Bioremediation: Eco Technology of Cultured Water Using Efficient Microbes (ECO Microbes)

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The emerging role of bacteria in the field of bioremediation eco- technology with countless new genes and biochemical pathways using antagonistic compounds and other useful molecules has generated new interest in them. After testing all the physical, chemical and biological means of bioremediation it has been found that for management of contaminated waters the best option is microbiological treatment which is more efficient economically and consumes less energy. "*In situ*" bioremediation is the cleanup approach where the dissolved and absorbed contaminants are in contact with microorganisms. The microorganisms act well only when the waste material helps them to generate energy and nutrients to build up more cells. Lack of biodegradation capacity of native microorganisms can be overcome by addition of selective substrates during. Insitu bio remediation or manipulate the native microorganisms for efficient and speedy biodegradation.

Advantages of *in situ* bioremediations are minimum exposure to site personnel, minimal site disruption, simultaneous treatment of soil and ground water and low costs. Disadvantages are seasonal variations of microbial activity due to direct exposure to environmental factors and also lack of control of these factors. Time consuming processing when compared to other bioremediation processes and probiotic application of treatment additives, nutrients, surfactants, oxygen etc. Several species of microorganisms are reported by Colwell and Walker (1977), Maeda and Liao (1992), Moriarty (1998) which have diverse metabolism and ability to degrade the recalcitrant organic compounds.This article highlights application of specific "EM" flora developed for the treatment of aquaculture pond water, like bacillus, photosynthetic bacteria, actinomycetes a yeast which can maintain neutral pH in pond water resulting in the occurrence of pathogenic vibrio's in pond. Water quality and control of disease are independent and linked to microbial activities in ponds. Only 17% feed is utilized. Microbial food webs are an integral part of all aquaculture ponds and have direct impact on productivity. If we can quantify productivity of bacteria we can make informed judgments of the three functional roles of bacteria and thus improve pond management to optimize productivity are as follows:

- Oxygen content of water is governed by bacterial activity. Respiration is often predominantly due to microbial processes affect water quality factors such as O_2, NH_3.
- In all extensive, semi-intensive and in some intensive aquaculture system bacteria also contributes significantly to the food web. They may be eaten directly by tilapia or mullet (adult producer) or by small animals. E.g.

Larvae and juveniles of penaeid prawn. Feeding may be impaired by formation of aggregates or by saline products.

- Through the activity of heterotrophic decomposers nitrogen and phosphorous are recycled to stimulate primary production.

Recent advances in microbial ecology now enable us to study community composition and activities of the bacterial members of aquatic systems. The results are now being applied to intensive aquaculture. This is an application of bioremediation eco technology which is essential for management of grow out ponds if harvests are to be sustainable, seasonal, quantitative, estimation and qualitative distribution pattern was studied for 2 years in the project FEM/ MB/1.

Heterotrophic bacteria consume oxygen and release CO_2 and ammonia while oxidizing organic matter.

In contrast the autotrophic, nitrifying and sulphur bacteria consume O_2 an CO_2 while oxidizing ammonium, nitrite or sulphide. The end products are nitrate and sulphate.

Feed contains high protein content. Ammonium release by the heterotrophic bacteria and cultured animals is inevitable. It must be taken up by algae or oxidized again by nitrifying bacteria rapidly to prevent toxicity to the cultured animal in the aquaculture pond. If nitrate concentrations become too high it will create problem at the end of the grow-out. In pond bottom detritus layer: oxygen diffusion is limited and thus oxygen is rapidly depleted due to consumption by bacteria and other microbes and animals. In the absence of O_2, nitrate present is used in place of O_2 for respiration producing nitrite, ammonia and nitrogen gas.

Fermenting bacteria also become active releasing acids like lactic acid, acetic acid, butyric acid, pyruvic acid, alcohols, CO_2 and H_2 which are then used by aerobic sulphate reducing bacteria. An oxygen debt is built up which may not be balanced during grow out stage, leading to an increase in reduced sediment on pond bottom. The ammonia and sulphide may not be present in toxic oxygen concentrations do not fall below 4pp in the water column.

