SALT POND BIOTECHNOLOGY RESOURCES: CURRENT RESEARCH TRENDS AND APPLICATION OF BRINE SHRIMP

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Abstract: Large numbers of solar saltworks are located in the coastal line of 18,000 km of China. The total area of the costal saltworks was expanded to 408,367 ha in 2004. The particular ecological and climatic conditions of the solar saltworks created a diversity of living organisms which are rich in salt ponds, brine shrimp Artemia is one of these organisms and plays an important role in the biological system of solar saltworks. And it has been exploited as a food source for aquaculture during past several decades. The current research trends and application of the brine shrimp Artemia was introduced in this paper.

Keywords: brine shrimp, salt pond biotechnology, live food, biological balance

INTRODUCTION

The genus Artemia, brine shrimp, a small branchiopod crustacean, common saying "Feng Nian Chong" in Chinese (a animal creating an abundant year), is in phylum Arthropoda, class Crustacea, subclass Branchiopoda, order Anostraca, family Artemiidae, genus Artemia. Brine shrimp Artemia is distributed widely over the world and is found in about 500 natural salt lakes and man-made salterns scattered throughout the tropical, subtropical and temperate climatic zones, along coastlines as well as inland.

BIOLOGY OF ARTEMIA

Brine shrimp Artemia reproduces on a special reproduction mode and life circle. Under the optimal conditions (e.g. salinity, temperature, food etc), brine shrimp is in the ovoviviparous reproduction, the fertilized eggs normally develop into free-swimming nauplii inside ovary, which are directly released into the water by the mother animals, hence results in a rapid expansion of Artemia population. Under the extreme conditions (e.g. high salinity, low oxygen levels), brine shrimp change its reproduction mode to oviparous reproduction. Fig.1 shows a typical schematic diagram of solar salt operation with natural occurrence of Artemia. Ovoviviparous reproduction is dominant in low and intermediate salinity ponds. Brine shrimp change its reproduction mode to oviparous reproduction and release resting eggs (winter eggs or Artemia cysts) when salinity reaches to 15%. Hydrated cyst has round shape, brown color and is covered by a thick shell that can resist extreme temperature and drought and make the cyst survive over a long winter season in salt ponds. When the water temperature rises up in spring, these cysts hatch into nauplii in salt ponds, in which they grow up to adults that reproduce on a viviparous reproduction for a season. The first generation of Artemia population appears in a period from the end of April to the beginning of May in northern saltworks of China, and Artemia population gradually disappears in the end of October when air temperature is decreasing. A nauplius grows into adult through about 15 molts. Normally it takes 10 to 15 days according to water temperature and food availability. The optimal temperature for brine shrimp ranges from 20 to 30°C, temperature above 35°C and below 10°C may cause death of Artemia. Brine shrimp adult reproduces at rate of up to 300 nauplii or cysts every 4 days.

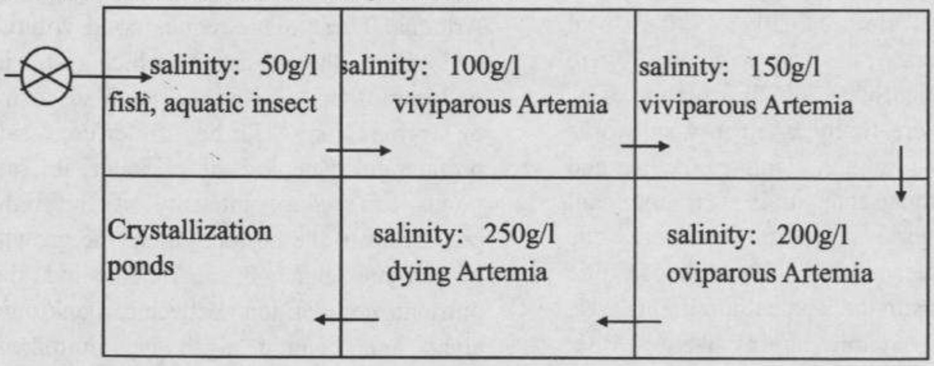

Fig. 1 Schematic diagram of solar salt operation with natural occurrence of Artemia
(from Sorgeloos et al.,1986)

