AQUACULTURE AND FISHERIES ENVIRONMENT

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AND
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Bioremediation
A Novel Tool for Environment Friendly Shrimp Aquaculture

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ABSTRACT

Organic matter contains three main energy nutrients, viz., proteins, carbohydrates and fats which microorganisms efficiently utilize to synthesize their cell structure and the energy for their life processes. Microorganisms play an important role in nutrient recycling in aquatic environment. N_2 - Cycle, C - Cycle, S – Cycle and P - Cycle are the major nutrient cycling process going on in the aquatic ecosystem. However, under most circumstances the appropriate species of microorganism for purifying water/ sediment and appropriate physico-chemical conditions are not always present in the aquatic environment for speedy mineralization of organic matter. The newest attempt being made to improve water quality in intensive shrimp culture is bioremediation which involves manipulation of microorganisms in ponds to enhance mineralization of organic matter and get rid of undesirable waste compounds. Bioremediation involves both intrinsic and engineered bioremediation. Engineered bioremediation includes Biostimulation and Bioaugmentation. Bioremediation (bioaugmentation) is applied in shrimp culture, but success varies greatly, depending on the nature of the products used and competition between species or strains of bacteria. The bacteria that are added must be selected for specific functions that are amenable to bioremediation and added at high enough population density, and under the right environmental

conditions. Commercial bioremediators products for aquaculture use are available in plenty and a few of these are AQUA.BACTA.AID; ACCELOBAC; Epicin; Bactaclean-ALGAE, Type 2; Alken Clear-Flo®; Sanjiban Microactive; NS Series Super SPO; Bioklean-MX 1, etc. However, their efficacy and success rates are variable as suppliers of such products often overrate their potential.

Keywords: Bbioaugmentation, bioremediation, commercial bioremediators, mineralization, nutrient cycle, shrimp culture.

INTRODUCTION

Aquaculture plays a vital role in world economy and is fast emerging as a major food producing industry. Aquaculture is the only hope of meeting the growing need of fish for the increasing world population, as the yield from capture fisheries have come to stagnancy. Of all the kinds of aquaculture practices, the outlook for shrimp aquaculture appears quite promising all over the tropical and subtropical countries. Asia holds a predominant position in the world shrimp production by culture. This has been possible due to the spectacular technology development and the favourable environment for the utilization of farming technology in the South East Asia over the last 10 to 15 years.

Water quality and disease control are interdependent and are linked to the microbial activities in aquaculture system. Microbial processes affect water quality factors such as the levels of dissolved oxygen, NH_3, NO_2^- and sulphide (Moriarty, 1996). One of the most important factors affecting the shrimp production is the build up and toxicity of NH_3 with the intensification of shrimp culture. As with many other industries, the intensive/rapid growth in shrimp aquaculture has brought with it the problem of environmental pollution. Shrimp culture all over the world has therefore been frequently affected by the viral and bacterial diseases (Lightner, 1993). The high microbial productivity coupled with stress and unfavourable environmental conditions lead to the outbreak of shrimp diseases.

Microorganisms not only act as autotrophs (primary producers) but also as saprophytes and heterotrophs, thereby helping in rapid recycling of dead and decaying animals and plants (organic matter), which in turn keeping the aquatic ecosystem alive. The autotrophic community is limited to few photo and chemoautotrophic bacteria, diatoms and cyanobacteria. Photoautotrophic bacteria belonging to the group green sulphur bacteria and purple sulphur bacteria help in carbon dioxide (CO_2) fixation but require anaerobic conditions, sufficient light and hydrogen donors like hydrogen sulphide (H_2S) and organic acid. Chemoautotrophic bacteria like nitrifying bacteria, bacteria involved in sulphur cycle, iron and manganese cycle contribute to primary production to a small extent (Rheinheimer, 1992). Heterotrophic microorganisms consisting of bacteria and fungi, help in degradation of organic

matter, if optimum conditions are prevalent, to simpler forms like CO_2 and H_2O. Moriarty (1986) observed that the heterotrophic microbial numbers in the water column were higher in most ponds receiving organic matter (feed pellets and chicken manure). Most heterotrophic bacteria were between 0.4 and 0.8 μm in diameter and 0.5 and 1.5 μm in length. Their average cell volume was 0.14 μm^3. Novitsky (1983) showed that the heterotrophic activity in the soil-water interface region was several times greater than that in the water column above and twice as high in the sediment immediately below.

Microorganisms play a major role in cleaning up the environment through rapid mineralization of organic matter present in culture ponds. Generally, in pond environment the organic matter content will be high compared to natural environment due to extraneous input like feed, excreta, fertilizer, etc. The micro organisms present in the pond such as bacteria, fungi, protozoa etc., carry out active decomposition of left our feed and metabolites to inorganic forms such as ammonia, hydrogen sulphide, carbon dioxide etc., through the process of mineralization. These nutrients will be utilized by algae for their growth and in term produce oxygen, which the microorganisms need for decomposition of organic matter. Such a natural process is called "self purification" process (Anon, 1993). In a way micro organisms and algae exist as symbiotic partners in ponds.

Many a times the appropriate species of micro organisms for purifying water/ sediment and appropriate physico-chemical conditions may not be always present in the pond to promote rapid growth and speedy mineralization. In this situation, seeding of micro organisms or manipulation of micro flora could hasten the mineralization process and bringing about rapid purification. The term bioremediation can be used to describe the process of reducing the hazardous organic wastes to environmentally safe levels through use of micro/macro organisms in ponds. Bio-remediation can broadly be classified into Engineered and Intrinsic bioremediation. Engineered bioremediation can again be divided into biostimulation and bio augmentation (Atlas and Unterman, 1999). Few of the micro organisms, which help in this process, are bacteria like *Bacillus sp, Acinetobacter sp, Pseudomonas sp, Nitrosomonas sp, Rhodopseudomonas sp* etc. As the micro organisms are fast growing (shorter generation time) and bring down levels of toxic products such as NH_3, H_2S etc, to insignificant levels, they are preferred over the micro organisms like algae, mussels, sea cucumber etc.

A variety of commercial bioremediators have been used in shrimp aquaculture to increase shrimp productivity but with varying degrees of success. There are number of reports on the positive and negative effects of the use of bioremediators in shrimp culture ponds (Boyd *et al.,* 1984; Moriarty, 1996).

DEGRADATION OF ORGANIC SUBSTANCES BY MICROBES IN SHRIMP CULTURE SYSTEMS

Microorganisms efficiently utilize the organic matter to synthesize their cell structure and the energy for their life processes. The breakdown of organic matter or mineralization is the major role played by micro organisms. If micro organisms would not have helped in degradation, the problem due to organic matter pollution would have been magnified. Even though the micro organisms can utilize organic matter, they need optimum conditions such as Temperature, pH, O_2, Oxidation, Reduction potential (Eh), proper carbon (C): Nitrogen (N_2) ratio, etc as these are major limiting factors for their growth. Organic matter usually contains three main energy nutrients, viz., Proteins, Carbohydrates and Fats.

PROTEINS

They are plenty of proteolytic micro organisms, which can utilize protein as source of energy. The decomposition of proteinaceous materials to soluble amino acids and other compounds is necessary for assimilation of this material into bacterial protoplasm. The breakdown of protein is also important for the release of nutrients from refractory compounds. Thus, generation of nitrogenous compounds is achieved. *Enterobacter, Pseudomonas* and other eubacteria and various fungi can carry out proteolyses. Proteins are primarily hydrolysed to polypeptides, oligopeptides by exo-enzymes of micro organisms. Later, they are taken up by cells, broken down, then utilized for body building, and lastly deaminated with liberation of NH_3. Zobell and Upham (1944) found that out of sixty strains of bacteria tested all could broken down peptone to NH_3 and forty seven could liquefy gelatin.

Sepers (1981) reported on number of bacteria, which can utilize amino acid as sole C, N_2 and energy source. According to him, 83% of the tested organisms were capable of utilizing 50-83% of the applied organic compounds as sole carbon and energy source. The amino acids most resistant to bacteria are methionine, taurine, threonine and glycine.

Barat and Jana (1987) studied the protein mineralizing bacteria and ammonifying bacteria in culture tanks and reported that seasonal changes of protein mineralizing bacteria were less pronounced with relatively low numbers in July than in the remaining months of the year. The ammonifying bacteria showed a small peak in autumn. The increase in intensification of the culture systems lead to increased metabolite production which supported higher population of protein mineralizing bacteria (Barat and Jana, 1987).

CARBOHYDRATES

Many eubacteria as well as actinomycetes and numerous fungi are able to degrade simple sugars to 3C compounds and finally to CO_2 and water (H_2O) under aerobic conditions. Under anaerobic conditions only fermentation is possible.

