

Credit seminar
On
Recent advances in Bioremediation in
aquaculture systems

Submitted by

S.J. Abisha Juliet Mary [MFT 16085]

Department of Fish pathology and Health Management



தமிழ்நாடு டாக்டர்.ஜெ.ஜெயலலிதா மீன்வளப் பல்கலைக்கழகம்
TAMIL NADU Dr.J.JAYALALITHAA FISHERIES UNIVERSITY

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What is Bioremediation?

Bioremediation consists of using living organisms (bacteria, fungi, actinomycetes, cyanobacteria and to a lesser extent, plants) to reduce or eliminate toxic pollutants. These organisms may be naturally occurring or laboratory cultivated. The physical, chemical and biological conditions of the culture environment have an influence on the shrimp to toxins like hydrogen sulphide, ammonia and carbon dioxide leads to stress and ultimately disease. Wastes produced in aquaculture farms differ in quality and quantity of components depending on the species farmed and the farming practices adopted. The wastes in aquaculture farms can be categorized as residual food and faecal matter, metabolic by-product, residues of biocides and biostats, fertilizer derived wastes and wastes produced during moulting and collapsing algal blooms. The current approach to improving water quality in aquaculture is the application of microbes/enzymes to the ponds known as 'bioremediation'. When macro and microorganisms and/or their products are used as additives to improve water quality, they are referred to as bioremediators or bioremediating agents. The newest attempt being made to improve water quality in aquaculture is the application of probiotics and enzymes to the ponds is known as bioremediation, which involves manipulation of microorganisms in ponds to enhance mineralization of organic matter and get rid of undesirable waste compounds.

Problems in Aquaculture- Why need for Bioremediation

The increasing demand of food consumption and improving the food security system have led to aquaculture development worldwide. FAO predicts that this industry is likely to boom in the near future due to world population growth. The decline of the fishing industry has demanded underlining challenges to fish farming practices in order to ensure the availability of fish as an important source of protein. On the other hand, the aquaculture industry has responded to a number of environmental challenges such as water shortage and quality, land degradation,

high costs of land hiring, environmental impact and diseases that has promoted the development of intensive aquaculture crop oriented.

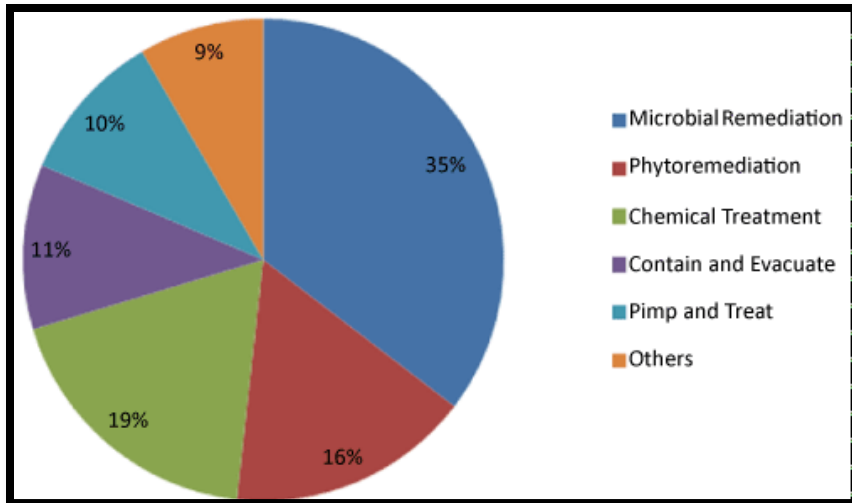
In intensive and semi-intensive aquaculture practices, high stocking densities of fish along with intense feeding and fertilization often leads to the deterioration of water quality and proliferation of pathogens. These are the major constraints in fish culture.

In aquaculture ponds, the quality of water during the culture period will deteriorate mainly due to the **accumulation of metabolic wastes, decomposition of unutilized feed and decay of biotic materials**. Water quality deterioration potentially leads disease development.

The current approach to **improving water quality in aquaculture** is the application of microbes / enzymes to the ponds known as '**bioremediation**'. When macro and microorganisms and /or their products are used as additives to improve water quality, they are referred to as **bioremediators or bioremediating agents**

Current scenario

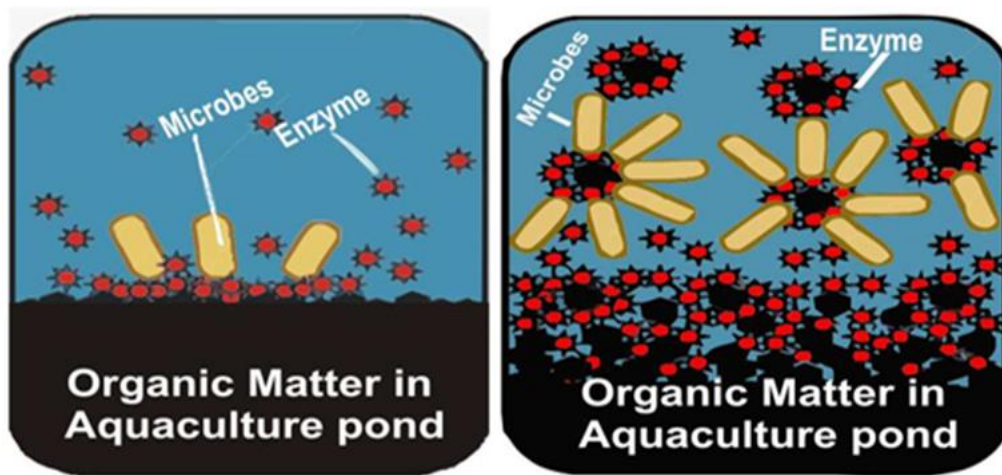
In fisheries and aquaculture, although perhaps not as much as in livestock production and crops, some biotechnologies have been used in developing countries. As mentioned earlier, use of biotechnologies in fisheries is very limited whilst in aquaculture biotechnologies are represented in a few fields such as genetic improvement, disease control, feeds and nutrition and environmental improvement. Global survey has examined the use of bioremediation Technologies in 2014 in all over the world. There were respondents from all continents (except Antarctica). This reports says that Major population prefers Microbial remediation for bioremediation.



