

Bioremediation: an environmental friendly approach for sustainable aquaculture

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Abstract

Aquaculture productivity needs to be improved to cater the ever-increasing demand, no doubt, but simultaneously a proactive role for environment protection is required. Bioremediation, an integral part of environmental biotechnology program, can be defined as a biological mechanism to destroy, transform or immobilize environmental contaminants to protect potential sensitive receptors. It is achieved by using a macro or microorganism that specifically targets particular pollutants. These microbes range from those which having a natural affinity towards hydrocarbons to others, which through competitive exclusion limit the presence of pathogenic microorganisms in aquacultural environments. Bacterial species belonging to genera *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Cellulomonas*, *Rhodoseudomonas*, *Nitrosomonas* and *Nitrobacter* are known to help in mineralization of organic wastes. This technology is conceptually simple and relies on actively promoting nutrient processing by bacteria into forms suitable for uptake by aquatic plants or animals. Now, when aqua culturist has to follow the effluent discharge guidelines as stipulated by the Environmental Protection Agencies (EPA), bioremediation or bioaugmentation can be a great aid. The substrate based aquaculture, the concept of biofilm, biocapsules, probiotics and microbial mats are being increasingly recognized in commercial aquaculture. In addition to that with the vast genomic data and genetic engineering it is now possible to redesign the microbes to produce molecular biosensors and bioreporters (which can recognize pollutants) and genetically modified bacteria to utilize organic waste. This review attempts to describe the underlining principles, present status and future prospects of bioremediation for sustainable aquaculture practice.

Keywords: Bioremediation, aquaculture, environment, sustainable, biocapsules, biofilm, genetically modified bacteria

Introduction

Bioremediation, an integral part of all Environmental Biotechnology Program, explore the use of biological mechanisms to destroy, transform or immobilize environmental contaminants to protect potential sensitive receptors. The use of living organisms (primarily microorganisms and plants) is emerging as one of the most useful alternative technologies for removing contaminants from the environment, restoring contaminated sites, and preventing further pollution. The natural biological processes can be explored to remediate nutrient-rich water by converting nutrients in to forms that can be more easily removed.

Aquaculture interacts with the environment. While this sector is world's fastest growing food sector for nearly 20 years since 1984 with an average compound growth rate of 11% (FAO report) and very important for food security, sustainability is at risk if related environmental problems including effluent discharge is not taken care of. As restricted or no use of antibiotics are encouraged and vaccination and chemotherapy has its own limitation in

aquaculture production microbial intervention including bioremediation for health management and /or effluent treatment is fast emerging as an alternative therapy.

Industrial units including that of aquaculture sector are becoming increasingly aware of the political, social, environmental and regulatory pressures to prevent escape of effluents into the environment. The occurrence of major incidents (such as the minamata and itai itai disease crisis, the progressive deterioration of the aquatic habitats and conifer forests in the Northeastern US, Canada, the Exxon Valdez oil spill, the Union-Carbide (Dow) Bhopal disaster, large-scale contamination of the Rhine River, and parts of Europe, or the release of radioactive material in the Chernobyl accident, etc.) and the subsequent environmental problems has highlighted the potential for imminent and long-term disasters.

Remediation continues to be improved, with use of microorganisms (both genetically modified organisms (GMOs) and naturally occurring ones, including extremophiles) to clean contaminated areas (David et al. ,1995; Tebo, 1995). Offshore or other contamination of marine ecosystems resulting from toxic organics may be amenable to *in situ* remediation by naturally occurring consortia of microorganisms in sediments (Deming, 1998). Plants, too, offer useful applications in bioremediation (Blaylock et al. , 1997; Huang et al., 1997).

As the aquaculture industry develops, efficient, cost-effective and environmentally friendly preventive and bioremediation methods of improving effluent water quality prior to discharge into receiving waters of sensitive areas will be necessary (Jones et al., 2001). In this review we have focused on the present status and future prospects with updates in environmental monitoring through bioremediation especially in aquatic environment.

Microbial intervention in aquaculture system:

In aquaculture the microbial preparation can either be antagonistic to pathogen (biocontrol and probiotics) depending on it's presence in GI tract. or improves the water quality (bioremediation) (fig 1). Probiotics are living microbial cells administered as dietary supplements with the aim of improving health. Most probiotics proposed as biological control agents in aquaculture belong to lactic acid bacteria (*Lactobacillus*, *Carnobacterium*) to genus *Vibrio*, *Pseudomonas* or *Bacillus*, which can also influence the immune response. Again the heterotrophic probiotic bacteria when added to water can eliminate NH_3 , H_2S and organic acids by oxidation, nitrification, denitrification, sulfur and nitrogen fixation. The use of microbial preparations as biological control agent is a kind of risk insurance that could reap benefit in critical moments.

