamoto, 1988; Lohman et al., 1992; Ghosh and Gaur, 1994). Mattila and Raeisaenen (1998) found that periphyton biomass and productivity can be used as an indicator of eutrophication in natural waters. In the most freshwater studies, phosphorous was identified as the limiting nutrient (Ghosh and Gaur, 1994; Vymazal et al., 1994), but sometimes nitrogen (Barnese and Schelske, 1994) and carbon (Sherman and Fairchild, 1989) may also act as limiting factors. Aquaculture ponds are fertilized with phosphorus and nitrogen, which serves as important source of nutrients for biofilms as well.

Action of nitrogen and carbon, as limiting factor, depends on the algal species and on other environmental factors such as hardness and acidity. High silicon: phosphorus (Si:P) and nitrogen: phosphorus (N:P) ratios favoured diatoms while low N:P and Si:P ratios favoured cyanophytes in a reservoir in Patagonia (Baffico and Pedroso, 1996). Similarly, high Si:N or Si:P ratios favoured diatoms but low N:P ratios favoured cyanophytes and high N:P ratios favoured chlorophytes in periphyton of the Baltic Sea (Sommer, 1996). Dissolved organic matter may play a role in determining the structure of the biofilm. Biofilm communities treated experimentally with dissolved organic carbon contained less mucilage than untreated controls (Wetzel et al., 1997). Molobela et al. (2010) reported that nutrients boost the biofilm cells growing in the medium which enhance extracellular polymeric substance (EPS) production.

Effect of grazing

Grazing is one of the most important determinants of biofilm biomass. Bacterial abundance is controlled by the grazing exerted by flagellates and ciliates (Thompson et al., 2002). Grazing has an overriding effect on biomass while the effect of nutrients has much less apparent effect due to the ability of the periphyton to recycle and utilize nutrients from the substratum (Hill et al., 1992; Steinman et al., 1992; Pan and Lowe, 1994). On plastic substrates in tilapia cages in Bangladesh, filamentous Chlorophyceae and Myxophyceae dominated the periphyton before fish stocking, whereas after stocking of the fish, diatoms became more abundant (Huchette et al., 2000), indicating the effect of grazing. Biofilms provide easy availabilityof food to the cultured organisms and thus help in enhancement of fish production.

Suitable species for biofilm-based aquaculture

It has been noticed that consumption pattern and feeding efficiency on biofilm depend on the grazing efficacy of cultured species. However, fishes are found to be fast grazers than crustaceans (Asaduzzaman et al., 2010). Biofilm has already been considered an important food source for Nile tilapia (Shrestha and Knud-Hansen, 1994) and carp (Ramesh et al., 1999). Azim et al. (2001) demonstrated that rohu (*Labeorohita*) and orangefinlabeo (*Moruliuscalbasu*) are more suitable candidates for periphyton based aquaculture than kurialabeo (*L. gonius*). In penaeid shrimp culture, consumption of periphyton developed over submerged substrate significantly improved growth of penaeid shrimp, *Fenneropenaeus paulensis* (Ballester et al., 2007; Thompson et al., 2002), *Penaeusesculentus* (Burford et al., 2004), *L. vannamei* (Audelo-Naranjo et al., 2011; Moss and Moss, 2004) and *P. monodon* (Anand et al., 2012; Khatoon et al., 2007). The culture performance of the freshwater prawn *Macrobrachium rosenbergii* improves in the presence of artificial substrata (Tidwell et al., 1998). Similar grazing activity was reported by Erler et al. (2004) who found that *Farfantepenaeus merguiensis* grazed on epibiota on AquaMats® and significantly reduced the attached biomass. *P. monodon* grown over periphytic microalgae attained significant improvement in body weight compared to the control (Anand et al., 2012; Arnold et al., 2009). Bourne et al. (2006) applied biofilm within a larval rearing tank of the tropical rock lobster (*Panulirusornatus)*. Biofilm can be used to promote the growth of bottom feeder fishes like *Cirrhinusmrigala* (Bharti et al., 2013; Mridula et al., 2006). The biofilm was considered a good tool during *F. brasiliensis* nursery phase, mainly due to enhancement of survival through maintenance of water quality (Viau et al., 2013).