Where the rate of feeding is very high as in the case in later stages of grow out excess of leached material in sediment surface leads to increased heterotrophic bacterial growth. Which inevitably causes anoxic conditions close to the sediment surface where bacterial oxygen requirement exceed the oxygen diffusion rate into the sediments? Thus the cultured animal will be exposed to sub-lethal concentrations of toxic compounds that is sulphate, nitrite and ammonia. Disease resistance is known to decrease in fish under such circumstances when pH is acidic and redox potential and this pathogens can invade. It is important therefore, to ensure that organic detritus and slime do not build up on the pond bottom as sludge. All faeces, excess feed and dead algae must be rapidly decomposed preferably in the water column itself. Regulation of bacterial growth;

Species composition:

In natural ecosystem, bacterial growth rates and biomass are controlled – by the slow rate at which large compounds or polymers such as proteins, starch or cellulose or fats are broken into smaller units. These particles are present as dead algae, uneaten food, faeces of animals.

Bacteria– efficient in hydrolyzing polymers would have a selective advantage and dominate– provided other conditions like O_2, pH, nutrients like phosphate are not limiting.

Bacillus:

Gram – positive bacteria bacillus group produce a wide range of exoenzymes efficicent at breaking down polymers so they are very useful in ponds. Normally, they are not present in high proportions in the water column. Their natural habitat is the sediment. Where there exoenzymes are held close to the cells by the sediment matrix and provide direct benefit to the cell and colony that secretes them. When certain bacillus strains are added to water sufficiently frequently and at high enough diversity – they do make an impact on the available organic matter. The bacterial strains must be adapted to the particular conditions in a pond. Eg. Strains that produce exoenzymes can degrade the particular bio chemicals in the organic detritus.The added bacteria then compete with the bacterial flora naturally present for the available organic matter such as leached or excess feed components and faeces. The results are that there is less accumulation of slime, slime cannot be completely eliminated. Lower numbers of potentially pathogenic bacteria and less accumulation of organic matter on the pond bottom. Thus there is better penetration of O_2 into sediment making a better environment into which cultured animals can burrow.

Competitive exclusion:

It is an ecological process that can be manipulated to modify the species composition of a soil or water body or other microbial environment. Small changes in factors that affect growth or mortality rates will lead to changes in species dominance. We are still a long way from knowing all the factors that control bacterial species, growth rate, and even the complete species composition in natural environments best enough is known that it is possible to change species composition by making use of competitive exclusion principles.

Species composition

Partly determined by chance; Partly determined by physiological factor, that allow a species to grow and divide more rapidly than others. Thus dominate numerically. Dilution, adsorption, aggregation, sedimentation etc contributes to the decrease in pathogenic bacterial contents. Chance follows: Those organisms that happen to be in the right place at the right time to respond to a sudden increase in nutrients. Eg: from the lysis of algal cells or decomposition of feed pellets that fall around them. So the farmer can manipulate the species composition by seeding large numbers of desirable strains of bacteria or algae. In other words : by giving chance a helping hand.

Bio- remediation:

The practice of bioremediation or bio augmentation is applied in many areas, but seems varies greatly depending on the nature of the products used and the technical information available to the end user. Selection of suitable ones doing specific functions that are amenable to bioremediation and added in a high enough heterotrophic bacillus density and under the right environmental conditions will enhance the productivity. Bio augmentation by DMS and the use of probiotics are significant management tools. But efficacy depends on understanding the nature of competition between species or strains of bacteria. They rely on the same concepts as used successfully for soil bio- remediation and probiotic usage in the animal industry. Criticism of this practice – bacterial products did not work as claimed. Products did not contain a large number of the right strains of bacteria to be effective or the bacteria were not viable. Suppliers of bacterial products must be aware of the physiological and ecological requirements of their bacteria. For eg: some contain purple sulphur bacteria that will remove sulphide only when conditions are anaerobic and light is present. Such conditions would be lethal to the cultured organism and would obviously not be present in a well-managed productive pond. The efficacy of the products containing nitrifying bacteria that were supposed to control ammonium concentration was also examined. Ammonium and nitrite oxidation is however a different process from control of pathogens and organic matter concentration by bacillus.

Nitrobacter and Nitrosomonas, Autotrophs

The nitrifiers are autotrophic. They need CO_2 for their carbon source. They use energy from oxidation of inorganic compounds such as ammonia. They are very difficult to maintain in a storage condition. Shipment and transport as commercial prepration cannot be done in nitrifiying bacteria. Thus the bacteria may not be viable or the number of bacteria added could have been too low to be effective.

Factors affecting effective usage of 'DMS'

Slow growing organisms require oxygen; Built up of nitrite and ammonia in pond which will inhibit their activity. Autothonus nitrifiers are best maintained by pond management practices. For eg: ensuring that there is a continual supply inorganic nutrients together with O_2 allowing natural populations of Nitrosomonas and Nitrobacter to grow to keep in balance with ammonium supply.

