# Bioremediation in Shrimp Culture Systems

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## Abstract

Aquaculture generates considerable amount of wastes, consisting of metabolic by-products, residual food, faecal matter and residues of prophylactic and therapeutic inputs, leading to the deterioration of water quality and disease outbreaks. Bioremediation, the application of microbes/enzymes to the ponds, is the method currently in use for improving water quality and maintaining the health and stability of aquaculture systems. Bioremediation involves organic matter mineralization to carbon dioxide, maximising primary productivity that stimulate shrimp production, nitrification and denitrification to (1) eliminate excess nitrogen from ponds and (2) maintain diverse and stable pond community where the pathogens are excluded from the system and desirable species get established. Apart from organic matter degrading (detritivorous) heterotrophic bacteria, nitrifying, denitrifying and photosynthetic bacteria are generally employed in bioremediation.


## Introduction

Aquaculture is the world's fastest growing food production sector (Moriarty 1999). It was once considered an environmentally sound practice because of its traditional polyculture and integrated systems of farming based on optimum utilization of farm resources, including farm wastes. Increased production is being achieved by the expansion of land and water under culture and the use of more intensive and modern farming technologies that involve higher usage of inputs such as water, feeds, fertilizers and chemicals. As a result, aquaculture is now considered as a potential polluter of the aquatic environment and a cause of degradation of wetland areas (Pillay 1992).

## Waste Production in Aquaculture

The physical, chemical and biological conditions of the culture environment have an influence on the health and productivity of shrimp. Exposure of shrimps to toxins like hydrogen sulphide, ammonia and carbon dioxide lead to stress and ultimately disease (Ravichandran and Jalaludin 2001). The types of wastes produced in aquaculture farms are basically similar. However, there are differences in quality and quantity of components depending on the species cultured and the culture practices adopted. The wastes in hatcheries or aquaculture farms can be categorized as: (1) residual food and faecal mater; (2) metabolic by-products; (3) residues of biocides and biostats; (4) fertilizer derived wastes; (5) wastes produced during moulting; and (6) collapsing algal blooms (Sharma and Scheeno 1999).

The current approach to improving water quality in aquaculture is the application of microbes/enzymes to the ponds, known as 'bioremediation'. When macro and micro organisms and/or their products are used as additives to improve water quality, they are referred to as bioremediators or bioremediating agents (Moriaty 1998). They result in a lower accumulation of slime or organic matter in the pond bottom, better penetration of oxygen into the sediment and a generally better environment for the farmed stock (Rao and Karunasagar 2000). The isolation and development of indigenous bacteria are required for successful bioremediation (Jameson 2003). A successful bioremediation involves: optimising nitrification rates to keep low ammonia concentration; optimising denitrification rates to eliminate excess nitrogen from ponds as nitrogen gas; maximising sulphide oxidation to reduce accumulation of hydrogen sulphide; maximising carbon mineralization to carbon dioxide to minimize sludge accumulation; maximising primary productivity that stimulates shrimp production and also secondary crops; and maintaining a diverse and stable pond community where undesirable species do not become dominant (Bratvold et al. 1997).

## Bioremediators as Disease Controlling Agents

In recent years, there has been growing interest in biocontrol of microbial pathogens in aquaculture using antagonistic micro-organisms (Westerdahl et al. 1991; Maeda 1994). A study on the role of antagonistic bacteria, especially the co-existing bacteria, as biocontrol agents appears worthwhile in lieu of the negative impacts of antibiotics (Abraham et al. 2001). Most probiotics proposed as biological control agents in aquaculture belong to the Lactic Acid Bacteria (*Lactobacillus, Carnobacterium* etc.), *Vibrio* (*Vibrio alginolyticus*), *Bacillus*, and *Pseudomonas* (Singh et al. 2001). Abraham et al. (2001) studied *in-vitro* antagonistic activity of penaeid shrimp larvae associated bacterium, *Alteromonas*, against several opportunistic crustacean pathogens and found that the *Alteromonas* species suppressed the activity of *Vibrio harveyi* and improved the survival of *Penaeus indicus* larvae *in-vivo.* Beneficial microbes, such as non-pathogenic isolates of *Vibrio alginolyticus*, can be inoculated into shrimp culture systems to suppress the pathogenic vibrios like *Vibrio harveyi, Vibrio parahaemolyticus* and *Vibrio splendens* and reduce the opportunistic invasion of these pathogens in shrimps (Jameson 2003).

