Winter School on

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

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Course Manual

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ENVIRONMENTAL MICROBES AND ITS ROLE IN HEALTH MANAGEMENT

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Microorganisms

Microorganisms are of major important in aquaculture and industrial wastewater treatment. They reside in the sediment and other substrates, and in the water of aquaculture facilities, as well as in and on the cultured species. Microorganisms may have positive or negative effects on the outcome of aquaculture operations. Positive microbial activities include elimination of toxic materials such as ammonia, nitrite, and hydrogen sulfide, degradation of uneaten feed, and nutrition of aquatic animals such as shrimp, fish; production of aqua-farmer. These and other functions make microorganisms key players in the health and sustainability of aquaculture. Yet, microorganisms are among the least known and understood elements in aquaculture. Like other areas in aquaculture, microorganisms require management and Manipulation.

Sub Contents:

- **Major Microbial Groups**
- **Viruses**
- **Algae**
- **Fungi**
- **Protozoa**
- **Bacteria**
- **Microbial Process**
- **Aerobic Microbial Process In Aquaculture**
- **Anaerobic Microbial Process In Aquaculture**

Major microbial groups

The world of microorganisms is made of bacteria, fungi, algae, protozoa, and viruses. They are grouped together only because of their small size, and not by their function. If, for example, the same taxonomical rules were applied to larger animals, some fish, shrimp, green plants, birds and mammals would be grouped together. Some microorganisms such as viruses, bacteria, and protozoa are notoriously small, under one mm. Others, like algae and fungi, have large size relatives (such as the brown algae that is among the largest living organisms). Unlike larger organisms, the morphology of microorganisms is relatively poor and is confined to few shapes and colors. However, their poor morphology is compensated by great physiological versatility.

Viruses

Viruses are very small, ranging between 0.01 and 0.03 microns, and only visible by using an electron microscope. They cannot live independently, and only multiply inside the cells of other organisms. However, their demand for a host is fairly specific. For example, it is unlikely that a crustacean virus will attack humans or fish. Viruses are also the simplest of all organisms and are made of nucleic acid (either DNA or RNA), frequently coated with a protein layer. Whenever not infected, they remain as crystals in solutions.

Algae

Algae are photosynthetic organisms (contain chlorophyll) and obtain their energy from the sun and their carbon from carbon dioxide. Their size ranges from one micron to many meters. All organisms that use carbon dioxide for their carbon requirement are called autotrophs. Algae are generally beneficial in aquaculture by supplying oxygen and a natural food base for the cultured animals. such as dinoflagellates that cause the red ties.

Fungi

Fungi are similar to algae, but they do not contain chlorophyll and require pre-formed organic matter as energy and carbon sources (e.g., sugars, fat, protein, and other carbohydrates). Such organisms are called heterotrophs. Fungi, ranging in size from a few microns to several centimeters, grow either independently by feeding on decaying matter, or in association with plants and animals.

Protozoa

Protozoa are heterotrophs, mostly free-living, feeding mainly by devouring smaller microorganisms. Their size ranges between two and 200 microns. A large group of protozoa, the Sporozoa, are parasites. Small numbers of protozoa contain chlorophyll and can switch between autotrophic and heterotrophic modes of feeding, based on light conditions.

Bacteria

Bacteria range in size from 0.1 to 15 micron, with some " giants "that may reach half a millimeter. They make up the most metabolically diverse group of living organisms. Although some are parasitic to animals and plants, the majority of bacteria are free-living, having either a neutral or beneficial relationship with humans and other animals and plants. Their metabolic versatility is incredible: while most are heterotrophs, using either light or chemical energy. One of their most remarkable characteristics is their ability to multiply rapidly, with generation times usually ranging between minutes to hours.

Microbial process

Bacteria and other microorganisms, most notably fungi, are able to metabolize and transform numerous organic and inorganic compounds. Therefore, man has used them for thousands of years for making yogurt, pickles, bread, cheese, wine, and more recently for making probiotics and immunostimulants waste purification and wastewater purification. Processes controlled by microorganisms can occur aerobically (in the presence of oxygen) or anaerobically (with no oxygen present). The starting materials and the end products of such processes vary based on the microorganisms' capabilities (as reflected in their genetic makeup), and the environment in which these processes occur (e.g., availability of oxygen, temperature, salinity, pH, etc.