Pie chart of preferred bioremediation methods

Principles and concept of Bioremediation

The goal in bioremediation is to stimulate microorganisms with nutrients and other chemicals that will enable them to destroy the contaminants. This is the main principle involving in the bioremediation process.



Conventional bioremediation

Enhanced bioremediation

Probiotics –in bioremediation

The term probiotic has been defined as “**a mono- or mixed culture of live microorganisms** that when applied to animals, humans affect beneficially the host by improving the properties of the indigenous microflora”. Moriarty (1998) extended the definition for aquaculture to include the addition of natural bacteria to tanks and ponds in which the animals live.

The application of beneficial bacteria (probiotics) is not only associated with gut health (feed probiotics), but also with bioremediation that improves the environment (water and soil) in which the animals are reared. Probiotics reduce the level of infection and mortality in aquaculture organisms. Probiotics compete with the disease-causing bacteria for food. Customarily, ponds have indigenous bad bacteria that leisurely eat on an abundance of undigested food, faecal matter, and dead algae. When farmers suddenly introduce a large quantity of these good bacteria -- probiotics -- into a pond, the newcomers eliminate the pre-existing bad bacteria out of the nutrient queue. The old bacteria, often-bad bacteria, never having had to compete for food, cannot keep pace with the aggressive probiotics.

Application of probiotics in aquaculture is such a technology and research on probiotics as bioremediation and biocontrol agents is increasing with the demand for eco-friendly aquaculture. Probiotic bacteria improve the health of shrimp or fish by controlling pathogens and improving water quality by modifying the microbial community composition of the water and sediment. Probiotic bacteria enter the gut or attach to external surfaces of the animals either directly from the water or via attachment first to food or other ingested particles. Thus, they are used in aquaculture both as water and sediment quality conditioners and as feed supplements.

Probiotics are generally administered as live microbial feed supplements which affect the host animal by improving the intestinal microbial balance to optimize

the presence of non-toxic species. A stable gut microflora helps the host resist pathogenic invasions, particularly via gastro-intestinal tract. Antibiotics reduce specific or broad-spectrum gut microflora and probiotics may have post-antibiotic treatment potential for restoring the microbial balance. Probiotics are widely used in animal health.

There are increasing reports of potential probiotics for shrimp aquaculture, which has been plagued by opportunistic bacteria, such as the luminescent *Vibrio harveyi*, and, in some cases, probiotics have been reported to significantly reduce antibiotic use in shrimp hatcheries. Suppression of proliferation of certain pathogenic bacteria (e.g., *Vibrio* spp.) in shrimp hatcheries has been achieved by introducing (inoculating) non-pathogenic strains or species of bacteria that compete for microbial metabolite resources. This procedure shows promise to be effective and economical. However, further refinement of administration and concentration loads required for effective pathogen suppression is required. Effective and economically viable probiotics also require greater research into optimal strains of probiotic micro-organisms and stringent evaluation under field conditions of their economic feasibility.

Enzymes at work

In the bioremediation process, enzymes play the role of catalysts that accelerate biochemical reactions in pond soil and water. When added to culture water or spread on top of pond soil, enzymes are able to degrade the major organic constituents normally found in shrimp and fish ponds. Each enzyme has its mode of action and is very specific in the chemical reaction it catalyses.

Enzymes are also naturally produced and excreted by some microbes. These extracellular enzymes, such as cellulase, protease and amylase, are produced during the aerobic fermentation of organic matter by micro-organisms, for example by some *Bacillus* species.

Enzyme	Substrate
Amylase	Starch
β -Glucosidase	β -Glucoside
Cellulase	Cellulose
Lipase	Lipids and fat
Protease	Protein
Xylanase	Xylan, Hemicellulose
Pectinase	Pectin

A diverse range of enzymes used as bioremediation agents in aquaculture.

For all these bioremediation processes catalysed by enzymes, the presence of beneficial bacteria is important as well. Enzymes accelerate microbial processes by breaking apart large sludge particles, thus creating more surface areas which can then be attacked and fermented by microbes. This reduction of sludge and dead organic matter can be seen visually not only with better water quality, but also better soil quality.

Coastal shrimp/ Fish Farm

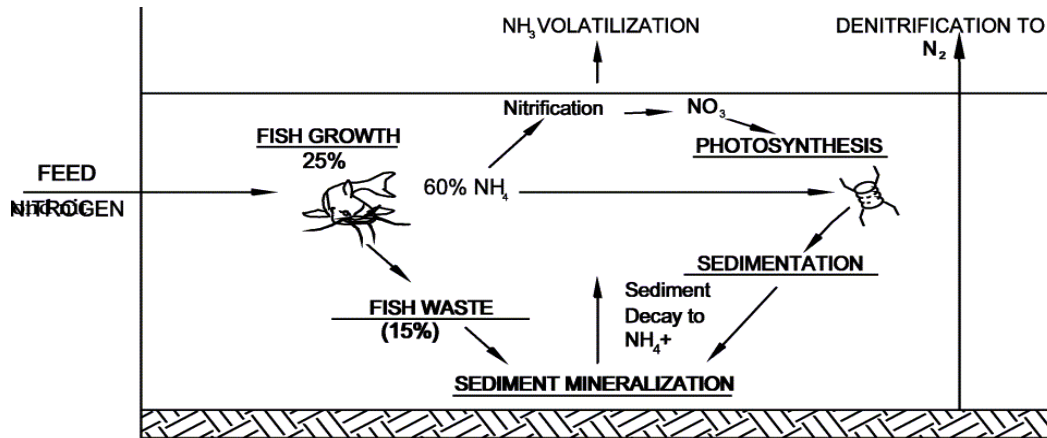
During the past 20 years, aquaculture industry has been growing tremendously, especially that of marine fish, shrimps and bivalves in addition to freshwater fish and prawn. Penaeid shrimps are among the most important and extensively cultured crustaceans in the world (> 60 countries). Shrimp culture all over the world has been frequently affected by viral and bacterial diseases inflicting huge losses and annual losses due to disease has been estimated to be more than US \$ 5 billion. Although diseases pose a serious threat to penaeid shrimp aquaculture, the production of these highly valued crustaceans continue to grow. For preventing and controlling diseases, particularly in aquaculture, the best method is the improving health of culture organisms and elimination of pathogens by improving aquatic environment.