NATURAL DISTRIBUTION OF ARTEMIA

Artemia populations are found in about 500 natural salt lakes and man-made salterns scattered throughout the tropical, subtropical and temperate climatic zones, along coastlines as well as inland over the world. Different geographical strains have adapted to widely fluctuating conditions with regard to temperature (6-35°C), salinity and ionic composition of the biotopes (e.g. chloride, sulfate and carbonate). Brine shrimp can survive in natural seawater, but do not possess an anatomical defense mechanism against predation; consequently they are an easy prey for carnivorous species (e.g. fish, crustaceans and insects). Therefore, Artemia is not existence of a biotope of natural seawater. Thanks to a most efficient osmoregulatory system of brine shrimp, they are capable to synthesize very efficient respiratory pigments (haemoglobin) to cope with the low oxygen levels that prevail at high salinities, therefore Artemia are only found at salinities where their predators can not survive (more than 7%). As a result of extreme physiological stress and water toxicity Artemia dies off at salinities close to NaCl saturation, i.e. 250 $g.l^{-1}$ and higher. As Artemia is incapable of active dispersion, Artemia could not migrate from one biotope to another through ocean, the Artemia populations are not found in every high saline biotopes and its distribution is discontinuous.

China is one of the countries rich in Artemia resources, 77 Artemia biotopes have been documented (Ma, 1993). The inland salt lakes with existence of Artemia are mainly distributed in Inner Mongolia, Qinghai, Xinjiang, Tibet, Nixia, Shanxi (Xin etc al., 1994). These Artemia biotopes have a striking physical, chemical and biotic diversity, which results in various strain characteristics (Zhang et al., 1998). The lakes of Gahai, Xiaocaidan in Qinghai province, Aibi and Balikun in Xingjiang province, Xiechi in Shanxi province, and several lakes in Tibet and Inner Mongolia have been exploited commercially. With further investigation of the Chinese Artemia resources, more Artemia resources will be exploited.

Large numbers of solar saltworks are located in a coastal line of 18,000 km of China. The parthenogenetic Artemia strain was natural existence in these saltworks, this strain is characterized as a large cyst diameter ranging from 260 to 270 microns. Due to the special salt production process and heavy rainfall in southern saltworks of China, the southern saltworks are usually small, the brine is kept at shallow and with a short retention time in the evaporation ponds, the water temperature are extreme high during summer time. Therefore, brine shrimps hardly survive in such environment. The coastal Artemia resources are mainly distributed in the saltworks located in the north of Yangtze River, most in Liaodong Bay, Bohai Bay and Laizhou Bay. Before early 1990's local Artemia species in the saltworks were parthenogenetic. Since 1992 inoculation of A. franciscana were firstly taken in 4 saltworks of Bohai Bay (Tanggu, Hangu, Luannan and Nanpu), A. franciscana strain were dominant in these saltworks in several years after the inoculation, later on due to the using of exotic Artemia strains in the aquaculture in this area, the bisexual Artemia strains were further expanded to other saltworks, field observation indicated that the dominant Artemia population in saltworks of Bohai Bay are mixture of bisexual strains and parthenogenetic strain (Bossier et al., 2004).

ARTEMIA CULTURE

Since 70's of last century, more Artemia resources have been exploited to meet the increasing demand of Artemia cysts in the market. But natural production of the cysts is fluctuated largely with the changes of the environmental conditions in the production sites. Since 80's, the culture techniques of Artemia have been studied for stabilizing cyst production. The techniques adapted to the different geographic and climatic conditions were described as follows:

Culture of Artemia in salt ponds of solar saltworks

The salt ponds of solar saltworks are important biotopes for brine shrimp. As the main purpose of saltworks is for salt production, two aspects must be considered when the measures are applied in salt ponds to increase Artemia production: the measures should be to meet optimal conditions for Artemia growth, but not to interfere salt production and salt quality. The following measures are usually applied to increase the Artemia production in solar saltworks:

