Winter School on

'RECENT ADVANCES IN DIAGNOSIS AND MANAGEMENT OF DISEASES IN MARICULTURE'

7th to 27th November, 2002

Course Manual

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BIOREMEDIATION IN PRAWN GROW OUT SYSTEMS

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Introduction

In any aquaculture systems there exist a continuous exchange of substances between the bottom sediment and the overlying water, and between the two and the reared animal body. This exchange is strongly influenced by the inputs made continuously in to the system. As a matter of fact the bottom soil conditions and water quality in ponds are very much closely interrelated. Obviously, the water quality in ponds is very much influenced by the nutrient inputs, organic matter content, primary productivity and dissolved oxygen. Any aquaculture system has a finite capacity to assimilate nutrients, organic matter and the byproducts of degradation to a level congenial for the animals to grow. Bioremediation aims at the maintenance and enhancement of this finite capacity of the pond in favour and well being of the stock. Bioremediation in principle use microorganisms to transform/ decontaminate toxic pollutants in the environment. The process has two basic methods 1. Bioaugmentation, 2. Biostimulation. One of the main concerns of bioremediation is that the degradation products, whatever it may be, should be non toxic to the stocked animals. Major advantages of bioremediation are 1. It can be done on site (*in-situ*) 2. The process does not lead to any site disruption, 3. There is every possibility for permanent waste elimination, 4. Being a biological process it will be comparatively too inexpensive and 5. Can be effectively coupled with other treatment technologies

Events in a Prawn grow out systems

In prawn grow out systems, requirement of supplementary feeding is a reality. In all such situations large part of the feed gets wasted without contributing to the growth of the animals. Bacteria degrade the organic matter and it leads to low dissolved oxygen tension in the water column during night. Besides there is accumulation of faeces and also dead phyto and zooplankton in the pond bottom. All these lead to the development of anaerobic processes in pockets. Simultaneously, as the excretory metabolites of animal and also as the product of bacterial de-amination copious ammonia also gets built up.

Microbiology of anaerobic processes

Anaerobic digestion is accomplished by microbes, which can use molecules other than oxygen as terminal electron acceptor. From the thermodynamic considerations the electron acceptors are preferred by microbes in a definite order as Oxygen, Nitrate, Iron, Sulphate, Carbon Dioxide and Organic matter. Anaerobic decomposition therefore progresses in stages as 1.Hydrolysis, 2.Acid production, 3. Nitrate reduction/ de-nitrification, 4. Iron reduction 5. Sulphate /sulphur reduction and 6. Methane production.

Hydrolysis of polysaccharide results in the generation of hexose and pentose sugars, lipids in the formation of fatty acids and glycerol and proteins in the release of aminoacids.

These monomeric substances pass through the cytoplasmic membrane in to the cell by active transport and enter in to several degradative pathways. Monosacharides enters

in to Embden Meyer Hoff Parnas pathway resulting in the formation of acetic acid or ethanol, CO_2 and water. Energy is stored as ATP and NAD is the Co-enzyme often involved in hydrogen transfer reaction, which must be regenerated with coupled reactions.

It is generally agreed that the anaerobic degradation of long chain fatty acids proceeds primarily via β - oxidation in which two carbon atoms at a time are split from the chain. The sequence shows the removal of one acetate unit which combines with reduced Co-enzyme A (CoA – SH). Acetic acid can then either be liberated from Co-A in a subsequent reaction or the acetate can be transferred to other functional compounds. FAD is the prosthetic group for a wide variety of enzymes associated with the hydrogen transfer reactions.

During anaerobic decomposition of aminoacids some aminoacids may be fermented individually by pathways specific to that compound. For example from a molecule of glutamic acid pyruvic acid and a molecule of acetic acid are formed. In Stickland reaction pairs of aminoacids are fermented- one aminoacid acts as the electron donor (oxidized) while the other as the electron acceptor (reduced).

Organisms involved in the hydrolysis and acid production are *Clostridium, Acrobactor, Bacillus, Escherichia, Micrococcus, Panacolobacterium, Proteus, Pseudomonas, Sarcinia, and Streptococcus*

Reduction of Nitrate

In the absence of oxygen, for the smooth conduct of hydrolysis and acid production, nitrate plays the role of electron acceptor and gets itself reduced which can be either Dissimilatory nitrate reduction or Denitrification. During dissimilatory nitrate reduction nitrate is reduced to nitrite where nitrate serves as the electron acceptor. During reduction of nitrite to ammonia there is dumping of excess energy in the molecule by the organism. Denitrification on other hand leads to the reduction of nitrate to nitrogen through nitrous oxide where nitrate serves as the electron acceptor.

Reduction of Ferric ion (Fe^{3+}) to Ferrous ion (Fe^{2+})

When nitrate is completely used up ferric ion takes up the position of nitrate as electron acceptor and become soluble. This means that when nitrate is present the redox potential will not drop low enough for ferric ion to be reduced. Ferric ion must then be expended before hydrogen Sulphide (H_2S) is produced by reduction of sulphate.