Enhancement of production

Shrimp showed a higher final weight in the tanks with biofilm, leading to higher biomass at the end of the experiments (Thompson et al., 2002). Ramesh et al. (1999) observed that easily biodegradable sugarcane bagasse, having more fibre and surface area favoured better growth of fish through bacterial biofilm than paddy straw and *Eichhornea*. The growth of rohu (*L. rohita*) in the presence of sugarcane bagasse, paddy straw and dried *Eichhornea*for settlement of biofilm, was higher by 47.5, 29.1 and 17.6%, respectively than the control. Similarly, they observed the growth of common carp (*Cyprinuscarpio*) in the presence of substrata like sugarcane bagasse, paddy straw and dried *Eichhornea* was higher by 47.4, 32.9 and 20.7%, respectively than the control. Umesh et al. (1999) conducted an experiment with sugarcane bagasse as substratum and found that the growth of fish was remarkably high in the treatments with an average of 50% over the controls. Natural biodegradable substrata like paddy straw and sugarcane bagasse favoured better growth of the fish than non-biodegradable substrata like plastic sheet and tile, with paddy straw turning out to be the best substratum for aquaculture of *C. mrigala* (Bharti et al., 2013). Keshavanath et al. (2012) evaluated the culture of rohu and common carp in the presence of periphyton developed on different types of substrata namely sugarcane bagasse, palm leaf, coconut leaf and bamboo mat in poultry-manured ponds and found that all the four substrata induced significantly high growth, production and survival rate in both fish compared to substrate free culture system, indicating the importance of biofilm in aquaculture. The growth of rohu under sugarcane bagasse, palm leaf, coconut leaf and bamboo mat treatment was 93.69, 103 and 44% higher, respectively than that of the control, while common carp performed 98, 74, 100 and 20% better than the control in the same treatments, respectively.

Schveitzer et al. (2013) observed that the final biomass of *L. vannamei* was 31.4% more and the survival rate was also significantly high in the tank with substrata. Asaduzzaman et al. (2008) found that the addition of biofilm on substrata increased net yield of prawn from 370 to 456 kg ha^{-1} 120 day^{-1} that is 23% higher yield than absence of biofilm.

Azim et al. (2002a) found the highest net yield of 2098 kg ha^{-1} in bamboo treatment, followed in diminishing order by 2048 kg ha^{-1} (jutestick), 2032 kg ha^{-1} (kanchi), 1960 kg ha^{-1} (feed) and 1226 kg ha^{-1} (control). Azim et al. (2001) and Keshavanath et al. (2001) have shown that the use of various substrata contributed to the growth and production of different aquaculture species in freshwater ponds through the development of biofilm on the substrata. Tidwell et al. (1998) reported that adding substrate in freshwater ponds increased prawn production by 20% as well as average size by 23%. Easy availability of food through biofilm as well as its positive impact on water quality helps in attaining better growth and higher production of finfish and shellfish.

Improvement in water quality

Biological nitrification can be accomplished in two types of systems like suspended and attached growth. Under a suspended growth environment, the microorganisms are freely mobile in the liquid media being in direct contact with the bulk water. In an attached growth system, on the other hand, microorganisms are grown in a viscoelastic layer of biofilm that are attached on the surface of a solid support medium. Thus, this process is also called a fixed film process in which the individual bacteria are immobilized. Attached growth on a fixed biofilm system offers several advantages when compared to suspended growth processes, such as handling convenience, increasing process stability in terms of withstanding shock loading and preventing the bacterial population from being washed off (Fitch et al., 1998; Nogueira et al., 1998). Pradeep et al. (2003b) observed improved water quality with application of probiotic and biofilm during the culture of fingerlings of *Catlacatla*. Lower level of total ammonia concentrations in the substrata based treatments was recorded compared to feed and control treatments. This might be due to higher nitrification rates in substrata treatments (Azim et al., 2002a).