1) Regulation of water level at different seasons and different salinity ponds: In low salinity ponds (5-8°Be'), benthic filamentous algae blooms up easily during summer time, which is not able to graze by Artemia. These algae reduce pond volume and excrete the substances which result in increase of brine viscosity. High viscosity of brine may further interfere salt production. Keeping deep water in salt ponds can reduce intensity of the light penetrated to the bottom where the growth of benthic algae is restricted, and the nutrient competition between planktonic algae and benthic algae is minimized. Through the regulation of the water depth at different seasons, the Artemia production can be enhanced. In spring and autumn when water temperature is low, the water is kept at a level of 30-40cm, the algae can efficiently use the light for the growing. In summer time water level is increased to 50-80cm, which can avoid too high water temperature and limit the growth of benthic algae.
2) The Artemia production can be improved through introducing of a new Artemia strain which is more suitable for local environment. A. franciscana is a Artemia strain which can survive at the conditions of low temperature and high salinity. The introduction of A. franciscana in northern saltworks of China may prolong existing time and area for Artemia population.
3) Fertilization: fertilizing salt ponds can enhance the primary productivity providing abundant food to sustain large population of Artemia. The varieties and amounts of the fertilizers applied in salt ponds should be regulated to avoid contamination of salt.
4) Density control of the Artemia: density of the Artemia in salt ponds is controlled to reduce the grazing pressure, and avoid the crashes of Artemia population due to food shortage.

Extensive culture of Artemia

Since 90's of last century, the culture of Artemia was expanded rapidly due to the high price of Artemia cysts, Artemia culture ponds of approximately 30,000ha were constructed in the coastal area of Bingzhou and Dongying in Shandong Province. These ponds were dug along with topography of the coastal fields; seawater was pumped into the ponds through a channel. The size of ponds is various in a range from several to hundreds of hectare. Seawater is pumped into the ponds in the early spring, and gradually evaporated up to salinity of 8-12°Be′ in April, salinity and nutrient are regulated by introducing new seawater during the culture season. This extensive culture is mainly used for production of Artemia cysts. Artemia production can be increased by mean of fertilizing the ponds with inorganic fertilizers and chicken manure that can help the algal growth. As these big ponds are hardly managed, the Artemia production is fluctuated largely and mainly depends on the climatic condition. The output of cysts is usually low (0.2-4kg per mu). The further study of techniques for production of Artemia in large ponds is still needed.

Semi-intensive culture of Artemia

The culture of Artemia was first developed in the SE Asian countries such as: Thailand, Philippine and Vietnam. Culture ponds were reconstructed from salt ponds and were usually small (100-1000m^2). The culture system was divided as two independent production units for algae production and Artemia production respectively. Firstly algae bloom up both in algae ponds and Artemia ponds by means of fertilization, then Artemia nauplii are introduced in Artemia ponds at a density of 20 individuals per liter, later algae water was pumped into Artemia ponds from algae ponds regularly to feed Artemia. Algae ponds are kept at a lower salinity, which make the algae bloom up easily. The production of Artemia in Vietnam is during the dry season from December to May, the Artemia is introduced once in the beginning of the season, Artemia cysts are produced as a main product, and the output is around 80kg/season/ha. Vietnam is the most successful country for semi-intensive culture of Artemia. A. franciscana was first successfully introduced in the salt ponds in 1982. The cyst production was increased from several tons in the beginning to several ten tons. As the Artemia strain inoculated in the ponds are controlled well, the high quality cysts (small diameter, high hatching ability) are produced and already accepted in world market. Recently, the techniques for semi-intensive culture of Artemia were improved by use of a technique of multi cycle production instead of one cycle production, in the process of multi cycle production the whole production season was divided as 3 cycles, inoculation of Artemia nauplii is taken place in the beginning of each cycle. The production of each cycle lasts 5-6 weeks, then Artemia were killed and the ponds were cleaned, fresh brine was introduced into the ponds to start another cycle. This technique may increase the output of the cysts up to 40%.

