

Bioremediation of Waste Waters and Application in Aquaculture - A Mini Review

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Abstract

Aquaculture production has increased steadily in recent years and is the fastest growing food production sector and has become a valuable component of national development and poverty reduction plans in many areas of the world. Increased fish 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, feed, fertilizer and chemicals. As a result, aquaculture is now considered as a potential polluter of the aquatic environment. In this context, Bioremediation is most cost effective and environmental friendly treatment option for many environmental pollutants. Bioremediation consists of using living organisms (bacteria, fungi, actinomycetes, cyanobacteria and to a lesser extent, plants) to reduce or eliminate toxic pollutants. Wastes produced in aquaculture farms differ in quality and quantity of components depending on the species farmed and the farming practices adopted. 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. 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.

1. Introduction

Bioremediation is the process of using microorganisms to transform biodegradable compounds in contaminated soil or sediments, to harmless end products. Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions. Bioremediation technology was invented by George M. Robinson in 1960's. Bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. For bioremediation, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity. Its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. Bioremediation is most

cost effective and environmental friendly treatment option for many environmental pollutants.

2. Bioremediation Process

- Microbes releases enzyme to break down the contaminant into digestible pieces.
- The contaminant of organic substances is ingested and digests as food along with other energy source by the cell.

3. Factors Affecting Engineered Bioremediation

Bioremediation process is a complex system of many factors, which are as follows;

- Existence of a microbial population capable of degrading the pollutants.
- Availability of contaminants to the microbial population.
- Environment factors like type of soil, temperature, ability of microorganisms to degrade pollutants, pH, presence of oxygen and nutrients.
- The outcome of each degradation process depends on biomass concentration, enzyme activities and population

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diversity of microbes.

4. Steps in Bioremediation

- Isolation and characterization
- Culturing
- Studying the catabolic activity of these microorganisms in contaminated material through bench scale experiments
- Monitoring and measuring the progress of bioremediation.

5. Types of Bioremediation

Different techniques are used depending on the degree of saturation and aeration of an area.

a. *In situ* techniques

b. *Ex situ* techniques

(a) *In situ* techniques

The *in situ* techniques involve the use of organisms or enzymes to remove pollutants. *In situ* bioremediation is highly dependent on site conditions and soil properties. *In situ* techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. These techniques are the most desirable options due to lower cost and lesser disturbances, since they provide the treatment in place avoiding excavation and transport of contaminants.

The most important land treatments are as follows:

- **Bioventing:** Bioventing is the most common *in situ* treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface.
- **Biosparging:** Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system.
- **Bioaugmentation:** Bioremediation frequently involves the addition of microorganisms indigenous or exogenous to the contaminated sites. Two factors limit the use of added microbial cultures in a land treatment unit (i) Non indigenous cultures rarely compete with an indigenous population to develop and sustain useful population levels and (ii) Most soils with long-term exposure to biodegradable waste have indigenous microorganisms that are effective degraders if the land treatment unit is well managed.

- **Bioslurping:** Its works in two ways such as vapour extraction that remove high volatile vapours from the vadose zone and bioventing stimulate biodegradation of less volatile hydrocarbons in unsaturated and capillary zones. Vacuum extraction slurp tube connected to vacuum pump that remove free product along with ground water.

- **Phytoremediation:** Phytoremediation is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and/or destroy contaminant in the soil and ground water. In natural ecosystems, plants act as filters and metabolize substances generated by nature. Some plants have capacity to take up and accumulate selected metals in their shoots. Some plants have developed symbiotic association with microbes that can degrade certain pollutants to compounds which are non- hazardous to the environments.

(b) *Ex situ* techniques

Ex situ techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation of soil or pumping water. These techniques involve the excavation or removal of contaminated soil from ground. Cost associated with excavation, screening, mixing, homogenization and disposal.

- **Land farming:** Land farming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous bio-degradative microorganisms and facilitate their aerobic degradation of contaminants. It stimulates indigenous microbes and requires less monitoring and maintenance cost.

- **Composting:** Composting has been used to degrade solid waste materials such as leaf litter, sewage sludge and food wastes. Composting is a technique that involves combining contaminated soil with nonhazardous organic amendants such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature of composting.

- **Biopiles:** Biopiles are a hybrid of land farming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of land farming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms.

6. Bioremediation of Aquaculture Waste Waters

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 (Pillay, 1992). Biotechnological solutions by using beneficial microorganisms, micro algae and bioprocessing methods for the treatment of wastewater ensure the sustainable development of modern aquaculture activities.