Several researchers have demonstrated that the presence of nitrifying bacteria in the biofilm decreased ammo-nium level in the cultured water (Bharti et al., 2013; Langis et al., 1988; Ramesh et al., 1999). In fact, decrease in ammonium and parallel augmentation in nitrite and nitrate concentrations in the experiment indicates that nitrifying bacteria present in the biofilm play a significant role in water quality management (Kaiser and Wheaton, 1983). On the other hand, ammonium is also absorbed by the microalgae that use this element to produce new biomass (Thompson et al., 2002). Nitrogen uptake by a biofilm may help to reduce the occurrence of pathogenic bacteria, since these microorganisms normally occur in situations where nitrogenous compounds reach extremely high values (Austin and Austin, 1999; Brock and Main, 1994). The direct discharge of large amount of waste water from hatcheries may cause eutrophication in rivers and coastal waters, but use of different periphytic microalgae in aquaculture can significantly reduce ammonia and nitrite levels from the system (Ziemann et al., 1992). In *Oscillatoria* tanks, total ammonia nitrogen (TAN), NO_2-N and soluble reactive phosphorus (SRP) levels were reduced more than 80% against approximately 60% in *Amphora*, *Cymbella*, and *Navicula* tanks in spite of no water exchange during the 16-day culture period.

The use of biofilm to reduce excess nutrients in hatchery tanks does not only maintain the water quality, but also reduces the risk of pathogen introduction since the system does not require water exchange (Khathoon et al., 2007). They observed that the water was very clear in the experimental tanks even without water exchange compared to the control tanks which was turbid. Biofilm can reduce phosphorous (Bratvold and Browdy, 2001; Hansson, 1989) and other nutrients in the water. In addition, biofilm communities reduce water turbidity by trapping organic matter in the column (van Dam et al., 2002). Anand et al. (2013) used bamboo as substratum for the culture of *P. monodon* and found the water quality parameters across the treatments were within desired range and were optimum for growth of cultured shrimp. The low concentration of nitrite observed during the culture period with practice of raceway system indicates the complete oxidation of ammonia to nitrate (Cohen et al., 2005). The low concentration of ammonia and nitrite in the aquaculture without exchange of water is accomplished by microbial activities which remove these compounds through nitrification process (Ebeling et al., 2006).

The addition of substrate in freshwater fish ponds improved water quality by lowering total ammonia concentration through the biofilm formation on substratum (Dharmaraj et al., 2002; Ramesh et al., 1999). Azim et al. (2002b) reported that the average total ammonia concentration in substratum-based freshwater aquaculture ponds (0.56 mg L^{-1}) was significantly lower than the substratum-free ponds (0.95 mg L^{-1})and biofilm on substratum reduces the nitrite-nitrogen concentration of the water column as well (Asaduzzaman et al., 2008). Natural and biodegradable substratum in aquaculture provides favourable water quality parameters for the culture of carp (Keshavanath et al., 2012). The nitrite concentration was significantly high in the feed treatment compared with biofilm (Viau et al., 2013).

Biofilm as food source

Many trials in fish culture ponds have demonstrated the utility of submerged substratum in enhancing fish production (Azim et al., 2005; Asaduzzaman et al., 2008; Jana et al., 2004; Keshavanath et al., 2002). Microalgae and probiotic bacterial products are well-known for their nutritional benefits (Ju et al., 2009) and widely used as dietary stimulants to shrimp juvenile (Ju et al., 2009; Wang, 2007). Burford et al. (2004) showed that epiphytes contributed substantially (39–53%) to shrimp requirements of carbon and nitrogen. Azim and Wahab (2005) reported that in substratum-based freshwater fish ponds, periphyton served as an additional food source. Khatoon et al. (2007) observed that the specific growth rate of shrimp post-larvae increased 28% in the presence of substratum. Ballester et al. (2003) determined that growth and survival of *F. paulensis* post-larvae did not enhance in the presence of artificial substrata that had their biofilm periodically removed, indicating the importance of biofilm as food. Therefore, though there may be a synergism of physical and biological aspects related to the use of artificial substrata, it seems that the nutritional role of the biofilm is most likely the important aspect affecting the culture of *F. paulensis* post-larvae in cages. The biofilm formed on the substrata is composed of organisms that belong to the natural diet of penaeid shrimp and serve an additional source of nutrition for the post-larvae having a high quality diet (Ballester et al., 2007).

