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The dinamics of water quality on tiger shrimp (*Penaeus monodon*) cultivation using probiotic in semi intensive pond

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***Abstract*— *Tiger shrimp (Penaeus monodon) is one of the aquaculture commodities which is still be Indonesia leading export commodity. However, since the outbreak of white spot syndrome virus (WSSV), tiger shrimp cultivation began to leave by farmers and switch to vannameiwhich is more profitable and more resistant to disease. This condition should be avoided considering tiger shrimp are native shrimp of Indonesia whose market demand is still quite high. Therefore, some efforts to improve tiger shrimp cultivation is necessary. One of the efforts to improve tiger shrimp cultivation is by improving culture medium in pond using RICA probiotics. The study was conducted on experimental pond installation in Takalar regency. The treatments were: A) liquid RICA probiotics, B) powdered RICA probiotics. Data of water quality i.e: phosphate, nitrate, nitrite, organic matter, ammonia, TSS,alkalinity and temperature, salinity, DO, pH were analyzed descriptively. The results of the study showed that the use of liquid RICA probiotics had average of phosphate 0.3531 mg/L, nitrate 0.2164 mg/L, nitrite 0.0090 mg/L and pH 8.11 whereas by using powdered RICA probiotics the average phosphate was 0.1851 mg/L, nitrate 0.0573 mg/L, nitrite 0.0069 mg/L and pH 8.51. Application of liquid and powdered probiotics in semi-intensive tiger shrimp could effectively maintain water quality parameters like NH_3-N, NO_2-N, NO_3-N and total organic matter**

Keywords*— probiotic, semi intensive, tiger, shrimp, water quality.

I. INTRODUCTION

Since 2000, probiotics began to be used for overcoming crop failure. Many types of probiotics on the market, one of which functions to improve the pond bottom by decomposing organic material by the bacterium *Bacillus sp* (Moriarty, 1997; Poernomo, 2004). According to Hirota, *et. al*., 1995 in Maeda (1999), the presence of *B. subtillis* in an anaerobic sedimentary layers can cause sulfide concentrations to decrease, therefore the potential redox (*Eh*) increases which indicates an enhancement in the pond sediment condition quality.

The cause of the decline in the pond waters environment quality is the disposal of aquaculture wastewater during operations containing high N and P elements, discharged into the surrounding environment (Boyd, 1999; Horowitz & Horowitz 2000; Montoya & Velasco, 2000). According to Teicher Coddington, *et al*. (1996), states that the waste load increases along with the increasing of shrimp stocking densities and feed conversion ratio (FCR).

Various efforts can be done to increase the shrimp production from the shrimp aquaculture in ponds, and one of them is the fulfillment of disease-resistant superior seeds. A seed production technique, using environmental improvements by using probiotics, needs to be done to get the disease-resistant seeds. A seed production technology through the use of probiotics has been successfully carried out at Tiger Shrimp Hatchery Installation in Barru Regency, however, it is necessary to do enlargement on the pond to see further performance.