7. Waste Production in Aquaculture

The wastes in hatcheries or aquaculture farms can be categorized as (1) residual food and faecal matter (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. The use of bioremediators results 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. A successful bioremediation involves:

- Optimizing nitrification rates to keep low ammonia concentration
- Optimizing denitrification rates to eliminate excess nitrogen from ponds as nitrogen gas
- Maximizing sulphide oxidation to reduce accumulation of hydrogen sulphide
- Maximizing carbon mineralization to carbon dioxide to minimize sludge
- Accumulation
- Maximizing primary productivity that stimulates shrimp production and secondary crops
- Maintaining a diverse and stable pond community where undesirable species do not become dominant.

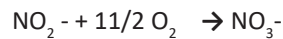
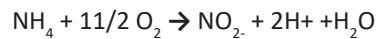
8. Bioremediation of Organic Detritus

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. Members of the genus *Bacillus*, like *Bacillus subtilis*, *B. licheniformis*, *B. cereus*, *B. coagulans* and of the genus *Phenibacillus*, like *Phenibacillus polymyxa* are good examples of bacteria suitable for bioremediation of organic detritus. *Lactobacillus* is also used along with *Bacillus* to break down the organic detritus.

9. Bioremediation of Nitrogenous Compounds

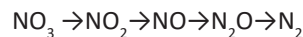
Nitrogen applications in excess of pond assimilatory capacity can lead to deterioration of water quality through the accumulation of nitrogenous compounds like ammonia and nitrite with toxicity to fish and shrimp. The principal sources of ammonia are fish excretion and sediment flux derived from the 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 process as follows:



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 oxidizers 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. 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).

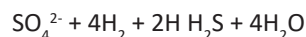
The vast majority of aquaculture ponds accumulate nitrate, as they do not contain a denitrifying filter. Denitrifying filters helps to convert nitrate to nitrogen. It creates an anaerobic region where anaerobic bacteria can grow and reduce nitrate to nitrogen gas (Rao, 2002). Nitrate may follow several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



10. 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 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. 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 H₂S is produced by a series of microbially mediated reductions (Boyd, 1995).

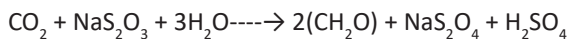
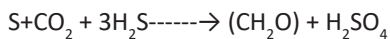
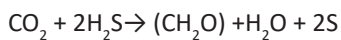


Organic loading can stimulate H₂S production and reduction in the diversity of benthic fauna (Mattson and Linden, 1983). H₂S is soluble in water and has been suggested as the cause

of gill damage and other ailments in fish (Beveridge, 1987). Un-ionised 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 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 purple and green sulphur bacteria obtain reducing electrons from H_2S at a lower energy cost than H_2O splitting photo-autotrophs and thus require lower light intensities for carrying out photosynthesis.

The general equation of this reaction is as follows:



The family Rhodospirillaceae 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). The importance photosynthetic bacteria in aquaculture are *Rhodospirillum*, *Rhodopseudomonas*, *Rhodomicrobium*, *Chromatium*, *Thiocystis*, *Thiosarcina*, *Thiospirillum*, *Thiocapsa*, *Lamprocystis*, *Thiodictyon*, *Thiopedia*, *Amoebobacter*, *Ectothiorhodospira*, *Chlorobium*, *Prosthecochloris*, *Chloropseudomonas*, *Pelodictyon*, *Clathrochloris* (Haung, 2003).

12. Bioremediators as Disease Controlling Agents

Most probiotics proposed as biological control agents in aquaculture belong to the Lactic Acid Bacteria (*Lactobacillus*, *Carnobacterium* etc.), *Vibrio* (*V. alginolyticus*), *Bacillus*, and *Pseudomonas* (Singh et al., 2001). Beneficial microbes, such as non-pathogenic isolates of *V. alginolyticus*, can be inoculated into shrimp culture systems to suppress the pathogenic vibrios like *V. harveyi*, *V. parahaemolyticus* and *V. splendens* and reduce the opportunistic invasion of these pathogens in shrimps (Jameson, 2003).

