Use of disinfectants in shrimp farming

Ind. Agr. Alexander Varela, Patologhy and Parasitology Laboratory of Crustaceans. Nicoya (Costa Rica).
Eng. Nelson Peña Navarro, Directorate of research, Universidad Técnica Nacional Sede del Pacífico, UTN (Costa Rica).

Introduction

The world aquaculture has shown an alarming increase in the rate of infections by pathogens of different aetiologies, among which, they are considered by their impact caused by virus and bacteria, such as the most important (Lightner, 1996; Morales, 2004; Peña et al., 2013; Varela and Peña, 2013).

The appearance of new diseases of bacterial origin, has resulted of particular relevance, for instance the EMS or Early Mortality Syndrome or the AHPND Pathogenesis of acute hepatopancreatic necrosis disease (Loc Tran et al., 2013; Lightner et al., 2013), which has produced damages in Asia and in Mexico recently (Lightner et al., 2013; Loc Tran et al., 2013; Varela, 2013). Accordingly, it implies a potential risk of an outbreak of the disease in Costa Rica as well as in other countries. Meanwhile and on a recurring basis, the outbreaks of viral illnesses have severely continued affecting the aquaculture production with particular influence on the shrimp productions. (Lightner, 1996; Morales, 2004; Morales and Cuellar-Anjel, 2008; Varela and Peña, 2013).

With the goal of reducing the microorganisms charge in the surfaces and environments, a variety of chemical and physical agents have been traditionally used, whose efficacy has been shifting, and affected by the amount of present factors and variables in this kind of aquatic environments possibly.

Among the biocidal substances which have been used widely in the control of microorganisms, the oxidizing chemicals are found, (García, 2004), such as halogens and peroxides. This author marks that in case of the chemical and antimicrobial agents, the concentration used is essential, because with less quantities to the required ones, may cause the lost of effectiveness of the agent against pathogens.

In view of the above, the use of OX-VIRIN® and OX-AQUACULTURE® is proposed as an aim for the present research in a shrimp farming placed in the Gulf of Nicoya, with the goal to know its efficacy and the possible application in the shrimp farming field.

Test design

For this test, two ponds of identical sizes were selected. The ponds belonged to a production company of shrimp, placed in the Costa Ricans Pacific coastline. One of the ponds called “On trial pond”, received an initial application of **OX-VIRIN®** and a weekly dosage of **OX-AQUACULTURE®**, under the protocol as further indicated. In the other pond, called “Control pond”, the previous indicated biocidal products were not applied.

Both ponds had similar topography and an extension of 1.5 hectares. Using the same type and origin of post-larvae, also similar diets with the aim to minimize the variables, they were sown the same day.

On a weekly basis, samples of 10 animals caught from both ponds and collected by chance, were processed through analysis in fresh (according to Varela (2007) and Morales (2013)). Simultaneously, records of the physical-chemical parameters and graphically represented were kept, in order to observe their temporal behaviour.

With the difficult to carry out studies about the viral load of the waters, the performing of bacteriological analysis such as the control parameter to verify the effectiveness of the OX-products tested was chosen. These analyses were applied to the processing of samples that were taken weekly, using Agar TCBS which is a selective and differential culture medium for *Vibrio spp*. This culture medium is composed of Thiosulfate, Citrate, Bile and Sucrose. Finally, samples of water were processed using Agar TSA (Tryptic Soy Agar) such as culture medium, medium of general growth.

Furthermore, with the aim to determine the possible relation between the concentration of the biocidal OX products and their efficacy, tests with different concentrations of the disinfectants were carried out.

Disinfection protocol applied to the "On trial pond"

- Initial disinfection of the pond with OX-VIRIN®:

Three days before the shrimp farming, the pond was filled until cover the bottom of the tank. During filling, two litres of **OX-VIRIN®** per hectare were applied and the product was left to take action for two days before to continue the filling.

-Filling the pond and refills, using OX-AQUACULTURE®:

Two litres per hectare of **OX-AQUACULTURE®** were applied, they were diluted in sea water and were applied by dripping at the entrance of the channel towards the testing pond. In order to obtain the effective mix with the water entry, during the entrance of water refills.
OX-VIRIN® and **OX-AQUACULTURE®** are 100% biodegradable products which show in their composition the specific OX-CORES which guarantee their efficacy and stability.