Feeding with substrate based biofilm had a significant effect on the production of *Tor khudree* and *L. fimbriatus*, as net production that was 30–59% greater than that of fish in tanks without substratum (Keshavanath et al., 2002). The growth response of *Heteropneustes fossilis,* in the presence of sugarcane bagasse as a substratum, was observed to be high in comparison to absence of substratum (Radhakrishnan and Sugumaran, 2010). Similarly, Bratvold and Browdy (2001) reported high shrimp production and low feed conversion ratio (FCR) during the culture of *L. vannamei* in a high density culture system with artificial substrata (Aquamats™). They also reported that artificial substrata increased the nitrification in the tanks, which resulted in decreased concentrations of NH_3 nitrogen. Moss and Moss (2004) reported enhancement in the production of *L. vannamei* post-larvae stocked at different densities in a flow-through system provided with Aquamats™. The authors suggested that increased shrimp growth in the presence of substrata was due to the availability of attached particulate organic matter and that the use of artificial substrata could lessen the negative effects of high stocking density during the nursery phase.

Nutritive quality of biofilm

It has been documented that nutritional composition of biofilm can be broadly considered as appropriate to fish dietary needs (Azim et al., 2002a; Dempster et al., 1993; Makarevich et al., 1993). Proximate composition of biofilm varied from 23-30% for protein, 2-9% for lipid, 25-28% for NFE and 16-42% for ash (Azim et al., 2005; Thompson et al., 2002; van Dam et al., 2002). Most fish farmers use complete diets comprising protein (18-50%), lipid (10-25%), carbohydrate (15-20%), ash (<8.5%), phosphorus (<1.5%), water (<10%), and trace amounts of vitamins and minerals (Craig and Helfrich, 2002). This indicates that nutritional quality of biofilm can be used as dietary supplement in the culture of fish and shrimps. *P. monodon* juvenile needs 35 to 40% protein (Alava and Lim, 1983; Shiau, 1998) and up to 10% lipid (Akiyama et al., 1992). The optimum requirement of protein for Indian major carps is 30% (Renukaradhya and Varghese, 1986). The protein supplementation has been observed by the microbial communities of biofilm (Buford et al., 2004; Wasielesky et al., 2006).

Biofilms are considered as good quality protein source (Oser, 1959). Therefore, biofilm attributes better growth in fish as well as shrimps (Anand et al., 2013). Apart from being a source of macronutrients, microalgae and heterotrophic bacteria are rich source of immune enhancers (Supamattaya et al., 2005), growth promoters (Kuhn et al., 2010), bioactive compounds (Ju et al., 2008) and dietary stimulants (Xu et al., 2012) which can enhance growth performance of cultured shrimp. Hence, it can be inferred that these beneficial effects of algae and microbes in biofilm might have attributed to improved growth response in tiger shrimp juvenile. Fish and shrimp larvae are very sensitive to the deficiency of certain fatty acids (FA) such as the n-3 poly unsaturated fatty acids (PUFA) (Sorgeloos and Lavens, 2000; Watanabe et al., 1983). This essential nutrient is ultimately derived from the natural food sources such as the phytoplankton, zooplankton and macro-invertebrates (Parrish, 2009). Even bacteria are abundant in the natural food sources and available as a potential food source for cultured species (Azim and Wahab, 2005; Burford et al., 2004;