When to add bacterial products:

Sometimes – conditions develop that leads to a decrease in the density of nitrifiers with corresponding increase in nitrite or ammonium concentration. A rapid response is necessary. Increasing the aeration only would be insufficient because nitrifiers have long generation time. In such a case rapid response could involve adding a large inoculum of the nitrifiers together with an increase in aeration and mixing.

Criticism of bioaugmentation with "DMS"

For example in agriculture rhizobium inoculation for leguminous plants and more general inoculation of plant root zones with growth promoting bacteria such as bacillus species is widely practiced with high beneficial results. Shrimp farms in Indonesia that use the DMS range of bacillus do not have problems from disease caused by luminescent vibrio species. The number of luminous vibrio was inversely correlated to the addition of particular strains of bacillus. In intensive aquaculture however time is important. A good way to achieve rapid response and to reduce lag time and to low floral density is to add sufficient numbers of desired bacillus bacteria. The application of bioremedial technology is feasible and necessary. Instead of asking how bacterial abundance is controlled by nutrient availability or grazing we are asking what is the best way to change bacterial density of a particular bacterial species at a given time. The research field of microbial ecology will advance with the demand that the aquaculture industry is placing on it. In aquaculture the concept of microbial food chain can be adapted. The addition of low concentration of organic matter promotes the growth of fish. Certain species of bacteria like spore forming bacillus may be added with live feed like algae, artemia or rotifers which can enhance survival rate of fish significantly as they are excellent bioconverters.

Bioconvertors and bioprobiotics

Bioconvertors is a new technology that allows to keep aquaculture ponds clean to improve zooplznkton growth. All the 5 types of gases methane, ammonia, nitrate and H2S gases that are harmful to aquaculture producing acidic waters reducing the immune potential of the animals. In the bioconverter technology the degraders converts these gases water and carbon di oxide and free nitrogen and release the same into atmosphere air. Thus bioconverters reduces pond pollution and improve zooplankton and increase their immune potential.

Pond water is classified as neutral if the pH is 6.5 to 7.5.

6.5 – 6 is designated as slightly acidic.

6 – 5 is moderately acidic

5 – 4 is strongly acidic

< 4 is extremely acidic

Whereas pH 7.5 – 8 is slightly alkaline

8 – 9 is moderately alkaline

9 – 10 is strongly alkaline

>10 as extremely alkaline

Ingredients for EM solutions

A mixture of bacterial microbes was used as EM by Takashi Kyan and Terno Higa (1996). The first creation is known as EM – 1 containing yeast photosynthetic bacteria and lactic acid bacteria. EM technology is PRO EM is a liquid probiotic supplement that suppots healthy digestive and immune system, supports weight loss, improves absorption of food nutrients and aids in controlling yeast infections. Dr. Higa's brilliant discovery has been introduced in 120 countries worldwide. Teraganic is exclusive distributor of EM technology products. Carotenoid pigments including hydrocarbons such as â- carotenoid or xanthophylls such as lutein and zeaxanthine are widely distributed in nature where they play an important role in protecting cells and organisms against the harmful effects of light, air and sensitizer pigments. This process has been demonstrated in bacteria algae, plants, animals and even humans in the light sensitive diseases, erythropoetic protoporphy. The primary mechanism of action of this phenomenon appears to be the ability of carotenoids to quench excited sensitizer molecules. Caroteinoids can also sense as antioxidants under conditions other than photosensitzation. Antioxidant action can be documented in both enzymic and non- enzymic systems, and has been reported as subcellular, cellular and animal studies. Their anticarcinogenic properties may be related to their ability to interact with and quench various radical species that can be generated within cells.

Concluding remarks

Contamination of cultured water will have economic impact and the bioremediation of cultured water is acheviable only with microbial metabolism. Autocthonous microorganisms alone cannot do the bioremediation if the load of wastes is too heavy for them and remediation percentage after supplement application (microbes or nutrients) were generally higher than control. It was evident that nutrients (organic and inorganic) could also promote bioremediation. A successful implementation of a remediation regime required a consideration of the indigenous biota, nutrient availability as well as environmental factors necessary to achieve optimal results. A combination of technologies regulated with stringent conditions and allowed enough time will prove definitely successful bioremediation culture water. Interdisciplinary approach is required for bio remediation with a combination of biotechnology, microbiology, biochemistry, genetic engineering as bio remediation tools. Ecological risk analysis has to be done. EIA is a prerequisite before commercial application of EM. Probiotic use require responsible care for sustainability an option to uplift the livelihood of fish farmers.

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