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Applications of Environment Biotechnology in Aquaculture-Review

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ABSTRACT

This paper explains all the chances of environmental biotechnology techniques useful in aquaculture and reviews its various areas together. Biotechnology has played a vital role in boosting production in the aquaculture sector. Considering the number of issues which deteriorate the ecological conditions in an aquaculture system, the field of environmental biotechnology has shown new possibilities to enhance the terms. Since the environment turns to be a vital segment in the survival of aquatic flora and fauna its day by day deterioration via pollution and toxic substances are need to be checked. The distinct role of environmental biotechnology within the sector of aquaculture emphasized finding the opportunities to contribute with new solutions and directions in remediating the contaminated environments, minimizing future waste release and creating pollution prevention alternatives.

Highlights

- Environment biotechnology is still an unexplored sector, with many new interventions to come in future.
- Water is environment for aquactic living beings , new approach to rectify its deterioration for better survival is the need of hour.
- Sustainable development of aquaculture field can be achieved with eco-friendly aquaculture practices.
- Combining aquaculture and biotechnology can help is overall sustainable growth.

Keywords: Environment Biotechnology, Bioremediation, Bioindicators, Biosensors

In the present scenario, aquaculture is accelerating its position in the food production sector and has played an essential role towards the food/nutritional security, livelihood/employment generation and reduction of poverty in many areas of the world. The increase in production is most significant in developing countries which accounts for about 93 per cent of aquaculture production. Polyculture and integrated system of farming were once considered an environmentally sound practice because of its optimum utilization of farm resource, including farm wastes. But with escalating demands of the population, expansion of land and water under culture and the use of more intensive and modern farming technologies which involve higher usage of inputs such as water, feed, fertilizer and chemicals resulted in the degradation of the aquatic environment. Aquaculture, which has a significant impact on world food security, is now also considered as a potential factor for the pollution of vast marine resources. Sustainable development of the aquaculture sector can be achieved by adopting eco-friendly aquaculture practices by minimizing the impact on the surrounding environment.

The era of the 21st century has found biotechnology emerging as a key enabling technology for sustainable environmental protection and stewardship (Cantor 2000). Environmental biotechnology is one of the gears of biotechnology which is an evolving technology in the context of environmental protection, since rapid industrialization, and modern lifestyle have threatened the clean environment. It is not a new area of interest, because some of the issues of concern are familiar with examples of "old" technologies, such as wastewater treatment. The environmental biotechnology has taken birth from chemical engineering, but later, other disciplines (biochemistry, ecological engineering, environmental microbiology, biology, ecology) also contribute to environmental biotechnology development (Hashim and Ujang 2004). This review focuses on the achievements of biotechnological applications for environmental protection and control, with new solutions and directions in remediation and monitoring of contaminated environments, minimizing future waste release and creating alternatives for better and efficient aquaculture practices.

Role of Biotechnology in Aquaculture

Biotechnology, the utilization of biological systems or living organisms in the production process, according to FAO (2005), has a wide range of useful applications in fisheries and aquaculture.

The rising global fish production reached about 171 million tonnes in 2016, with aquaculture representing about 47 per cent of the total. Aquaculture has been responsible for the impressive growth in the supply of fish for human consumption (SOFIA, 2018). India, as the second-largest country in aquaculture production after China, has the share of inland fisheries and aquaculture in total fish production of about 8.90 million tonnes (mt) recently with 12.59 million tonnes (mt) of total fish production (Handbook of Fisheries Statistics, 2018). Freshwater aquaculture contributes to over 95 per cent of the total aquaculture production. Aquaculture production has increased dramatically since the first 1980s. With an increment in demand for fish products, capture fisheries harvest reaches to lag phase or maybe decline as the expansion of the human population is soaring. With the advantage of more biomass produced per unit surface area than that for terrestrial animals, aquaculture can be the key to providing global food security. Therefore, biotechnology holds many potentials in the field of aquaculture for increasing the production via enhanced husbandry procedures, improved nutrition, improved disease diagnosis and therapies and the application of genetics to production traits.

Natural populations are perhaps the simplest gene banks, a critical resource for genetic variation for present and prospective application in genetic improvement for farmed species and specialized sport-fish applications (Biswas and Maurye 2017). Though, there are several improved aquaculture species through the use of genetics, which significantly contribute to production efficiency, enhancing production and increasing sustainability. Quite an ample amount of the genetically improved strains cultured in the aquaculture field developed through traditional selective breeding (selection, crossbreeding, and hybridization). However, modern technologies for genetic manipulation seem to take 10-20 years from being established until applications affect the industry. Gonadotropin-Releasing Hormone (GnRH) is now the simplest available biotechnological tool for the induced breeding of fish. GnRH is that the principal regulator and central initiator of reproductive cascade altogether vertebrates (Bhattacharya *et al.* 2002). As it is challenging to breed fish in a captive state, the development of GnRH technology has resulted in more efficient and successful induced breeding processes these days.