Probiotic bacteria are able to accelerate the breakdown of organic waste into minerals that are useful for phytoplankton in a pond so that the regeneration process of nutrients has been applied faster in Indonesia (Poernomo, 2004). Furthermore it is said that probiotics applied in a pond must be able to live in a pond, grow, breed, and work actively in their respective fields as expected (Muliani, *et al*. 2004; Muliani, *et al*. 2006), reported that several international researchers have isolated probiotics from the culture environment and from organisms that have been maintained, then examined the use of these probiotics to improve the cultivation environment (Muliani, *et al*., 2008), to increase survival of pet organisms (Aly, *et al*., 2008; Markidis, *et al*., 2008), to improve digestion of pet organisms (Kumar, *et al*., 2006), to stimulate growth and immune systems of pet organisms (Aly, *et al*., 2008) and to countermeasure shrimp larvae disease (Tjahyadi, *et al*., 1994: Haryanti et al., 2000). Cruz, *et al*. (2012), reported that the use of probiotics in a practical aquaculture system could increase disease resistance, increase the growth of aquatic organisms, and increase the feed efficiency. Matiasi, *et al*. (2000), reported that the use of certain commercial probiotics in Malaysia has the potential to improve water quality, and be able to increase shrimp production from aquaculture ponds. Furthermore Wang, *et al.* (2005), reported that the use of probiotics was able to increase the density of ammonification bacteria, *Bacillus sp*, and Protein Mineralizin Bacteria (PMB) significantly, therefore the concentration of nitrogen and phosphorus decreased, resulting in increased shrimp production. Furthermore Gunarto, *et al*. (2006), states that the use of probiotics can improve the pond environment such as improving the redox potential value of the pond sediment, reducing the concentration of ammonia, the total organic matter (TOM), and suppressing the growth of *Vibrio sp* population in pond water.

The RICA 1, 2, and 3 probiotics are the probiotics produced by the Brackishwater Aquaculture Research and Development Center in Maros Regency, which are isolated from pond, marine, and mangrove leaf sediments. These probiotics have been tested in several ponds in South Sulawesi, even in East Java on traditional plus tiger shrimp cultivation, and the results are proven to increase shrimp survival and production (Atmomarsono, *et al*., 2010: Susianingsih and Atmomarsono, 2014). However, to complete the information about the application of RICA probiotics, a semi-intensive study of tiger shrimp ponds was conducted, comparing the liquid form RICA probiotics with powder probiotics. This research aims to obtain data and information about the effect of using different probiotics on the dynamics of water quality in tiger shrimp aquaculture in ponds.

II. METHODS AND MATERIAL

This research was conducted in the Punaga experimental pond, the Brackish Aquaculture Fisheries Research Institute, in Takalar District, South Sulawesi, using four ponds with an area of 1000 m^2 each. The ponds construction was made of concrete with a drainage system using a central drainage system. Each plot was equipped with a two leaf mill, thus the water quality remained excellent. The water preparation used the main reservoir which functioned as sedimentation of seawater Sterilize the container by using a 40 ppm chlorine solution equally across the surfaces of the ponds, then washed with clean sea water. The water filling in the ponds was carried out in step by step until the depth reached 1 m.. The growth of natural feed was with urea fertilizer at 150 kg/ha, and SP-36 fertilizer at 75 kg/ha. This research used two treatments, namely: A = liquid RICA probiotics, and B = RICA powder probiotics; each of two replications. Application of probiotics at a dose 0.5-1 mg/L per week (Atmomarsono et al., 2014). The applied density was 20 shrimp/m^2. The test animals were black tiger shrimp seeds with PL 20 in size, free from *White Spot Syndrome Virus* (WSSV) based on *Polymerase Chain Reaction* (PCR) observation. During the ongoing maintenance, the shrimp was fed by 3-20% dosage of commercial feed, decreased along with the increasing of the shrimp biomass. The feeding frequency was 2-4 times a day evenly. The water replacement began after entering the 30^{th} day as much as 5-10%. The maintenance time was estimated at 120 days. The observed water quality was Total Organic Matter, ammonia, alkalinity, nitrite, nitrate, phosphate, and Total Suspended Solid. The samples were taken as much as 500 mL in each plot, analyzed in the laboratory. Specifically for temperature, salinity, pH, and oxygen were measured *in situ* on the ponds. The water quality data during the research were analyzed and discussed descriptively.