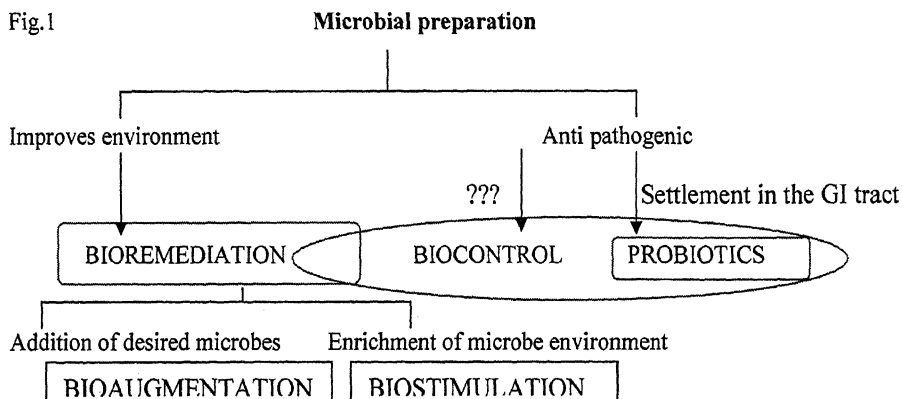


Fig. 1 Microbial treatments practiced in aquatic environment

Aquaculture effluents & Environmental monitoring programme:

Effluent from aquaculture ponds may add to receiving waters high levels of biochemical oxygen demand (BOD), inorganic particulate matter, living and dead particulate organic matter, dissolved organic matter, ammonia, nitrite, nitrate, phosphate, and other potential contaminants. Therefore, regulatory agencies have developed standards and criteria for the aquaculture industry concerning effluents (Yoo and Boyd, 1994). Marine organisms are capable of extracting compounds from nutrient rich residual flows (phosphate and nitrate), or from contaminated residual flows. Following bioremediation through macroalgal biofiltration, decrease in ammonia, total nitrogen, and total phosphorus concentrations were observed (Kinne et al., 2001).

Briggs and Funge-Smith (1994) estimated the amount of added nitrogen and phosphorus to the intensive shrimp ponds through feed and fertilizers as being 95% and 71% of total amount of nitrogen and phosphorus in the natural environment, respectively, while harvested shrimp accounted for only 24% nitrogen and 13% of phosphorus loaded into the pond. Thus, the portion of nutrients in the feed consumed by shrimp and converted to shrimp flesh is relatively small and a greater portion is wasted in the water column.

Aerobic heterotrophic bacteria decompose organic matter in the water column and sediment surface in aquaculture pond systems (Moriarty, 1997). Therefore, the need for biotechnological intervention arises (Cole, 1993) particularly in situations where the indigenous bacterial activities are limited by prevailing ecological variables.

Accelerated nitrification and rapid decomposition of organic solids using bacterial consortia in aquaculture ponds are reported. The aquaculture mats convert ammonia and organic materials from fish wastes into mat cellular protein that can be subsequently used as tilapia feed (Phillips et al. 1994). These prolific fish may be a food source for carnivorous fishes (Bender et al., 2004), resulting in a closed system whereby fish waste is transformed into consumable fish products. Again, the 'polyculture' approach of planting seaweeds contributes to biodiversity and better management of marine resources while also producing a commercially valuable crop.

Microbes involved in bioremediation:

The ubiquitous presence of microbes in the air, water, soil significantly affects day to day life as well as industrial activities. Bacterial species belonging to genera *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Cellulomonas*, *Rhodoseudomonas*, *Nitrosomonas* and *Nitrobacter* are known to help in mineralization of organic wastes (Thomas et al., 1992). The concept of using bacterial products needs further research to determine the factors contributing to their effectiveness in augmenting production. Lots of new microbes are being identified as having bioremediation potential in different ecosystem and the list is ever increasing.