Results and discussion

→ **Analysis in fresh:**

The results of the analysis in fresh did not report significant differences between the "On trial pond" and the "Control pond", because during the testing, bacterial or viral infections were apparently not found in any of the two ponds. This fact did not allow evaluating the behaviour of the tested products against confirmed infections, so this aspect will be required in subsequent studies.

→ **Physico-chemical parameters:**

Given the nature of the aquaculture, the evolution of some parameters such as the temperature or pH, is independent of the presence or absence of biocidal products.

Among the parameters which could be linked directly to the use of disinfectant agents for their effects on the microbiota in the pond, by virtue of its importance, the dissolved oxygen was studied, whose evolution is graphically showed then.

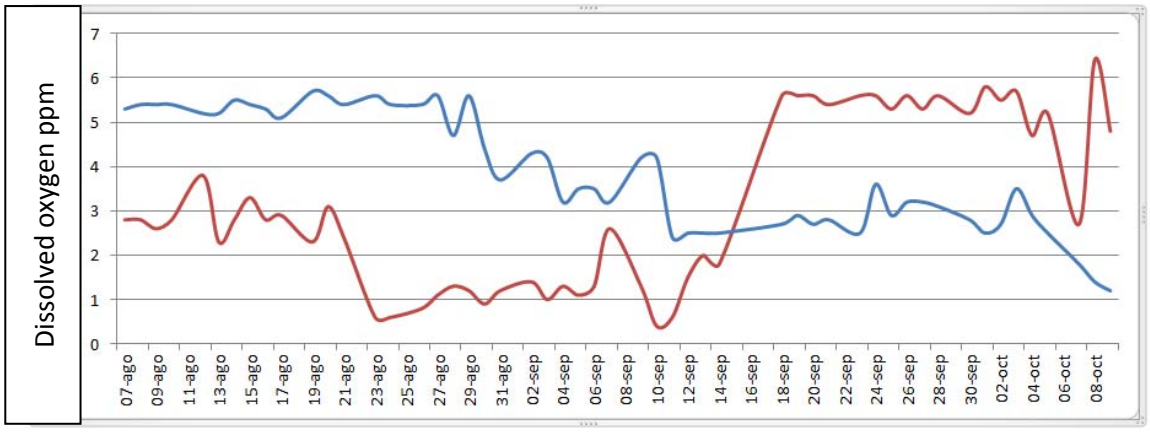

Figure 1. Graphic of the records of dissolved oxygen.
The red line refers to the "On trial pond" and the blue one to the "Control pond", showing the morning values of the dissolved oxygen registered (mg/L) during the two months of testing.

As noted in the previous graphic, the behaviour of the two ponds showed different tendencies. In the "Control pond" the dissolved oxygen levels were higher initially and between the third and fifth week started to drop, possibly due to an increase in the organic matter in the bottoms.

This fact is usually connected to the culture ponds, as a consequence of the rise of the diets. Moreover, the "On trial pond" showed low readings at the beginning, which could be related to the application of the strong oxidizing products during the preparation and filling of the ponds. However, an increase in the readings of dissolved oxygen was reported in the fifth week.

→ **Bacteriological parameters:**

The results displayed by the seeding in Agar TCBS, showed promising trends, recording lower bacterial counts in the "On trial pond" (Table 1). This suggests a higher stability in the microbiota of the pond.

The referred pond treated with OX-products had a lowest general microbial average and a lesser relation between the saccharolytic and non-saccharolytic bacteria, which is relevant regarding some species of high-pathogenicity for shrimps are non-saccharolytic, such as *Vibrio harveyi* and *Vibrio parahaemolyticus* (Martin et al., 2004). It is worth mentioning, as previously stated, a strain of this last specie has been identified such as the cause of the Early Mortality Syndrome.

On the other hand, the "Control pond", greater general average of microbial counts and the relation between the saccharolytic and non-saccharolytic bacteria were higher, even more than the unit in one of the samples. The standard deviation was equally increased, with a bigger variability between samples, indicating lesser stability in the microflora.

Table 1. Observed results after seeding in the Agar TCBS, specified for *Vibrio spp.*

Pond	Date	Saccharolytic Bacteria (CFU/ml)	Non-saccharolytic Bacteria (CFU/ml)	Total Bacteria (CFU ml)
Control	12 October	80	130	210
	17 October	30	<10	30
	24 October	40	10	50
	Average	**50**	**46,7**	**96,67**
	Stand. Devi.	**26,46**	**72,34**	**98,66**
On trial	12 October	70	<10	80
	17 October	30	30	60
	24 October	20	<10	20
	Average	**40**	**10**	**53,3**
	Stand. Devi.	**26,46**	**17,32**	**30,55**

In the Table 2, the test result averages performed in treated water with different concentrations of **OX-AQUACULTURE®** and using as a culture medium the AGAR TSA, which allows the total concentration of aerobic organisms present to be known. These trials were carried out by triplicate.