Few bacteria are capable of breaking down disaccharide such as sucrose, lactose and maltose and polysaccharides such as mannitol, rhamnose and xylose. These include *Azotobacter, Desulfovibrio, Clostridium, Klebsiella* and *Enterobacter* (Herbert, 1975; Lakshmanaperumalsamy, 1975). Starch is an important food reserve in plants. It is polysaccharide, which is utilized by only 10% of bacteria as C, N and energy source (Sepers, 1981). *Pseudomonas, Bacillus, Actinomycetes* and higher fungi can hydrolyse starch by means of exo-enzymes (Amylase, Maltase) into glucose under aerobic conditions, where as *Clostridium* utilize starch under anaerobic conditions. Cellulose is decomposed by Myxobacteria (*Cytophaga* and *Sporocytophaga*) and higher fungi (*Ascomycetes* and *Deuteromycetes*) under aerobic conditions (Rheinheimer, 1992).

Agar and alginic acids are product of red and brown algae, respectively and are degraded by the action of many bacteria, Viz., *Achromobacter, Agarobacterium, Flavobacterium, Cytophaga, Alginomonas alginovorus, A. alginica* and others (Rheinheimer, 1992). Chitin, a skeletal component of many lower animals, fungi and crustaceans are broken down by bacteria of the genera *Pseudomonas, Vibrio* and by fungi (Rheinheimer, 1992).

FATS

Fats are esters of fatty acids with glycerol, contained in plants and animals and also in water and sediment. Zobell and Upham (1944) isolated 13 species of lipolytic bacteria belonging to genera *Pseudomonas, Vibrio, Sarcina, Serratia and Bacillus*. Bianchi et al (1992) reported that out of 20 isolates of NH_3 and Nitrite (NO_2) - oxidizing bacteria, 49% and 21% could utilize fatty acids as carbon and energy source.

ROLE OF MICRO ORGANISMS IN NUTRIENT CYCLES OF SHRIMP CULTURE SYSTEMS

Micro organisms help not only in the production and break down of organic matter but also in nutrient recycling. Nutrient cycling is an essential process in the aquatic ecosystem. N_2 - Cycle, C - Cycle, Sulphur (S) - Cycle, Phosphorus (P) - Cycle are major nutrient cycling process going on in the aquatic ecosystem and these play key role in the formation of organic materials. Carbon is the prime substance of all the organic materials; nitrogen is necessary for the synthesis of amino acids, nucleic acids and amino sugars; sulphur is essential in sulfhydryl groups of amino acids and their polymers and phosphorus is contained in nucleic acid, phosphate esters, sugar phosphates and adenosine triphosphate (Austin,1988).

NITROGEN CYCLE

Nitrogen is a major constituent of proteins, the building block of all living matter. N_2 cycle, therefore, occupies an important place in organic matter recycling. It involves N_2 fixation, ammonification, nitrification and denitrification processes carried out by different microorganisms.

Biological N_2 fixation transforms molecular N_2 to NH_3 or organic N_2 and through this process, the atmospheric N_2 enters the biosphere and gets involved in N_2 cycle in aquatic environments. It is carried out by prokaryotes referred to as "diazotrophs". Stal *et al.* (1984) cited evidence of N_2 fixation for 18 blue green algae belonging to the genera *Anabaena, Calothrix, Microchaete, Nostoc, Nodularia, Rivularia* and *Trichodesmium*. The occurrence of *Azotobacter, Clostridium, Desulfovibrio and* photosynthetic N_2 fixing bacteria in marine sediments has been documented of which *Desulfovibrio* plays an important role (Sisler and Zobell, 1951; Pschenin, 1963; Truper and Genovese, 1968; Wyne Williams and Rhodes, 1974). The nitrogenase activity is light stimulated and to some degree inhibited by O_2 (Stal *et al.,* 1984).

Green plants utilize NH_3 and Nitrate (NO_3^-) as source of N_2 for synthesis of protein (Rheinheimer, 1992). The complex proteinaceous matter is converted to free NH_3 or ammonium ion (NH_4^+) depending on pH first by protein mineralizing bacteria and then by ammonifying bacteria such as *Pseudomonas, Bacillus and Vibrio*. This process is called ammonification and is the dominant mechanism for NH_3 production (Fry, 1987). Ammonification can take place either aerobically or anaerobically in water and sediment (Fry, 1987).

Ammonia is also produced from NO_3^- by nitrate dissimilation, which is important in anaerobic sediments. Herbert (1982) showed that *Aeromonas, Vibrio, Klebsiella, Escherichia and Clostridium* were very active in NO_3^- dissimilation and they contained an enzyme NO_3^- reductase whose activity reaches maximum under anaerobic conditions. In aerobic sediments and in the water column, NH_3 gets oxidized to NO_3 by Nitrification process. The organisms involved in nitrification have been fully described (Watson *et al.,* 1981) and consist of two genera that use different respiratory mechanisms. The NH_3 oxidizers convert NH_3 to NO_2^- (nitritation); there are 5 genera of which 2 are aquatic, viz., *Nitrosomonas* (rod shaped 1xl.5 μm) and *Nitrosococcus* (coccoid, 1.5-2 μm). The NO_2^- oxidizers convert NO_2^- to NO_3^- (nitratation) and all are aquatic, viz., *Nitrobacter* (pearl shaped rod, 0.7×1.5 μm) *Nitrococcus* (coccoid, 1.7 μm) and *Nitrospina* (rod shaped 0.35x3 μm). The activity of *Nitrosomonas* and *Nitrobacter* was reported to be affected by light (Olson, 1981) with *Nitrobacter* being the most sensitive. They are also highly sensitive to sudden changes in temperature, pH below 6, reduction in availab1e nutrients and several chemicals used for treating diseases in aquatic ecosystems (Burrows and Combs, 1968; Scott and Gillespie, 1972; Collins *et al.,* 1975; Spotte, 1979; Smith *et al.*, 1981; Bower and Turner, 1982). Nitrification, denitrification and nitrogen fixation are threatened also by contaminants such as heavy metals (Bouwman and Bloem, 2000).

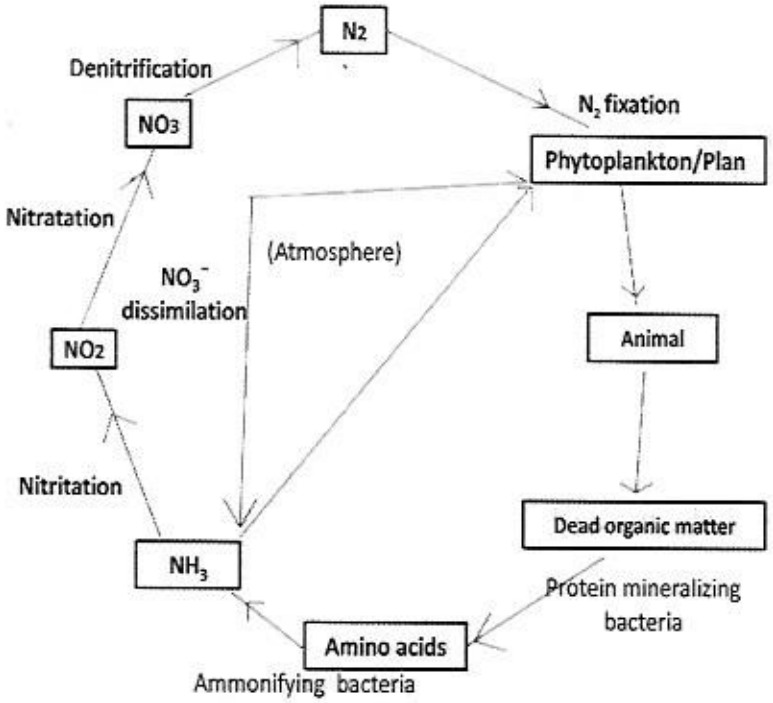

Fig 8.1: Nitrogen Cycle

Nitrosomonas sp

$$55\ NH_4^+ + 5\ CO_2 + 76\ O_2 \longrightarrow C_5H_7O_2N^- + 54\ NO_2^- + 52\ H_2O + 109\ H^+$$

NH_3-monooxygenase

Nitrobacter sp

$$400\ NO_2^- + 5\ CO_2 + NH_4^+ + 195\ O_2 + 2H_2O \longrightarrow C_5H_7O_2\ N^- + 400\ NO_3^- + H^+$$

The generation times of the autotrophic nitrifying bacteria are in the range of 10-30 h. Engel (1958) summarized that heterotrophs are also able to carry out nitrification, but to a lesser extent. The co-culture of a heterotroph *Arthrobacter* sp increased the nitrifying activity of an autotrophic *Nitrosomonas* strain possibly by reducing its lag phase (Kuenen and Gottschall, 1982; Kaplan, 1983). Bianchi *et al.* (1992) reported the ability of pseudomonads and asporogenous gram-positive rods isolated from an enclosed shrimp rearing facility to utilize NH_3. Joye and Hollibaugh (1995) reported that nitrification was rapidly and substantially reduced when 60-100 µm hydrogen sulphide (H_2S) was added to sediment slurries.