For example, stimulation of self purification in the field of eutrophic bottom environment could be achieved by two promising bacterial strains isolated from the study site, named *Enterobacter* sp. 9410-O and *Pseudomonas* sp. W-4 when introduced by absorbance onto porous substrates in the sediment-bottom water complex system (Karim et al., 2003). The use of a psychrophilic or psychrotolerant bacterium that can be active at low temperatures may be a possible way to enhance heterotrophic activities during the winter season (Fukami et al., 1999). *Pseudomonas* sp. W-4, which is psychrotolerant, was isolated from bottom water of the study site in the coldest season (December) and had high growth rates and proteolytic activities even at low temperatures such as 10°C.

In nature, microbial mats are laminated heterotrophic and autotrophic vertically stratified communities typically dominated by cyanobacteria, eukaryotic microalgae like diatoms, anoxygenic phototrophic bacteria and sulfate reducing bacteria (D'amellio et al., 1989). The microbial product applied in the ponds containing *Bacillus* spp., nitrite oxidizing bacteria and low numbers of ammonia oxidizing bacteria, sulphur oxidizing bacteria, sulphur

reducing bacteria and a yeast, *Saccharomyces* sp. with a total plate count of 3×10^8 cfu mL⁻¹ (colony-forming units) is reported to give better result.

Evaluation of the microbial diversity in nature could be studied easily done by 16S rRNA based PCR technique, Fluorescent in-situ hybridization (FISH) or other molecular typing methods like Pulsed field gel electrophoresis (PFGE), ribotyping, random amplification of polymorphic DNA (RAPD).

Microbial mats, biofilm and substrate based aquaculture:

Microbial mats are laminated, cohesive microbial communities, composed of a consortium of bacteria dominated by photoautotrophic cyanobacteria (also referred to blue-green algae) (Nisbet and Fowler, 1999). Mats generally include anoxygenic photoautotrophs (purple bacteria) and sulfur-reducing bacteria. They are embedded in a negatively charged polymeric matrix of gel. Mats are ubiquitous in nature, commonly found over the sediment surfaces or as floating masses in marine waters. The treatment via constructed microbial mats (Paniagua-Michel and Garcia, 2003) 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. Lee et al., 1996 showed that mats reduced dissolved organic carbon from 125 to 10 mg l⁻¹ in an aquaculture system. Microbial mats have been used as a feed for tilapia, *Oreochromis niloticus* (formerly *Tilapia nilotica*) (Phillips et al., 1994) and as an effluent filtering system, transforming nitrogenous fish wastes into benign products (Bender et al, 2004; Lee et al., 1996). Mats have been shown to sequester heavy metals (Phillips and Bender, 1998) and radionuclides (Bender et al., 2000), as well as degrade recalcitrant toxic organic contaminants (Fig. 2). Cyanobacteria have been tested as an amendment to enhance soil fertilization via nitrogen fixation (Fernandez Valiente et al., 2000). The use of microbial mats in energy production is speculative. Mats produce hydrogen (Hill, 2001) because of various cyanobacteria. Based on this concept the substrate based aquaculture is a simple and efficient technology to increase productivity from aquaculture system by putting some easily degradable biological substance like water hyacinth, paddy straw or bagasse which can provide good surface area for periphyton (Nandeesh, 2003). The economic advantage by adopting such system could be up to 50 % more profit. Additionally, biofilms are being used to explore better productivity.

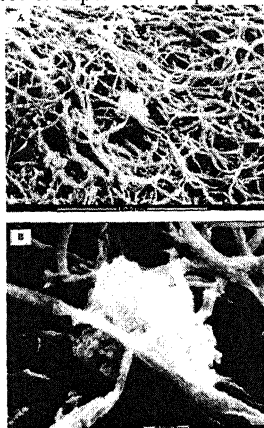


Fig. 2. Filamentous cyanobacteria with deposit of elemental selenium (Se⁰) (center)—57 MM=10 μ m

(B) Enlargement of Se deposit—25 MM=10 μ m
(Source-Bender and Phillips. 2004)

Mechanism of bioremediation:

There are range of microbes which have a natural affinity towards hydrocarbons to others which, through a primary mechanism of control known as competitive exclusion, limit

the presence of pathogenic microorganisms in aquacultural environments. Still others, through biological nutrient removal, reduce the overload of organic matter in water, usually excess nitrogen, ammonia and phosphorous. Microbial intervention in aquaculture system works because of the competitive exclusion of the indigenous microflora and the antagonistic characteristics against potential pathogens

The mechanisms that likely relate directly to the removal of specific metals, oxyanions and organic contaminants are as follows (Fig. 2)

- i) Biostimulation - enriching the environment by adding substances that boost the capability of naturally occurring microbes to break down toxic substances.
- ii) Bioaugmentation - direct addition of the microorganisms that can break down contaminants and accelerate their destruction.