Table 1. Parameters and tools used for water quality analysis

Variable	Unit	Tools//Methods	Description
Temperature	^oC	DO meter	In situ
Dissolved Oxygen	mg/L	DO meter	In situ
pH		pH meter	In situ
Salinity	ppt	Handrefractometer	In situ
Ammonia (NH_3)	mg/L	Spektrofotometer/SNI	Laboratory
Nitrate (NO3)	mg/L	Spektrofotometer/SNI	Laboratory
Nitrite (NO2)	mg/L	Spektrofotometer/SNI	Laboratory
Phosfate (PO_4)	mg/L	Spektrofotometer/SNI	Laboratory
Total Organic Matter	mg/L	Gravimetri/ SNI	Laboratory
Alkalinity	mg/L	Gravimetri/ SNI	Laboratory

III. RESULTS AND DISCUSSION

Water quality is very influential on the growth and survival rate of shrimp (Fast & Lester, 1992; Prihutomo, 2013). In addition, quality parameter influences the life cycle of organisms, and are a limiting factor for the spread of species (Rochmady, 2011; Susiana, *et al.*, 2014). In environmental parameter, water quality plays an important role in the metabolic processes of aquatic organisms (Susiana, 2011, 2015; Rochmady, *et al.*, 2016).

Table 2. Range of water quality on tiger shrimp maintenance during the experiment

Variable	Treatment	Observation time	Range value	Standard deviation	Optimal Range/ References
Temperature (^oC)	A	Morning	27.00-31.77	0.00-0.20	26.6-30.0 (Atmomarsono *et al.*, 2003)
	B		27.05-31.83	0.21-0.23	
	A	Afternoon	26.90-31.85	0.07-0.14	
	B		26.50-31.05	0.21-0.71	
pH	A	Morning	8.05-9.61	0.05-0.06	7.5-9.0 (Tharavathy,2014)
	B		7.96-9.45	0.17-0.47	
	A	Afternoon	6.12-8.99	0.25-1.06	
	B		6.44-9.00	0.08-0.18	
Dissolved Oxygen (mg/L)	A	Morning	3.35-5.47	0.18-0.38	4-7 Mangampa *et al* (2003)
	B		3.58-5.47	0.15-0.29	
	A	Afternoon	3.98-7.80	0.18-0.51	
	B		4.12-7.33	0.76-1.00	
Salinity (ppt)	A	Morning	28.64-36.68	0.11-2.53	10-35 Murdjani (2007)
	B		28.25-36.26	0.11-2.18	
	A	Afternoon	28.74-36.84	0.05-2.14	
	B		28.26-37.29	0.26-2.16	

Temperature is an energy or a limiting variable which has an important role for life growth, and accelerates the metabolic processes of aquatic animal organisms. In Table 2, the water temperature observation results in the morning range from 27.00 to 31.77°C in Treatment A, and 27.05 to 31.83°C in Treatment B; whereas in the afternoon it ranges from 26.90 to 31.85°C in Treatment A, and 26.50 to 31.05°C in Treatment B. The water quality temperature in the morning is higher than in the afternoon. The optimum water temperature for the fish and shrimp aquaculture in ponds is 28 to 32°C (Effendi, 2003). According to Chanratchakool, *et al.* (1995), water temperature influences the shrimp feeding response when the temperature is higher than 32°C, and the shrimp appetite will decrease by 30% to 50% if the water temperature is lower than 25°C; the optimal temperature for the shrimp growth ranges from 26 to 39°C (Atmomarsono, 2003). The metabolic rate from several aquaculture cultivation research is strongly influenced by water temperature factor, apart from the activity, size, and age of the species (Suther and Rissik, 2008). The optimal temperature range for shrimp growth is 25 to 32°C (Fast & Lester, 1992; Sutanti, 2009).

