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Biofloc new technology and shrimp disease in super-intensive aquaculture

[1] Mazdak Aalimahmoudi, [2] Hamid Mohammadi Azarm, [3] Yasaman Mohamadi

[1] Departments of Fisheries and Aquaculture, Khorramshahr University of Marine Science and Technology, Khorramshahr, khouzestan, Iran

[2] Department of Fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, khouzestan, Iran

[3] Departments of Fisheries, faculty of Agriculture and natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran

Abstract

Disease remains a limiting factor for the aquaculture industry including fresh waters aquaculture, shrimps culture and maricultur. With respect to the shrimp culture industry, disease outbreaks have been the primary cause of production loss during the last two decades. Disease outbreaks not only result from the mere presence of a pathogen in the system, a compromised health status of the cultured animals in combination with suboptimal environmental conditions are also factors facilitating disease outbreaks. Therefore, disease prevention and control should not only focus on implementing biosecurity measures, but must be performed in an integral approach involving, among others, adequate nutrition, enhancing the immunity of the cultured animals and maintaining a good water quality. The basic principle of the biofloc system is to recycle waste nutrients, in particular nitrogen, into microbial biomass that can be used in situ by the cultured animals or be harvested and processed into feed ingredients. So far, very few studies investigated the immunological potential of the biofloc technology although it is widely known that microorganisms, their cell components or their metabolites can act as immunostimulants that enhance the shrimp innate immune system and provide improved protection against pathogens.

Keywords: biofloc, shrimp, viruses, pathogen, disease, super-intensive

1. Introduction

Disease remains a limiting factor for the aquaculture industry including fresh waters aquaculture, shrimps culture and maricultur (FAO, 2012) [26]. With respect to the shrimp culture industry, disease outbreaks have been the primary cause of production loss during the last two decades (FAO, 2012) [26]. Disease outbreaks not only result from the mere presence of a pathogen in the system, a compromised health status of the cultured animals in combination with suboptimal environmental conditions are also factors facilitating disease outbreaks. Therefore, disease prevention and control should not only focus on implementing biosecurity measures, but must be performed in an integral approach involving, among others, adequate nutrition, enhancing the immunity of the cultured animals and maintaining a good water quality. (De Schryver *et al*., 2014) [15].

Biofloc technology (BFT) has been studied at several occasions and contributes to the maintenance of good water quality in the system and to the nutrition of the cultured animals (Avnimelech, 1999) [3]. The basic principle of the biofloc system is to recycle waste nutrients, in particular nitrogen, into microbial biomass that can be used in situ by the cultured animals or be harvested and processed into feed ingredients. Heterotrophic microbial aggregates are stimulated to grow by steering the C/N ratio in the water through the modification of the carbohydrate content in the feed or by the addition of an external carbon source, so that the bacteria can assimilate the waste ammonia for new biomass production. Biofloc systems have been shown not only to maintain ammonia below toxic levels and to improve the feed nutrient utilization efficiency of the cultured animals, but also to provide extra nutrients and exogenous digestive enzymes. Biofloc application can also lead to increased growth, survival and reproductive performance of the cultured animals (Avnimelech, 2012; crab *et al*., 2010) [2, 12].

So far, very few studies investigated the immunological potential of the biofloc technology although it is widely known that microorganisms, their cell components or their metabolites can act as immunostimulants that enhance the shrimp innate immune system and provide improved protection against pathogens (Vazquez *et al*., 2009) [46]. Xu and Pan (2013) [50] reported that the total haemocyte count and phagocytic activity of the haemocyte of the shrimp from biofloc containing culture units were significantly higher than those of the shrimp in the non-biofloc control group. Furthermore, the authors also noted that shrimp grown in a biofloc environment harbored a higher total antioxidant capacity both in the plasma and hepatopancreas. A recent study reported that the expression of six selected genes (prophenoloxidase [ProPO1 and ProPO2], serine protease [SP1], prophenoloxidase activating enzyme [PPAE1], masquerade-like serine protease [mas] and Rat-sarcoma-related nuclear protein), directly and indirectly related to the shrimp immune response, were significantly upregulated in biofloc-grown shrimp. Immune stimulation may thus be a very important feature in biofloc-grown shrimp contributing to disease control. It could for example (partly) explain the lower prevalence of acute hepatopancreatic necrosis disease

(AHPND) and White spot syndrome virus (WSSD) observed in farms that apply BFT. AHPND is currently causing very large problems in the culture of shrimp post larvae in Asia (Ekasari *et al*., 2014) [29].