Culture of Artemia in Brazil

The introduction of the brine shrimp A. franciscana in Brazil is one of the successful examples. A. franciscana was firstly introduced to just one saltern in Macau in 1977, a few years later it had already been dispersed by local water fowl from Macau to most of the saltworks of NE Brazil. As this Artemia strain is well adapted to local environment, high production of the cysts (24600kg in dry weight) was obtained in the following year (1978), this good production lasted for 4 years (1978-1981), and then was declined in another 3 years (1982-1984), the production was dropped to only 1240 kg dw in 1984, which is only 5% of the production in 1978. The decline of the cyst production was caused by a continuous heavy drought during1979-1983, which restricted the growth of phytoplankton, and led to a shortage of mangrove water (rich in nutrient and excellent culture medium for Artemia). Therefore, extensive culture to produce

Artemia as by-products in big ponds is known as unstable model, semi-intensive culture system was then developed in Brazil. The economic depression in the end of 60's, and mechanization in big salt ponds led to depression of salt industry, many small salt ponds were abandoned. Those salt ponds were reconstructed as semi-intensive system for Artemia culture, water from mangrove fields was used as culture medium, high cyst production (1-5kg dw/ha) is obtained under proper management.

UTILIZATION OF ARTEMIA IN AQUACULTURE

The use of various live foods such as rotifer, Artemia and copepods led to the success of marine larviculture in the past decades. These live foods are suitable for the marine fish larvae in the aspects of variation of particle size and nutrition (Sorgeloos, 1994). Free-swimming nauplii can directly be fed as a nutritious live food source to the larvae of marine and freshwater organisms, with the advantages of the most convenient to use, less pollution to the water, and containing digestive enzymes which is absent in the dry diets. Different geographical strains are characterized as various nutritional value and nauplus size which adapted to requirements of different organisms and larval stages. Since Artemia nauplii were firstly used as a food for marine fish larvae in 1933, they have been used in the larviculture of more than 50 fish and shellfish species. Now over 2000 metric tons of dry Artemia cysts are marketed worldwide annually, in which 1000 tons of the cysts is consumed within China. At present the cysts are partly exploited from inland salt lakes and saltworks in Bohai Bay in China. Despite this, a large part of the cyst market is still supplied by harvests from the Great Salt Lake (GSL), USA and the salt lakes of CIS countries. GSL is the largest producer with a annual production of 1000-3000 tons. This situation makes the market still extremely vulnerable to climatological and/or ecological changes in this lake, which has been illustrated by the unusually low cyst harvests in the seasons 1993-1994 and mainly 1994-1995. This made a fluctuation of the cyst price in the market; the cyst price in 1996 was 3-4 times higher than normal season due to low cyst production of GSL. To solve this problem, more Artemia resources are further exploited in inland salt lakes, sustainable exploitation is used to avoid over harvesting of the Artemia cysts. In coastal saltworks new strain of Artemia that adapted to local environment is introduced to increase Artemia production.

The annual production of Artemia biomass was estimated up to 10 thousands of tons in the world. Several thousand tons of Artemia biomass is produced every year in northern saltworks of China. In nutritional value, adult Artemia is superior to freshly hatched nauplii. On the dry weight base the protein content increases from 50% in nauplii to 60% in adults. Furthermore, adults are richer in essential amino acids and carotenoids. The exoskeleton of adult Artemia is extremely thin, which facilitates digestion of the whole animals by predator. Improved growth, developmental rate and survival have been reported for numerous fish and crustacean species when adult Artemia are supplied as a transition food between nauplii and dry feed. In lobster and sturgeon farming adult Artemia is used as a starter feed in larval rearing. In coastal area of China Artemia biomass can also be used as a main source of feed throughout the entire life cycle of penaeid shrimps. Artemia biomass is still live when they are treated and transferred to shrimp ponds from saltworks properly during the transportation. Feeding Artemia biomass to shrimp ponds can eliminate the pollution caused by low quality artificial diets. The recent finding shows a diet of adult Artemia may induce maturation in various Penaeus spp. A field investigation of 30 shrimp farmers in Tanghai, Hebei Province indicated that Artemia biomass was fed to shrimp at amount of one quarter of total shrimp feed in 1992. The reason why Artemia bomass was not replaced artificial diets completely is only due to its higher price. If more Artemia biomass were used in shrimp ponds, the crashes of the shrimp culture in Bohai Bay would be probably avoided. In addition to its application as a food source, Artemia biomass can also be used as a valuable dietary ingredient or gustatory attractant in artificial diets. The flake based on Artemia biomass was already formulated and well accepted in the market. Artemia biomass can be directly frozen. But frozen Artemia biomass is not suitable for long distance transportation and the nutrient leakage of frozen Artemia is also a problem, therefore the market of frozen Artemia biomass is still small. In fact the techniques of encapsulation provides opportunity for using Artemia not only as food but also a carrier of essential nutrients, pigments, prophylactics, therapeutics, hormones, etc. to the predator larvae.