## Bioremediation of Organic Detritus

The dissolved and suspended organic matter contains mainly carbon chains and is highly available to microbes and algae. A good bioremediator must contain microbes that are capable of effectively clearing carbonaceous wastes from water. Additionally, it helps if these microbes multiply rapidly and have good enzymatic capability. Members of the genus *Bacillus,* like *Bacillus subtilis, Bacillus licheniformis*, *Bacillus cereus*, *Bacillus coagulans,* and of the genus *Phenibacillus*, like *Phenibacillus polymyxa,* are good examples of bacteria suitable for bioremediation of organic detritus. However, these are not normally present in the required amounts in the water column, their natural habitat being the sediment. When certain *Bacillus* strains are added to the water in sufficient quantities, they can make an impact. They compete with the bacterial flora naturally present for the available organic matter, like leached or excess feed and shrimp faeces (Sharma 1999). As a part of bio-augmentation, the *Bacillus* can be produced, mixed with sand or clay and broadcasted to be deposited in the pond bottom (Singh et al. 2001). *Lactobacillus* is also used along with *Bacillus* to break down the organic detritus. These bacteria produce a variety of enzymes that break down proteins and starch to small molecules, which are then taken up as energy sources by other organisms. The removal of large organic compounds reduces water turbidity (Haung 2003).

## Bioremediation of Nitrogenous Compounds

Nitrogen applications in excess of pond assimilatory capacity can lead to deterioration of water quality through the accumulation of nitrogenous compounds (e.g., ammonia and nitrite) with toxicity to fish and shrimp. The principal sources of ammonia are fish excretion and sediment flux derived from the mineralization of organic matter and molecular diffusion from reduced sediment, although cyanobacterial nitrogen fixation and atmospheric deposition are occasionally important (Ayyappan and Mishra 2003). Nitrification proceeds as follows:

$$NH_4^+ + 1^1/_2\, O_2 \rightarrow NO_2^- + 2H^+ + H_2O$$
$$NO_2^- + 1^1/_2\, O_2 \rightarrow NO_3^- \quad (1)$$

Bacteriological nitrification is the most practical method for the removal of ammonia from closed aquaculture systems and it is commonly achieved by setting of sand and gravel bio-filter through which water is allowed to circulate. The ammonia oxidisers are placed under five genera, *Nitrosomonas, Nitrosovibrio, Nitrosococcus, Nitrolobus* and *Nitrospira,* and nitrite oxidisers under three genera, *Nitrobacter, Nitrococcus* and *Nitrospira.* There are also some heterotrophic nitrifiers that produce only low levels of nitrite and nitrate and often use organic sources of nitrogen rather than ammonia or nitrite. Nitrifiers in contaminated cultures have been demonstrated to nitrify more efficiently. Nitrification not only produces nitrate but also alters the pH slightly towards the acidic range, facilitating the availability of soluble materials (Ayyappan and Mishra 2003).

The vast majority of aquaculture ponds accumulate nitrate, as they do not contain a denitrifying filter. Denitrifying filters helps to convert nitrate to nitrogen. It creates an anaerobic region where anaerobic bacteria can grow and reduce nitrate to nitrogen gas (Rao 2002). Nitrate may follow several biochemical pathways following production by nitrification.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (2)$$

Unlike the limited species diversity of bacteria mediating nitrification, at least 14 genera of bacteria can reduce nitrate. Among these, *Pseudomonas, Bacillus* and *Alkaligenes* are the most prominent numerically (Focht and Verstraete 1977).

## Bioremediation of Hydrogen Sulphide

Sulphur is of some interest in aquaculture because of its importance in anoxic sediments. In aerobic conditions, organic sulphur decomposes to sulphide, which in turn get oxidised to sulphate. Sulphate is highly soluble in water and so gradually disperses from sediments. Sulphide oxidation is mediated by micro organisms in the sediment, though it can occur by purely chemical processes (Boyd 1995). Under anaerobic conditions, sulphate may be used in place of oxygen in microbial metabolism. This process leads to the production of hydrogen sulphide gas (Midlen et al. 1998). The $H_2S$ is produced by a series of microbially mediated reductions (Boyd 1995).

$$SO_4^{2-} + 4H_2 + 2H^+ \rightarrow H_2S + 4H_2O \quad (3)$$

(Djurle 2003).