Vibrio bacteria often dominate when algal blooms die and shrimp numbers are high. Oxygen diffusion is limited in organic detritus on the pond bottom, especially when the feeding rate is high and thus oxygen is rapidly depleted. Fermenting bacteria, which include Vibrio, become active and release organic acids, some of which are toxic to shrimp and which are used by sulphate-reducing bacteria in shrimp ponds. It is important to ensure that organic detritus and slime do not build up on the pond bottom. All faeces, excess feed and dead algae must be decomposed rapidly. Aeration by itself is not enough. Active bacterial populations must be changed to species that are adapted to rapid degradation of complex organic molecules. The Bacillus group produce a wide range of exo-enzymes that are very efficient at breaking down large molecules such as protein and fats. Use of bacillus species improved water quality, survival, growth rates and the health status of juvenile *Penaeus monodon* and reduce the pathogenic vibriosis. High stocking density or crustaceans in ponds usually exacerbates problems with water quality and sediment deterioration. There are no specific signs and symptoms of EHP infection in shrimp. It was discovered in slow growing shrimp and also from shrimp that exhibited mortality associated with white faeces syndrome. There occurs no drugs for this EHP treatment as they suggest to follow BMP. So there occurs again a problem of water quality deterioration

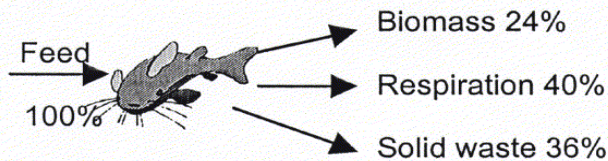
Aquaculture pond dynamics

Aquaculture ponds are a living dynamic systems they exhibits continuous and constant fluctuations. The pond undergoes a vast collection of both chemical reactions and physical changes. Exchange of atmospheric gases including Oxygen (O₂), nitrogen (N₂) and Carbon dioxide (CO₂) with the pond water are vital to the process of fish metabolism and plant photosynthesis. Inorganic substances (minerals) dissolve from the pond walls and bottom while precipitation of dissolved minerals occurs. Physicals exchanges between the pond its surroundings include absorption of sunlight (radiant energy) to fuel photosynthesis and supply oxygen

with in the pond, heat exchange and volume changes caused by evaporation and precipitation (rain).



Fish Oxygen Demand



Changes in the volume of a pond are very important as they affect the concentration of dissolved substances and correspondingly requirements for treatment. Hence, the pond dynamics not only depend on its own characters and conditions but also on the surrounding atmospheric weather conditions. Good production from aquaculture ponds can be achieved when the pond and surroundings make chemical and physical exchanges at a steady state. When all of the processes balance, a state of equilibrium is achieved.

Activities of bioremediators

- The microflora of the sediment and water in which the cultured shrimp or fish live is influenced by the microbes released from faeces of all the animals in their environment. If a pathogen is present, its population density can be magnified through interactions in the intestinal tracts of the animals and in the

faeces. When food for aquatic animals is added to the water, it adsorbs or absorbs bacteria from the water before it is eaten.

- However, when beneficial bacteria are added to ponds or tank water and are adsorbed to feed, they enter the intestinal tract and compete with pathogens. Thus the farmer can manipulate the species composition by seeding large numbers of desirable strains of bacteria or algae.
- The bioremediators can decompose the excreta of shrimp, prawn or fish, remaining food materials, remains of the plankton and other organic materials to CO₂, nitrate and phosphate. These inorganic salts provide the nutrition for the growth of micro algae while the bacteria grow rapidly and become the dominant group in the water, inhibiting the growth of the pathogenic microorganisms. The photosynthesis of the micro algae provide dissolved oxygen for oxidation and decomposition of the organic materials and for the respiration of the microbes and cultured animals. This kind of cycle may improve the nutrient cycle, and it can create a balance between bacteria and micro algae, an maintaining a good water quality environment for the cultured animals.
- Bacterial excretions make the pond medium less inhabitable for bad bacteria. Not only do probiotics bacteria have terrific appetites; they excrete enzymes -- exoenzymes -- as a natural byproduct of their metabolic activity, just as human's sweat. The enzyme excretions infuse and spread throughout the pond medium, changing its chemistry resulting destroy of bad bacteria. Hence, probiotics therapy greatly improves pond water quality and reduces the pollution level of effluent before its release into the environment.
- Bioremediators speed the breakdown of organic waste fragments (dissolved proteins and unused feed), thus lessening sludge build-up. If sludge is not removed or does not decompose, dangerous concentrations of sulfide, nitrite, ammonia and various organic acids can occur. While the exo enzymes of good bacteria go to work on larger waste particles and breaking them down

through chemical reaction. As a result, there are fewer harmful chemicals in the medium.

- Bioremediators balance algae growth through providing nutrients by their biodegradation activity. Probiotics utilize phosphate for their body metabolic activities and hence prevent overgrowth (heavy blooms) of algae by limiting the phosphate. Dead algae are one of bacteria's favorite foods. Also, probiotics diminish nutrients normally consumed by algae and result in less slime in the ponds.
- Bioremediators make a better habitat for culture organisms. With less accumulation of organic matter on the pond bottom, more oxygen can penetrate the sediment. For eg. Prawn and shrimp characteristically burrow in the sediment. By loosening the sediment, probiotics make this burrowing easier. Moreover, they diminish the toxin level in the sediment itself.
- Bioremediators optimize the immune systems of culture organisms, increasing their resistance to disease. By upgrading the pond environment, augmenting the food supply, and making habitat more comfortable, probiotics fortify fish, prawn and shrimp immune systems.