ROLE OF ARTEMIA IN SOLAR SALTWORKS

In recent years there has been a growing awareness of hydrobiological aspects of the solar production process, which may be largely influenced by organic contributions of microorganisms (mainly planktonic algae and halophilic bacteria) present in the brines. The studies of biological system in saltworks have already been documented (Davis, 1980, 1990, Sorgeloos, and Tackaert, 1990). The solar saltworks have a biological system that consists of algae, bacteria, protozoa, nematodes, brine shrimp, sea grasses and fish. This system can either improve or reduce salt production. Characteristics of biological system that help salt production of communities 1) formation of bottom communities to prevent water leakage and trap nutrients, 2) development of water colors that aids solar energy absorption and water evaporation, and 3) the ability to rid the salt works of a large part of the organic matter produced by the living organisms. Characteristics of biological systems that reduce salt production are associated with excessive production and accumulation of organic matter. These substances decrease pond volumes, evaporation and interfere the crystallization, which finally decrease the quantity and quality of salt. With proper management, a biological system can by made to aid salt production.

In many saltworks the natural conditions ensure a maximal salt production (e.g. in France, Brazil, and South Africa), in other locations, however, proper biological management is needed (e.g. in India, Italy, Australia, Bahamas and Venezuela). Algal blooms, induced by natural availability of organic and inorganic nutrients, are generally beneficial since they ensure increased solar heat absorption, resulting in faster evaporation and increased yields of salt. However, algal excretion and decomposition products act as chemical traps and consequently prevent early precipitation of gypsum that will decrease salt quality. Furthermore, such organic impurities as algal agglomerations, which turn black on oxidation, may contaminate the salt and reduce the size of the crystals and hence the salt quality. In the worst situations, high water viscosities may completely inhibit salt crystal formation and precipitation. In a biological balanced system brine shrimps graze much of planktonic algae, these animals clear the water and oxidize most of the organic matter they digest, and deposit their waste in fecal pellets that drop to the bottom of the pond. In the ponds of high salinity, most of brine shrimp, as well as the other suspended organic matter, are converted to a large population of red halophilic bacteria. In the process, the bacteria color the brine red, a color that greatly aids solar energy absorption and water evaporation, which improve salt production. Brine shrimp and halophilic bacteria ride much of suspended matter, and decrease viscosity of the brine, lower viscosity levels promote the formation of larger salt crystals, and thereby improve salt quality. In many solar saltworks, a sufficient Artemia population in present in low salinity ponds, the Artemia population in intermediate and high salinity ponds is mainly come from low salinity ponds. Natural recruitment of Artemia from cysts dispersed by wind and water birds assures the presence and development of sufficient numbers of brine shrimp for optimal salt operation. In some situations, however, the salt producer should not rely on this opportunistic dispersion of Artemia. In saltworks with short retention times in their evaporation ponds, a rapid dilution may wash away the Artemia populations, a hurricane or season of exceptionally heavy rainfall may eliminate or so reduce the local population that it can not effectively cope with the algae blooms. Some saltworks may be completely isolated from natural source of Artemia dispersion. In these saltworks and a new saltworks salt producers should optimize the hydrobiological activity in the evaporation ponds through a controlled introduction of brine shrimp.