In the microbial mat or biofilms the mechanisms can possibly be accounted for by (1) the presence of a variety of microorganisms that contrast in their anaerobic/aerobic functions (2) the distinct zonation within the macrostructure of the mat (3) the mediation of the chemistry in the surrounding water column (4) releases of negatively charged bioflocculants and (5) filamentous cyanobacteria with negative surface charge.

Ecological recovery through Bioremediation:

Ecological recovery requires, in addition to the remediation of oil or other contaminants, the establishment or recovery of appropriate microbial, plant and animal communities to create a functioning ecosystem (Haimi, 2000). In the case of the *Exxon Valdez* spill, the largest and most thoroughly studied application of bioremediation in the field, the application of fertilizer increased rates of biodegradation three to five times, as indicated by increased carbon dioxide evolution, oxygen consumption and microbial biomass (Swannell et al., 1996).

Biomonitors and bioreporters can be an integral part of the Environment Monitoring Programme also for assessing ecological recovery.

Biomonitors: These are a set of organisms whose behavior, growth, survival and histopathological changes could be monitored to assess environmental changes. Aquatic and pulmonate snails were evaluated for their suitability as biomonitors for the habitat recovery following experimental oil spill in a freshwater marshland to assess the impacts of crude oil, rates of natural recovery, and the efficacy of bioremediation treatments to enhance the bacterial degradation of residual oil in the sediments. Snail survival, growth, and histopathological changes were monitored. The use of sponges for marine bioremediation in a farming scenario has been investigated (Milanese et al., 2003) focusing on *Chondrilla nucula*. One square meter patch of this sponge can filter up to 14 l/h of seawater retaining up to 7×10^{10} bacterial cells/h.

Bioreporters: Genetically modified bioreporter bacteria are being designed to measure concentrations of polluting or toxic chemicals. The underlying principle is that the microbial cells (bacteria and yeast) and eukaryotic cell lines can detect specific analytes and report an analytically useful signal and its performance could be improved by improving transcriptional regulation, molecular signaling, microbial physiology and detection methodology. As microorganisms are living beings, their 'measurements' reflect a bioavailable concentration rather than the total concentration that many chemical methods assess. Among other reporter proteins, now green fluorescent proteins (GFP) have great advantage. When the contaminant binds to the receptor and turns on the reporter gene (luciferase, GFP etc) in bacteria the expression intensity is measured by fluorescence microscope or by PCR amplification method.

Bioremediation of organic-rich sediment:

The selection of indigenous bacteria able to degrade a wide range of natural organic compounds (e.g., lipids, proteins and carbohydrates) constitutes a new approach to study the potential of bioaugmentation in eutrophicated environment (Jones, 1998) and has thus far not

been assessed. In some case the microbial processes employed in remediation strategies aim at the removal of excessive organic input through the mobilisation of elements (e.g., C, N, P) in the accumulation areas (Fabiano et al., 2003).

Vezzulli et al. (2004) described a conceptual model for application of bioremediation to face the problem of sediment eutrophication after a field trial experiment to assess the potential of bioremediation for mobilisation of carbon in organic-rich sediments. They tested both bioaugmentation (bio-fixed microorganisms) and biostimulation (oxygen release compounds—ORC) protocols and the response of the bacterial community to assess the baseline for bioremediation potential (Fig. 3). In contrast to classical approach that basically aims at the degradation of chemicals, bioremediation of organic-rich sediments aims at the mobilisation and removal of biological elements (e.g., C, N, P). Mobilisation can be achieved both by bioaugmentation and biostimulation of the microbial community while removal can be either 'physical' (e.g., open systems) or 'biological' (e.g., enclosed and semi-enclosed systems). The latter implies net carbon removal via respiration process (CO_2) and biomass removal as proposed recently in bioremediation studies (Hiroaki, 2003)