13. Nutrient Removal in Aquaculture Waste Using Aquatic Plants

Macrophytes (aquatic floating or rooted plants growing in wetlands) are commonly used in artificial wetland constructed for treatment of wastewater domestic sewage treatment. This plant is capable of removing organic matter,

suspended solids and nutrients such as nitrogen and phosphorus from water. Many species of macrophytes are used for wastewater treatment. Selection of species depends mainly on their availability and tolerance to the wastewater. Seaweed, *Gracilaria fisheri*, is capable of assimilating NH_3 , NO_2 , NO_3 and PO_4 from shrimp-farming effluents. Other seaweed, such as, red seaweed (*Gracilaria salicornia*), green seaweed (*Caulerpa macrophysa*), and brown seaweed (*Sargassum polycystum*), also assimilated waste nitrogen (NH_3 and NO_3^-) from shrimp pond effluent efficiently.

14. Probiotics in Aquaculture Bioremediation

The newest attempt being made to improve water quality in aquaculture is the application of probiotics and/or enzymes to the ponds, which involves manipulation of microorganisms in ponds to enhance mineralization of organic matter and get rid of undesirable waste compounds.

15. Microbial Mat in Aquaculture Waste Water Treatment

Microbial mats are laminated, cohesive microbial communities composed of bacteria dominated by photoautotrophic cyanobacteria. In aquaculture applications, it is important to consider several simultaneous activities performed by the mats in relation to nitrogen and carbon management. Ammonia is converted to nitrate by nitrifying bacteria, which take up residence under the cyanobacteria. Some nitrogen is also assimilated by mats, producing a thick, protein-rich product. Others have demonstrated that nitrate is removed by three cyanobacterial strains of the genus *Synechocystis*. The bioremediation using microbial mat is easier and provides a cleaner environment when compared to other conventional methods. Constructed microbial mats offer an interesting alternative for shrimp culture effluents.

16. Micro Algae in Bioremediation

Several researchers in the United States developed the processes to remove nutrients (primarily nitrogen and phosphorus) from wastewater by growing any of several strains of microalgae in the water. While nutrient removal can be accomplished by a variety of biological and chemical processes, microalgae holds promise of being able to accomplish nutrient removal with a net energy savings to the water treatment system. The nutrients, instead of being waste, become feed for the algae, which in turn become either a feed or a fuel source. The treated wastewater is of a quality suitable for many industrial applications.

17. Microalgae in an Integrated Aquaculture System

Portuguese researchers investigated the potential to use microalgae to process fish-farm effluents (primarily inorganic nitrogen and phosphorus) in seawater and use the microalgae as food for the *Tapes decussates* bivalve clam. The nutrient removal efficiency for ammonium and nitrite-nitrogen was in the range of 80%-100%, for nitrate 41%-100%, and for

phosphorus 21- 99%. After treatment, the water is similar in quality to fresh seawater. Researchers note the importance of this process, since fish farm effluent nutrient levels are generally too low for removal using standard bacterial systems (Borges *et al.*, 2005).

18. Case Studies on Bioremediation

- Effect of constructed microbial mat on water quality performance of *Liptopenaeus vannamai* post larvae.
- Bioremediation of shrimp culture effluent using constructed microbial mat.
- Use of *Chlorella vulgaris* for bioremediation of textile waste water.
- Ammonia – Nitrogen and orthophosphate removal by immobilized *Scenedesmus sp.* In municipal waste waters (Endong Zhang *et al.*, 2008).
- Bioremediation of shrimp culture effluent using constructed microbial mat.
- Nitrate and phosphate removal by immobilized *Scenedesmus*.
- Efficiency of ammonia and phosphorus removal from agro industrial waste by *Chlorella vulgaris* and *Scenedesmus*.
- Bioremediation of integrated fish culture ponds using *Gracilaria*.
- The Red algae (*Gracilaria lichenoides*) is also used to alleviate nutrient pollution in shrimp (*Litopenaeus vannamei*) and fish (*Epinephelus awoara*) culture ponds.
- The green seaweed (*Cladophora porifera*) is used for the uptake of ammonia deriving from aquaculture system and it is a candidate species for bioremediation of aquaculture wastes.

19. Advantages of Bioremediation

- Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated water. The residues for the treatment are usually harmless products and include CO₂, water and cell biomass.
- Bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material.
- Instead of transferring contaminants from one environmental medium to another, the complete destruction of target pollutants is possible.
- Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste

off site and the potential threats to human health and the environment that can arise during transportation.

- Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste

20. Disadvantages of Bioremediation

- Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.
- There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.
- Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.
- Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids, and gases.
- Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration.

21. Conclusion

Bioremediation is a technological tool that holds great value for the future as scientist learns more about its capabilities, it is likely to become one of the best technologies used to clean up and protect our environment.

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