Due to heavy population and industrialization of coastal areas in China, it is an environment where the natural ecosystem is heavily under stress with accumulation of nutrients and sediments. To solve this problems, in addition the waste discharged to the sea should be in the control, a beneficial biological system built in the coastal saltworks is extreme important, Artemia plays an important role in this system. A parthenogenetic Artemia population is existence of northern saltworks of China during a growing season of May to October, due to slow colonization, population growth and poor salinity resistance, the local population remains relatively small especially during early spring and is only survive in the ponds of low and intermediate salinities. As a result, it is not able to remove sufficient amounts of the rapidly proliferating algae during early spring when half amounts of the salt is produced, which result in a disjoint of salt production and Artemia growth. In recent years the introduction of A. franciscana in saltworks of Bohai bay shows a month earlier appearance of Artemia population in these saltworks, and this strain Artemia can thrive well in high salinity ponds and is more suitable for salt production than parthenogenetic Artemia.

RESEARCH TRENDS OF ARTEMIA IN FUTURE

As the larvae rearing techniques are improved and more Artemia resource is exploited, the shortage of the cysts is not a problem in the future. However, the market of high quality cysts will be gradually developed with the expansion of marine fish larviculture. Due to the low quality cysts from most of production sites in China (large size of the cysts and poor hatching synchrony), these cysts could not compete with GSL and SFB cysts. High quality cysts produced in the coastal area through introducing high quality strain should be focused in the future. Furthermore, a better knowledge of the biology of Artemia was at the origin of the development of other Artemia products, such as disinfected and decapsulated cysts, various biomass products, which presently have application in hatchery, nursery and broodstock rearing. All these developments resulted in optimized and cost-effective applications of this live food in hatchery production.

References

ABATZOPOULOS, T., KAPPAS, I., BOSSIER, P., SORGELOOS, P., BEARDMORE, J.A., 2002. Genetic characterization of Artemia tibetiana (Crustacea: Anostraca). Biol. J. Linn. Soc. **75**: 333-344.

BOSSIER, P., XIAOMEI, W., CATANIA, F., DOOM, S., VAN STAPPEN, G., NAESSENS, E., SORGELOOS, P., 2004. An RFLP database for authentication of commercial cyst samples of the brine shrimp Artemia spp. (International Study on Artemia LXX). Aquaculture **231**: 93-112.

Cai, Y.N., 1988. A new species of brine shrimp (Artemia sinica). Artemia newsletter **10**: 40.

Davis, J.S., 1980. Experiences with Artemia at solar saltworks. In: The brine shrimp Artemia. Vol.3 Ecology, culturing, use in aquaculture. Persoone, G., Sorgeloos, P., Roels, O., Jaspers, E. (Eds). Universa. Press, Wetteren, Belgium, 456pp: 51-55.

Davis, J.S., 1990. Biological management for the nutrient-rich Chinese sole salt works. International Symposium on biotechology of saltponds. Tanggu, Tianjin, China.

Sorgeloos, P., Lavens, P., Léger, P., Tackaert, W., Versichele, D., 1986. Manual for the culture and use of brine shrimp Artemia in aquaculture. Gent: ARC. 319pp.

Sorgeloos, P., Tackaert, W., 1990. Role and potential of Artemia in costal saltworks. Proceedings of International Symposium on biotechnology of saltponds; Sept. 18-21, 1990, Tanggu, Tianjin, PR China.; Cheng, L. (Ed.) Salt Research Institute, Tanggu, Tianjin, PR China.

VAN STAPPEN, G., SUI, L., XIN, N., SORGELOOS, P., 2003. Characterization of high-altitude Artemia populations from the

Qinghai-Tibet Plateau, PR China. Hydrobiologia **500**: 179-192.

XIN, N., SUN, J., ZHANG, B., TRIANTAPHYLLIDIS, G.V., VAN STAPPEN, G., SORGELOOS, P., 1994. International study on Artemia. LI. New survey of Artemia resources in the People's Republic of China. International Journal of Salt-Lake Research **3**: 1-8.

Ma, Z., 1993. Study on geographic distribution of brine shrimp in China. Recent Fishery Information **8(9)**: 19-26.

Zhang, B., Xin, N., Yu, X., Sui, L., 1998. Strain characterization of Artemia from nine inland salt lakes of China. Journal of Lake Science **10(3)**:19-24.