Denitrification involves reduction of NO_3 to NH_3 free N_2. Bacteria capable of denitrification are predominantly facultative anaerobes. Jetter and Ingraham (1981) listed 73 genera capable of denitrification including common aquatic heterotrophs, viz., *Pseudomonas, Vibrio and Alcaligenes*. Denitrification rates are highest in early summer and freshly anaerobic water (Nedwell, 1984). Considering denitrification to be a two step process with methanol as C and energy source, the following reactions can be written:

$$CH_3OH + 3NO_3 \longrightarrow 3NO_2^- + CO_2 + H_2O$$

$$2NO_2^- + CH_3OH \longrightarrow N_2 + CO_2 + H_2O + 2OH^-$$

The optimum pH for denitrifying bacteria lies between 7 and 8. They are sensitive to sudden changes in temperature. Most of the N_2 cycle process occurs simultaneously in aquatic ecosystem. A well balanced microbial load would help in efficient cycling of N_2 in environment.

SULPHUR CYCLE

Sulphur is one of the most abundant elements in our planet, present at approximately 520 mg/l level in the earth's crust (Goldschmidt, 1954). Sulphur is assimilated by many microorganisms and is the second most abundant anion in sea water (Austin, 1988).

Sulphate is one of the most common forms of sulphur found in habitats. In marine sediments, sulphate (SO_4^{2-}) and H_2S are constantly recycled between oxidation and reduction steps, predominantly carried out by two main groups of bacteria, viz., SO_4^{2-} reducers and sulphide (S^{2-}) oxidizers. Sulphate (SO_4^{2-}) is assimilated by bacteria and primary producers when they grow and incorporated mainly into sulphur containing amino acids of proteins. A variety of putrefying bacteria belonging to the genera *Proteus, Mycobacterium, Chromobacter, Bacillus, Micrococcus, Flavobacterium and Vibrio* produce H_2S by degrading the sulphur containing amino acids (Wetzel, 1983).

The H_2S is also produced directly from SO_4^{2-} by sulphate reducing bacteria (SRB) (Fry, 1987). These bacteria are all strict anaerobes and use SO_4^{2-} as terminal electron acceptor to oxidize organic compounds. The SRB in marine sediments have been reported to mineralize 25 - 50% of C. These include bacteria of the genera *Desulfovibrio*, (rod, curved shaped or spiral), *Desulfotomaculum* (spore forming rod) and *Desulfococcus* (coccoid). The primary habitat of SRB is the sediment with redox potential of -100 mV or below and that SRB are active within detrital particles of 100 µm thickness (Jorgensen, 1977a, b). Sulphate (SO_4^{2-}) reduction was reported to be highest in summer and lowest in winter (Fry, 1987). Suplee and Cotner (1996) found that new ponds initially had lower levels of SRB than old ponds, but the difference was lost by 17th week of grow out.

Once formed, the H_2S is either reoxidized to SO_4^{2-} or precipitated with iron to form insoluble ferrous sulphide. Reoxidation of S^{2-} is carried out biologically by a wide range of sulphide oxidizing bacteria (SOB). The H_2S oxidizers mainly include two groups of bacteria, viz., colourless sulphur bacteria and photosynthetic bacteria.

The colourless sulphur bacteria are all aerobic or microaerophilic and oxidize H_2S to S, which they store as S globules within their cells and they can use this S later to obtain energy when H_2S is unavailable (Austin, 1988). They include bacteria of the genera *Macromonas* (rod or bean shaped, 9×20 µm), *Thiovulum* (ovoid, 20

μm), *Thiospira* (spiral 2×50 - 10 μm), *Thiobacterium* (non motile rod, 1×2 μm), *Beggiatoa* (filaments, 1-55 μm), *Thioplaca* (sheathed, 1-55 μm) and *A. chromatium* (ovoid, 30-50 μm). There are a second group of colourless sulphur bacteria that oxidize H_2S and other inorganic sulphur to produce energy and form SO_4^{2-} but, there was no intracellular S deposition in these bacteria. They include bacteria of the genera *Thiobacillus* (rod, 0.5×1-4 μm), *Thiomicrospira* (spiral, 0.2- 9.3 × 1-2 μm), *Thiosphaera* (coccoid), *Thiodendron* (vibrioid, 0.15-0.25 μm) and *Acidiphilium* (rod, 0.3-1 .2 × 0.6-4.2 μm). *Thiobacillus denitrificans* can grow in anaerobic conditions by converting NO_3^- to N_2. There are again a third group of colourless sulphur bacteria that require optimum temperature above 55°C for growth and thus, are of lesser importance in S cycle. They include members belonging to genera *Thermothrix* (rod), *Sulfolobus* (spherical) and *Acidianus* (spherical) (Fry, 1987).

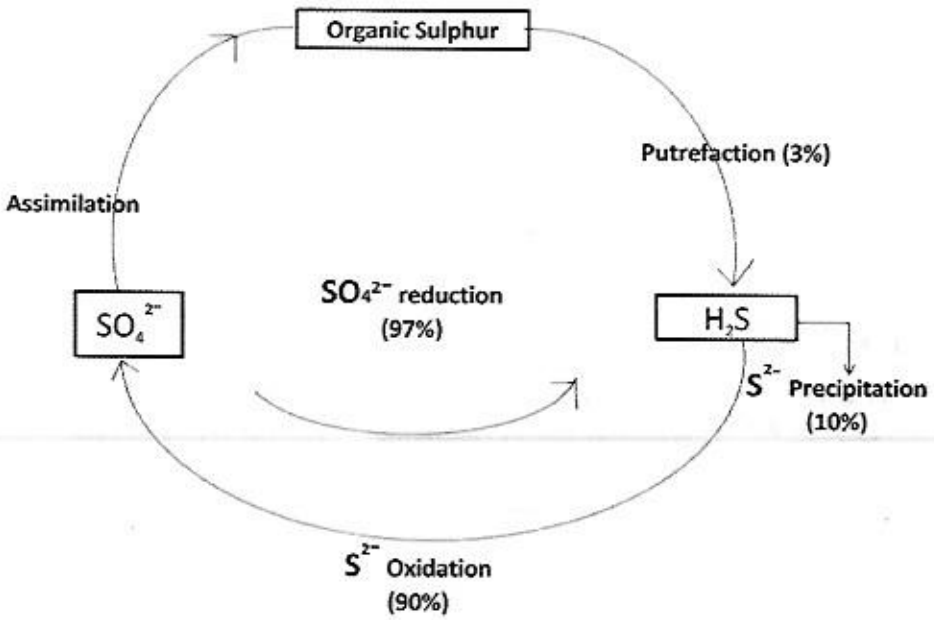

Fig. 8.2: Sulphur Cycle

Under strict anaerobic conditions and in the presence of light, photoautotrophic bacteria of the family *chlorobiaceae* [green sulphur bacteria, e.g., (i) *Chlorobium* - a non motile rod of 0.3-1.1 × 0.7-2.7 μm and (ii) Pelodictyon - a rod of 0.8 × 1.8 μm] and *Chromatiaceae* [purple sulphur bacteria, e.g. (i) *Chromatium* - an avoid rod of 1-6 × 2-15 μm and ii) *Thiopedia* - a non motile rod of 1.5 × 2 μm] oxidize H_2S efficiently to fix CO_2 phototrophically (Fry, 1987). The green sulphur bacteria grow at lowest light intensities cannot tolerate O_2 but can tolerate high H_2S concentrations. The purple sulphur bacteria need more light, are O_2 tolerant and H_2S sensitive, they always grow in a thin band just above the green sulphur bacteria and even may penetrate the oxygenated part of the H_2S/O_2 interface. Phototrophic bacteria are also found in sediments where light penetration and H_2S accumulation meet.

CARBON CYCLE

Carbon, one of the major constituents of all organic matter, undergoes recycling in nature, at the center of which stands CO_2. The earth's atmosphere contains about 0.032% (2.3×10^2 tons) by volume of CO_2 (Rheinheimer, 1992), but in sea water 50 times this amount is in solution. The cycle is very complex in water because many organisms are involved and many pools of different carbon compounds can be envisaged.

The C - Cycle can be divided into assimilation, i.e., synthesis and transformation of organic material into multitude of natural C compounds and dissimilation which is the stepwise breakdown of all these substances by respiration by heterotrophic plants and animals (Rheinheimer, 1992). The C-fixing bacteria including cyanobacteria, photo and chemo autotrophic bacteria, etc. synthesize organic matter by fixing CO_2 and using light and other chemical substances such as NH_3, NO_2, NO_3^- and S as their energy source (Fry, 1987). Some heterotrophs are also able to fix CO_2 in the dark and some predominantly autotrophic bacteria can use organic compounds as source of energy and C, these types are often called mixotrophs (Fry, 1987).