Keshavanath and Gangadhar, 2005). Various studies have suggested that bacteria acted as an important nutritional source for penaeid shrimp in promoting grazing ability, growth and survival when occurring as biofilm on structures in semi-intensive and extensive ponds (Azim and Wahab, 2005; Bratvold and Browdy, 2001; Keshavanath and Gangadhar, 2005). The presence of protozoans and nematodes in the biofilm is probably an important nutriational increment for shrimp growth (Ballester et al., 2007). These organisms have a higher protein to energy ratio and, due to their ability to synthesize long chain polyunsaturated fatty acids, they enrich the quality of microbial aggregates, such as the biofilm (Zhukova and Kharlamenko, 1999). Lipid contents were high in periphyton derived from bamboo and kanchi and low in jutestick substrata. The ash content was high in periphyton from jutestick, followed by bamboo and kanchi. The energy content of periphyton was similar in bamboo and kanchi treatments and higher than jutestick treatment, while bottom sediments contained 93–95% ash and negligible amounts of protein and lipid (Azim et al., 2002).

Autotrophic and heterotrophic biomass in biofilm

Herbivore food chain reduces the loss of energy during transfer from one trophic level to another. Therefore, selection of herbivore fish and enhancement of autotrophic biomass is one of the best strategies to boost the fish production in aquaculture by reducing the cost of production. Biofilm serves as a good source of autotrophic and heterotrophic biomass because several types of phytoplankton as well as zooplankton are attached with it. The harvesting of energy from the attached biomass by fish and shrimp is easier as compared to planktonic form. Qualitative analysis of biofilm by Anand et al. (2013) indicated that the harvested biofilm was composed of 37 genera of algae belonging to Bacillariophyceae (13 genera), Cyanophyceae (10), Chlorophyceae (11) and Euglenophyceae (3) and 5 genera of zooplankton belonging to Rotifer (3) and Crustacea (2). Viau et al. (2013) reported that the chlorophyll-a concentration in water was higher in biofilm and feed based treatment (1.36 µg L^{-1}) than the feed treatment (0.49 µg L^{-1}) alone.

The biofilm bacterial density on substratum per unit weight was 100 times more than the water (Ramesh et al., 1999). Increasing the C:N ratio raised the total heterotrophic bacterial population in the water column, sediment and periphyton (Asaduzzamanet al., 2008). Karunasagar et al. (1996) found the highest cell density on the plastic surface followed by cement slab and steel surface. On contrast to this, King et al. (2008) used six different types of substrata for growing of biofilm in recirculating aquaculture. Even, after three days of introduction of substrata, they reported the numbers of bacteria remained constant throughout the experiment and at the same time they found that there was no significant difference in bacterial count on these different substrata.

Substrata enhance survival rate

Abdussamad and Thampy (1994) observed high levels of shrimp damage in high density rearing systems due to cannibalism and reported increased chance of cannibalism in the case of newly moulted specimens. The introduction of substrata in the culture system is one of the ideal methods to increase the survival through minimizing the cannibalism. Substrata, apart from providing biofilm, provide shelter and protection from predators. This alone is an interesting feature since several studies have showed an inverse relation between stocking density and shrimp growth (Martin et al., 1998; Preto et al., 2005; Wasielesky et al., 2001). Khatoon et al. (2007) showed the presence of post-larvae inside the PVC coated pipes which could have served as refugium to the moulting post-larvae leading to enhanced survival as a significantly high survival (51–60%) was found compared to the control (37%). Sandifer et al. (1987) reported high survival (24%) in the nursery rearing of *L. vannamei* in the tanks where fiberglass window screens were provided. In addition to increasing food supply, the presence of substratum appears to reduce stress by acting as a shelter or hiding place for fish (Keshavanath et al., 2002). Ju et al. (2009) recorded significantly high growth and survival in *L. vannamei* with diet supplemented with microalgae.