Organic loading can stimulate $H_2S$ production and reduction in the diversity of benthic fauna (Mattson and Linden 1983). $H_2S$ is soluble in water and has been suggested as the cause of gill damage and other ailments in fish (Beveridge 1987). Unionised $H_2S$ is extremely toxic to fish at concentrations that may occur in natural waters as well as in aquaculture farms (Bonn and Follis 1967). Bioassays of several species of fish suggest that any detectable concentration of $H_2S$ should be considered detrimental to fish production (Boyd 1979).

The photosynthetic benthic bacteria that break $H_2S$ at pond bottom have been widely used in aquaculture to maintain a favourable environment (Singh and Radhika 2001). These bacteria contain bacterio-chlorophyll that absorb light (blue to infrared spectrum, depending on type of bacterio-chlorophyll) and perform photosynthesis under anaerobic conditions (Haung 2003). They are purple and green sulphur bacteria that grow at the anaerobic portion of the sediment-water interface. Photosynthetic purple non-sulphur bacteria can decompose organic matter, $H_2S$, $NO_2$ and harmful wastes of ponds. The green and purple sulphur bacteria split $H_2S$ to utilize the wavelength of light not absorbed by the overlying phytoplankton. The purple and green sulphur bacteria obtain reducing electrons from $H_2S$ at a lower energy cost than $H_2O$ splitting photoautotrophs and thus require lower light intensities for carrying out photosynthesis. The general equation of this reaction is as follows:

$$CO_2 + 2H_2S \rightarrow (CH_2O) + H_2O + 2S$$

$$S + CO_2 + 3H_2S \rightarrow (CH_2O) + H_2SO_4$$

$$CO_2 + NaS_2O_3 + 3H_2O \rightarrow 2(CH_2O) + NaS_2O_4 + H_2SO_4 \quad (4)$$

Chromatiaceae and Chlorobiaceae are the two families of photosynthetic sulphur bacteria that favour anaerobic conditions for growth while utilizing solar energy and sulphide. Chromatiaceae contain sulphur particles in cells but Chlorobiaceae precipitate them out. The family Rhodospirillaceae is not of any use for $H_2S$ removal as they mainly utilise organic material, such as lower fatty acid, as source of hydrogen. But they can be used as efficient mineralizers at pond bottom as they grow in both aerobic and anaerobic conditions as heterotrophic bacteria even in the dark without utilizing solar energy (Singh and Radhika 2001). Photosynthetic bacteria of importance in aquaculture are the following (Haung 2003):

Rhodospirillaceae →
*Rhodospirillum, Rhodopseudomonas, Rhodomicrobium*

Chromatiaceae →
*Chromatium, Thiocystis, Thiosarcina, Thiospirillum, Thiocapsa, Lamprocystis, Thiodictyon, Thiopedia, Amoebobacter, Ectothiorhodospira.*

Chlorobiaceae →
*Chlorobium, Prosthecochloris, Chloropseudomonas, Pelodictyon, Clathrochloris.*

For bioremediation of $H_2S$ toxicity, the bacterium that belongs to Chromatiaceae and Chlorobiaceae can be mass cultured and can be applied as pond probiotic. Being autotrophic and photosynthetic, mass culture is less expensive and the cultured organisms can be adsorbed on to the sand grains and applied so that they may reach the pond bottom to enrich the hypolimnion and ameliorate $H_2S$ toxicity (Singh and Radhika 2001).

Table 1. Organisms used as bioremediators.

| Identity of the bioremediator | Source | Used on | Method of application | References |
|---|---|---|---|---|
| GRAM-POSITIVE BACTERIA | | | | |
| *Bacillus* sp. 48 | Common snook | *Centropomus undecimalis* | Added to water; reduced salinity | Kennedy et al. 1998 |
| *Bacillus* sp | Commercial product | Penaeids | Water | Moriarty 1998 |
| *Bacillus* sp | Commercial product | Channel catfish | Spread in pond water | Queiroz and Boyd 1998 |
| Mixed culture, mostly *Bacillus* sp. | Commercial product | *Brachionus plicatilis* | Mixed with water | Hirata et al. 1998 |
| GRAM-NEGATIVE BACTERIA | | | | |
| Aeromonas media | Unknown | *Crassostrea gigas* | Mixed with water | Gibson et al. 1998 |
| Aeromonas CA2 | Unknown | *Crassostrea gigas* | Mixed with water | Douillet and Langdon 1994 |
| *Photorhodobacterium* sp. | Unknown | *Penaeus chinensis* | Mixed with water | Xu-per communication 1997 |
| *Pseudomonas fluorescence* | *Onchorhynchus mykiss* | *Onchorhynchus mykiss* | Mixed with water to $10^5$ or $10^6$ cells $ml^{-1}$ | Gram et al. 2001 |
| *Pseudomonas* sp. | *Onchorhynchus mykiss* | *Onchorhynchus mykiss* | Mixed in water | Spanggaard et al. 2001 |
| *Roseobacter* sp. BS 107 | Unknown | Scallop larvae | Mixed in water | Ruiz-Ponte et al. 1999 |