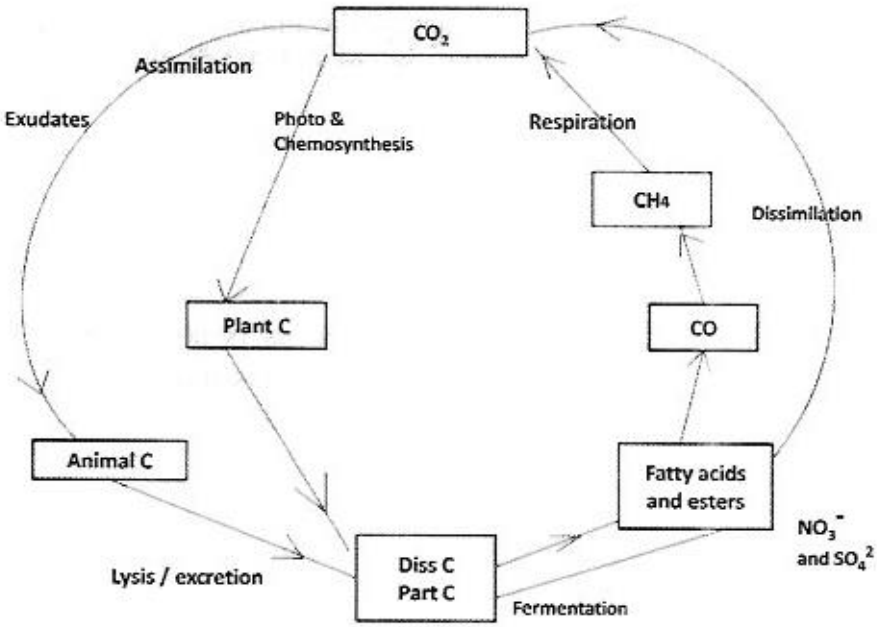

Fig 8.3: Carbon Cycle

The decomposition of primary producers, when they die by microorganisms contributed to both the dissolved organic and particulate organic carbon compound pools. Heterotrophic bacteria grow on the particulate organic carbon and secrete exoenzymes that decompose it and the decomposition products enter the dissolved organic carbon pools. Most of the dissolved organic carbon is respired by heterotrophic microorganisms to CO_2 .The wide range of heterotrophic bacteria involved in the entire process from decomposition of primary producers to production of CO_2 mainly

belong to the genera of *Flavobacterium, Pseudomonas, Vibrio, Aeromonas and Alcaligenes*. Pike (1975) reported that 90-95% of bacteria in oxidation ponds are *Pseudomonas, Achromobacter and Flavobacterium,* thus demonstrating their predominance in these systems. The SRB and denitrifying bacteria are also known to mineralize carbon. The zone near the surface containing the redoxycline, is often the site of most bacterial activity when gross measures are used (Fry, 1987).

A portion of the dissolved organic carbon will be converted to methane (CH_4), probably mainly through acetate by methanogens. The CH_4 producing bacteria are morphologically diverse but physiologically similar group and most of the 7 genera are rod shaped (*Methanobacterium*) or coccoid (*Methanogenium*) but one genus (*Methanospirillum*) have spirally shaped members (Fry, 1987). All are anaerobes and grow best at redox potential of -200 mV or below. However, their growth is limited by SRB as they complete with them for acetate and hydrogen (Nedwell, 1982; 1984).

The CH_4 produced is not oxidized anaerobically in sediments by methanogens but rises into the water column and once it reaches the oxygenated layer is rapidly oxidized by methanotrophic bacteria, *Methylomonas*, *Methylcoccus* and *Methylosinus* (Fry, 1987). They are microaerophilic and use CH_4 carbon and energy source to produce CO_2 (Cappenberg, 1972; Rudd and Hamilton, 1975).

PHOSPHORUS CYCLE

Phosphate (PO_4^{3-}) is one of the most important limiting factors for plant life in many waters. Phosphorus as a vital element for all organisms is present in phospholipids, phosphorylated sugar, phytin, ATP etc and also particularly as a constituent of nucleic acids. Phosphorus cycle involves conversion of inorganic phosphorus to organic and vice versa. Phosphorus is taken up by plants as pyrophosphates, that is changed to organic P compounds and from these, PO_4^{3-} are released mainly due to action of microorganisms (Rheinheimer, 1992). During cycling P may get immobilized due to adhesion to clay particles or formation of ferric or aluminium phosphates.

Solubilization of inorganic phosphates is carried out by a wide range of micro organisms, viz., *Pseudomonas, Achromobacter, Flavobacterium, Arthrobacter, Streptomyces and Aspergillus* (Botto, 1988). This solubilized PO_4^{3-} taken up by phytoplankton and plant for production of organic substances. Mineralization of organic P is carried out by a variety of microorganisms such as *Arthrobacter, Proteus, Serratia, Streptomyces, Aspergillus* and *Rhizopus* (Botto, 1988). Although many organisms have the ability to hydrolyze phytate in vitro, this form of organic PO_4^{3-} has a very strong affinity for adsorption on clay particles, which prevent accesses by the phytases produced by the organisms. Consequently, phytate tends to accumulate and is the major form of organic phosphorus found in most soils (Botto, 1988).

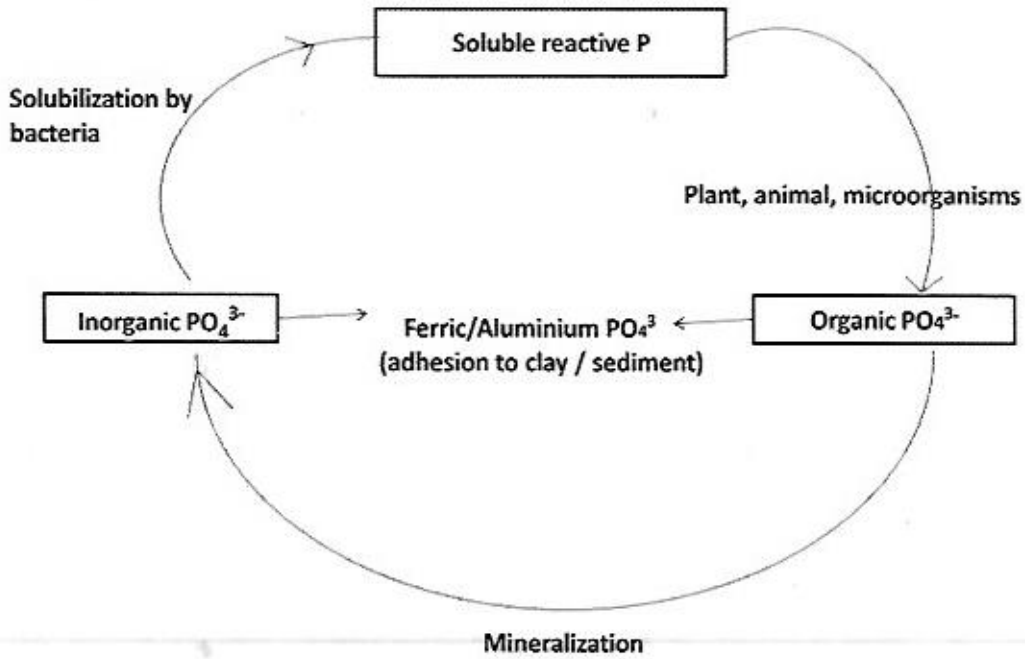

Fig. 8.4: Phosphorus Cycle

BIOREMEDIATION: A NEW CONCEPT

Bioremediation has almost become a household word these days, implying the use of biological agents to control problems of environmental pollution. A prominent example of bioremediation, often cited in popular press and scientific magazines, is that of Exxon Valdez Oil Spill, where indigenous microorganisms were supplied with an oleophilic fertilizer and allowed to proliferate and consume the spilled oil (Chakrabarty, 1992).

Bioremediation is defined as the process by which microorganisms are stimulated to rapidly degrade hazardous organic contaminants to environmentally safe levels in soils, subsurface materials, water, sludges and residues (Thomas 1992). Stimulation is achieved by the addition of nutrients and a terminal electron acceptor usually O_2, because most biological reactions occur faster under than anaerobic conditions. Under anaerobic conditions, NO_3^- has been as the terminal electron acceptor.

Bioremediation is a pollution treatment technology that uses biological systems to catalyze the destruction or transformation of various chemicals to less forms (Atlas and Unterman, 1999). Bioremediation is cost effective, environmentally sound and increasingly the preferred choice of remedial technology for clean up operation.

The objective of bioremediation programme is to immobilize contaminants (reactants) or to transform them to chemical products no longer hazardous to human health and environment. The end products of effective bioremediation are non-toxic and can be accommodated without harm to the environment and living

organisms (Atlas and Unterman, 1999). The selection of most effective bioremediation strategy is based on - a) characteristic of the contaminants (toxicity, molecular structure, volatility, solubility and susceptible to microbial attack) b) the contaminated site (hydrology, geology, soil type and climate and the legal, economic and political pressures felt by the site owner and c) the microbial process that will be exploited, such as pure culture, mixed culture, their respective growth conditions and supplements.