Dissimilatory Sulphate/Sulphur reduction

Dissimilatory sulphur reducing bacteria are able to oxidize acetate completely to carbon dioxide using elemental sulphur as the electron acceptor (eg. *Disulphuromonas*). Meanwhile the sulphate reducing bacteria are capable of reducing sulfate, thiosulphate or any oxidized sulphur compounds as electron acceptors leading to the production of H_2S (eg. *Desulphovibrio*)

Methane production stage

Methane gets generated from the low molecular weight acids by methanogenic bacteria. These organisms are capable of coupling organic oxidation to reduction of CO_2. Methanogens are subdivided as 1. Hydrogenotrophic methanogens, and 2. Acetotrophic bacteria. Hydrogenotrophic methanogens utilize Hydrogen chemolithotrophically and

convert CO_2 to methane. Meanwhile acetoclastic bacteria cleave acetate in to methane and CO_2

Bioremediation requirements

In any aquaculture system bioremediation should focus on the removal/ transformation of 1. Detritus, 2. Ammonia and 3. Hydrogen sulphide by way of bio-augmentation and bio-stimulation.

1. Bioremediation of detritus

It is a myth that organic matter is continuously accumulated and transfered from one crop to another. Instead, what happens is that the anaerobic conditions at the pond bottom is being generated due to accumulation of fresh organic matter during the current crop. The first option is to try for the aerobic degradation of organic matter by way of re-suspending it by using aerators/ agitators. While doing so care should be taken not to re-suspend soil particles and the subsequent disturbance of hypolimnion. Aeration by way establishing uniform water current over the bottom is good option. During the aerobic degradation ammonia gets liberated which may get accumulated to toxic level if nitrification is not set in.

It has to be remembered that oxygen cannot move down to soil as the interstitial spaces are filled with water. In this situation soil microbes revert to anaerobic respiration producing H_2S, NH_3^+, N_2, H_2 and CH_4. If the hypolimnion remains intact, the organisms in this film of oxygenated sediment in the sediment water interface oxidize the above elements and compounds and prevent their diffusion in to the water column. As the order of preference of electron acceptors next to oxygen is nitrate the best option is to supply nitrate to the soil as the electron acceptor and stop the progress of rest of the anaerobic process. In such a situation the end product will be N_2, a harmless gas. Another advantage of the situation is that the phosphorus from water column gets precipitates from water columns as ferric phosphate. This will help in controlling phytoplankton bloom. The main concern of nitrate supplementation is not to allow the redox potential falls below – 150mV.

As part of bio-augmentation specific microorganism can be used for the faster degradation of the detritus. The organisms thus selected must be able to degrade organic matter faster than the native flora. They must be highly versatile to get adapted to the dynamic pond bottom and must be able to use NO_3 as the electron acceptor in the event of oxygen depletion. Two species of *Bacillus* such as *B.subtilus* and *B.lichiniformis* are best candidate species for bioremediation. They can be mass-produced, mixed with sand or clay and broadcasted to be deposited in pond bottom. As a matter of caution care must be taken to avoid heavy oxygen demand which may arise during application. As a matter of fact regular application will be required for sustained detritus removal.

2. Bioremediation of Ammonia

Biological nitrification is a natural process carried out mainly by chemolithotrophic bacteria, which derive energy by oxidizing NH^+ to NO_2 and NO_3. CO_2 serves as the carbon source. Nitrifiers are slow growers with a generation time of 10 to 60 hours. Temperature optima range from 28-30^{0}C and pH 7.6 to 8.5. Visible, long wavelength and UV light are lethal due to photo oxidation of Cytochrome C

The bioremediation protocol includes mass production of appropriate nitrifying cultures/ consortia, application in water at sunset, aeration during night, maintenance of good phytoplankton bloom and monitoring NH_3 and NO_2 level.

3. Bioremediation of Hydrogen Sulphide

Hydrogen Sulphide is toxic to aquatic animals as it binds with enzymes and blocks the oxidative process. It subsequently reduces the oxygen carrying capacity. Hydrogen Sulphide is soluble to waters in 4000ppm. Aeration can take care of H_2S only if the concentration is less than 2.0ppm. Gas absorbents like zeolite can give only temporary relief. Only option is to adopt biological method.

Anoxyphotobacteria splits H_2S into elementary sulphur and hydrogen ion during photosynthesis. They grow at sediment water interface in regions where oxygen tension is < 0.1mg/L and visible light of long wavelengths, not absorbed by phytoplankton and microalgae reach. Anoxyphotobacteria have three families such as Rhodospirillaceae, Chromataceae and Chlorobiaceae. Rhodospirillaceae utilize organic matter as source of hydrogen (non-sulphur photobacteria). They are efficient mineraliser at pond bottom as they grow in both anaerobic and aerobic conditions even in dark without utilizing solar energy. Chromataceae are purple sulphur bacteria, which hold sulphur particles in cells. Chlorobiaceae are green sulphur bacteria which precipitates sulphur particles out.

These organisms can be mass cultured and applied at pond bottom. Being autotrophic and photosynthetic mass cultures become less expensive. Cells can be adsorbed on to sand grains and broadcasted

Closed culture systems - a way out

Effective implementation of the above bioremediation programmes prawn grow out systems will lead to successful development of closed culture systems. Such systems are ideal in the scene that the entry of potential pathogens from the surrounding water bodies can be prevented. Besides, the impact of culture system on the outside aquatic environment also would be marginal as the residue of management chemicals used in the culture system also are not allowed to go out, instead sufficient time is given for their bio-degradation. Bioremediation coupled with the introduction of closed culture system will be the next phase of development in the aquaculture industry world over.

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