Aerobic microbial process in aquacultutre

Generally, aerobic microbial processes yield compounds which can be beneficial, and are either not toxic or have lox toxicity levels in aquaculture ponds or tanks. Oxidation of organic matter to carbon dioxide, a process which is the main consumer of oxygen in aquaculture ponds or tanks. Oxidation of ammonia to nitrate via nitrite, which also consumes large quantity of oxygen Oxidation of reduced sulfur compounds (such as hydrogen sulfide and elemental sulfur) to sulfate, a process that generally has low oxygen demand in aquaculture. Conversion of carbon dioxide to biomass by autotrophic bacteria (such as the nitrifying bacteria) with a relatively small amount of biomass produced in aquaculture facilities, when compared to the conversion of carbon dioxide to biomass by algae. 30% of primary productivity only comes from bacteria using bacteriochlorophyll.

Conversion of carbon dioxide to biomass by algae depending on the availability of light. Excluding feeding, the photosynthetic process in aquaculture is the main input of carbon source and natural food for aquatic animals.

Anaerobic microbial processes in aquaculture

Microbial anaerobic processes, if not controlled, can produce compounds and gases like CO_2, NH_3, H_2S, CH_4 that are highly toxic to cultured animals. These processes include:

Consumption of organic matter, without the utilization of free oxygen, resulting in products which are usually not fully oxidized (such as alcohols, organic acids). Reduction of nitrate and nitrite, which can yield either nitrogen gas or ammonia. In aquaculture, due to the toxicity of ammonia and nitrite, ammonia production is not good, while nitrogen gas production is beneficial. However, in agriculture, the reverse is true - the conversion of nitrate and nitrite to nitrogen gas result in a loss of fertilizers. Reduction of sulfur compounds to hydrogen sulfide as a final product, a compound, which is toxic to most animals even at very low concentrations.

Microbial bio-technology & sustainable aquaculture

So far, aquatic microbiological activity studies in the country was confined to SANITATION AND POLLURION ASPECTS. Only in recent years studies were initiated with reference to culture productivity. Water quality and control of disease are inter dependent and linked to microbial and culture animal activities in ponds

Microbial food webs → emerge as the first link joining the living world with abiotic factors.
→ are an integral part of aquatic culture pond
→ has direct impact on primary productivity

For eg: RESPIRATION : predominantly due to bacteria heterotrophic decomposers

Microbial processes : Affect water-quality factors like
(1) Content of O_2
(2) Toxic metabolites NH_3, nitrite & sulphide.

NUTRIENTS like Nitrogen, Phosphorus } are recycled to stimulate primary production, the processes are known as transformation and regeneration.

Recent advances in microbial ecology → enable to study the bacterial community
→ study to activity of bacterial genera.

The results of these studies are Probiotics and Immunostimulants being applied to INTENSIVE Aquaculture. This is an application of microbial bio-technology which is essential for management of grow-out ponds if harvests are to be sustainable.

Processes mediated by aquatic bacteria

HETEROTROPHS consume O_2 release → Co_2 → Dissipatesin to atmosphere
NH_3 While oxidizing organic matter.

In contrast, the autotrophic, nitrifying and sulphur bacteria consume O_2 and Co_2
While oxidizing ammonium, nitrite or sulphide

The end products are → nitrate
→ Sulphate

Only 17% of the feed is consumed by cultured animals. 83% of the feed is fermented by native microbes. As feed contains high protein content ammonium release is inevitable. It must be rapidly taken up by algae or oxidized by nitrifying bacteria to prevent toxicity to the cultured animal. Eg. Shrimp. If nitrate produced is too high, there is a problem towards the end of the grow out. In organic detritus, the pond bottom oxygen diffusion is limited thus O_2 is depleted rapidly. Any nitrate present is used first by many aerobic bacteria in place of O_2 for respiration in the absence of O_2 producing nitrite, ammonia and nitrogen gas.

Importance of redox potential (Eh)

Fermenting bacteria is active releasing → acetic acid
→ alcohol
→ Co_2 & H_2

'O_2 debt" is built up. Sulphate-reducing bacteria is activated when Eh is -- 100 m volts,"SRB"Increased in reduced sediment and Redox potential increases. Poising capacity of the water and sediment is lost. Ammonia & sulphide is not toxic directly if O_2 concentration do not fall below 4ppm in water column. When rate of feeding is high-as in later stages of grow-out excess of leached material in the sediment surface leads to increased heterotrophic bacterial growth, which inevitably causes anoxic condition close to the sediment surface. When O_2 requirement of bacteria exceeds that O_2 diffusion rate in to the sediment. Thus, the cultured shrimp would be exposed to sub-lethal-stressful concentration of toxic level.