The general approaches to bioremediation are:

1. **Intrinsic bioremediation**: Intrinsic bioremediation is the management of contaminant biodegradation without taking any engineering steps to enhance the process. It uses the innate capabilities ofnaturally occurring microbial communities to metabolize environmental pollutants. Because intrinsic bioremediation occurs in the landscape where both indigenous microorganisms and contaminants reside, this type of bioremediation necessarily occurs in situ. It may be used along or in conjunction to other remediation techniques. For intrinsic bioremediation to be effective, the rate of contaminant destruction must be faster than the rate of contaminant migration.
2. **Engineered bioremediation**: Engineered bioremediation, either accelerates intrinsic bioremediation or replaces it completely through the use of modification procedures that allow concentration of nutrients, electron acceptors, or other materials to be managed in a manner that hastens biodegradation reactions. Engineered bioremediation may be chosen over intrinsic bioremediation because of considerations of time, cost and liability. It falls into 2 categories:
   (a) Biostimulation refers to the addition of specific nutrients to a waste situation with the hope that the correct, naturally occurring microbes are present in the waste sufficient numbers and types to breakdown the waste effectively. This assumes that every organism needed to accomplish the desired treatment results present.
   (b) Bioaugmentation involves the addition of specifically formulated micro organisms to a waste situation. It allows one to control the nature of the biomass. It ensures that the proper team of microbes is present in the waste in sufficient type, number and compatibility to attack the waste constituents effectively and break them down into their most basic compounds (Burlage *et al.*, 1999)

Biodegradation of naturally occurring and synthetic organic compounds requires or is faster when several species of microorganisms are present. In instances where the indigenous microflora fails to degrade the target compounds or has been decimated by the presence of toxicants, microorganisms with specialised metabolic capabilities may be added (Thomas *et al.*, 1992).

In bioremediation, the emphasis, so far, has however, been on the use of microorganisms rather than genetically manipulated ones, because of the adverse public perception on the release of genetically engineered microorganisms as well as various regulatory constraints on their use (Chakrabarty, 1992). The major reason for using genetic selection in the decontamination of polluted environment is the fact, that in many cases, natural microorganisms have not evolved the genetic competence to utilize a synthetic compound. To generate new degradative capability against a newly made synthetic compound, a micro organism must evolve the appropriate genes encoding enzymes that would have high affinity for the target chemical or its intermediate products as substrates. Bioremediation has some definite advantages over other treatment technologies in that it can be done at site, facilitates permanent elimination of waste, biological systems are cheaper, evokes positive public acceptance, minimum site disruption, eliminates transport cost and liability and can be coupled with other treatment techniques.

The bioremediation microorganisms frequently identified as active members of microbial consortium are *Alcaligenes denitrificans, Arthrobacter globiforms, Arthrobacter sp, Bacillus sp, B. megaterium, Flavobacterium sp, Mycobacterium, M. vaccae, Methanobacteriaceae, Nitrosomonas europaea, N. corallina, N. erythropolis, Pseudomonas sp, P. aeruginosa, P. putida , P. cepacia, P. fluorescens , P. glatheri, P. mendocina, P. methanica, P. paucimobilis, P. testosteroni and P. vesicularis* (Baker and Hersan, 1994).

The requirements for an effective bioremediation is illustrated (Cookson, 1995) pyramidically as follows.

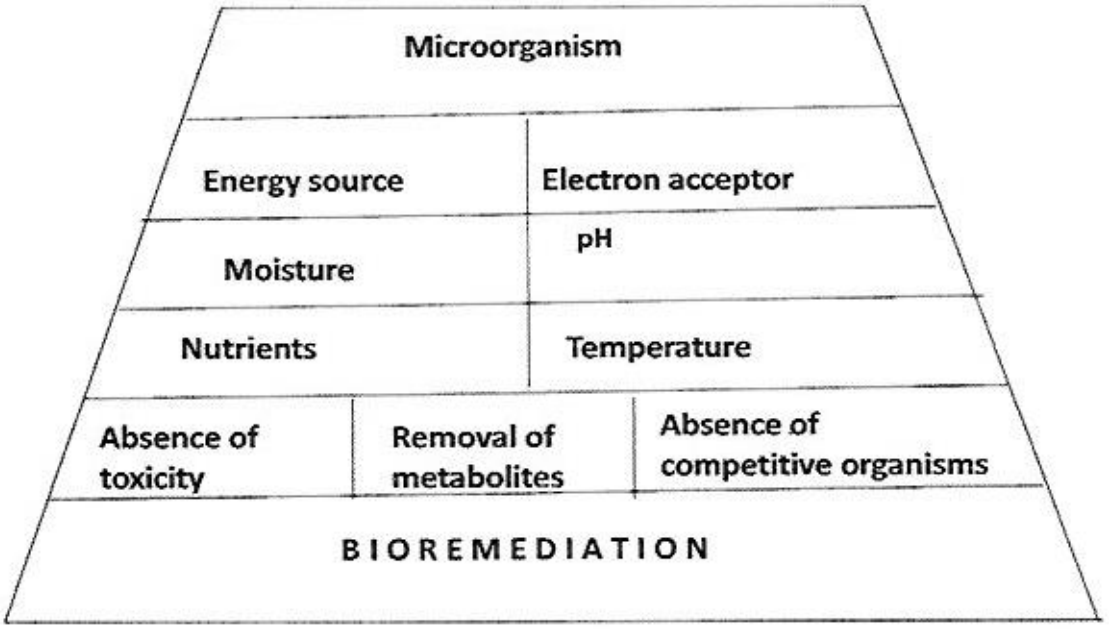

Fig. 8.5: Requirements for an Effective Bioremediation

The spectrum of compounds susceptible to bioremediation are naturally occurring, have simpler molecular structure, is non-toxic and serves as a growth substrate for aerobic microorganisms. A few examples of such compounds are inorganic ones as NO_3^-, SO_4^{2-}, PO_4^{3-} and NH_4 compounds and petroleum (xylene, toluene, benzene, ethylbenzene, alcohols and ketones). In compounds that are resistant to microbial metabolism, have complex molecular structure, low water solubility, and strong sorptive interactions, toxic and do not support the growth of microorganisms. A few examples of such compounds are halogenated aliphatic and aromatic compounds (Burlage *et al.*, 1999).

BIOREMEDIATION IN SHRIMP AQUACULTURE

The Need/Necessity

It is a golden rule that successful intensive shrimp culture requires intensive management to maintain good pond water quality. The pond water quality changes quickly because of the input of large quantities of high quality feeds. Most of these feeds eaten by shrimps are eventually excreted as metabolic wastes that add inorganic nutrients and organic matter to the bottom of ponds. According to Briggs and Funge-Smith (1994) only 21% of nitrogen and 13% of phosphorus of the feed input (at a conversion rate of 2) gets incorporated into flesh of shrimp. On the other hand, Primavera (1994) has reported only 17% incorporation of feed input by shrimp. The ponds, thus, become eutrophic with active decay and assimilation of left over feed and metabolic wastes carried out by microorganisms. As a result of microbial activity under aerobic conditions the organic matter is converted to inorganic compounds such as PO_4^{3-}, NH_3 and CO_2. The microbial process of converting organic matter to inorganic compounds is called mineralization. Some of these organic compounds serve as nutrients to stimulate algal growth, which in turn produce oxygen required for decomposition of organic matter.

Many a times the appropriate species of microorganism for purifying water/ sediment and appropriate physico-chemical conditions may not be always present in the pond to promote rapid growth and speedy mineralization. The newest attempt being made to improve water quality in intensive shrimp culture is the application of bacteria or enzymes to the ponds. This type of biotechnology is known as 'bioremediation' which involves manipulation of microorganisms in ponds to enhance mineralization of organic matter and get rid of undesirable waste compounds (Anon, 1993). Beneficial, ecofriendly bacteria are a must for healthy prawn culture. Moreover, water treatment with chlorine, iodophores and antibiotics kill the beneficial autochthonous microbes as well as pathogenic allochthonous microbes, reducing the fertility of water. Microorganisms are known to play an important role in nutrient recycling in any aquatic environment (Rheinheimer, 1992). Water quality in aquaculture system is, to a large extent, controlled by microbial biodegradation of

organic residues (Avnimelech et al., 1995). Therefore, attempts are being made to improve water quality in intensive shrimp culture ponds through application of bacterial population capable of degrading organic matter in the ponds.