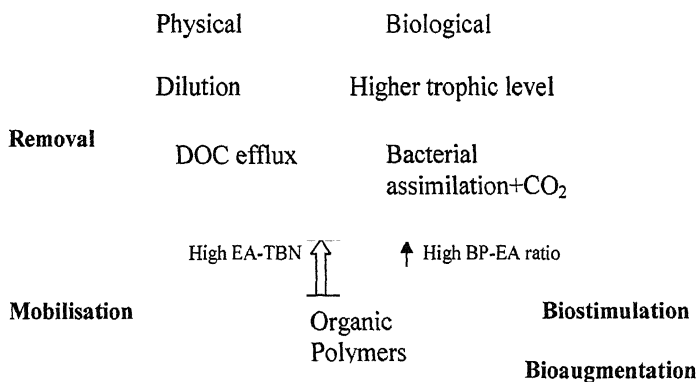


Fig. 3 Conceptual model for bioremediation of organic-rich sediment. descriptors such as EA:TBN (bacterial functional stress) and BP:EA (bacterial efficiency) can be proposed as candidate indices to describe the key processes involved in bioremediation of organic-rich sediments. EA: extra cellularenzymatic activities; BP: Benthic bacterial production; TBN number of bacteria. (Source Vezzulli et al., 2004)

Genetically engineered microorganisms

Bacteria can be altered to produce certain enzymes that metabolize industrial waste components that are toxic to other life, and also new pathways can be designed for the biodegradation of various wastes. Research is underway to engineer or enhance bioremedial enzyme pathways, using genes from microbes able to survive and compete effectively in environments contaminated by metals and/or radionuclides. Since the effluent from one industry contains mostly one toxic chemical, it may apply one or a few genetically modified bacterial strains to get rid of its major toxic waste. However, it may be important to contain the "waste-eating" bacteria within the manufacturing plant, and not release these with the wastewater. In such cases, filter installations will have to be built to separate the bacteria from the effluent. In some cases, where genetically modified organisms were utilized,

bioaugmentation improved pollutant-biodegradation rates in the environment due to the establishment of transconjugants capable of degrading the pollutants rather than the direct contribution of the inoculated organisms. The effects of key environmental parameters on regulation and expression of these genes can be explored.

Recombinant PCB (polychlorinated biphenyl)-degrading microorganisms with improved stability and survivability in mixed populations of soil organisms is already been developed. The same company also has developed a naturally occurring bacterium that degrades trichloroethylene (TCE) in the presence of toluene, a toxic organic solvent killing many other microorganisms.

Potential to engineer microbes at a genetic level to improve their ability to immobilize metals and radionuclides is also enormous. Dewitt (2000) mentioned about the *Deinococcus radiodurans* –the most radiation resistant organism yet known which was engineered to express an *Escherichia coli* enzyme that converts ionic mercury to a less toxic form and a pseudomonas enzyme that breaks down toluene. Again the isolation of novel bacterium that destroys vinyl chloride, the breakdown product of chlorinated hydrocarbon as a part of its energy metabolism raises the prospect of effective bioremediation for contaminated water reservoir (Jianzhong et al., 2003). Seaweed species are genetically tailored to have high growth rate and other characters, which can be planted in the coastline for pollution control. Recombinant microorganisms with expanded bioremediation capacity are increasingly accepted, though application in aquaculture sector is yet to take shape.

Commercialisation products and process:

Currently, there are several microbial products in the market for aquaculture use. In addition to live bacterial cells, enzyme preparations, plant and yeast extracts are also used in aquaculture ponds. To mention few of them like Biostart, Licalife, Pond pro VC, Nitro clear and Eutro clear and many probiotic formulations, which are marketed the elucidation that bacteria, which improve water quality, may be good for animal health. In aquaculture, heavily stocked ponds can become hypereutrophic. Using a combination of nitrifying bacteria with probiotic formula will address ammonia and reduce the organic sludge within the system. Similarly many designs or assemblages are being engineered for effectively inducing the bioremediation process. For instance-an underwater device, able to favour the sea auto-cleaning capacities, was described by Cattaneo-Vietti et al., (2003). This system, called MUDS (marine underwater depuration system), consists of a percolating filter and is placed at sea over an urban sewage outflow of a submarine pipeline. Rich microbenthic communities develop on the MUDS, both interstitially, inside the filter, and on the structure.

Conclusion:

Microorganisms are nature's original recycler. Utilizing microorganisms to detoxify effluents, soils, etc. is getting such wide acceptance that "bioremediation" has now become a common buzzword in waste management. When development and intensification of aquaculture sector is inevitable for food security and social well-being, it should be within the framework of sustainability and environmental friendly approach. With the evolving genetically modified microbes this environmentally responsible alternative to chemical treatments becomes more fascinating. The physical or chemical remediation process might be replaced or supplemented with this biological process, which are cost effective and eco-friendly. Certainly, bioremediation holds great promise for dealing with aquaculture system and other effluent processing, however much of it is yet to be realized.

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