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MICROBIAL BIOREMEDIATION: A POTENTIAL TOOL FOR SUSTAINABLE AQUACULTURE

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ABSTRACT

Microbes the oldest inhabitants of the earth, versatile and adaptive to the changing environment will be the cost-effective components to combat the present problems in aquaculture. These mini creatures have the novel capability of degrading the nitrogenous and organic matter causing self -pollution of the system and thus maintaining the water quality, to enhance immune system of cultured aquatic animals and to produce bioactive compounds such as vitamins, hormones and enzymes that stimulate growth, thus to decrease the FCR of feed .This paper aims to review the development of scientific concepts of microecology and the role and functions of beneficial microorganisms in aquaculture, preventing aquatic pollution thus aiming towards sustainability.

INTRODUCTION

Whether as an economic criteria for developing countries, or as one of the most environmentally destructive food industries, aquaculture has come under increasing scrutiny and criticism as the world tries to supply food for a population exceeding six billion. Aquaculture the farming of aquatic organism such as fish, molluscs,crustaceans and plants, is the fastest growing food production sector in the world. It has been heralded for its potential to meet the increases in seafood demand, and to take pressure off the wild fisheries. It was 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 as an option to cope with the world food demand (Craig Emerson, 1999). As a consequence of space and resource constraints traditional aquaculture has been intensified into reticulated systems with high stocking densities (Seri Inta *et al.*, 2005). Thus intensification of the system started leading to a condition of self-pollution of the system itself due to less self- purification capability. It resulted in an artificial environment that has the propensity for supporting the growth of pathogenic bacteria and the accumulation

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of waste metabolites in aquaculture systems. The indiscriminate release of spent aquaculture wastes into the surrounding environments is also problematic leading to stressful environmental conditions such as poor water quality and leading to situation such as eutrophication (Bhatnagar and Singh, 2010). The application of antibiotics is of no use due to emergence of antibacterial resistance and increased disease resistance in aquatic pathogens. It damaged or harasses normal microflora of culture environment and caused microdysbiosis, as double pollution, but it also made antibiotic residue accumulated in aquatic products to be harmful for human consumption (Qunlan Zhou *et al*., 2009). Control of water quality is often the bottleneck in intensive culture practices (Amiya Panigrahi *et al.*, 2009). As an alternative measure to control the self- pollution and disease within the system the use of beneficial microbes as a biological tool for sustainable and eco-friendly aquaculture is gaining momentum (Moriarity, 2005). They could maintain the eco equilibrium, inhibit the proliferation of harmful organisms and disintegrate harmful chemical substances in ecological environment such as *Bacillus thuringiensis* in the system (Qunlan Zhou *et al*., 2009). In aquaculture pond culture microorganisms have major roles, particularly with respect to productivity, nutrient cycling, the nutrition of the cultured animals, water quality, disease control and environmental impact of the effluent. However management of the activities of microorganisms in food webs and nutrient cycling in ponds is necessary for optimising production (Ekubo and Abowei, 2011). Microbes and their diverse metabolic enzymes are typically employed for safe removal of environmental contaminants either through direct destruction or indirectly through a transformation of the contaminants to a safer intermediate and it can be self-sustaining and inexpensive (Pandey *et al*., 2014). Bioremediation here proves to be an innovative technology which is defined as the process whereby organic wastes are biologically degraded under controlled conditions. It generally involves three different types of organisms: plants, natural microorganisms and transgenic (genetically-modified) organisms (Jaap van Rijn, 2010).