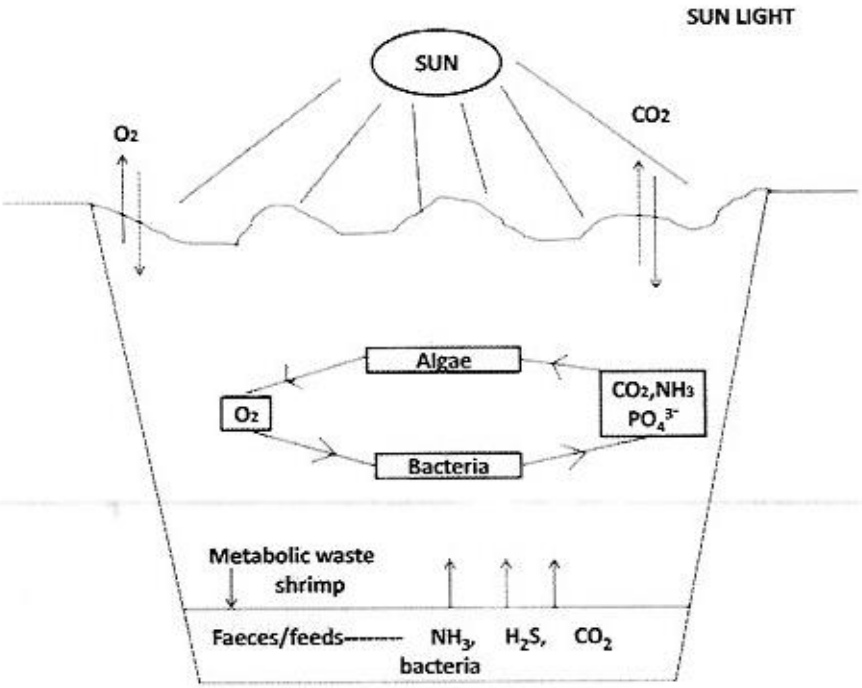

Fig. 8.6: Role of Microorganisms in the Metabolic Cycle in Shrimp Ponds

Many scientists feel that addition of bacterial seed stock is pointless. Their beliefs are based on misinterpretation of ubiquity principle. The principle of ubiquity states that bacteria may be found anywhere, it does not state that all bacteria are found everywhere all the time. The underlying assumptions of the following misconceptions are not true: (i) that the appropriate species for water purification and organic sediment decomposition are always present; (ii) that appropriate physico-chemical conditions are always present to permit rapid growth; and (iii) that bacterial growth is not limited by process such as predation (Ehrlich *et al.*, 1988). Bird and Kalff (1984) reported that addition of specific microbial mixture or manipulation of the microflora of the deteriorated environment may hasten the process of mineralization, thereby bringing about rapid purification.

The practice of bioremediation (bioaugmentation) is applied in shrimp culture, but success varies greatly, depending on the nature of the products used and the technical information available to the end users. The bacteria that are added must be selected for specific functions that are amenable to bioremediation, and added at high enough population density and under the right environmental conditions. Bioaugmentation is a significant management tool, but its efficacy depends on understanding the nature of competition between species or strains of bacteria.

APPLICATION OF BIOREMEDIAL PRODUCTS IN SHRIMP AQUACULTURE

There are many reports on the success as well as unsuccessful results of using bacterial products in aquaculture.

In the 1980's Alken-Murray Corporation changed the economic feasibility of bacterial treatment by offering highly concentrated, non-pathogenic and cost effective Alken-Clear Flo ® formulae to degrade excess nutrients, chemical pollutants and NH_3 in traditionally high volume shrimp producing pond waters. Alken-Clear Flo ® 1000 and 1002 include basic spore forming, waste degrading strains of *Bacillus* to reduce organic loadings in the water column, preventing a built of sludge on pond bottom by 60%. Alken-Clear Flo® 1100, 1200 and 1400 contain *Nitrosomonas europaea*, which degrade NH_3 to NO_2^- and *Nitrobacter winogradskyi*, which degrade NO_2^- to NO_3^- in the aerobic environment of the water column and to N_2 gas in the anaerobic environment of the bottom sludge or gravel.

Boyd *et al.* (1984) studied the effect of commercial bacterial suspension (AQUA. BACTA. AID) and found that it did not have any significant effect on water quality parameters such as total ammoniacal nitrogen (TAN) concentration, NO_2-N concentration, NO_3-N concentration, total phosphorus concentration, biological oxygen demand (BOD) and chemical oxygen demand (COD). On the other hand, Ehrlich *et al.* (1988) reported positive effects of the same bacterial consortium in its ability to accelerate nitrification, increase decomposition of organic solids (10-12 cm/month), reduce excessive algal growth, facilitate oxygenation and aid in transformation of agricultural wastes into faunal biomass.

Sanjiban Microactive is a liquid stimulator developed from complex fermentation process. It is an organic extract enriched with natural enzymes that activates and rapidly multiplies healthy organisms already present in the effluent system. In shrimp ecopond system under warm climatic conditions, the typical removal efficiency of various pollutants that have been achieved are: total suspended solids (TSS):- 80-95%, BOD: - 85-98%, COD: - 80-93%, NH4-N: - 85-95%, phosphorus: - 90-95% and E. coli: - 99%. Furthermore, production of 500-1000 kg shrimp/ha and 5000-6000 kg fish/ha can also be harvested.

Porubcan (1991a; b) reported on two attempts at bacterial treatments to improve water quality and production yield of *Penaeus monodon* - (i) floating biofilters pre-inoculated with nitrifying bacteria decreased the amounts of NH_3 and NO_2 - in the rearing water. This treatment also increased shrimp survival (Porubcan, 1991a) and (ii) the introduction of *Bacillus* sp in close proximity to pond aerators reduced COD and increased shrimp harvest (Porubcan, 1991b).

Chiayvareesajja and Boyd (1993) studied the effect of a bacterial product (ACCELOBAC) on TAN concentration and reported that treatment of pond water with up to 40 mg/1 of ACCELOBAC caused no change in TAN concentration over

a 10 day period. Tucker and Lloyd (1985) found no benefits of bacterial augmentation in lowering TAN concentration or improving any other aspects of water quality.

An intensive shrimp culture pond in Thailand which use a commercial bacterial product throughout the culture period got good production of 6806 Kg/ha (FCR 1.4 and survivality 80%), demonstrating the possibility of using bacterial products to maintain good water quality (Anon, 1993). The use of 'EPICIN' a commercial bacterial product of EPICORE Network in shrimp ponds in Indonesia has produced the largest harvest ever recorded in the ponds involved and profits were up to 5 times greater than ponds not treated. The major and probably most significant effect of 'EPICIN' on water quality was in its ability to reduce NH_3 concentration. Following 'EPICIN' application, concentration of other nutrients including NO_2-, NO_3 - and H_2S were also reduced to well below than that in untreated ponds (Anon, 1995). Funge-Smith and Hawthorn (1996) tested 5 commercially available bacterial products for their efficacy in improving water quality under laboratory conditions. They reported that none of the products had significant effect on TAN as well as NO_2 -N concentrations.

Shrimp farms in Indonesia that use the Detritus Management System (DMS) - range of *Bacillus*, do not have problems from diseases caused by luminescent *Vibrio* sp (Moriarty, 1996). Chandrika (1999) reported on bioaugmentation with 10^9/g of DMS- Bacillus to mineralize and reduce the faecal matter of shrimps and left over feed in intensive aquaculture. Anon (1999) studied the effect of a bioaugmentor, viz., Bioklean MX - 1 (bacterial product) for removal of toxic NH_3 from shrimp culture systems and reported that Bioklean @ 12 ppm was effective in reducing the concentration of NH_3. They also studied the efficacy of *Pseudomonas* (1×10^6/ml) on removal of NO_2 from shrimp culture systems and reported that 5 ml/ l of Pseudomonas was effective in decreasing NO^2 concentration. They further studied effect of plant by-products and extracts on removal of NH_3 from shrimp culture system. According to them, neem seed oil @ 100 ppm, neem leaf extract @ 90 ppm and custard apple seed oil @ 90 ppm were all effective in reducing NH_3 concentration.

In China, Li Zhuojio et al. (1997) reported on the application of a mixture of several strains of photosynthetic bacteria (*Rhodomonas* sp) to improve the shrimp culture water and have achieved remarkable results. There was a total elimination of NH_3-N, H_2S and organic acids coupled with improvement in water quality and balancing of pH resulting in increase in body length and weight of shrimps. They concluded that the bacterial population might have chemical actions such as oxidation, nitrification, ammonification, denitrification, N_2 - fixation and sulphurication.

An alternative way to maintain high water quality in intensive shrimp culture is biological treatment based on the use of filters with a high surface/volume ratio, pre-colonized by microorganisms that absorb excess nutrients from the water.

A biological filter for filtration of shrimp culture water has been developed recently by Bioworld. The filter occupying a volume of 11% of water volume under production provides a large surface area (20 m^2) for many biological processes: ammonification, nitrification and denitrification. The bioremedial products offered by New China Limited, are very useful to those raising shrimp in ponds. They create larger, healthier shrimps and lessen mortality by avoiding NH_3 build up; thus increasing profits. Bacta Clean - ALGAE, Type 2 is a bioremedial product used for shrimp aquaculture pond maintenance as it prevents NH_3 build up, slime formation and algal growth. Moreover, it reduces NO_3^- added to pond water by shrimp faeces and scavenges bottom sludge materials.

Prabhu *et al.* (1999) studied the effect of a commercially available probiotic (NS series Super SPO) on the water quality parameters of 4 ponds in a shrimp farm. The product was soaked in pond water @ lg/200ml and activated by vigorous aeration for 4 h. After activation, the liquid containing the slurry was sprinkled uniformly over the surface water in each pond. The results of the experiment showed a marked decrease of NH_4-N in the concentration 3 experimental ponds with progressive days of culture (DOC); while there was a marked increase in NH_4-N concentration in the control pond. The total heterotrophic bacterial count increased by 10^4 (from 10^3 to 10^7) cfu/ml in water and by 10^5 (from 10^3 to 10^8 colony forming units (cfu)/g in sediment in the control pond, which is much more when compared to the increase by 10^3 (from 10^3 to 10^6 cfu/ml in water and 10^3 (from 10^4 to 10^7) cfu/g in sediment of experimental tanks.