Also the study by de Schryver *et al*. (2009) [16] showed that biofloc containing poly-b-hydroxybutyrate (PHB) is between 0.9 to 16%, which is sufficient to meet the needs of the fish will PHB which is not more than 1%. PHB is an intracellular polymer products produced by various species of microorganisms as a form of energy savings and carbon (Defoirdt *et al*., 2007) [19]. This polymer is believed to have the effect of prevention and treatment of infection with Vibrio and benefits of prebiotics in aquaculture (Defoirdt *et al*., 2007; de Schryver *et al*., 2008) [19, 13].

2. Immune system in Crustaceans

Crustaceans mainly depend on a non-specific immune system that includes circulating hemocytes and various active substances released into hemolymph such as the prophenoloxidase (proPO) system, antibacterial peptide, lectin, proteinase inhibitor, etc (Soderhall and Cerenius, 1992)[42]. It is well documented that neuroendocrine and immune systems communicate and operate to form a network to achieve homeostasis in non-mammalian vertebrates, particularly in fish (Yada and Nakanishi, 2002) [51]. The neuroendocrine-immunoregulatory network is a mechanism of structures and processes involving the physiological and biochemical interactions between the neuroendocrine system and immune system which protect organism from stress and diseases (Besedovsky and DelRey, 1996) [4]. During stress responses, the neuroendocrine-immune network controls reactions to many external and internal stressors (Aladaileh and Raftos, 2008) [1].

Corticotrophin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), which control the release of biogenic amines in both vertebrates and invertebrates, are secreted during these regulatory processes as the primary response to physiological stress in teleost (Ottaviani and Franceschi, 1997) [38], while the subsequent induction of hyperglycemia and suppression of immunity are secondary responses(Chang *et al*., 2007) [7]. Activated G protein-coupled receptors transmit the signal of biogenic amine to intracellular trimeric GTP binding (G) proteins. Once activated, the G proteins either stimulate or inhibit specific target proteins, e.g., adenylyl cyclase and phospholipase C. This causes changes in the concentration of intracellular second messengers, such as cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), inositol 1,4,5- trisphosphate (IP3), and diacylglycerol (DAG) (Blenau and Baumann, 2001) [5]. Finally, second messenger-dependent enzymes are activated and modify the properties of various immune responses (Schneider Igelmund and Hescheler, 1997; Clapham and Neer, 1997) [41, 8]. Although crustaceans have simpler endocrine/neuroendocrine and immune systems than vertebrates, they have similar robust stress responses that include the release of stress hormones/neurohormones (Li *et al*., 2005) [34]. The release of stress hormones is believed to be a proto-stress response. It has been reported that salinity stress could lead to fluctuations in the concentrations of dopamine (DA), noradrenaline (NE) and 5-hydroxytryptamine (5 HT) in crustacean hemolymph (Pequeux *et al*., 2002) [39].

3. A Short Review on Infectious Viruses in Cultural Shrimps (*Penaeidae Family*)

Viruses are the most common biological agents in the marine environment and it is known that they infect Fish, Shrimp and other aquatic animals. Marine crustaceans can be simultaneously infected by more than one type of virus (Flegel, 2001; Sritunyalucksana *et al*., 2006) [25, 44]. The major viruses of concern in shrimps and fresh water shrimp are mention in the following (Ganjoor, 2015; Claydon *et al*., 2010; Flegel, 2006; Lightner, 2001; Sudhakaran *et al*., 2006) [27, 9, 25, 35, 45]**:**

1) White-spot syndrome virus (WSSV or PmNOBII a mistake name which called for WSSV).
2) Monodon baculovirus (MBV).
3) Yellow-head virus (YHV).
4) Hepatopancreatic parvovirus (HPV).
5) Related Australian lymphoid organ virus (LOV).
6) Gill associated virus (GAV).
7) Infectious hypodermal and hematopoeitic necrosis virus (IHHNV).
8) Taura syndrome virus (TSV).
9) Mourilyan virus (MOV).
10) Laem Singh virus (LSNV).
11) Baculovirus midgut gland necrosis virus (BMNV).
12) Monodon slow growth syndrome (MSGS).
13) Infectious myonecrosis virus (IMNV).
14) Macrobrachium rosenbergii nodavirus (MrNV).
15) Extra small virus (XSV).