Oxygen plays an important role as a water quality indicator, since dissolved oxygen plays a role in the oxidation and reduction of organic and inorganic materials. In Table 2, the lowest oxygen measurement result in Treatment A ranges from 2.98 to 7.80 mg/L, however, in Treatment B it is higher, which ranges from 4.12 to 7.33 mg/L in the afternoon, and in the morning ranges from 3.35 to 5.47 mg/L in Treatment A, and in Treatment B ranges from 3.58 to 5.47 mg/L. The value of the oxygen range in the administration of liquid probiotics in the afternoon decreases, ranging from 2.98 - 7.80 mg/L, however, in Treatment B it arises, ranging from 4.12 to 7.33 mg/L. According to Pushparajan and Soundarapandian (2010), the minimum dissolved oxygen value is 3.9 mg/L, and the maximum value is 4.9 mg/L during shrimp rearing in a pond. Shailender, *et al.* (2010), reported that the minimum dissolved oxygen value is at 4.5 mg/L, and the maximum value is at 5.5 mg/L in tiger shrimp maintenance, plus for 140 days can support the growth of tiger shrimp to reach 40.2 g/shrimp.

The salinity during this research in each treatment was almost the same, which ranged from 28.64 to 36.66% in Treatment A, and 28.25 to 36.26 ppt in Treatment B in the morning, while in the afternoon was 28.74 to 36.84 ppt in Treatment A, and 28.26 to 37.29 ppt in Treatment B. For tiger shrimp aquaculture, the salinity ranges from 10 to 30 ppt (Chanratchakool, *et al.*, 1995), and 10 to 35 ppt for an intensive maintenance (Murdjadi, *et al*., 2007). In this research, the salinity was quite high. At high salinity, a lot of energy transformation is needed for the osmoregulation process rather than for flesh formation, thus it can affect the shrimp growth.

pH level of pond water during the study ranged from 8.05 to 9.61 in Treatment A, and 7.96 to 9.45 in Treatment B in the morning. and in the afternoon from 6.12 to 8.99 in Treatment A, and 6.44 to 9.00 in Treatment B. To increase the shrimp growth, a pH range of 6.8 to 8.7 is needed (Suwoyo and Sahabuddin, 2017). The water with a pH of 7.5 to 9.0 is considered a suitable value for shrimp production, a pH below 5.0 can inhibit the shrimp growth. Usually a low pH is followed by a high content of accumulated organic matter, and no perfect oxidation occurs (Anonymous, 1985). Low pH can result in the shrimp reduced appetite, unstable alkalinity, and easy stress. This can be anticipated by applying lime, both during the pond preparation period, and during cultivation. The pH value is very determining the suitability of a marine environment for shrimp (Ratnawati, 2008; Nengsih, 2015).

The phosphate content in Treatment A on the second observation was higher than Treatment B (Figure 1A). In Treatment A, the phosphate content increased to 0.8516 mg/L, whereas in Treatment B it decreased to 0.1621 mg/L. The phosphate content in the Treatment A and B in the third observation decreased until the end of the research. In a shrimp aquaculture activity, the suitable phosphate content ranges from 0.05 to 0.5 mg/L (Kasnir, *et al*., 2014). Phosphate is a form of phosphorus that can be utilized by high and low level plants (microalgae), thus affecting the level of aquatic productivity.

During the observation, the nitrite content (Figure 1B) fluctuated and tended to increase even though it was very small. The nitrite content in Treatment A and Treatment B in the first and fourth observation was smaller than 0.001 mg/L. Adiwijaya, *et al.* (2003), that the optimal range of nitrite for aquaculture is 0.01 to 0.05 mg/L. For shrimp aquaculture, nitrite content is < 0.25 mg/L (Kasnir, *et al*., 2014). Nitrites are formed in the process of nitrification, that is the oxidation of ammonia becomes nitrite by the bacterium Nitrosomonas (Wetzel, 1983). The detection result of water nitrite content in ponds must be less than 0.01 mg/L (Puryaningsih, 2003).

The nitrate content had a different pattern in Treatment A and Treatment B (Figure 1C). Treatment A in the third observation was much higher than Treatment B. Treatment A was 0.5427 mg/L, and Treatment B was 0.0901 mg/L. This is as a result of the performance of the bacterium *Bacillus sp* in A to convert NO_2 – N water into

NO_3 – N. Madigan, *et al*. (1997), in Mustafa and Mangampa (1990), stated that the bacterium *Bacillus sp* is one type of bacteria that can convert NO_2 – N to NO_3 – N. According to Clifford, (1994) that the optimal nitrate content for shrimp aquaculture ranges from 0.4 to 0.8 mg/L.