Toxic compounds → Sulphide
→ Nitrite
→ Ammonia

At this stage disease resistance decreases in animals and thus, pathogens invade. It is very important to ensure that organic detritus and slime-should not build up in bottom.

In other words all feaces, excess feed, dead algae must be rapidly decomposed preferably in the water column itself. An increase in oxygen concentration & redox potential enhance aerobic oxidative bacterial proliferation from reductive anaerobic flora.

In natural aquatic systems, regulation of bacterial growth, species composition, bacterial growth rate and biomass are controlled by slow rate at which large compounds and polymers such as proteins and starch, cellulose and fats are broken into smaller units.

Dissolved oxygen concentration, pH, essential nutrients like phosphate controlling factors of polymer hydrolysis. At this stage Probiotics will be useful. BACILLUS- Gram –positive, spore-forming microbe with the candidate species being *Bacillus subtilis,B. coagulans* etc. Wide-range of exoenzymes are most efficient in breaking down polymers. Bacillus strains are useful in ponds and hatcheries.

Production, consumption and decomposition are the 3 important eco regulatory and balancing process in a culture pond. However Bacillus are not present in water column as their original "niche" is only sediment. Where exoenzymes are held together close to cells by sediment matrix, which provide direct benefit to the cell colony that secretes them. When certain bacillus are added to water sufficiently-frequently at high enough density, they do make an impact on the available organic matter. The bacterial strains must be adapted to the particular conditions in the pond.

Eg. Strains that produce exoenzymes can degrade the particular biochemicals in the organic detritus. The added bacteria then compete with native bacterial flora, naturally present for the available organic matter- such as leached or excess feed components and faces.

The results is that there is

(1). Less accumulation of slime. (slime can never be completely eliminated)

(2). Lower the number of other bacteria, especially potentially pathogenic bacteria.

(3). Less accumulation of organic matter on the pond bottom-sediment water interface.

Thus there is better penetration of O_2 into the sediment making a better environment in which shrimps can burrow themselves.

Competitive exclusion is one of the ecological processes that can be manipulated to modify the species composition of a sediment and water body and other microbial environment. Small changes in factors that affect growth and mortality rates will lead to changes in species dominance.

We still don't know all the factors that control (1) bacterial species growth rates.

(2) Complete species composition in the natural environments. But,enough is known to suggest that it is possible to change species composition by making use of competitive exclusion principles. The species composition of a microbial community (such as that in a pond) will be determined partly by environmental factors and partly by physiological factors.

Environmental factors favours those organisms that happen to be in the right place at the right time to respond to a sudden increase in nutrients eg: Lysis of the algal cells and decomposition of feed pellets that fall around them. So species composition can be manipulated by seeding large numbers of desirable strains of bacteria and algae.

Bioremediation

Large areas of water and land can be enhanced for their microbial density of a particular number of species and biochemical activities. Bio augmentation is applied to many areas but success varies greatly depending on the natural products used and technical information available to the end-user. The bacteria that are selected for specific functions must be amenable to bioremediation, added at a high enough density under the right environmental conditions. Bioaugmentaion and the probiotics are significant management tools. But the bioconverters efficacy depends on the understanding of the nature of competition between species and strains of bacteria. They rely on the same concept as used successfully for soil bioremediation and probiotics in the animal industry.

Criticism of this practice has been made by various scientists in the world around Saying that Bacterial products tried did not work as claimed. Probably it did not Contain probiotics of right strains in enough number and intensity to be effective or the strains may not be viable. Many suppliers are unaware of the physiological and ecological requirement of the bacteria. For eg: some contain purple sulphur bacteria that will remove sulphide only when conditions are anaerobic and light is present. Such anaerobic conditions will be lethal to cultured animals and should not be present. In a well managed productive pond. The efficacy of products containing nitrifying bacteria was supposed to control ammonium concentrations.