Induced triploidy a widely accepted procedure because of the best method for producing sterile fish for aquaculture and fisheries management. The detailed description of the methods used to induce triploids and other kinds of chromosome set manipulations in fishes and also the applications of these biotechnologies to aquaculture and fisheries management have well-scripted (Purdom 1983; Chourrout 1987; Thorgaard 1983; Pandian and Koteeswaran 1998). Tetraploid breeding lines are of potential benefit to aquaculture, because of producing large numbers of sterile triploid fish through simple interploidy crosses between tetraploids and diploids (Chourrout *et al.* 1986 and Guo *et al.* 1996). Although tetraploidy is successfully induced in many finfish species, the viability of tetraploids was low in most instances (Rothbard *et al.* 1997). Transgenesis technology is another excellent opportunity for modifying or improving the genetic traits of commercially important fishes, molluscs and crustaceans for aquaculture. The idea of producing transgenic animals became popular by Palmitter *et al.* (1982). The first transgenic fish was created by Zhu *et al.* (1985) in China, which claimed the transient expression in putative transgenics.

However, they gave no molecular evidence for the mixing of the transgene. There have been many pieces of evidence showing that this technology has resulted in higher growth rates and gave a boost to the aquaculture industry.

The thrust area in the aquaculture industry is the feed and nutrition, since last a decade, many advances in this sector has shown progress in sustainable aquaculture with use of nutritionally balanced and complete formulated feeds. Alternative and biotechnologically improved feed ingredients alongside improvements in pond management and manipulation of pond productivity can bring the boost to general productivity. As feed results in the 40 to 50 per cent of the cost of production, there is an alternate path to utilize plant-based protein than a fish meal can help to cut short the production cost. Use of biotechnology to produce alternative plant-based protein sources is suitable for production in aquaculture. However, the use of plant-derived materials like legume seeds, differing kinds of oilseed cake, leaf meals, leaf protein concentrates and root tuber meals as feed ingredients often limited by the presence of a wide variety of anti-nutritional substances (Fournier *et al.* 2005). One of the most exciting technological developments has come from the ability to manipulate the plant genome to produce products economically for use in aquaculture. The use of genetically modified crops to eliminate anti-nutritional factors and increase specific nutrients (limiting amino acids, n-3 fatty acids, etc.) is now possible. The packaging of genetically engineered proteins in corn seed to produce very inexpensive oral vaccines is also used (Singh et al., 2008). Another trend is to formulate diets on digestible amino acid levels, thereby reducing the requirement for protein. So far lysine and methionine have been used as supplements. Lysine produced by microbial fermentation and methionine also is chemically synthesized. Genetically enhanced microorganisms utilized to create threonine and tryptophan on a commercial basis, and soon other essential amino acids also would become available. The concept of a perfect protein blend from G.M. feedstuffs and feed additives (such as amino acids and enzymes) will significantly help with decreasing the quantity of nitrogen excreted in animal waste (Halver 2002). Probiotics are probably one of the most critical research developments in recent times. For ecological consideration, the antibiotics are substituted with probiotics, as it strengthens both the internal as well as the external microbial environment (Gildberg *et al.* 1997).

Enhancement of natural growth rates for fish in aquaculture has been extensively explored, with gains arising from improvements in husbandry, nutrition and genetic selection (Pennel and Barton 1996). Advantages of growth enhancers in aquaculture are shortening production times, improved feed conversion efficiency, and controlling product availability. Endocrine approaches to managing growth have also been extensively explored, principally applications of somatotropins such as growth hormone (G.H.), prolactin, and placental lactogen, insulin-like growth factor-1, thyroid hormones and sex steroids (Mclean and Devlin 2000). Nutrigenomics is the study of molecular relationships between nutrition and the response of genes, so to extrapolate how can it affect animal health. Nutrigenomics emphasizes on the genome, proteome, and metabolome and the effect of nutrients on them. Nutrigenomics has also been defined because of the application of high-throughput genomic tools in nutrition research (Müller and Kersten 2003). Nutrigenomics involves the characterization of gene products and the physiological function and interactions of these products. Though nutrigenomics will ultimately enable improved dietary requirements but its work still in its infancy. Integration of genetic and metabolic studies using the zebrafish as a model allows dissecting the fundamental pathways coordinating growth, nutrition and energy homeostasis. Goldsmith *et al.* (2006) revealed a critical transition in growth control during zebrafish tail fin development.