Ballester et al. (2007) reported that shrimp *F. paulensis* post-larvae reared in cages had a considerable increase in their biomass and survival with the addition of polyethylene substratum. During the experimental period, it was observed that shrimps were constantly occupying the substratum and feeding on the biofilm. Besides, the nutritional contribution provided by the biofilm, the physical presence of artificial substratum within the culture units promotes the enlargement of the area for shrimp distribution (Ballester et al., 2007). Bratvold and Browdy (2001) observed that the presence of artificial substratum delayed the negative effects of overcrowding, contributing to a better performance of *L. vannamei* reared in an intensive culture system. Furthermore, Tidwell et al. (1998) were able to improve the culture performance of the freshwater prawn *M. rosenbergii* as they utilized artificial substrata to reduce the negative effects derived from the strong territorial behaviour of this species.

Addition of periphyton substrata increased the survival of prawn 63 to 72% as compared to periphyton free treatment (Asaduzzaman et al., 2008). Survival of shrimp (*F. brasiliensis*) maintained in the biofilm was significantly higher than that observed in the feed treatment (Viau et al., 2013).

Use in vaccination

Natural resistant property of bacterial biofilms for development of effective oral vaccines is simple and cheap approach. Azad et al. (1999) reported that the oral route of vaccination is one of the simple, cheap and ideal techniques among the various methods of vaccination for mass administration to fish of all sizes. Oral vaccination is regarded beneficial in aquaculture as it is non-stressing and accessible to fish of any size, age and numbers (Quentel et al., 1997).

However, direct administration of antigen during the oral vaccination showed poor response due to digestive degradation of antigens in the fore-gut before the vaccine reached immune-responsive areas in the hind-gut and other lymphoid organs (Johnson and Amend, 1983; Rombout et al., 1985). Strategies developed for improvements of oral vaccination have attempted to avoid this gastric destruction especially by the use of encapsulated antigen microspheres (Dalmo et al., 1995; Piganelli et al., 1994).

Azad et al. (1997) developed and evaluated a biofilm of *A. hydrophila*for oral vaccination of carp which induced significantly higher antibody titres and protection compared to a free cell vaccine. Bacterial biofilm developed on substrata have been found to be resistant to antibiotics (Anwar and Costerton, 1990), phagocytosis and the killing effect of whole blood and serum (Anwar et al., 1992) due to presence of a protective layer of glycocalyx.

The glycocalyx of biofilm is a polymer of neutral hexoses which encapsulates and possibly protects the bacterial surface antigens even from digestion in the gut (Costerton and Irvin, 1981).

This property of biofilm vaccine is reported to facilitate longer retention of antigens in the gut and lymphoid tissue and hence, might have resulted in the early and heightened primary antibody response (Azad et al., 2000). The importance of biofilm vaccine has more relevance for oral vaccination of carnivorous fish where stomach is well developed in the digestive system (Nayak et al., 2004). Bacillus spp. are prominent bacteria in biofilm (Pradeep et al*.,* 2004) and *B. subtilis* has been shown to possess antitumor and immunomodulatory activities in fish (Cohen et al., 2003). Some studies have demonstrated that *B. subtilis* and spores of *B. subtilis* act as probiotics since they promote growth and viability of the beneficial lactic acid bacteria in the intestinal tracts of humans and some animals (Hoa et al., 2000).

Alya et al. (2008) showed that *B. subtilis* and *Lactobacillus acidophilus* inhibited the growth of *A. hydrophila* in the *O. niloticus*. The *B. subtilis* inhibited the establishment of *P. fluorescens* in the *O. niloticus*. The two *Bacillus* strains, *B. subtilis* 2335 and *B. licheniformis* 2336 are well characterised and a number of clinical studies have been used to demonstrate probiotic effects (Bilev, 2002).

CONCLUSION

Improved water quality and nutrient availability through biofilm enhance the survival rate and growth of fish in the substrate based aquaculture. Heterotrophic bacterial load is very high in biofilm in comparison to water indicating vast scope for biofilm utilization in production of fish and shellfish in aquaculture. Biofilm based microorganisms enhance the immune response of aquaculture species through oral vaccination. Locally available cheap natural biomass may be used as substratum for biofilm formation in aquaculture so as to convert them into a valuable resource within a pond ecosystem with a view to promote sustainable aquaculture especially in developing countries.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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