The nitrifiers are autotrophic, and they need CO_2 for their carbon source. They use energy from oxidation of inorganic compounds such as NH_3. Nitrifiers are very difficult to maintain in a condition suitable for storing and for shipment as commercial preparations. The bacteria may not be viable and a number added may be too low, to be effective. They are also slow growing and require O_2. So the conditions that result is in build up of NH_3 and nitrite in pond may well inhibit shrimp activity. In general nitrifier micropulation are best maintained only by pond management.

Continuous supply of inorganic nutrient and O_2 only will allow natural microbes to keep in balance with ammonium supply. Decrease in nitrifiers occurs at times, which will increase in nitrite and ammonium concentration. Rapid response is necessary. Increasing aeration would be insufficient as autotropic nitrifiers have slow generation time. In such a case rapid response could involve adding large inoculums of nitrifiers together with aeration and mixing. All bacteria are present naturally in ponds. Thus adding them is unnecessary is the general criticism. The one's best adapted to the extent of bioaugmentaion conditions would be expected to be dominant and couldn't be replaced and displaced by inoculation. Such statements are not supported by the principles of microbial ecology in both aquaculture and agriculture. In agriculture rhizbium inoculation of legumes, Bacillus inoculation of plant root zones has beneficial results.

Use of DMS-range of bacillus do not have problems from disease caused by luminescent vibrios. The occurrence and distribution of luminous vibrio is inversely correlated to the addition of particular strains of bacillus. Growth Rate-is most important factor influencing the added Bacillus to a natural or semi natural environment will be beneficial and cost effective. Sediment bacteria can degrade any substance that is applied but it is not valid to argue that this is a reason for not adding selected bacteria as only a few indigenous bacteria are presented with the desired ability. Their growth rate may be slow or limited under the existing parameter. It may take several weeks to build up enough to have measurable effects in intensive aquaculture. A farmer-cannot afford to wait for natural process to readjust to new study state conditions. To overcome-the lag time due to low population density-we can add sufficient number of desired bacteria.

The application of biotechnology although not simple are indeed feasible and necessary. As bacterial abundance is controlled by nutrient availability or grazing.
Now Probiotics is the best way to change population density of a particular bacterial species at a given time.

The effectiveness of probiotics is dependent on range of environmental conditions, and we have to take care of side effects. Application of Probiotics to aquaculture is fraught with difficulties. Cross-reactions or resistance is to be studied. Taste of the final product will be different. Next we have to do research to improve the taste of fish and shrimps. The field of microbial ecology will progress with the requirements of the aquaculture industry

"El Nino" and shrimp farming

Every three or four years an "EL Nino" arises in tropical waters. The 1991-1993 'EL Nino' (of average intensity but unusually long) triggered major weather changes and disrupted shrimp farming on a global basis. Temporary weakening of trade winds and warming of surface waters in the Eastern and Central Pacific Ocean near the equator. These changes can lead to heavy rainfall and flooding in the Eastern Pacific, while at the same time cause drought conditions in the Western Pacific (Australia and Indonesia). Altered weather patterns are not guaranteed, but their likelihood increased during an EL Nino event.

In the past 98 years, there have been 23 EL Ninos. Four of the four strongest have occurred since 1980. But no one knows whether this indicates a trend on its supply a meaningless random clustering.

Heavy rains lowers the salinity, blue green algae flair up and when ingested by the shrimp produce an off-flavour called "Choclo".

A to Z in Aquaculture

A Assess and reduce the environmental impacts of aquaculture in grow out

B *Bacillus Subtilis*, can be used as probiotics and for dioremediation.

C Crop rotation is a good method for sustainability than crop holiday.

D Denitrification is a micro-aerophilic phenomenon.

E Emerging application of biotechnological methods & diagnostics environmental microbes as influenced aquaculture

F Fish farmer expects a feed with good 'FCR' potential

G Genes and genetic engineering has a role in aquaculture

H High stocking density leads to collapse of aquaculture programmes

I In intensive farming best studied Iron bacteria is the sheathed bacteria, that reduces the 'vibrio' infection

J " Jelly Fish" threat is heavy to Norwegian fish farming centers

K Kelp is very good for inducing Kanagana positive Vibrio by crop rotation technique

L Lactic acid bacteria are very good supplement in shrimp farming practices.

M 'Microcosm' like mangroves are also suitable for culture programmes.

N *Nitrobacter* and N*itrosomonas* are highly fastidious forms which are inhabiting the biofilter in aquaculture ponds.

O Oxygen is important to boost the immune system of culture animals.

P Purple sulphur photosynthetic bacteria ' PSB' increase indirectly the physiological activity of animals.