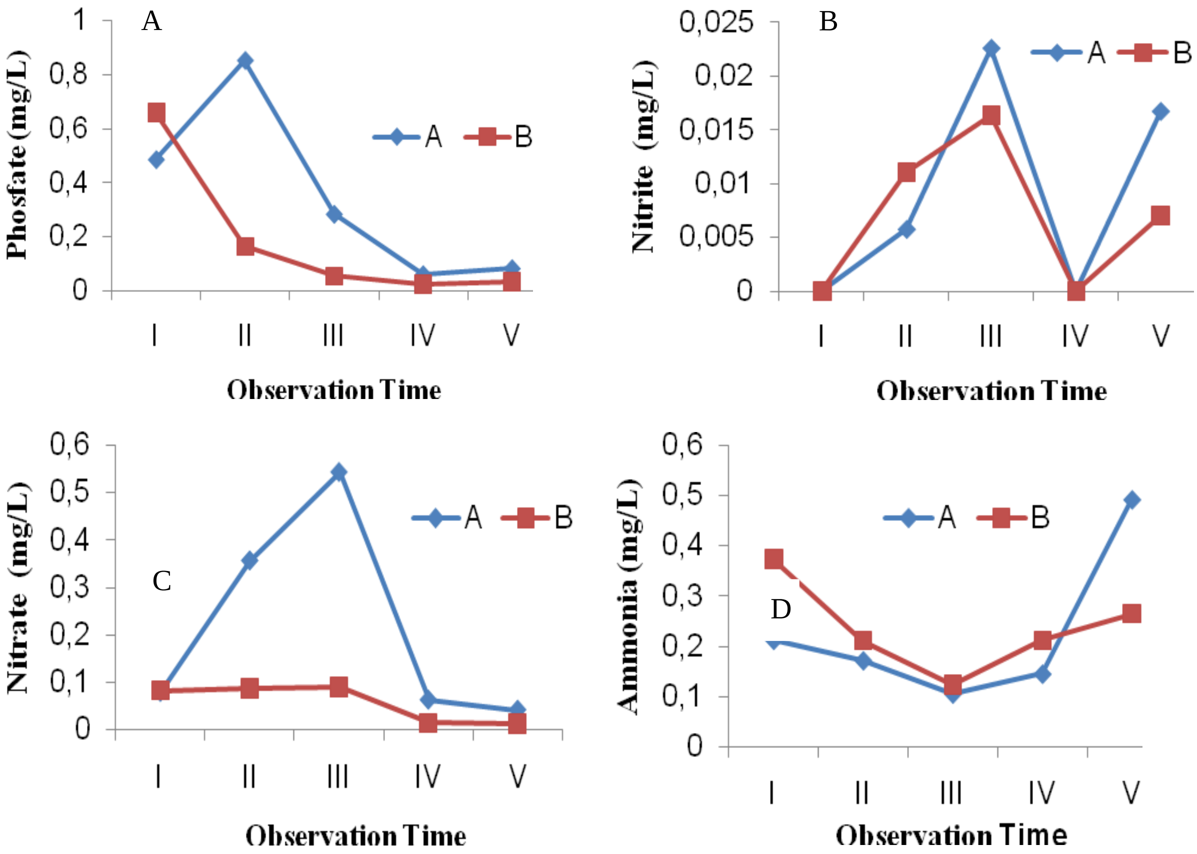

Fig.1: Fluctuation of Phosphate (A), nitrite (B), nitrate (C) and ammonia (D) in pond water during tiger shrimp (P.monodon) cultivation

The phosphate content in Treatment A on the second observation was higher than Treatment B (Figure 1.A). In Treatment A, the phosphate content increased to 0.8516 mg/L, whereas in Treatment B it decreased to 0.1621 mg/L. The phosphate content in the Treatment A and B in the third observation decreased until the end of the research. In a shrimp aquaculture activity, the suitable phosphate content ranges from 0.05 to 0.5 mg/L (Kasnir, *et al*., 2014). Phosphate is a form of phosphorus that can be utilized by high and low level plants (microalgae), thus affecting the level of aquatic productivity.