## Screening of Microbes for Utilization as Bioremediators

Microorganisms both Gram positive and Gram negative have been tested for their efficacy as bioremediators in aquaculture by various workers (Table 1). *Bacillus* is the most commonly used organism followed by *Aeromonas* and *Pseudomonas* .

## Commercial Products

Bioremediators commercially available in the market mainly include Nitrifiers, Sulphur bacteria, *Bacillus* sp. and *Pseudomonas* sp. (Table 2).

## Conclusion

There are several commercial products marketed for use in aquaculture to clean up the pond bottom, maintain good water quality and improve shrimp health, particularly for intensive aquaculture. The role of beneficial bacteria to control pathogens will become particularly important in aquaculture, especially in the light of the increasing number of antibiotic resistant strains of bacteria, strict government regulation of environmental treatments, and cost-effectiveness. Management of pond microbial ecology is an area where applied research can lead to important findings for improving the productivity and environmental "friendliness" of the shrimp farming industry worldwide, particularly in view of recent negative environmental impacts of shrimp farms. It seems likely that the use of bioremediators will gradually increase and the success of aquaculture in future may be synonymous with the success of bioremediators that, if validated through rigorous scientific investigation and used wisely, may prove to be a boon for the aquaculture industry.

Table 2. List of commercially available bioremediators for aquaculture applications.

| Product | Microbial content | Company / firm |
|---|---|---|
| ABIL nitrifying package | Nitrifiers | Tropical marine centre, London. |
| Alken clear-flo 1002 | *Bacillus* sp. | Alken Murray Corp., New York. |
| Alken clear-flo 1100 | Nitrifying bacteria | Alken Murray Corp., New York. |
| Alken clear-flo 1400 | 3 species of *Bacillus* + 2 species of Nitrifying bacteria | Alken Murray Corp., New York. |
| Ammonix | Nitrifying bacteria | Prowins Bio-Tech Pvt. Ltd., India. |
| Bactaclean | Nitrifiers | Enviro-Comp. Services, Inc., Dover, USA. |
| Biogreen | Bacillus subtilis | Activa Biogreen Inc., Wood Dale, USA. |
| Biostart | *Bacillus* sp. | Bio-CAT, Inc., Virginia, USA. |
| BRF-13A | Nitrobacter, Nitrosomonas | Enviro-reps., Ventura, CA, USA. |
| BRF-1A | Nitrifying bacteria | Enviro-reps. Ventura, CA, USA. |
| BRF-4 | Nitrobacter, Nitrosomonas | Enviro-reps. Ventura, CA, USA. |
| BRF-4 | Nitrifying bacteria | Enviro-reps. Ventura, CA, USA. |
| BZT ® Aquaculture | Nitrifiers | United-Tech, Inc., Indiana, USA. |
| Detrodigest | Bacillus sp. | NCAAH, CUSAT, India. |
| Eutroclear | Nitrifying bacteria | Bioremediate. Com, LLC, Atlanta. |
| Nitroclear | Nitrobacter, Nitrosomonas | Bioremediate. Com, LLC, Atlanta. |
| PBL - 44 | Nitrifying bacteria / Bacillus sp. | Enviro-reps. Ventura, CA, USA. |
| Probac BC | *Bacillus* sp. | Synergy Biotechnologies, India. |
| Pronto | *Bacillus* sp. | Hort-Max ltd., New Zealand. |
| Ps-1 | *Pseudomonas* sp. | NCAAH, CUSAT, India. |
| Remus | Nitrifying bacteria | Avecom, Belgium. |
| Super PS | Sulphur bacteria | CP aquaculture Pvt. Ltd., India. |

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