Q Quick results can be obtained by using Quinolones than Antibiotics

R Redox potential, oxygen tension, light and other factors influence the in black-mud formation in ponds.

S Standards for water quality in aquaculture is given by (ISI) Water Pollution Board

T Turbidity is the main factor affecting Blue-Green Microbes activity in surface waters and imbalance helps Leptothrix, Beggiatoa to establish themselves in sediment water interface.

U Ultraviolet sterilized water should be kept in dark otherwise pathogens DNA will be photoreactivated in light.

V Viral disease and Vibrio are the main reason for collapse of aquaculture programmes.

W WSSV Virus and White Sulfur bacteria 'Leptothrix' are prevalent in turbid waste water.

X Xenobiotic compounds are degraded by *Bacillus subtilis*.

Y Yeast is one of the probiotic for aquaculture progarmme

Z Zymogenous microbes are the only answer for bio-control management of aquaculture.

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GENERAL HATCHERY PROFILE FOLLOWED IN AND AROUND COCHIN.

I. General hatchery profile:

I. How many years have been in business
  a) 10 yrs
  b) b) <3 yrs

II. Species produced
  a) *Penaeus monodon*
  b) *Penaeus indicus*

III. Production capacity
  a) 5 million/month
  b) 10 million/month
  c) 20 million/month
  d) 50 million/month
  e) Others

IV.

A. Number of larval rearing tanks have been used.
  a) 10-20
  b) 20-50
  c) >50

B. Capacity of larval rearing tanks.
  a) 5 m^3
  b) 5-10 m^3
  b) 11-15 m^3
  c) 16-20 m^3
  e) 20-50 m^3

V. Maturationg and spawning source
  a) Brood stock maturation and spawning
  b) In house breeding programme
  c) Mature females from wild

VI.

A. Water treatment methods followed
  a) Chlorination & UV irradiation
  b) Ozomation

B. Whether disinfection method by bacterial plate counts are used or not
  a) Yes
  b) No

VII. Discharge treatment monitoring
  a) Sedimentation/oxidation ponds
  b) Mechanical filtration
  c). Injection wells
  d) Discharge to mangrove areas

e) None of the above methods

VIII. Disease sanitation

A. Viral screening practices

a) Yes
b) No

If yes, which of the following viruses are being checked.

1. White spot syndrome virus
2. Taura syndrome virus
3. Infectious hypodermal and haematopoetic necrosis virus
4. Yellow head virus
5. Hepatopancreatic parvo like virus

B. Methods for viral screening

a) Dot Blot test
b) PCR

IX. Routine production, sanitation

A. Whether drying out the tanks between each production cycle.

a) Yes
b) No

B. Disinfection of Nauplii by

a) Rinsing nauplii in clean sea water, before stocking in L.R.T
b) External disinfection of nauplii by iodine compounds.
c) Prophylactic nauplii treatments.

C. Nauplii density/litre.

a) <50
b) 50-100
c) 100-125
d) 125-150
e) 151-200
f) >200

X. Product quality assessment

A. Methods have been used to assess PL quality

a) Stress test
b) Evaluation of gill development
c) PL size
d) External fouling
e) Activity
f) Muscle to gut ratio
g) Evaluation for deformities and gut lipid content

XI. Biosecurity application methods by

a) Controlling movement of potential disease carriers
b) Disinfection of water and equipment in a multiple points of use.
c) Preventing contamination by workers through use of special clothing and foot dip.
d) Controlling airflow between hatchery sections.

XII. Feeding practices

1. A. Micro algae used
   - *a) Chaetoceros*
   - *b) Thalassiosira* or *Tetraselmis*
   - c) *Isochrysis* and *dunaliella*

B. Methods of algal culture practiced
   - a) Batch culture of algae
   - b) Continuous culture method
   - c) Alage paste concentrates

2. Artemia

1. Concentration of artemeia cysts used for producing million PLs
   - a) 1-3 kg/million PLs
   - b) 4-6 kg/million PLs

3. Replacement diets

A. Types of replacement diets have been used.
   - a) Microencapsulted diets
   - b) Micro particulate diets
   - c) Liquid diets
   - d) Flake diets

B. Enrichment routs have been applied for replacement diets
   - a) With highly unsaturated fatty acids (HUFA)
   - b) With immunostimulants
   - c) With antibiotics.