Waste generation in the culture ponds

As the days of culture increase the biomass and feed input increases, as such the pond bottom gets deteriorated by left over feed due to excess feeding, fecal matter and dead algae. With this increase, the culture environment becomes unstable and prone to rapid changes in water quality conditions. The system becomes susceptible for the growth of pathogens such as *Vibrio*, *Pseudomonas* and *Aeromonas* sp. (Shanmugam *et al*., 2009). Metabolic waste comes in two forms: dissolved and suspended waste and occurs in many forms: ammonia, nitrite, nitrate (i.e.; nitrogen), phosphorus, biological oxygen demand (BOD), chemical oxygen demand (COD) and fatty acids such as oleic acid (Milva Pepi *et al*., 2013). Fish and shrimp accumulate about 20-25% of protein and the rest is released to the pond as ammonium and organic nitrogen (Bhatnagar and Singh, 2010). These proteinaceous wastes result in development of total ammonia nitrogen (TAN) and biochemical oxygen demand (BOD). Total ammonia-nitrogen (TAN) is composed of unionized (NH_3-N) and ionized forms (NH^4+). The unionized ammonia is most toxic to aquatic organisms as it can readily diffuse through cell membranes and is highly lipid-soluble. Other toxic metabolites that form are nitrite, hydrogen sulphide, carbon di oxide etc (Milva Pepi *et al*., 2013).The three nitrogen conversion pathways traditionally used for the removal of ammonia–nitrogen in aquaculture systems are photoautotrophic removal by algae, autotrophic bacterial conversion of ammonia-nitrogen to nitrate – nitrogen and heterotrophic bacterial conversion of ammonia–nitrogen directly to microbial biomass. Nitrite (NO_2) an intermediate product of nitrification is also one of the toxic forms of nitrogen that can be found in aquaculture ecosystems. It is common in aquatic chemistry to express inorganic nitrogen compounds in terms of the nitrogen they contain, i.e., NH_4+–N (ionized ammonia–nitrogen), NH_3–N (un-ionized ammonia–nitrogen), NO_2^- –N (nitrite–nitrogen), and NO_3- N (nitrate– nitrogen) (Joel and Amajuoyi, 2010). Hydrogen sulfide (H_2S) is excreted by bacteria during anaerobic decomposition of waste products on the pond bottom. Organic loading can stimulate H_2S production and reduction in the diversity of benthic fauna. H_2S is soluble in water and has been suggested as the cause of gill damage and other ailments in fish .Unionized H_2S is extremely toxic to fish at concentrations that may occur in natural waters as well as in aquaculture farms (James *et al*., 2006).

Role of microbes in aquaculture ponds

Beneficial microorganisms play a great role in natural and man-made aquatic ecosystems based on the co-evolution theory in living biosphere on earth.