During the observation, the nitrite content (Figure 1.B) fluctuated and tended to increase even though it was very small. The nitrite content in Treatment A and Treatment B in the first and fourth observation was smaller than 0.001 mg/L. Adiwijaya, *et al.* (2003), that the optimal range of nitrite for aquaculture is 0.01 to 0.05 mg/L. For shrimp aquaculture, nitrite content is < 0.25 mg/L (Kasnir, *et al*., 2014). Nitrites are formed in the process of nitrification, that is the oxidation of ammonia becomes nitrite by the bacterium *Nitrosomonas* (Wetzel, 1983). The detection result of water nitrite content in ponds must be less than 0.01 mg/L (Puryaningsih, 2003). This shows that application of probiotics is quite effective in reducing nitrite levels. This shows that application of probiotics is quite effective in reducing nitrite levels. Both forms of probiotics could effectively maintain water quality parameters like total organic matter, NH_3-N, NO_2-N, and TBV/TPC ratio (Atmomarsono, 2020)

The nitrate content had a different pattern in Treatment A and Treatment B (Figure 1.C). Treatment A in the third observation was much higher than Treatment B. Treatment A was 0.5427 mg/L, and Treatment B was 0.0901 mg/L. This is as a result of the performance of the bacterium *Bacillus sp* in A to convert NO_2 – N water into NO_3 – N. Madigan, *et al.* (1997), in Mustafa and

Mangampa (1990), stated that the bacterium *Bacillus sp* is one type of bacteria that can convert $NO_2 - N$ to $NO_3 - N$. According to Clifford, (1994) that the optimal nitrate content for shrimp aquaculture ranges from 0.4 to 0.8 mg/L.

The ammonia content (Figure 1.D) in both treatments, from the initial shrimp rearing until the third observation, decreased from 0.3740 mg/L to 0.1241 mg/L in Treatment B, and decreased from 0.2129 mg/L to 0.1061 mg/L in Treatment A; after the fifth observation, the ammonia content rose to 0.4920 mg/L. Kumar, *et al*. (2016), that the value of ammonia nitrogen content in shrimp rearing, using probiotics and without probiotics, respectively are 0.32 to 0.71 mg/L and 2.1 to 2.7 mg/L; and further stated to maintain the culture media and stabilize ammonia, it is recommended to use probiotics. Mangampa (2010) states that the direct effect of high ammonia levels, but not deadly, will damage the gill tissue, then the gill sheet will swell (*hyperplasia*), therefore the function of the gills as a means of breathing will be disrupted in terms of the oxygen binding of water. High levels of ammonia can increase the concentration of ammonia in the blood, thereby reducing blood activity (*hemocyanin*) in oxygen binding. In addition, high levels of ammonia can also increase the susceptibility of shrimp to disease.