More than 15 viruses have been reported to infect marine shrimp (Claydon *et al*., 2010; Bonami, 2008) [9, 6]. They cause disease in shrimp specially penaeid shrimp family as species as (*Penaeus monodon*), (*Litopenaeus vannamei*), (*Fenneropenaeus indicus*), (*Litopenaeus stylirostris*), (*Marspenaeus japonicas*) and etc (Loh *et al*., 1997; Sritunyalucksana *et al*., 2006) [37, 43]. Nine viruses are responsible for main considerable economic losses. These include white spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), monodon baculovirus (MBV), hepatopancreatic parvovirus (HPV), yellowhead virus (YHV), gill-associated virus (GAV), Taura syndrome virus (TSV), infectious myonecrosis virus (IMNV), and Mourilyan virus (MoV) (Claydon *et al*., 2010)[9]. Although these viruses were no cause for alarm to human health, authorities find that they were economically crippling for Asian shrimp farmers (Flegel, 2006) [23]. Initially, *Penaeus monodon* was the main cultivated species in Asia but this has changed markedly since 2002 when *Litopenaeus vannamei* (formerly called *Penaeus vannamei*) started to be cultivated in many Asian countries. Since 2004, it has been the main cultivated species in the world (Flegel, 2006) [23]. Viral infection found not only in cultivated shrimp but also in wild shrimp. Different viruses have found in wild shrimps for example at a research which done in Brunei waters, Over 270 *Penaeus monodon* were collected from the South China Sea, screened and spawned. Of the nine viruses assessed, infectious hypodermal and hematopoietic necrosis virus (IHHNV) was most commonly detected (19.6%), followed by monodon baculovirus (MBV) (7.4%), hepatopancreatic parvovirus (HPV) (3.8%), and Mourilyan virus (MoV) (0.9%). The only multiple viral infections found were a combination of IHHNV and MBV (2.2%). Two most infectious viruses for *P. monodon*, white spot syndrome virus (WSSV) and yellowhead virus (YHV) were not distinguished in any shrimp (Claydon *et al*., 2010) [9]. Additional research in Thailand display number of viral infection in 42 shrimp samples from central and southern areas of Thailand using multiplex RT-PCR technique. Percentage of infection in examined shrimp with different viruses was**:** HPV 4.8%, TSV 7.1%, YHV 2.4%, MBV 2.4%, IHHNV 2.4%, WSSV 40.5%, and Mix-infection 2.4% (Khawsak *et al*., 2008) [30].

White Spot Syndrome Virus (WSSV) is the causative agent of widespread disease related with high mortality rate in cultured shrimp (Lightner and Redman, 1998) [36]. It causes up to 100% mortality within 10 days in commercial shrimp farmhouses, resulting in huge losses to the shrimp farming industry (Flegel, 1997)[27]. About 4–6 billion US$ of economic losses have been estimated in Asia and more than 1 billion US$ in America, between 1992 and 2001 and presently the disease has spread worldwide. Conventional control strategies such as improvement of environmental conditions, stocking of specific pathogen free (SPF) shrimp post-larvae and augmentation of disease resistance by oral immune stimulants, are currently employed to contain WSSV infections. However, extreme virulence of this virus and its wide host range including many other crustaceans make the transmission control and prevention to be problematic (Wongteerasupaya *et al*., 1995b; Rout *et al*., 2007) [49, 40].

4. Pathogenic DNA viruses in shrimp

DNA viruses which cause infection in shrimp contain**:** IHHNV, HPV, WSSV, MBV and BP.

5. Pathogenic RNA viruses in shrimp

RNA viruses which are infectious for shrimp contain**:** YHV, GAV, LOV, TSV, IMNV, MOV, MrNV, XSV and LSNV. Several positive sense RNA (+ssRNA) viruses have been reported from shrimp. Most notably, these include yellow head complex viruses (YHV), and Taura syndrome virus (TSV) (Sritunyalucksana *et al*., 2006; Rout *et al*., 2007) [43, 40].