Recently, Oppenheimer Biotechnology, New York, USA is co-operating with the Philippines Company Envirogenics, Inc. to evaluate the use of Oppenheimer Formula 1 product to enhance production in shrimp pond culture. The application of Oppenheimer Formula 1 to sediments and water of 2-5 acre ponds have been shown to double the normal production in the same time period. There is also evidence that microbes may reduce the mortality caused by other competing microorganisms, control algae, decrease BOD and COD, decrease NH_3, NO_2^- and NO_3^-.

Shan and Obbard (2001) from the Department of Chemical and Environmental Engineering, National University of Singapore isolated cultures of nitrifying bacteria from intensive prawn aquaculture water and enriched them using continuous and batch enrichment techniques. Cultures were immobilized on to porous clay pellets to enhance cell density and when applied to water with high TAN concentrations have been found to exhibit high TAN removal rates.

Moriarty (1996) has summarized the reasons for inefficiency of few bacterial mixtures under field conditions. According to him, the bacterial products might have lacked the sufficient number of right strains of bacteria to be effective or it was possible that the bacteria were not viable. It is apparent that many suppliers of bacterial products are unaware of the physiological and ecological requirements of

their bacteria. For example, some contain purple sulphur bacteria that will remove S^{2-} only when conditions are anaerobic and light is present. Nitrifying bacteria are autotrophic and need CO_2 as their carbon source and oxidize NH_3 for their energy. They are very difficult to maintain, require oxygen and are slow growers. If these conditions are not provided, the activity of these bacteria will be inhibited.

The use of macroorganisms as effective bioremediators in shrimp culture system has led to the development of new culture models such as shrimp-shellfish (molluscs/oysters) shrimp-fish and shrimp-algae. The seaweed *Gracilaria* is an attractive species to be grown as part of polyculture with molluscs in a biological treatment system because it can remove soluble nutrients, nitrogen and phosphorus, which are not absorbed by molluscs. The culture of shrimp with fish is found to be the most successful for preventing disease occurrence. It is believed that predatory fish may eat sick or morbid shrimps, thereby eliminating the spread of diseases in shrimp culture pond. Mangroves have also been suggested to treat shrimp pond effluents in that it acts as biological filters by trapping pollutants, i.e., excess nutrients, suspended solids, heavy metals, toxic hydrocarbons, etc. (Babu *et al.*, 1998).

FUTURE DIRECTION

The significance of special groups of microorganisms with varied physiological characteristics in aquaculture systems is not well documented and also their ecology. Future studies should focus on monitoring the levels of these microorganisms with different physiological characteristics involved in nutrient cycling in different aquaculture systems. Also efforts should go into the development and evaluation of suitable bioremedial products using indigenous microflora of the culture system.

CONCLUSION

Bioremediation and its efficacy are debatable topics. However, they have potential applications in aquaculture. The challenge in maintaining a viable culture of indigenous bacteria at high cell density in active growth phase is a key factor in providing an effective treatment for shrimp culture pond water. A thorough and detailed investigation is, however, necessary to understand the behaviour as well as environmental requirements of beneficial microbes that exist in shrimp ponds. Large scale laboratory and field studies are required to clearly demonstrate the ability of the microbes as bioremediators. Viability and economics are the other vital aspects, which have to be considered before adopting these methods.

REFERENCES

Anon (1993): Bioremediation Technology for Shrimp Culture – Does it Work?. *Asian Shrimp News*, 16(4): 3.

Anon (1995): Water Treatment Booster Profits for Indonesian Shrimp Farmers. *Fish Farming International*, 22 (12): 10-11.

Anon (1999): Soil and Water Quality and Productivity Management for Sustainable Shrimp Farming. *Annual Report 1997 – 1998, Central Institute of Brackishwater Aquaculture, Chennai*, pp: 50.

Atlas, R.M. and R. Unterman (1999): Bioremediation, In: Manual of Industrial Microbiology and Biotechnology. *ASM Press, Washington DC.*, pp: 666-680.

Austin, B. (1988): Marine Microbiology. *Cambridge University Press, Cambridge*, pp: 221.

Avnimelech, Y., Mozes, N., Diab, S. and M. Kochba (1995): Rates of Organic Carbon and Nitrogen Degradation in Intensive Fish Ponds. *Aquaculture*, 134: 214-216.

Babu, B.T. and B.V.S.S.R. Subba Rao (2000): Application of Bioremediation in Sustainable Shrimp Culture. *Fishing Chimes*, 20 (7): 25-27.

Baker, K.H. and D.S. Hersan (1994): Bioremediation. *Mc-Graw Hill, New York*, pp: 375.

Barat, S. and B.B. Jana (1987): Effect of Farming Management on the Distribution Pattern of Ammonification Rates, Protein Mineralizing and Ammonifying Bacterial Population in Experimental Culture Tanks. *Bamidgeh*, 39(4): 120-132.

Bianchi, M., Perfettini, J. and A. Bianchi (1992): Marine Heterotrophic Bacteria Associated with Enrichment Culture of Nitrifying Bacteria Planned for Closed Aquaculture System. *Aquat.* Living *Resour.*, 5(2): 137-144.

Bird, D.F. and J. Kalff (1984): Empirical Relationship Between Bacterial Abundance and Chlorophyll Concentration in Fresh and Marine Waters. *Can. J. Fish. Aquat. Sci.*, 41: 1015-1023.

Botto, K.G. (1988): The Phosphorus Cycle, In: Mangrove Microbiology. *UNDP/UNESCO Regional Project, Research and its Application to the Mangroves of the Asia and the Pacific* (RAS/86/120).

Bouwman, L.A. and J. Bloem (2000): Microbial Indicators of Soil Quality, In: Abstracts of Intercost Workshop on Bioremediation. *Sorrento*, pp: 53-55.

Bower, C.E. and P. Turner (1982): Effect of Seven Chemotherapeutic Agents on Nitrification in Closed Seawater Culture Systems. *Aquaculture*, 29: 331-345.

Boyd, C.E., Hollerman, W.D. and Plumb, J.A. and M. Saeed (1984): Effect of Treatment with a Commercial Bacterial Suspension on Water Quality in Channel Catfish Ponds. *Prog. Fish Culturist*, 43: 36-40.

Briggs, M.R.P. and S.J. Funge-Smith (1994): A Nutrient Budget of Some Intensive Marine Shrimp Ponds in Thailand. *Aquacult. Fish. Management*, 25: 789-811.

Burlage, R.S., Atlas, R., Stahl, D., Geesy, G. and G. Sayler (1999): Theoretical and Applied Aspects of Bioremediation, In: Techniques on Microbial Ecology. pp. 356-362.

Burrows, R.E. and B.D. Combs (1968): Controlled Environment for Salmon Propagation. *Prog. Fish Culturist*, 30: 123-136.

Cappenberg, T.E. (1972): Ecological Observation on Heterotrophic, Methane Oxidizing and Sulphate Reducing Bacteria in a Pond. *Hydrobiologia*, 40: 471-485.

Chakrabarty, A.M. (1992): Bioremediation: How does the Environment Modulate Microbial Gene Evaluation?, In: Harnessing Biotechnology for the 21st Century. *American Chemical Society Conference Proceedings Series, American Chemical Society, Washington DC.*, pp: 422-426.

Chandrika, V. (1999): Incidence of Antagonistic *Bacillus* sp – an Ecofriendly Aquatic Probiotic from Aquaculture Ponds, In: Fourth Indian Fisheries Forum Proceedings. pp: 147-150.

Chiayvareesajja, A. and C.E. Boyd (1993): Effects of Zeolite, Formalin, Bacterial Augmentation and Aeration on Total Ammonia Nitrogen Concentrations. *Aquaculture*, 116: 33-45.

Collins, M.T., Gratzek, J.B., Shotts, E.B., Dawe, D.L., Campbell, L.M. and D.R. Senn (1975): Nitrification in an Aquatic Recirculating System. *J. Fish. Res. Board Canada*, 32: 2025-2031.

Cookson, J.T. (1995): Bioremediation Engineering – Design and Application. *Mc-Graw Hill, New York.*

Ehrlich, K.F., Cantin, M.C. and Horsefall (1988): Bioaugmentation: Biotechnology for Improved Aquaculture Production and Environmental Protection, In: Aquaculture Engineering Technologies for the Future. pp. 329-341.

Engel, H. (1958): Nitrification, In: Handbuch der pflangen Physiologic. *Springer, Heidelberg*, pp: 1107-1127.

Fry, J.C. (1987): Functional Roles of the Major Groups of Bacteria Associated with Detritus, In: Detritus and Microbial Ecology in Aquaculture. *ICLARM Conference Proceedings, Manila, Philippines*, pp: 83-122.