Biotechnological tools such as gene probes and polymerase chain reaction (PCR) are showing great potential in analyzing and stabilizing the health of fishes. Gene probes and PCR based diagnostic methods have been developed for several pathogens affecting fish and shrimp (Karunasagar and Karunasagar 1999). Biotechnology not only has a direct positive impact on many of the main elements of fish health management but also has a knock-on effect on other vital issues. Rapid detection and identification of pathogens are crucial for effective disease management which allows less use of antibiotics and chemicals in the environment; prevention of disease by vaccination also reduces antibiotic use. Moreover, the spreading via movement of infected fish needs to be controlled to prevent the spread of disease; therefore, regular screening for the presence of pathogens is also important (Hill 2005). Significant contributions have been made in the past decade in the development of vaccines for fish (Thompson *et al.* 2004) in addition to diagnostic probes and tests (Cunningham, 2004). Many novel technologies are now available to assist in the improvement of fish health, and offers benefits to the aquaculture industry.

Though these interventions helped in boosting productivity, a detrimental effect on the environment has created a menace. New findings in biotechnology with a motive to protect and conserve the environment have been beneficial for sustainable growth. Currently, the intensification in aquaculture practices has caused an adverse and negative impact on the environment. They are associated with both uncontrolled use of feed and massive production of waste which if released into the environment untreated, it deteriorates the water quality, leads to eutrophication, causes diseases and pollution. Other impacts include destruction and alteration of natural habitats, depletion of wild stocks, and salinization of adjacent soils and change of biodiversity.

At least four key points need to be kept in mind to improve the environment;

- to detect with the help of biosensors and biomonitoring
- to improvise manufacturing process, by substitution of traditional methods, single process steps and products with the use of new bio and gene technology in various industries
- to control and to remediate the emission of pollutants into the environment by ensuring the degradation of harmful substances during water/wastewater treatment, soil decontamination, treatment and management of solid waste (Gavrilescu 2009).

Environmental biotechnology can also contribute to pollution reduction are the production of biomolecules (proteins, fats, carbohydrates, lipids, vitamins, amino acids), yield improvement. The production processes can assist in the reduction of waste and minimization of pollution by the so-called clean technologies with biotechnological tools involved in reuse or recycle waste streams, generate energy sources, or produce new, viable products (Gavrilescu 2009). Many mitigation methods are taken into account for more profitable culture practice along with taking into account the health of the surroundings such as environmental bioremediation, waste minimization, ecological biomonitoring and maintenance.

Bioremediation

Bioremediation techniques are known to be an environment-friendly, healthy, efficient and cost-effective in improving the quality of aquaculture by minimizing waste and reducing environmental damage. Bioremediation, as defined by U.S. Environmental Protection Agency (USEPA) is "a managed or spontaneous practice in which microbiological processes are used to degrade or transform contaminants to less toxic or nontoxic forms, thereby remediating or eliminating environmental contamination" (USEPA, 1994). The primary principle is to biologically degrade the organic waste, under controlled conditions into an innocuous state or to the levels below concentration limits. As bioremediation is feasible only in manipulated environmental conditions which permit microbial growth. The presences of nutrient fertilizers such as nitrogen and phosphorus, as well as oxygen, boost the capability of the microorganism of breaking down the organic pollutant. Aerobic bacteria responsible for oxidizing processes can only function well in an ample amount of oxygen. The utilization of macro and microorganisms and their products as additives to enhance water quality is known to be as bioremediation or bioremediating agents (Moriarty 1998).

Wastewater Biotreatment

Wastewater generation in aquaculture may be a result of its operation from hatcheries and farming systems. The quantity and quality of wastewater from aquaculture operations vary according to the type and location of the aquaculture system. The wastewater from a hatchery is different from that of a production farm in terms of quality and quantity of waste (Ober- Dorff and Porcher 1994). In intensive commercial aquaculture operations, the significant sources of wastewater are uneaten food and fish faeces. Advances in biotechnology have tendered most methods of wastewater treatment technology effective in the aquaculture industry. Biotechnology also offers one strategy to reduce waste production in water through the process of oxygen injection, automated feeding, on-site re-pelleting technology and recirculation technology (Mayer and Mclean 1995). The first treatments of wastewater involve solids removal, ammonia oxidation, aeration and disinfection.

