# **ARTEMIA CULTURE AND CYST PRODUCTION**

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# INTRODUCTION

The brine shrimp, *Artemia* is a unique marine organism which can remarkably withstand and survive in a wide range of salinity. The eggs of the animal remain viable in the form of dry cysts for many years and can produce tiny nauplii within 24 hours when immersed in sea water. It is widely distributed and more than 50 strains have so far been recorded from about 300 natural biotopes spread over the five continents. The importance of the brine shrimp was realized during the 1930s when its five nauplii were identified as ideal feed for the larvae of crustaceans. Since then the demand for the cysts of brine shrimp is increasing and with the expansion of aquaculture activities and establishment of more and more hatcheries, the demand for the cysts is exceeding the supply. At present, mostly the cysts harvested from the wild are being used in various hatcheries. However, as the cysts harvested from the wild are inadequate to meet the growing demand due to the expansion of aquaculture activities, effort is being made in different parts of the world to produce the cysts under controlled conditions.

Apart from the nauplii obtained from the cysts which are used as feed for the larvae of crustaceans and fishes, the pre-adults and adults of brine shrimp are valued as protein rich food for the juveniles. It is in this context the culture of brine shrimp for the production of both cyst and biomass assumes great significance in the field of aquaculture. In India the distribution of brine shrimp has been reported from Tamil Nadu, Maharashtra, Gujarat and Rajasthan. As the salt pan area in Tuticorin (Tamilnadu) is one of the natural biotopes of the brine shrimp, the Tuticorin Research Centre of the Central Marine Fisheries Research Institute is actively engaged in the intensive culture of brine shrimp with the primary objective of producing cysts and biomass under controlled environmental conditions

### REPRODUCTION

A knowledge of the reproductive biology of the animal is essential to carry out the culture operation successfully. There are two types of reproduction in brine shrimp viz. i) viviparous and ii) oviparous. The eggs develop in the paired ovaries which are situated on either side of the digestive tract behind the thoracopods. Under normal environmental conditions the eggs develop into nauplii within the ovary and are set free by the mother. This is called viviparous reproduction. However, under extreme environmental conditions such as high salinity and low oxygen levels, the embryonic

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development stops at gastrula stage and the eggs are surrounded by a thick shell or chorion. The cysts thus formed within the ovary of the animal are released into the water. The cysts normally float on the surface and are washed ashore in the salt lakes and salt pans by wave action. These cysts remain dormant "(diapause) as long as they are in dry condition. When such cysts are hydrated with sea water, embryonic development starts and free-swimming nauplii emerge by breaking the shell-membrane. The first instar of brime shrimp nauplius which measures about 500u. moults into instar II after about 12 hours. The animal moults about 15 times and reaches adult stage within 2 weeks. Thereafter, it lives for several months and reproduces about 300 cysts or nauplii every five days depending upon the environmental conditions.

# **BRINE SHRIMP ECO-SYSTEM**

As environmental factors play an important role in the mode of reproduction of brine shrimp, an understanding of the same is necessary to achieve optimum production of biomass and cysts under natural as well as controlled environmental conditions. In one of the natural ecosystems at Tuticorin some hydrographical features namely, salinity, dissolved oxygen and pH were studied in detail for a period of 17 months. During the entire period of observation the salinity ranged from 46 to 138 ppt. dissolved oxygen from 0.5 to 3.5 ml/1 and pH from 7.5 to 9.0. A large number of nauplii and juveniles were found swarming the pond in several points during November and December when the salinity was less (46-55 pt)

### PRODUCTION

At Tuticorin, production of biomass and cysts of brine shrimp under controlled conditions is being carried out by following Batch culture method and Flow-through culture method.

### **Batch Culture Method**

In the Batch culture system, the culture operation is carried out in a rectangular tank with a central partitioning. In order to provide optimum water circulation the central partitioning should be arranged in such a way that it is not closer to the walls of the small side than the width of the channel. Further, the partitioning should also 'be kept about 5 cm above the bottom. This may be done by suspending the partition with the help of a wooden bar resting on the walls of the tank. Commonly available cheap materials like asbestos sheets may be used to construct the raceway system.

The water in the tank is aerated by air-water lift system which may be constructed with PVC pipes and elbows. This system ensures both horizontal and vertical movement of the water resulting in a screw-like flow pattern in the culture tank. It is advisable to prevent formation of tiny air bubbles as they may affect the animals. For the same reasons air stones should be avoided in the brine shrimp culture tanks. It has been reported that optimum circulation and aeration are obtained when AWL pipes are installed at about 30 cm intervals. In order to assure the maximum water lift effect, the diameter of the AWL pipes should be related to water depth. For instance, if the water level is 40 cm the inner diameter of the AWL should be 40 mm which will provide 6.6 litre of air per minute to displace 12.5 litres of water per minute. To ensure the best water-lift effect, the aeration lines should extend as deep as possible in the AWL system.

#### Flow-through culture method

This resembles the Batch culture method. The main difference in this system is that the medium is continuously renewed as against the former system where the same water is used. In the flow through system the water change results in the removal of all particulate dissolved materials thus providing a better environment for higher production.

#### Inoculation

Adult brine shrimps where initially collected from a salt pan alongwith brine having a salinity of 190 ppt. and transferred to a cement tank. The salinity of the brine was gradually reduced to induce viviparous mode of reproduction. Within few days good concentration of nauplii was noticed. The nauplii were reared to adult stage by feeding them with rice bran extract. As this and the subsequent generations also underwent viviparous mode of reproduction resulting in a large number of nauplii, the animals were segregated to different tanks containing brine of various salinity levels for largescale production of biomass and cysts under controlled environmental conditions.