Funge-Smith, S. and S. Hawthorn (1996): The Effect of Bacteria Remediation Products on Water Quality in the Presence of Shrimp Sediments. *Abstracts of World Aquaculture Society*, pp: 133.

Goldschmidt, V.M. (1954): Geochemistry. *Clarendon Press, Oxford, UK.*

Herbert, R.A. (1975): Heterotrophic Nitrogen Fixation in Shallow Estuarine Sediments. *J.Experiment. Mar. Biol. Ecol.*, 18: 215-225.

Herbert, R.A. (1982): Nitrate Dissimilation in Marine and Estuarine Sediments, In: Sediment Microbiology. *Academic Press, London*, pp: 53-72.

Jetter, R.M. and J.L. Ingraham (1981): The Denitrifying Prokaryotes, In: The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria. *Springer-Verlag, Berlin*, pp: 913-925.

Jorgensen, B.B. (1977a): The Sulphur Cycle of a Coastal, Marine Sediment (Limfjorden, Denmark). *Limnol. Oceanogr.*, 22: 814-832.

Jorgensen, B.B. (1977b): Bacterial Sulphate Reduction within Reduced Microniches of Oxidized Marine Sediments. *Mar. Biol.*, 41: 7-17.

Joye, S.B. and J.T. Hollibaugh (1995): Influence of Sulphide Inhibition of Nitrification on Nitrogen Generation in Sediments. *Science*, 270: 623-625.

Kaplan, W.A. (1983): Nitrification, In: Nitrogen in the Marine Environment. *Academic Press, London*, pp: 139-190.

Kuenen, R.D. and J.C. Gottschall (1982): Competition Among Chemolithotrophs and Methylotrophs and Their Interactions with Heterotrophic Bacteria, In: Microbial Interactions and Communities. *Academic Press, London*, pp: 153-188.

Lakshmanaperumalsamy, P., Chandramohan, D. and R. Natarajan (1975): Studies on the Nitrogen Fixation by Marine Nitrogen Fixing Bacteria. *Bull. Dept. Mar. Sci. Univ. Cochin*, 8: 103-116.

Li Zhuojia, Zhang Qing and Yang Huaquan (1997): The Effect of the Probiotics to the Shrimp Ponds. *Aquaculture of China*, 5: 30-31.

Lightner, D.V. (1993): Diseases of Cultured Penaeid Shrimp, In: CRC Handbook of Mariculture, 2nd edn. Vol. 1, Crustacean Aquaculture. CRC Press Inc., Boca Ratan, FL, pp: 393-486.

Moriarty, D.J.W. (1986): Bacterial Productivity in Ponds Used for Culture of Penaeid Prawns, Gelang Petals, Malaysia. *Microbiol. Ecol.*, 12: 259-269.

Moriarty, D.J.W. (1996): Microbial Biotechnology: A Key Ingredient for Sustainable Aquaculture. *Infofish International*, 4/96: 29-33.

Nedwell, D.B. (1982): The Cycling of Sulphur in Marine and Freshwater Sediments, In: Sediment Microbiology. *Academic Press, London.*

Nedwell, D.B. (1984): The Input and Mineralization of Organic Carbon in Anaerobic Aquatic Sediments. *Adv. Microb. Ecol.*, 7: 93-132.

Novitsky, J.A. (1983): Heterotrophic Activity Throughout a Vertical Profile of Seawater and Sediment in Halifax Harbour, Canada. *Appl. Environ. Microbiol.*, 45: 1753-1760.

Olson, R.J. (1981): Differential Photoinhibition of Marine Nitrifying Bacteria: A Possible Mechanism for the Formation of the Primary Nitrite Maximum. *J. Mar. Res.*, 39: 227-238.

Pike, E.B. (1975): Aerobic Bacteria, In: Ecological Aspects of Used-water Treatment, Vol. 1, The Organisms and Their Ecology. *Academic Press, London*, pp: 1-63.

Porubcan, R.S. (1991a): Reduction of Ammonia Nitrogen and Nitrite in Tanks of *Penaeus monodon* Using Biofilters Containing Processed Diatomaceous Earth Pre-inoculated with Nitrifying Bacteria, In: Programme and Abstracts of the 22nd Annual Conference and Exposition. *World Aquaculture Society, Baton Rouge, Louisiana.*

Porubcan, R.S. (1991b): Reduction in Chemical Oxygen Demand and Improvement in *Penaeus monodon* Yield in Ponds Inoculated with Aerobic *Bacillus* Bacteria, In: Programme and Abstracts of the 22nd Annual Conference and Exposition. *World Aquaculture Society, Baton Rouge, Louisiana.*

Prabhu, N.M., Nazar, A.R. Rajagopal, S. and S. Ajmal Khan (1999): Use of Probiotics in Water Quality Management during Shrimp Culture. *J. Aqua. Trop.*, 14 (3): 227-236.

Primavera, J.H. (1994): Environmental and Socio-economic Effects of Shrimp Farming: the Philippine Experience. *Infofish International*, 1/94: 44-49.

Pschenin, L.N. (1963): Distribution and Ecology of Azotobacter in the Black Sea, In: Symposium on Marine Microbiology. *Thomas Springfield, Illinois*, pp: 383-391.

Rheinheimer, G. (1992): Aquatic Microbiology. *4th Edition, John Wiley and Sons, Chichester*, pp: 363.

Rudd, J.W.M. and R.D. Hamilton (1975): Factors Controlling Rates of Methane Oxidation and the Distribution of Methane Oxidizers in a Small-stratified Lake. *Arch. Hydrobiol.*, 75: 522-538.

Scott, K.R. and D.C. Gillespie (1972): A Compact Recirculation Unit for the Rearing and Maintenance of Fish. *J. Fish. Res. Board Canada*, 29: 1071-1074.

Sepers, A.B.J. (1981): Diversity of Ammonifying Bacteria. *Hydrobiol.*, 83: 343-350.

Shan, S. and J.P. Obbard (2001): Ammonia Removal from Prawn Aquaculture Water Using Immobilized Nitrifying Bacteria. *Appl. Microbiol. Biotechnol.*, 57: 791-798.

Sisler, F.D. and C.E. Zobell (1951): Nitrogen Fixation by Sulphate Reducing Bacteria Indicated by Nitrogen/Argon Ratios. *Science*, 113: 511-512.

Smith, C.E., Piper, R.G. and H.R. Tisher (1981): The Use of Chinoptilolite and ion Exchange as a Method of Ammonia Removal in Fishing Culture Systems. *US Fish and Wildlife Service, Fish Cultural Development Centre, Bozeman Information Leaflet, Bozeman, Montana.*

Spotte, S. (1979): Fish and Invertebrate Culture: Water Management in Closed Systems. *2nd Edition, John Wiley and Sons, New York.*

Stal, L.J., Grossbarger, S. and W.E. Krumbein (1984): Nitrogen Fixation Associated with the Cyanobacterial mat of a marine laminated Microbial Ecosystem. *Mar. Biol.*, 82: 217-240.

Suplee, M.W. and J.B. Cotner (1996): Temporal Changes in Oxygen Demand and Bacterial Sulphate Reduction in Inland Shrimp Ponds. *Aquaculture*, 145: 141-158.

Thomas, G.M., Ward, C.H., Raymond, R.L., Wilson, J.T. and R.C. Loehr (1992): Bioremediation, In: Encyclopedia of Microbiology. *Academic Press, London*, pp: 369-385.

Truper, H.G. and S. Genovese (1968): Characterization of Photosynthetic Sulphur Bacteria Causing Redwater in Lake Faro. *Limnol. Oceanogr.*, 13: 225-232.

Tucker, C.S. and S.W. Lloyd (1985): Evaluation of Commercial Bacterial Amendment for Improving Water Quality in Channel Catfish Ponds. *Miss. State Univ. Res. Rep.*, 10: 1-4.

Watson, S.W., Valois, F.W. and J.B. Waterbury (1981): The Family of Nitrobacteriaceae, In: The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria. *Springer-Verlag, Berlin*, pp: 1005-1022.

Wetzel, R.G. (1983): Limnology. *2nd Edition, W.B. Saunders, Philadelphia*, pp: 767.

Wyn-Williams, D.D. and M.E. Rhodes (1974): Nitrogen Fixation in Seawater. *J. Appl. Bacteriol.*, 37: 203-216.

Zobell, C.E. and H.C. Upham (1994). A List of Marine Bacteria Including Description of Sixty New Species. *Bull. Scripps Inst. Oceanogr.*, 5: 230-292.

AQUACULTURE AND FISHERIES ENVIRONMENT

The present book mainly deals with aquaculture and fisheries environment and updates the subject matter and problems to incorporate new concepts and issues related to aquaculture and fisheries environment. The extensive use of illustration is intended to increase the understanding and the concepts in context of the modern scenario. The book includes chapters contributed by outstanding experts and scientists from recognized institutions. This book would be of immense benefit to researchers, scientists, academician, students, entrepreneurs and fishers working in the field of aquaculture, limnology, freshwater ecology, aquatic ecosystem, environmental pollution and fisheries.

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