Microbes in Aquatic ecosystem

Microbes include **microalgae, bacteria, fungi and virus**.

- ❖ **Microalgae:** absorb carbon dioxide and to supply oxygen in aquatic animal.
- ❖ **Bacteria and fungi:** decompose organic matter and keep the water clean.
- In **aerobic condition** algae, diatoms and cyanobacteria are predominant.
- In **anaerobic conditions** other photosynthetic bacteria are dominant.

Organic matter settles to the bottom and is decomposed by microorganisms.

- Metabolic products of **aerobic decomposition** are Carbon dioxide, water, ammonia and other nutrients.

- In **anaerobic sediment** some microorganism decompose organic matter by fermentation reactions that produce alcohols, ketones, aldehydes.

Organic Detritus & Bioremediation

The dissolved and suspended organic matter contains mainly carbon chains and is abundantly available to microbes and algae. A good bioremediation must contain microbes that are capable of effectively clearing carbonaceous wastes from water. Further it would be very supportive, when these microbes multiply rapidly and have good enzymatic capability. Members of the genus *Bacillus* like *Bacillus subtilis*, *B. licheniformes*, *B. cereus*, *B. coagulans* and species *Phenibacillus polymyxa* are good examples of bacteria suitable for bioremediation of organic detritus. However, they are not normally present in the required quantities 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. As a part of bio-augmentation, the *Bacillus* can be produced, mixed with sand or clay and broadcast to be deposited in the pond bottom. *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

Nitrogenous Compounds & Bioremediation

Nitrogen applications in excess of pond assimilatory capacity can lead to deterioration of water quality through the accumulation of nitrogenous compounds (ammonia and nitrite) causing toxicity to fish and shrimp. The principal sources of ammonia are excretion and sediment flux derived from the mineralization of organic matter and molecular diffusion from reduced sediment. Bacteriological nitrification is

the most practical method for the removal of ammonia from closed aquaculture systems and it is commonly achieved by the setting of sand and gravel bio-filters through which water is allowed to circulate. The ammonia oxidizers are placed under five genera Nitrosomonas, Nitrosovibrio, Nitrosococcus, Nitrolobus and Nitrospira. Nitrification not only produces nitrate but also alters pH towards the acidic range, facilitating the availability of soluble materials. The vast majority of aquaculture ponds accumulate nitrate, as they do not contain a denitrifying filter. Denitrifying filters help to convert nitrate to nitrogen. It creates an anaerobic region where anaerobic bacteria can grow and reduce nitrate to nitrogen gas. Nitrate may follow several biochemical pathways following production by nitrification.

Phosphorous & Bioremediation

Phosphorus normally has limitations in a freshwater environment. Any deviation from the normal NO_3/PO_4 ratio is believed to be dependent on which influence the rate of nitrification or bacterial regeneration of phosphorous, is available in organisms mainly as phospholipids and nucleoproteins. Phosphorous is generated from organic compound as PO_4 by certain bacteria that produce enzymes such as phosphatases and phytases. The solubility of inorganic phosphatases is primarily a function of pH. Bacteria are capable of liberating PO_4 from these compounds through the production of organic and mineral acids.

Hydrogen Sulphide (H_2S) & Bioremediation

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 gets oxidized 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. Organic loading can stimulate H_2S production and reduction in the diversity of benthic fauna. Hydrogen Sulphide is soluble in water and has been suggested as the cause of gill damage

and other ailments in fish. Unionised H_2S is extremely toxic to fish that may occur in natural waters as well as in aquaculture farms. Bioassays of several species of fish suggest that any detectable concentration of H_2S should be considered detrimental to fish production.

The photosynthetic benthic bacteria that break H_2S at pond bottom have been widely used in aquaculture to maintain a favorable environment. These bacteria contain bacterio-chlorophyll that absorb light and perform photosynthesis under anaerobic conditions. 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.

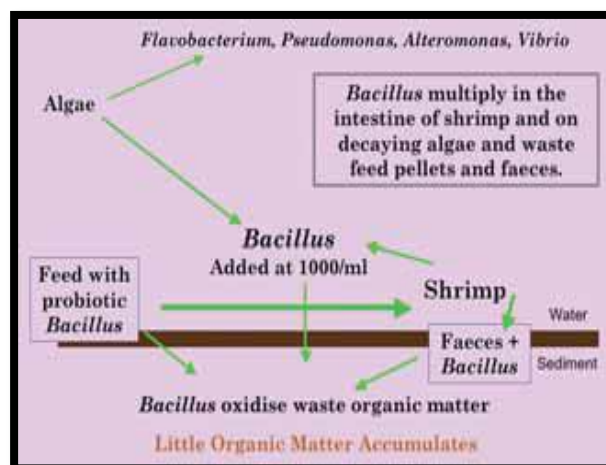
Chromatiaceae and Chlorobiaceae are the two families of photosynthetic sulphur bacteria that favour anaerobic conditions for growth while utilizing solar energy and sulphide. Chromatiaceae contain sulphur particles in cells but Chlorobiaceae precipitate them out. The family Rhodospirillaceae is not of any use for H_2S removal, 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. The common examples of photosynthetic bacteria of importance in aquaculture are Rhodospirillum, Rhodopseudomonas, Chromatium, Thiocystis, Thiospirillum, Thiocapsa, Lamprocystis, Thiodictyon, Thiopedia, Amoebobacter, Chlorobium, Prosthecochloris, Pelodictyon and 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 H₂S toxicity.

Bacillus – the true bioremediator for shrimp aquaculture

Gram positive Bacillus species are spore formers and produce a wide range of antagonistic compounds. They are suitable as commercial bacteria in aquaculture. Species such as *B. subtilis* and *B. licheniformis* occur naturally in fresh and sea water environments and are found naturally in the intestinal tracts of prawns. When selected Bacillus strains are added to ponds frequently and at high density, they degrade organic matter faster than in situations where only the natural populations are available. Denitrifying Bacillus, which breakdown organic waste and use nitrate when oxygen is depleted, are especially effective on the pond bottom. A product is now available on the market from INVE that contains specially selected bacteria to speed up degradation processes (Figure).