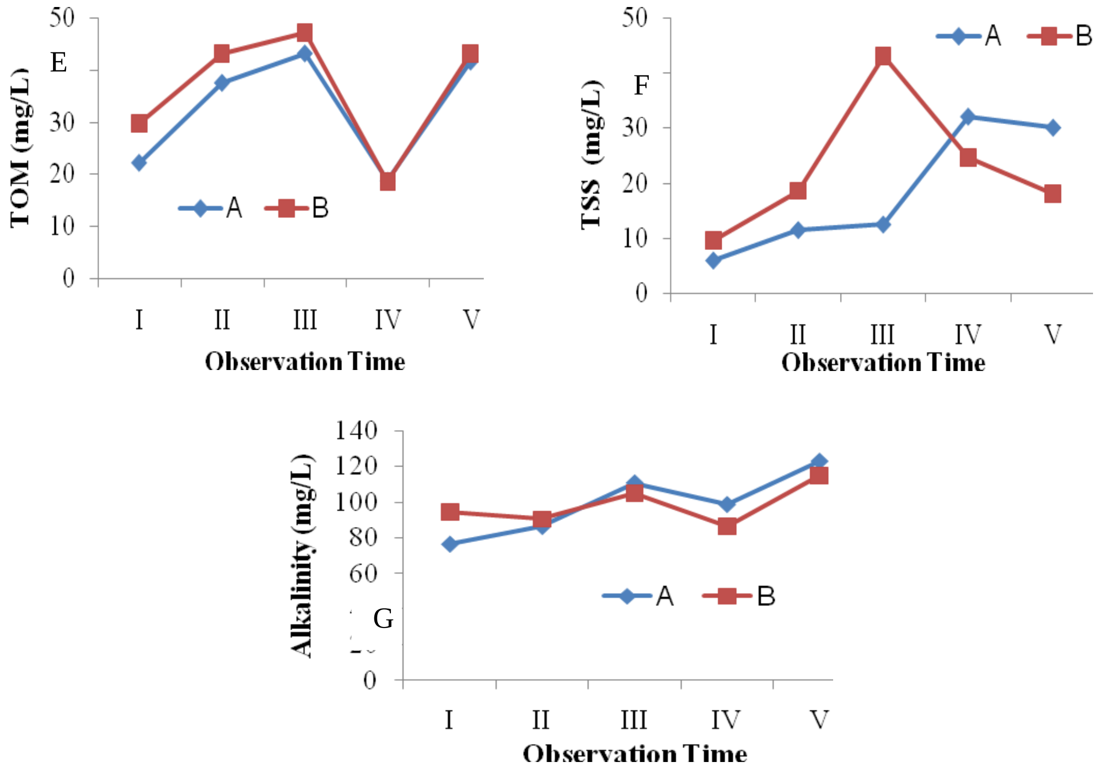

Fig.2: Fluctuation of TOM (E), TSS (F), and Alkalinity (G) in pond water during tiger shrimp (P. monodon) cultivation

The TOM and TSS content increased in both treatments, and decreased after the fifth observation. The average value of TOM in Treatment A was 32.65 mg/L, and treatment B was 36.35 mg/L. Gunarto, *et al*. (2006), states that the use of probiotics can improve the redox potential value of pond sediments, reduce the value of ammonia content, organic matter, and suppress the growth of *Vibrio sp* population in pond water. The TSS content in Treatment A was 18.4 mg/L, and Treatment B was 22.7 mg/L; the high TSS content in shrimp ponds is caused by artificial feed input in the shrimp aquaculture system which is not consumed (leftover), and the metabolism results in the form of feces that are dissolved in the waters. For the shrimp aquaculture that depends entirely on artificial feed (pellets), if the feed is given a lot that is not eaten by shrimp, then it can cause high organic matter (TOM). The average alkalinity obtained was 98.86 mg/L in Treatment A, and in Treatment B it was 36.35 mg/L. Alkalinity is determined by the amount of acid needed to reduce pH. According to Atmomarsono, *et al*. (2013), the value of water alkalinity in ponds is used as pH stabilization and normal growth of phytoplankton.

According to Adiwidjaya, *et al*. (2008), the optimal alkalinity for vannamei shrimp aquaculture activities ranges from 90 to 150 ppm. The alkalinity content of tiger shrimp pond water is recommended > 100 mg/L or in the range of 120 to 160 mg/L.

IV. CONCLUSIONS

Application of liquid and powder formed probiotics in semi-intensive tiger shrimp aquaculture ponds can maintain a better water quality stability.

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