Their functions are to adjust algal population in water bodies so as to avoid unwanted algal bloom; to speed up decomposition of organic matter and to reduce COD, NH_3-N and NO_2-N in water and sediments so as to improve water quality; to suppress fish/shrimp diseases and water-borne pathogens; to enhance immune system of cultured aquatic animals and to produce bioactive compounds such as vitamins, hormones and enzymes that stimulate growth, thus to decrease the FCR of feed (Rahiman 2010). The key to any bioremediation process is the application of specific microbes to achieve specific transformation. The bacteria that derive energy from chemical energy are called as chemotrophs and the bacteria that use organic compounds as the principle carbon source are called as heterotroph. Bacteria that have both traits are called as chemoheterotrophs and are the most important organism for bioremediation (Kamath and Ganesh, 2008). Phylogenetically there are two distinct groups of bacteria that collectively perform nitrification. These are generally categorized as chemosynthetic autotrophic bacteria because they derive their energy from inorganic compounds and heterotrophic bacteria that derive energy from organic compounds. Ammonia oxidizing bacteria obtain their energy by catabolizing un-ionized ammonia to nitrite and include bacteria of the genera *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus*, and *Nitrosovibrio* (Sheryl, 2010). Heterotrophic bacteria grow significantly faster than nitrifying bacteria and will prevail over nitrifying bacteria in competition for space and oxygen in biofilters, when concentrations of dissolved and particulate organic matter are high. Culture period after 60-65 shows increased organic sludge having high content of NH_3, NO_2, H_2S and high population of *Vibrio* pathogens. Aeration plays a significant role on nitrification in simulated aquaculture system. Aeration can also reduce the BOD and COD load (Barik *et al*., 2010). Bioremediators such as *Bacillus, Lactobacillus, Nitrosomonas, Nitrobacter, Rhodococcus*, prevent the accumulation of organic sludge thus controlling the *Vibrio* population and thereby maintaining the water quality and health of the shrimp pond (Moriarity, 2005). *Bacillus mucilaginosus* has a potential role in bioflocculation. These bacteria can utilize the nutrients in the culture medium to synthesize high molecular weight polymers internally within the cell under the action of specific enzymes and these polymers can be excreted and exist in the medium or on the surface of the bacteria as capsule. The action of bacteria converts the simple substances in their environment into complex polymers that can be used as flocculants. In wastewater treatment, flocculation is an easy and effective method of removing suspended solids (SS). Since bioflocculants can be nontoxic, harmless and without secondary pollution, they have a great potential for use in aquaculture systems (Ravi Kumar *et al*., 2013). The addition of *Lactobacillus* sp. and *Bacillus* sp. into the fish-rearing medium markedly reduced the mortality rate of ornamental fish such as *C. auratus* and *X. helleri*. The results showed *Bacillus* sp.and *Lactobacillus* sp. confer benefit to *C. auratus* and *X. helleri* when administered as probiotics in water. Both *Lactobacillus* sp. and *Bacillus* sp. exhibited pronounced *in vitro* inhibitory action on many of the opportunistic pathogens including *Aeromonas* sp. and *Pseudomonas* sp. by the production of bacteriocin-like substance (Abraham & Banerjee, 2007). Gram-positive *Bacillus* sp. offer an attractive solution to the challenges facing modern aquaculture. Advantages of this genus include the ability to grow rapidly, tolerate a wide range of physiological conditions and the ability to sporulate. The robust spores of Bacillus sp. are also amenable to simple and cost effective production processes and the end products are stable for long period (Shubhadeep Ghosh *et al*., 2010). *Bacillus* sp. is generally more efficient in converting organic matter back to CO_2 than Gram-negative bacteria, which would convert a greater percentage of organic carbon to bacterial biomass or slime (Purnima Dhall *et al*., 2013). The reduction of phosphate concentration in *C. carpio* culture systems has been demonstrated through addition of Bacillus species.) The improvement in bioavailability of bound phosphate, through solubilisation, is also thought to facilitate removal of phosphate and reduce the propensity of algal blooms (Lalloo *et al*., 2007). Studies have been conducted on the role of *Bacillus* showing better growth and survival of *Pterophyllumscalare* larvae under environmental stress (high temperature). The result showed that the larvae of angle fish had better tolerance to high temperature when Bacillus was included in the system. The finding is useful for the larviculture of this species (Ramin and Rehman, 2012). The bacterium *Cellulomonas* helps in the degradation of unutilized feed and compete in growth with *Vibrio* sp bringing down *Vibrio* population due to competitive exclusion. These beneficial bacteria have bactericidal effect, which targets specially *V. harveyi*. The organisms also produce exudates which keep the culture animals free from other secondary infection

(Shanmugam, 2009). *Lactobacillus* sp isolated from curd was known to outcompete the growth of pathogenic *Vibrio* spand Shigellasp in the post larval culture of *Macrobrachium rosenbergii* in a study. (Ramin and Rehman, 2012). *Bacillus* sp. are very versatile organisms. They produce a plethora of extracellular enzymes, like proteases, amylases or cellulases that are involved in the degradation of organic matter. They perform heterotrophic nitrification and denitrification, mechanisms that are relevant for bioremediation of contaminated water. Because of these properties *Bacillus* sp. are interesting for aquaculture applications, where accumulation of organic matter and ammonia nitrogen reduces water quality, which impairs animal health (Pazlarova, 1987). Currently there is strong tendency to combine the photosynthetic bacteria, *Bacillus*, nitrifiers and denitrifiers, therefore improving the remediation process in the system and thus can be applied to different culture species in various culture conditions (Zizhong Qi *et al.*, 2009).

Role of microbes in nitrification

Nitrification and denitrification are the two process involved in bioremediation of nitrogenous compounds. In nitrification process ammonia is first oxidized to nitrite and then nitrite is oxidized to nitrate. 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 (Vibha Kumari *et al.*, 2011). *Pseudomonas, Bacillus* and *Alkaligenes* are the most prominent microbes acting as nitrate reducters. There exist seasonal variations in the population of nitrifiers in freshwater fish ponds, suggesting them to be actively involved in nitrification processes. The physico-chemical parameters like dissolved oxygen level, pH, temperature and concentration of ammonia as well as nitrite play regulatory role in controlling rate of nitrification and abundance of nitrifiers in the ponds(Antony and Philip, 2006).