Bioaugmentation Agents in Super Intensive Marine Shrimp Farming

The usage of commercial bioaugmentation agents containing bacteria of the genus *Bacillus* sp. *Nitrosomonas* sp. *Nitribacter* sp. and *Lactobacillus* in intensive cultivation of marine shrimp *Penaeus monodon* and *L. vannamei* increased the

survival and reduced considerably the concentrations of *Vibrio sp.*, total organic carbon and total nitrogen in waters of cultivation. We can observe a decline of BOD and COD in cultured shrimp with application of products containing bacteria the genus *Bacillus sp.* *Nitrosomonas sp.* *Nitribacter sp.* and *Lactobacillus*.

Higher values in the concentration of nitrite in the first week may indicate the mineralization of organic matter immediately after the addition product bioremediation, while the concentration of dissolved mineral matter in the form of orthophosphate and nitrate did not differ between treatments claim reduction of ammonia and nitrite as a result of product application bioremediation in tanks of intensive cultivation of marine shrimp.

Mechanism of bioremediators

The resistance cause by probiotics to infections to shrimp, prawn and fish is due to

- **Bacterial antagonism,**
- **bacterial interference,**
- **barrier effect,**
- **colonization resistance and**
- **competitive exclusion**

Microbial antagonism is the method of using established cultures of microorganisms to prevent the intrusion of foreign strains. When introduced to an already-colonized environment, an invasive strain of bacteria tends not to thrive and may go completely extinct.

Bacterial interference refers to the antagonism between **bacterial** species during the process of surface colonisation and acquisition of nutrients.

Our bodies need certain types of **bacteria** to maintain proper functioning. However, many types of **bacteria** are pathogenic, and if they get within our bodies, acute, chronic, and fatal illnesses result. The human body has developed different **barriers** throughout evolution to prevent **bacteria** from entering

In ecology, the **competitive exclusion** principle, sometimes referred to as Gause's law, is a proposition named for Georgy Gause that two species competing for the same limiting resource cannot coexist at constant population values. I.e. the inevitable elimination from a habitat of one of two different species with identical needs for resources

Advances of bioremediation in Aquaculture in this century

Aquaculture systems

- ❑ **Biological methods**, including **Integrated Multi-trophic Aquaculture** are now gaining interest for increasing *In-situ* removal of nitrogen and other nutrients at sea cage sites.
- ❑ Several studies on **biological nitrogen removal** through nitrification, denitrification and anaerobic ammonium oxidation (anammox) have been reported and a number of bacterial groups active in this regard have been described.
- ❑ **Biofilters** (*Ex-situ* bioremediation) and also, the **macroalgae** *Gracilaria acaudata* which was used to remove nutrients from aquaculture effluents.

IMTA- Integrated Multi-trophic Aquaculture

A new project designed to encourage the development of integrated multi-trophic aquaculture (IMTA) in western Europe is beginning to take shape called INTEGRATE, it aims to deliver tools to effectively increase competitiveness in Atlantic IMTA, unlocking sectorial green growth and improving the quality and

public perception of aquaculture products. Eight organisations – from Spain, France, Ireland, Portugal and the United Kingdom – are involved.

The project seeks to improve the environmental performance of IMTA by making best use of the nutrient flow in aquaculture facilities by farming together species belonging to different trophic levels. The IMTA approach maximises resource efficiency while providing natural bioremediation at aquaculture farming sites.

Scotland's largest IMTA project, on Loch Fyne, features the co-culture of salmon, seaweeds, sea urchins, mussels, scallops, and oysters.

Multi-trophic aquaculture systems are a circular economy paradigm. They contribute to making aquaculture more sustainable and competitive. However, despite being encouraged by European Union (EU) policies such as the Blue Growth Strategy, the Atlantic Action Plan and RIS3, there still are socio-economic, administrative and legal bottlenecks hampering the development of IMTA to its full potential. To overcome these, INTEGRATE is supporting cooperation between academia, the corporate sector and relevant authorities, thus strengthening collaborative networks in the field of Atlantic IMTA. “INTEGRATE will deliver tools to effectively increase competitiveness in Atlantic IMTA, unlocking sectorial green growth and improving the quality and public perception of aquaculture products,” explains María del Mar Agraso, Technical Director at the Andalusian Aquaculture Technology Centre (CTAQUA), the project's lead partner.

INTEGRATE aims to capitalise the available knowledge in order to turn the innovative solutions provided by IMTA into a technical, legal and administrative reality. INTEGRATE is funded by the ERDF through the INTERREG Atlantic Area 2014-2020 Programme. CTAQUA (Spain) has joined forces with Agrocampus Ouest (France), the Scottish Association for Marine Science (United Kingdom), the Portuguese Institute for Sea and Atmosphere (Portugal), National University

of Ireland Galway (Ireland), Irish Seaweed Consultancy (Ireland), ALGAplus (Portugal) and the Centre for Study and Promotion of Algae (France).