Although brine shrimps are distributed worldwide, their cysts are commercially exploited only from a very few places viz. salt lakes in Sasketchewen, Canada; Great Salt Lake, Utah, USA and salt pan areas in the San Francisco Bay, California, USA. As cyst production under controlled conditions has not yet been achieved on a commercial scale in any part of the world due to various limitations, the wild cysts collected mostly from these places are sent to different parts of the world to meet the demand of various hatcheries. Although it is possible to produce cysts under controlled environmental conditions by subjecting the animals to salinity stress, the quantum of production depends to a very great extent upon the volume of brine used and the quality of the feed supplied besides the prevailing climatic conditions. It is for such reasons the production of cysts on commercial scale has not yet been accomplished although cysts have been produced successfully in various parts of the world in a limited scale under controlled conditions.

#### **Quality of Cysts**

As the cysts of brine shrimp are collected mostly from the wild, they may contain certain impurities viz. fine sand, salt crystals etc. Hence an assessment of the quality of the cysts in terms of its hatching efficiency (i.e. the number of nauplii hatched/g of cysts) as well as hatching percentage (percentage of cysts that actually hatch out) are important. The hatching efficiency may vary from strain to strain. For instance, the hatching efficiency of the cysts collected from Chaplin Lake, Canada may be as low as 65,600 nauplii/g.of cysts. But the cysts collected from Great Salt Lake may yield as high as 2,50,000 naupliiand Brazilian and Vietnamese cysts may have even higher efficiency of producing 3,00,000 nauplii. Hatching synchrony also must be high. When the cysts are incubated the first nauplius should appear within 16 hours and the last one within 24 hours. When the hatching synchrony is poor, the first nauplius would have consumed much of its energy before the last nauplius hatches out.

# **Hatching of Cysts**

To obtain maximum hatching rate the container used for hatching must be cylindroconical. The salinity of the medium should be slightly less than that of sea water. Optimum temperature for maximum hatching rate is 30°C. The pH value of the water should be in the range of 8-9. The dissolved oxygen content should be main-tained at saturation level. Continuous illumination of about 1000 lux is required. The quantity of cysts released for hatching should not exceed 10g/litre. The nauplii should be harvested as soon as they are hatched out because the dry weight and caloric value of the brine shrimp nauplii decrease by 20% and 27% respectively after second moulting which normally occurs within 24 hours.

#### **Decapsulation of Cysts**

The chorion of the brime shrimp cysts is removed without affecting the viability of the embryos by a process known as decapsulation. The decapsulated cysts can be supplied as feed for shrimp larvae. The process of decapsulation involves hydration of the cysts and removal of the chorion in a hypochlorite solution. For decapsulation of 10g of cysts, a solution is prepared by dissolving 5 g of bleaching powder in water and aerating it for about 10 min. Then either 7 g of sodium carbonate or 3 g of calcium oxide is added to the solution and it is aerated again for about 10 min. After keeping the solution overnight, the supernatant is taken for decapsulating the cysts. The decapsulated cysts have several advantages viz. i) they can be fed to the larvae directly, ii) the cysts get disinfected and iii) there is no need to undergo the complicated process of separating the cysts from the hatched nauplii.

#### **Conditions required for culture**

High levels of suspended solids in the medium affect the production of brine shrimps due to bacterial growth. The soluble waste products also spoil the medium as they result in the formation of toxic nitrogenous compounds. Hence, care must be taken to avoid these two factors while feeding the organisms. The brine shrimp is a non-selective particle filtering organism. It is a continuous feeder and can feed on microflora and any inert particle in the size range of 1 to 50 microns. Dried algal meal and nonsoluble waste products from agricultural crops or from the food processing industry (e.g. rice bran, corn bran) have been found to be very suitable feed source for high density culture of the brine shrimp. The optimum temperature for culture should be between 25 and 30°C; the salinity level between 80 and 130 ppt; pH between 6.5 and 8.0 and dissolved oxygen concentration in the range of 2.5 to 5 ppm.

### **DISEASES AND THEIR CONTROL**

The common diseases encountered in the brine shrimp culture systems are mostly caused either by dietary deficiency or by excess of nutrient rich feed which affects the medium. The "black disease" with black spots in the thoracopods and antennae appear due to dietary deficiency which affects the lipid metabolism. This disease can be controlled by giving the right quantity and quality of feed to meet the lipid requirements of the organism. On the other land, in nutrient rich medium the bacteria *Leucothrix* colonizes mostly on the thoracopods affecting the filtration rate initially and growth and moulting subsequently which ultimately results in the mortality of the organism. The colonization of the bacteria can be arrested by renewing about 25% of the medium every week.

# **PRODUCTION POTENTIAL**

As natural population of brine shrimp thrives well in salt lakes and salt pans, it is possible to culture them profitably in places like Tuticorin where hundreds of hectares of salt pans are in operation. In such areas the biomass and cyst of the brine shrimp can be produced by fertilizing the ponds and inoculating brine shrimp nauplii after making necessary modifications of the existing salt pans. The culture of brine shrimp in salt pan will assure an additional income to the salt pan owners. The rate of production from the salt pan may vary widely as the culture operation has to be carried out under natural environmental conditions which may fluctuate from year to year. But under controlled conditions a guaranteed production of 5 kg of biomass of water can be obtained in 2 weeks by feeding the animals with cheap agricultural byproducts under batch culture method. It has been reported that the production can be increased upto 25 kg/m3 of water/2 weeks by feeding with live algae under flow through culture method. However, the cost of input will be very high due to the additional expenditure involved in the production and maintenance of live algae. Hence it is suggested that the former method may be adopted to minimize the cost of input. As the brine shrimp grows from naplius to adult stage in a short period of 2 weeks and increases its weight by nearly 500 times by feeding on cheap feed materials, it is possible to carry out the culture operation profitably.



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