Role of microbes remediating organic detritus

Members of the genus *Bacillus*, like *Bacillus subtilis, Bacillus licheniformis, Bacillus cereus, Bacillus coagulans*, and of the genus *Phenibacillus*, like *Phenibacillu spolymyxa*, are good examples of bacteria suitable for bioremediation of organic detritus. These microbes are not normally present in the required amounts in the water column, their natural habitat being the sediment. When certain *Bacillus* strains are added to the water in sufficient quantities, they make an impact (David, 1997). *Bacillus* sp. is Gram-positive bacteria, it enhance the immune system of the animal and also act beneficially in improving the quality of the water system. *Bacillus* sp. acts more efficiently in converting organic matter into carbon dioxide in comparison to the Gram-negative bacteria, which converts a greater proportion of organic matter into bacterial biomass or slime (Shubhadeep Ghosh, *et al.*, 2010). The introduction of *Bacillus* sp in proximity to pond aerators reduces chemical oxygen demand. *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. These microbes when fed along with feed increased the larval survival rate (André nduwimana *et al.*, 2007). Recently, the bioremediation of aquaculture discharge using a combination of Nitrobacter culture solution and grass plant species, *Lotiumperenne* proved as a biofilter to improve water quality in aquaculture system is in acceptance (Raja *et al.*, 2014).

Role of microbes in remediation of hydrogen sulphide

In aerobic conditions, organic sulphur decomposes to sulphide, which in turn get oxidised to sulphate. Sulphate is highly soluble in water and so gradually disperses from sediments (Boyd, 2014). Sulphide oxidation is mediated by microorganisms 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 (Adnan Amin, 2013). In aerobic conditions, organic sulpur decomposes to sulphide, which in turn get oxidized to sulphate. Sulphate is highly soluble in water and so gradually disperses from sediments. The bacteria involved in this process 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 (Arunkumar Jha, 2011). 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 common examples of photosynthetic bacteria of importance in aquaculture are *Rhodospirillum, Rhodopseudomonas, Chromatium, Thiocystis, Thiospirillum, Thiocapsa, LamprocystisThiodictyon, Thiopedia, Amoebobacter, Chlorobium, Prosthecochloris, Pelodictyon* and *Clathrochloris* (Rajeev Ranjan *et al*., 2014).

For bioremediation of H_2S toxicity, the bacterium that belongs to Chromatiaceae and Chlorobiaceae can be mass cultured and can be applied as pond probiotic. Being autotrophic and photosynthetic, mass culture is less expensive and the cultured organisms can be adsorbed on to the sand grains and applied so that they may reach the pond bottom to enrich the hypolimnion and ameliorate H_2S toxicity (Moriarty, 1997).

Role of microbes in degrading oil and fatty acids

A great variety of marine molecules (produced by microorganisms) with surface-active properties known as biosurfactants (BS), offer the potential for developing innovations for the control of infectious diseases (Dinamarca, 2013). Preliminary studies evidenced capability of producing biosurfactant by the cells of certain bacterial strain of *P. aeruginosa* , suggesting its use in bioremediation processes, and highlighting a possible use of the native wastewaters of the aquaculture (Zheng Zhong Ming, 2009). Bioaugmentation processes with the addition of high biomass of microorganisms degrading fats and oils to wastewaters have been studied. Bacterial strains of *P. aeruginosa* isolated from aquaculturewastewaters showed the ability todegrade fatty acids, showing a reduction in their concentrationof more than 80 % in 2 days (Milva Pepi *et al*., 2013).

Microbial mats in aquaculture

In aquaculture microbial mats were shown to produce protein, via nitrogen fixation, and were capable of supplying nutrition to tilapia (*Oreochromisniloticus*). Research on examining the role of mats in the nitrification of nutrient enriched effluents from aquaculture is been carried on. A low-cost biosolar filter system, based on microbial mats and fluidized sand filters is developed. Which provide an excess of oxygen for the nitrifying bacteria on the fluidized sand filters, providing good conditions for ammonia removal . The microbial mats, composed of complex bacteria and dominated by photoautotrophic cyanobacteria, can transform nitrogenous wastes into cellular protein and rapidly metabolize other fish wastes (Brad, 1993) Waste effluent treatment system based on microbial mats for black sea bass *Centropristisstriat* are cycled-water aquaculture has been studied. These microbial consortiums in the constructed mat, act in concert and in synergistic function to capture inorganic and organic molecules from the bulk liquid providing nutrients for the cell growth within the mat (Judith Bender and Peter Phillips, 2004).