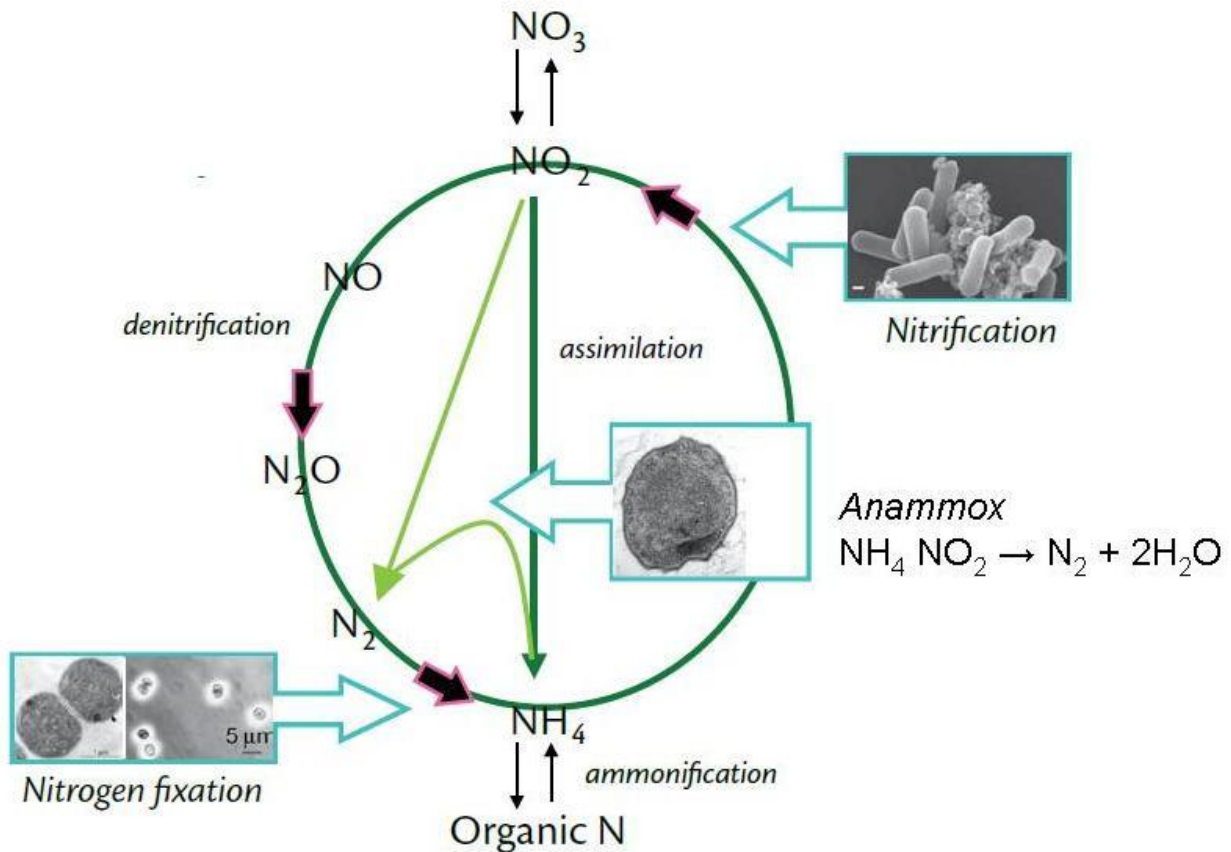
The three-year project expects to create an Atlantic IMTA platform for sectorial collaboration and three pilot actions to develop Atlantic IMTA technology and farming techniques. INTEGRATE shall capitalise its technical outcomes through best practice guidelines and the pilot actions will be open to academia and the aquaculture industry. The project will also launch Atlantic IMTA expert groups and dissemination events are envisaged to facilitate collaboration and knowledge transfer.

Anammox

Anaerobic ammonium oxidation (anammox) bacteria, which were discovered in waste-water sludge in the early 1990s, have the unique

metabolic ability to combine ammonium and nitrite or nitrate to form nitrogen gas. This discovery led to the realization that a substantial part of the enormous nitrogen losses that are observed in the marine environment. *Anammox*, a process of *Anaerobic Ammonium Oxidation*, is an innovative technological advancement in the removal of *ammonia nitrogen* in *wastewater*. This quite new process combines *ammonia* and nitrite directly into dinitrogen gas (SHIVARAMAN and SHIVARAMAN 2003) instead of passing through a two-stage process of *aerobic nitrification* and *anaerobic denitrification*. It was discovered about 15 years ago and has resulted in new opportunities for research and development of *sustainable nitrogen* removal systems. Compared to conventional *nitrification /denitrification* in activated sludge systems, *Anammox* eliminates the necessity of an *organic carbon* source for *nitrification*, reduces *energy* demand for aeration and has a smaller production of excess *sludge* and lower CO₂ emissions (BERTINO 2010). The *Anammox* reaction can be represented as $\text{NH}_4^- + \text{NO}_2^- = \text{N}_2 + 2\text{H}_2\text{O}$. This reaction is carried out by *Anammox bacteria* (*Candidatus Brocadia*

anammoxidans) belonging to the group of planctomycete (SHIVARAMAN and SHIVARAMAN 2003).



CIBAMOX

CIBAMOX, a water probiotic product has also been developed to mitigate the nitrogenous toxins in grow-out shrimp culture operations ensuring good water quality. In aquaculture ponds, nitrogenous wastes are contributed by uneaten feed and excretion due to host metabolism. The accumulating ammonia concentration may reach up to 4 ppm in intensive culture or even in less intensive shrimp farms that are poorly managed. Microbial nitrification and denitrification process naturally eliminates the nitrogenous wastes accumulating in shrimp culture ponds. In an effort to enhance this natural bioremediation process, the institute developed a probiotic product containing enriched consortia of nitrifying and denitrifying bacteria that are

well suited for use in brackishwater aquaculture systems. Administration of water probiotic product CIBAMOX has been shown to reduce the nitrogen and ammonia levels, thereby improving pond health. The product has successfully been tested in commercial shrimp grow out ponds in Gujarat, Tamil Nadu and Andhra Pradesh and has been proved to be effective in wide range of salinity. Both the WSSV kit and CIBAMOX products are offered for commercialization. These products will be available through Agri Business Incubator (ABI) at ICAR-CIBA offers services like, preparation of detailed project report, consultancy to establish production facility and technical training following the transfer of the technology. Several products are available in the market containing consortia of nitrifying and denitrifying bacteria for application in freshwater aquaculture operations. These microbes are highly sensitive to physio-chemical parameters of the culture environment and hence cannot effectively function in brackishwater environments with salinity ranging from 15 to 45 ppt. Through persistent research for a decade on environmentally relevant bacteria, the ICAR-CIBA has enriched consortia of nitrifying and denitrifying bacteria that are well suited for use in brackishwater aquaculture systems in India to manage toxic nitrogenous compounds in brackishwater aquaculture systems. CIBAMOX was developed through enrichment/characterization and development of consortia of naturally occurring chemolithotrophic ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) and denitrifying bacteria (DNB) from brackishwater ecosystems of India. The product had successfully completed field trials in 27 ponds in Surat and Navsari districts of Gujarat, 15 ponds in Nellore and Bhimavaram districts of Andhra Pradesh and 40 ponds in Kancheepuram and Thiruvallur districts of Tamil Nadu. Regular application of the product @ of 3 l/ha once in ten days from 45 days of culture onwards till the end of culture has shown to restrict the levels of ammonia below the maximum permissible limits.