6. The use of bioflocs as a biocontrol measure

In addition to the advantages of biofloc technology discussed above, Crab *et al*. (2010b) [10] have recently shown that biofloc technology constitutes a possible alternative measure to fight pathogenic bacteria in aquaculture. Intensive aquaculture of crustaceans is one of the fastest-growing sectors in aquaculture production (Wang *et al*., 2008) [48]. Despite its huge success, shrimp culture is facing severe outbreaks of infectious diseases, which have caused significant economic losses. Due to the haphazard mishandling of antibiotics in aquaculture, pathogenic bacteria are now becoming resistant to numerous antibiotics and as a result, antibiotics are no longer effective in treating bacterial disease (Defoirdt *et al*., 2011) [20]. The disruption of quorum sensing, bacterial cell-to-cell communication with small signal molecules (Defoirdt *et al*., 2008) [18], has been proposed as a new strategy to control bacterial infections in aquaculture as this cell-tocell communication mechanism regulates the expression of virulence factors (Defoirdt *et al*., 2004) [17]. Interestingly, we recently found that bioflocs grown on glycerol were able to protect gnotobiotic brine shrimp (*Artemia franciscana*) against pathogenic Vibrio harveyi, and that the beneficial effect was likely due to interference with the pathogen's quorum sensing system (Crab *et al*., 2010b) [10].

Indeed, survival of challenged nauplii increased 3-fold after the addition of live bioflocs. This complies with former research that revealed that primary production and promotion of in situ microbial populations, as is the case in biofloc technology, were found to be beneficial for shrimp (Lezama-Cervantes and Paniagua-Michel, 2010) [33]. The exact mechanism of the protective action of bioflocs and its selective action, however, needs further in-depth investigation. Another interesting feature of bioflocs to further investigate with respect to biocontrol effects is the capability to accumulate the bacterial storage compound poly β-hydroxybutyrate (PHB). PHB and PHBaccumulating bacteria have been shown before to protect different aquaculture animals from bacterial infections (De Schryver *et al*., 2010; Defoirdt *et al*., 2007; Dinh *et al*., 2010; Halet *et al*., 2007)[13, 19, 21, 28]. PHB-accumulating bacteria are present in bioflocs as we have measured PHB levels in bioflocs of between 0.5 and 18% of the dry matter (Crab, 2010; De Schryver and Verstraete, 2009) [11, 16].

The latter bioflocs contain a sufficient PHB level to protect cultured animals from infection by pathogenic bacteria (Halet *et al*., 2007) [28]. Numerous researches have noted that shrimp are healthiest and grow best in aquaculture systems that have high levels of algae, bacteria and other natural biota (Kuhn *et al*., 2009) [31]. Probiotics are viable microbial cells that have a beneficial effect on the health of a host by improving its intestinal equilibrium through improved feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic actions, growth-promoting factors, and an increased immune response (Verschuere *et al*., 2000) [47]. Since several research articles have been published on the benefits of using Bacillus to improve shrimp growth performance, survival, immunity, and disease resistance in aquaculture (Decamp *et al*., 2008; Tseng *et al*., 2009; Verschuere *et al*., 2000) [16, 45, 47] we inoculated biofloc reactors with a probiotic Bacillus mixture in an attempt to produce probiotic bioflocs. Our preliminary results showed that the water of shrimp tanks fed bioflocs inoculated with Bacillus had an on average 5 times lower Vibrio load when compared to the shrimp tanks fed an artificial feed (Crab, 2010) [11]. These results indicate that inoculating biofloc reactors with probiotic bacteria might have biocontrol effect toward Vibrio spp., but the inoculation of biofloc systems with specific desired microorganisms needs further investigation in order to confirm these beneficial effects. Other interesting fields of research regarding this subject are possible immunostimulatory features of the bioflocs. Enhancement of the innate immunity of cultured organisms may provide broad-spectrum resistance to infections. Existing immunostimulants include bacteria and bacterial products, complex carbohydrates, nutritional factors, animal extracts, cytokines, lectins, plant extracts and synthetic drugs such as levamisole (Wang *et al*., 2008) [48]. Bioflocs might also contain immunostimulatory compounds since biofloc technology deals with bacteria and bacterial products.

7. Conclusions

In conclusion, the many study showed that bioflocs have positive effects on the immune response of white shrimp leading to a higher resistance against disease challenge.

Overall, all studies has demonstrated that the potential of applying biofloc technology to achieve disease control and management in the shrimp culture industry. On the other hand a variety of beneficial features can be ascribed to biofloc technology, from water quality control to in situ feed production and some possible extra features. Biofloc technology offers aquaculture a sustainable tool to simultaneously address its environmental, social and economic issues concurrent with its growth. Researchers are challenged to further develop this technique and farmers to implement it in their future aquaculture systems. The basics of the technology is there, but its further development, fine-tuning and implementation will need further research and development from the present and future generation of researchers, farmers and consumers to make this technique a keystone of future sustainable aquaculture.

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