Research on the use of constructed microbial mats offering 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 shrimp culture effluent. The results indicate that average efficiencies of ammonia nitrogen removal from shrimp (*Litopenaeusvannamei*) 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 BOD_5) of shrimp culture effluents(Paniagua and Garcia, 2003).

Microbial mats are rich in nitrogen and can be used as feed for young tilapia and thus if tilapia are fed with microbial mats there is the possibility of a complete recycling of all nitrogen and carbon from the black sea bass wastes. This shows raising of black sea bass in a recirculating system with a microbial mat filter system a good promise (Judith and Peter, 2004). Microbial mats have been called ideal for bioremediation because they are immobilized ecosystems that allow easy harvest/removal. These conglomerations of species support both aerobic and anaerobic reactions, and are self-sustaining and highly resilient. It shows that the mature microbial mat consortium is a durable microbial community that may be applied in a variety of uses related to aquaculture, bioremediation of contaminants, agriculture and energy production (Brad, 1993).

Special Features of Bioremediation

i) It is a natural process, it takes a little time, as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded,the biodegradative population declines. The residues for the treatment are usually harmless.

ii) Bioremediation also requires a very less effort and 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.
iii) Bioremediation is also a cost effective process as it lost less than the other conventional methods that are used for clean-up of hazardous waste.

iv) It also helps in complete destruction of the pollutants, many of the hazardous compounds can be transformedto harmless products,this feature also eliminates the chance of future liability associated with treatment anddisposal of contaminated material (Shilpi Sharma, 2012).

v) It does not use any dangerous chemicals. The nutrients added to make microbes grow are fertilizers commonly used on lawns and gardens. Because bioremediation changes the harmful chemicals into water and harmless gases, the harmful chemicals are completely destroyed (Neori, 2008).

Limitations of Bioremediation

i) Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapidand complete degradation.

ii) There are some concerns that the products of biodegradation may be more persistent or toxic than the parentcompound. Biological processes are often highly specific. Important site factors required for success include thepresence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriatelevels of nutrients and contaminants.

iii) It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.

iv) Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complexmixtures of contaminants that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids and gases (Megharaj, 2011).

v) Bioremediation often takes longer than other treatment options, such as excavation and removalof soil or incineration.

vi) Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation (Bhatnagar and Reeta Kumari, 2013).

Chemical Dynamics of Bioremediation

Bioremediation success depends on the following: (a) the growth and survival of microbial populations; and (b) the ability of these organisms to come into contact with the substances that need to be degraded into less toxic compounds; (c) sufficient numbers of microorganisms to make bioremediation successful; (d) the microbial environment must be habitable for the microbes to thrive (Qunlan Zhou, 2009); (e) ecological process or processes to be changed, (f) species that are naturally dominant and the desirable alternative species that could be added.

CONCLUSION

Utilizing microorganisms to detoxify waste water, contaminated soils, etc., is getting wide acceptance that "bioremediation" has now become a common buzzword in waste water management.They are considered as nature's original recycler. 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 (Panigrahi *et al.*, 2009). Many compounds that are considered to be hazardous legally can be degraded without posing any potential threat to the human health and environment. Bioremediation is less expensive than other technologies that are used for clean-up of hazardous waste. However bioremediation is limited to those compounds only that are biodegradable (Lopes, 2011). Every biological forms has a different growth requirements (temperature, pH and nutrients) so we need to isolate those forms, which can be cultured easily in the lab, with minimal requirement and can be useful in treating variety of pollutants. Not all compounds are susceptible to rapid and complete degradation (Jiechao Cheng, 2014). Microbial ecology and biotechnologies have advanced in the last decade, to the point that commercial products and technologies are available for treating large areas of water and land to enhance population densities of particular microbial species or biochemical activities. The practice of bioremediation (or bioaugmentation) is applied in many areas, but success varies greatly, depending on the nature of the products used and the technical information available to the end user. Thus for intensive aquaculture to become a sustainable industry with minimal environmental impact microbial ecology is critically important as a scientific discipline (Moriarty, 1999). However there is no doubt that bioremediation is in the process of paving way to greener pastures.

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