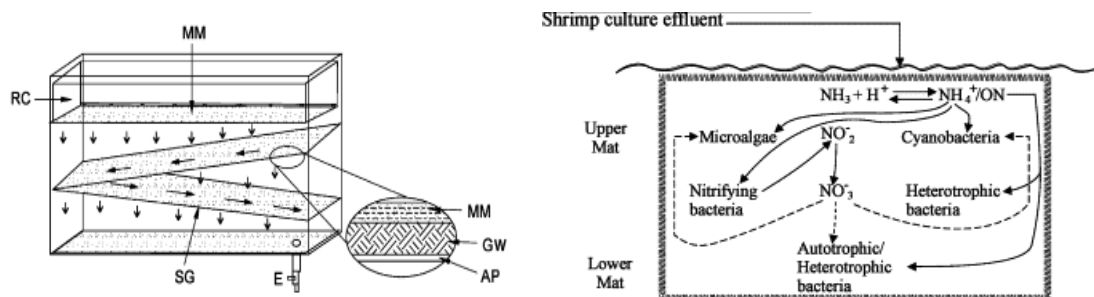
Insitu depuration

For **recirculation systems**, where reused water must be of adequate quality to maintain the culture organisms in a healthy and fast growing condition, it is

especially important to remove waste products as quickly as possible, an area with ample scope for **using immobilized microbial consortia technology** for *in situ* depuration.

Ex-situ bioremediation of shrimp culture effluent using constructed microbial mats

In coastal countries, marine shrimp industry poses a problem with respect to the waste effluent disposal into the ocean as well as the nearby area. Constructed microbial mats offer an interesting alternative for shrimp culture effluents. The treatment concept relies on the immobilization of natural marine microbial consortium on glass wool to mitigate the levels of dissolved nitrogen from a shrimp culture effluent. Our results indicate that average efficiencies of ammonia nitrogen removal from shrimp (*Litopenaeus vannamei*) effluent was 97% and 95% for nitrate nitrogen, over a 20 days period of treatment. This treatment via constructed microbial mats is a technically feasible method for simultaneously reducing effluent nutrient loading (especially nitrate and ammonia) and for reducing organic loading (especially BOD5) of shrimp culture effluents.



Schematic design of the bioreactor with constructed mats. RC, retention chamber; MM, microbial mat; SG, sprinklers; E, exit; GW, glass wool; AP, acrylic plate. Arrows indicate flow of water.

Oxidative bioremediation

Effect of Oxygen Depletion in ponds: Increases in oxygen consumption can be caused by an

- over-abundance of algae, "turnover" of a pond water
- increased organic waste entering the pond
- death and decay of organic matter
- by improperly dried pond having with sludge that remove oxygen directly from the water column.

The use of a combination of **Oxygen releaser and multi strain probiotics** including nitrifying bacterial formulation will address the water quality issues by controlling the ammonia and organic sludge within the pond system.

- ❖ **Oxidative bioremediation** is more than just simply adding oxygen and a certain species of bacteria.
- ❖ Oxidative (Aerobic) bioremediation involves **microbial reactions that require oxygen to go forward.**
- ❖ The bacteria use a **carbon substrate as the electron donor** and **oxygen as the electron acceptor.**
- ❖ Whereas anaerobic bioremediation involves microbial reactions occurring in the absence of oxygen and encompasses many processes, including fermentation, methanogenesis, reductive dechlorination and sulfate- and nitrate reducing conditions.
- ❖ Depending on the contaminant, a subset of these activities may be cultivated. In anaerobic metabolism, nitrate, sulfate, carbon dioxide, oxidized materials or organic compounds may replace oxygen as the electron acceptor and thereby the end products are toxic to shrimp and fish (EPA 2006).

Geosmin

Geosmin and MIB are terpene-type compounds produced in FW ponds by algae. Bacterial strains were isolated for their ability to grow on compounds having structures similar to these terpenes. While many of the newly isolated bacteria resemble previously strains in their ability to transform these compounds, some novel and important strains were identified. Of the bacteria tested, *Rhodococcus wratslaviensis* DLC-cam, *Pseudomonas putida* G1, and *Rhodococcus* sp. T1 were found to be the most promising by virtue of having the highest amount of transformation of geosmin or MIB. *Rhodococcus wratslaviensis* DLC-cam was shown to have constitutive activity against both compounds.

To improve the activity of these organisms, a key enzyme in the degradation of terpene-like compounds was recently crystallized. We have collaborated with this group and used molecular modeling tools to show that MIB will also bind to the active site of this enzyme. The work suggests that degradation of MIB differs from the degradation of the enzyme's normal substrate, but also suggests that modifications of the enzyme's structure might improve its ability to degrade MIB.

Initial tests were conducted to determine if these organisms had properties that would allow them to be used for bioremediation of off-flavors in aquaculture environments. In a preliminary round of testing, *Rhodococcus wratslaviensis* DLC-cam was found to not be pathogenic against fish, even at very high concentrations. All three bacterial strains were able to be grown to very high densities on inexpensive media, a condition necessary for mass production of the organism. All three strains were also able to be grown in minimal media with fish feed as the sole carbon source, a condition typical of aquaculture environments. Finally, all three strains can be frozen without significant loss in viability, which is desirable for storage and transport to a treatment area

Zeolite

Oxygen and ammonia are the two most important parameters in aquaculture. The oxygen is relatively easily controlled, but the ammonia is much more difficult. Ammonia, the un-ionized form of ammonia (ammonia gas) is produced from the gills and urine from the fish as well as from the bacterial decomposition of the unused food and fecal material. Fish utilize the nitrogen component of digested proteins, the amino group (NH₂) to build new proteins. However, when they utilize the proteins for energy, they cannot metabolize the nitrogen, and the amino group is split off as ammonia gas. Ammonia gas solubilizes readily in water to form ammonium ion. Ammonia is toxic, and it reduces the ability of the hemoglobin in the blood to hold oxygen. Additionally, ammonia damages the gill structure further impairing the fish in getting oxygen.