Membrane technology is one of the innovative and advanced techniques in water and wastewater treatment and reuses since it combines biological with physical processes (Bitton 2005). The combination of a membrane includes process like microfiltration or ultrafiltration with a suspended growth bioreactor (Ben Aim and Semmens 2003; Bitton 2005). Membrane bioreactors (MBR) is mostly used in the removal of dissolved organic substances with low molecular weights, which are not eliminated by membrane separation alone, instead of taken up, broken down and gasified by microorganisms and raising the quality of treated water (Yamamoto 2001). The main advantage of biological processes over the chemical oxidation is that there is no need to separate colloids and dispersed solid particles before treatment. Also, there is lower energy consumption, the use of open reactors results in lower costs, and no need for waste gas treatment (Wiesmann *et al.* 2007).

Toxic metals can damage the biological treatment process. However, there are microorganisms with metabolic activity resulting in insolubilization, precipitation, chelation, biomethylation, volatilization of heavy metals (Bitton 2005; Gerardi 2006). Metals from wastewater such as iron, copper, cadmium, nickel, uranium can be mostly complexed by extracellular polymers produced by several types of bacteria (*B. licheniformis, Zooglea ramigera*). Nonliving immobilized bacteria, fungi, algae can remove heavy metals from wastewater (Bitton, 2005).

Biosorption

Biosorption is a fast and reversible process for the removal of toxic metal ions from wastewater by live or dried biomass, which resembles adsorption and in some cases ion exchange (Gavrilescu 2009). The biosorption is an alternative measure for remediation of industrial effluents as well as the recovery of metals. Biosorbents are prepared from naturally abundant and waste biomass. It has been demonstrated that both living and non-living biomass may be utilized in biosorption processes, as they often exhibit a marked tolerance towards metals and other adverse conditions (Gavrilescu 2009). Biosorption of heavy metals by algal biomass is an appropriate and economically feasible method used for wastewater and waste clean-up, as it uses algal biomass which is waste from biotechnological processes (Feng and Aldrich 2004) or its availability in coastal areas makes it suitable for developing new by-products for wastewater treatment plants (Brinza *et al.* 2007).

Biofilters

Biofilters or biological filters allow the diffusion of oxygen and form adhesion sites for rapidly collecting natural aerobic bacteria involved in the nitrification and denitrification processes (Rijn 1996). According to Saidu (2009), biofilters are generally classified into two categories of emerged and submerged. In the emerged biofilters, the water cascades over the media to maximize the transfer of oxygen, by providing optimal conditions for efficient nitrification. The submerged biofilters are entirely in the bulk media and consist of a packing, liquid and biofilm phases. Biofilters are commonly used in semi-closed recirculating systems to treat aquaculture wastewater to be reused. The recirculating methods involve recirculating water between a culture facility and a water- treatment facility containing the biofilter. The waste is captured in concentrated effluents; it is thickened to sludge and then decomposed by the bacteria present in the biofilter (Rijn 2013). Badiola *et al.* (2012) reported that biofilters have an efficiency of reducing waste up to 90%. The most commonly used type of biofilters includes: microbial mats, activated sludge, trickling filter, rotating biological contactor and denitrifying screens.

Bioaugmentation

Bioaugmentation involves either addition of commercially prepared bacteria strains or addition of a blend of isolated enzymes or the combination of both the bacteria strains and the proteins (mostly preferred), which increase the natural degradation carried out by the indigenous bacterial population. Bacteria strains (most preferably indigenous ones) are carefully isolated, tested and applied as bioremediation agents. Presently, biotechnology uses some bacterial strains that have been genetically modified to increase their stability and survival in the aquatic environment. The most commonly used and commercially available augmenting agents belong to the genus *Bacillus* (mostly preferred due to its ability to secrete many different enzymes), which helps in mineralization and breakdown of proteins. The earliest documentation on the use of bioremediation in aquaculture described the use of different strains of *Bacillus* spp. in a *Penaeus monodon* farm (Porubcan 1991). In this experiment, bacteria were first pre-inoculated into the rearing water, which diminished the concentrations of the ammonia and nitrite, and then increased the shrimp survival.