As it was expected, the microbial counts decreased when the dosage of the biocide applied was increased and it not being possible to isolate the bacteria in the treated sample with a concentration of 100 ml of **OX-AQUACULTURE®** / m^3 of water.

These results apply to the analyzed sample, however given the mode of action of the disinfectant agent; it is expected to observe similar actions in other microbial groups such as fungi, protozoa and virus.

In this regard, it is important to consider that the recommended dosage of applying of the product may not be standardized, since the product is mainly oxidant, its effect could be affected by factors as the presence of organic matter in the water column and bottoms, the quantity of the applied food and others. Nevertheless, the reduction of counts is noticeable and it is recommended to perform tests and consult with the Technical Area of Grupo OX in order to know the required amounts of the different ponds, having into account their particular conditions.

Table 2. Observed results after seeding in the Agar TSA, suitable for the culture of aerobic microorganisms.

Concentration of OX-AQUACULTURE® (ml/m^3)	Total bacteria (CFU/ml)
0	1600
25	650
50	400
100	<10

Conclusions and final recommendations

The oxidizing chemical products may be used with efficacy in the disinfection of surfaces and equipment, as gillnets, acclimatization tanks, packets of feeding and footwear of the personnel. But the use of oxidizing biocides and their effectiveness will be influenced by the specific conditions of the tanks, mainly in the organic matter content, which has to be considered to adjust the recommended dosage.

This test confirms that the use of the oxidizing biocidal products as **OX-VIRIN®** and **OX-AQUACULTURE®,** allows controlling in an efficient manner the microbial growth in the shrimp farming tanks. This context represents an important factor in the biosafety program which must be applied in this type of productive facilities.

Bibliography

García, V. (2004) Introducción a la microbiología, 2ª Edición. San José, Costa Rica, Editorial EUNED.

Lightner, D.V. (1996) A Handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp. USA. World Aquaculture Society, Louisiana, (Interactive CD-ROM format).

Lightner, D., Redman, R.M., Pantoja, C., Noble, B.L., Nunan, L.M., Loc Tran., Gomez, S. (2013) Documentation of an Emerging Disease (Early Mortality Syndrome) in SE Asia & Mexico.

Loc Tran, Nunan, L., Redman, R., Mohney, L., Pantoja, C., Fitzsimmons, K., Lightner, D.V. (2013) Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Journal of Diseases of Aquatic Organisms, 105: 45–55.

Martin, G., Rubin, N., Swanson, E. (2004) Vibrio parahaemolyticus and V. harveyi cause detachment of the epithelium from the midgut trunk of the penaeid shrimp Sicyonia ingentis. Journal of Diseases of Aquatic Organisms, 60: 21–29.

Morales, M.S. (2004) Enfermedades del camarón: detección mediante análisis en fresco e histopatología. Editorial Trillas. México, D.F.

Morales, M.S. (2013) Camarón Análisis en Fresco, herramienta de diagnóstico. 1ª Edición. CIAD-OIRSA, pp 86.

Morales, V., Cuéllar-Anjel, J. (2008) Guía técnica - patología e inmunología de camarones penaeidos. Programa CYTED Red II-D Vannamei, Panamá, Rep. de Panamá.

Peña, N., Vargas, R., Varela, A. (2013). Productos naturales como estimuladores del sistema inmunológico de Litopenaeus vannamei, infectado con Vibrio parahaemolyticus. Rev. Agron. Mesoam. 24 (1):133-147.

Varela, A. (2007) Manual para la interpretación de resultados de laboratorio. Alicorp, Publicis- Asociados, Perú. 24 pp.

Varela, A. (2013) Enfermedades bacteriales en camarón de cultivo de mayor importancia para C. R. Taller Internacional de Patologías de camarón blanco (Litopenaeus vannamei) con fines de cultivos comerciales en Costa Rica. Dirección de Investigación, Universidad Técnica Nacional, Sede Puntarenas.

Varela, A. Peña, N. (2013) El Virus del Síndrome de las Manchas Blancas (WSSV): una revisión y su impacto en la camaronicultura costarricense. Rev. Ciencias Veterinarias, 28 (2): 51-69.