Zeolite is a naturally occurring deposit (i.e. rock!) that has a fairly unique structure which is highly porous whilst also allowing for rapid uptake and losing of charged particles. Therein lies the relevance of zeolite to aquaculture; when placed in water zeolite filters ammonia out of the water and holds it inside its' porous structure. Pores- act as filters

The natural filtration properties of zeolite can be used within aquaculture industries such as fish farming, aquariums, and transport. Zeolite can act as a medium to help to absorb nitrates, nitrites, and phosphates to maintain a high quality of water.

Chitosan-based biosorbents

Adsorption/biosorption process is one of the effective methods to remove heavy metals from water. Numerous studies have been carried out to create various suitable or new sorbents/adsorbents/ biosorbents for heavy metal removal from aqueous solution. Chitin is abundant naturally occurring basic polysaccharide, mainly from animals, existing in crustacean shells such as shrimps, prawns, crabs and lobsters, in insects such as beetles, in cuttlefish of bone, in fungi such as

mushrooms. Chitosan in its original form is not suitable for practical use due to its **relatively low mechanical strength** and **weak base soluble at acidic media**. Chitosan with amine groups in acidic solution is not stable due to formation of protonated chitosan. Moreover, chitosan in its original form usually does not own specific selectivity for a certain kind of heavy metals or very high sorption capacity from complex polluted water or wastewater, although chitosan with high content of amine and hydroxyl groups shows good sorption capacity for transition metals and no or little sorption for alkaline or alkaline earth elements.

Two main goals for the chitosan modifications for metal sorption are:

- (1) to increase the stability of the material in acid media (good mechanical properties, etc.), and
- (2) to enhance the metal sorption performance, including sorption capacity, metal selectivity, good diffusion and hydrodynamic behavior.

The easy separation from real effluent after treatment and the cost effectiveness of the sorbents preparation are also important. Thus, chitosan modification by introducing the desired properties in physical, chemical and mechanic properties is key issues in order to enhance its metal sorption capacity, to improve its affinity for the metal, to change the selectivity series for metal sorption, and to alter the optimum pH range.

- Chitosan - **relatively low mechanical strength** and **weak base soluble at acidic media**.
- Chitosan - **does not own specific selectivity** for a certain kind of heavy metals or very **high sorption capacity**
- Chitosan – with high content of amine and hydroxyl groups **shows good sorption** capacity **for transition metals** and no or **little sorption for alkaline or alkaline** earth elements.

Nitrifying Bioreactor

Oriental Aquamarine Biotech (India) P Ltd offers a patented NITRIFYING BIOREACTOR TECHNOLOGY FOR THE ESTABLISHMENT OF RECIRCULATING AQUACULTURE SYSTEMS (RAS). Scientists at the National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Kochi (www.ncaah.org) developed the technology with funding from the Department of Biotechnology, Government of India, New Delhi. This nitrifying bioreactor is commercialized by Oriental Aquamarine Biotech India Private Limited, Coimbatore, Tamil Nadu. The Nitrifying Bioreactor technology facilitates management of water quality in a hatchery with maximum consistency, and least incidence of diseases and offers reef quality oligotrophic water. Recirculating larval production systems, which could be developed by incorporating this technology, totally eliminates the application of chemical disinfectants, antibiotic and other chemotherapeutic agents, facilitating organic seed production in its truest form. The Nitrifying Bioreactor Technology for the establishment of Recirculating Aquaculture Systems (RAS) was developed after 16 years of research by implementing the following projects funded by arms of Government of India. The project was initiated in April 1995. This bioreactor is

- Patented technology
- First of its kind in the world
- Maintains reef-like conditions in aquaculture tanks
- Can be used for any level of salinity- Fresh Water/ Salt Water/ Brackish Water
- Can be used to farm a variety of marine animals- Shrimp/ Ornamental Fish/ Crabs/Lobsters

Advantages of Bioremediation

- It works on a variety of organic and inorganic compounds
- Can be done either on-site or off-site, easy to implement and maintain

- Low-cost compared to other treatment methods
- Environmentally-friendly and aesthetically pleasing
- Reduces the amount of wastes to be land filled

Disadvantages of Bioremediation

- It may take several years to remediate and depends on climatic conditions
- Restricted to sites with contamination near the roots
- Harvested plants may be classified as hazardous waste
- Consumption of contaminated plants may be harmful
- There may be harmful effects on the food chain

Beneficial effects of Probiotics

- i. Neutralization of toxin and suppression of viable count
- ii. Production of antibacterial compounds and competition for adhesion sites
- iii. Alternation of microbial metabolism, stimulation of immunity in the host
- iv. Accelerate the sediment decomposition by producing organic acids
- v. Production of hydrogen peroxide and enzymes

Application of Probiotics in Aquaculture

1. To regulate the microflora of aquaculture water and pathogenic microorganisms.
2. To enhance decomposition of the undesirable organic substances in aquaculture water and improve ecological environment of aquaculture by minimizing the toxic gases like ammonia, nitrite, hydrogen sulfide, methane etc.
3. To increase the population of food organisms improves the nutrition level of aquaculture animals and improve immunity of cultured animals to pathogenic microorganisms.
4. The frequent outbreaks of diseases can be prevented.

Conclusion

Constant efforts are being made to address the negative impacts food production systems including aquaculture have on the environment. The role of beneficial bacteria to control pathogens will become important in aquaculture, especially in the light of the increasing number of antibiotic resistant strains of bacteria. The 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. 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.

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