In India, a probiotic containing *Bacillus, Lactobacillus, Nitrosomonas, Nitrobacter* was experimented to study its effects on both populations of *Vibrio*, which is pathogenic to several different marine organisms, and toxic gases in shrimp ponds (Nakano *et al.* 1998). Results of this work showed the complete removal of all toxic gases present in the lakes, which in return improved the water quality, significantly reduced the concentrations of *Vibrio* species and eventually improved the shrimp health. Other agents include strains of *Paracoccus* spp., *Thiobacillus* spp. and *Aeromonas* spp. (Gibson *et al.* 1998). Strains of *Acinetobacter* spp. help in the reduction of the organic matter, *Cellulomonas* spp. helps to breakdown material, while *Nitrosomonas* and *Nitrobacter* are liable for the oxidation of ammonia and nitrite, respectively. Bacteria strains belonging to the Families *Chromatiaceae* and *Chlorobiaceae* have been proved to have excellent capabilities for biodegrading hydrogen sulphide and in reducing lousy odour.

Bacterial strains capable of biodegradation waste can be genetically engineered to produce specific enzymes suitable for degradation (Singh *et al.* 2001). Direct application of enzymes has shown more rapid action due to the immediate breakdown of the chemical bonds. Proteins are durable, and some remain unchanged even after the reaction, thus easy to recover and recycle. Whereas enzymes are only limited to enzyme-mediated responses which are less toxic than the substrate, also the detoxification process is reduced to a single step from the multistep process. Combination of bacteria strain and proteins have shown to counter the limitation related to the sole use of enzymes. For example, the bacteria strains are used for fermenting the sludge after the activity of the protein, which further creates large surface areas for a more comfortable bacterial action. This combination of microbes and enzymes ensures the entire reduction of the organic matter, thus improving the water quality. It also helps in diseases prevention, improving the survival of cultured organisms and thus increasing the production yields (Janeo *et al.* 2009).

Environmental Biotechnology Monitoring

Environmental monitoring deals with the assessment of ecological quality, primarily by measuring a set of selected parameters regularly. In general, two methods – physicochemical and biological – are available for measuring and quantifying the extent of pollution (Conti, 2007). The harmful effect of toxic chemicals on natural ecosystems has resulted in an increasing demand for early-warning systems so on detect those toxicants at deficient concentrations levels (Durrieu *et al.* 2006). The integration of environmental biotechnology with information technology has revolutionized the capacity to monitor and control at molecular levels (Hasim and Ujang 2004).

Bioindicators/Biomarkers

Most recently, environmental monitoring programmes have included the determination of contaminant levels in biota, along with the assessment of various responses of biological/ecological systems. Some organisms or communities may react to an environmental effect by responding in a measurable changed biological function and their chemical composition. This significant environmental change and their responses are referred to as bioindicators/biomarkers (Conti 2007). Three main types of indications can be obtained: on exposure, effect, and susceptibility. Biomarkers that have the potential for use in biomonitoring are: molecular (gene expression, DNA integrity), biochemical (enzymatic, specific proteins or indicator compounds), histo-cytopathological (cytological, histopathological), physiological and behavioural.

Biosensors

Research on biosensing techniques and devices for aquaculture, together with that in genetic engineering for sensor cell development has expanded in the latest time. Environmental biosensors are analytical devices composed of a biological sensing element or biomarker (enzyme, receptor antibody or DNA) in intimate contact with a physical transducer (optical, mass or electrochemical), which together relate the concentration of an analyte to a measurable electrical signal (Rodríguez-Mozaz *et al.* 2004). The biosensors exploit biological specificity to supply signals which will be wont to measure pollution levels. Biosensors based on a combination of a biological sensing element and an electronic signal-transducing element that offer high selectivity, high sensitivity, short response time, portability and low cost, are ideal for monitoring pollutants in the environment of an aquaculture system (Lam and Gray 2003)

CONCLUSION

With evolving environmental challenges, new technologies for environmental protection and control in aquaculture system are currently in infancy. Though new approaches continue to gain more and more ground in practice, harnessing the potential of bioremediation, enzyme engineering for improved biodegradation, evolutionary and genomic approaches to biodegradation, designing strains for enhanced biodegradation, reuse of treated wastewater, biomembrane reactor technology and design wastewater treatment based on decentralized sanitation and reuse. Since environmental biotechnology proved to have an enormous potential to contribute to the prevention, detection and remediation of environmental pollution and degradation, it is a sustainable way to develop clean processes and products, less harmful, with the reduced environmental impact on the different production systems in aquaculture. Since some new techniques make use of genetically modified organisms, regulation to guarantee the safe application of new or modified organisms in the environment is essential. Environmental and economic benefits that biotechnology can offer in manufacturing, monitoring and waste management are in balance with technical and financial problems which still need to be solved. All this is often being achieved with reduced environmental impact and enhanced sustainability. An evaluation of the consequences, opportunities and challenges of modern biotechnology is essential both for policymakers and the industry.

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