J. Mar. Biotechnol. Immunol
Vol. 2 No. 1 (2024)
ISSN 3026-1457 (Online)
ISSN 3026-5274 (Print)

Journal of Marine
Biotechnology and Immunology

Journal homepage : https://ejournal.immunolmarbiotech.com

Physico-Chemical Dynamics of Vanname Shrimp (*Litopenaeus vannamei*, Boone 1931) Cultivation Pond Water Quality with A Recirculation System

Aisyiyah Rasyidah[1], Sunaryo[2], Ervia Yudiati[2], I Nyoman Widiasa[1]*

[1]Department of Chemical Engineering, Diponegoro University, Jalan Prof. Soedarto, SH, Tembalang – Semarang
[2]Department of Marine Scince, Diponegoro University, Jalan Prof Jacub Rais, SH, Tembalang – Semarang

Article Info
Received: Desember 01, 2023
Accepted: January 14, 2024
Published: January 26, 2024
Available online: January 30, 2024

Keywords:
Litopenaeus vannamei
Recirculating Aquaculture System
Water Quality Dynamic
Ultrafiltration

*Corresponding Author email:
inyomanwidiasa@lecturer.undip.ac.id

Abstract

In 2021 Indonesia was experiencing an increase in shrimp exports. *Litopenaeus vannamei* shrimp is valuable in exports outlook with high resistance to environmental disturbances. The cultivation technique that can maximize production is a super-intensive technique that uses Recirculating Aquaculture system (RAS) in a closed aquaculture system. In this study, the RAS system was equipped with an external biological treatment (WWTP) of overflow water of the cultivation ponds and the use of ultrafiltration membranes as a tool for the inflow water treatment. This study observe the physico-chemical water dynamics that occur during one cultivation cycle (100 days of cultivation). It was observed that the dissolved oxygen of the pond decreased as the shrimp grew, the salinity increased during the cultivation process if there was no water replacement, the brightness decreased every day until it reached a specified point. Temperatures tended to be low due to weather factors, ammonia levels tended to decrease but nitrite and nitrate levels tended to increase due to the slower transformation rate of nitrite and nitrate. The operating system for this process was carried out by adding CaO and sugar. The addition of CaO ranges from 100-600 g/day. The effective addition of sugar if there was a spike in ammonia levels was 1.25 kg/day. The application of the RAS system seems to provide hope regarding the superintensive vannamei shrimp cultivation.

1. Introduction

Shrimp is one of the fishery export commodities in Indonesia. During this pandemic, demand for exports continues to increase, which can be seen with shrimp exports in 2021 which increased by 9.3% compared to the previous year (Emeria, 2022). One type of shrimp that has experienced an increase in exports is the *Litopenaeus vannamei* shrimp. In 2020 exports of *L. vannamei* experienced a significant increase, approximately 22.9% from the previous year (KKP, 2021).

It is estimated that around 80% of shrimp farms in Indonesia still use extensive techniques as a means of cultivation (Rubel *et al*., 2019). Low operational costs and lack of skilled personnel are one of the reasons for using extensive techniques. The land used for the extensive techniques is larger than for other techniques. In addition, several disadvantages are still attributed to these conventional systems, such as a lack of water quality management, biosecurity issues, and disease outbreak, which lead to unpredictable culture performance (Suantika *et al*., 2018).

Two emerging production strategies are recirculating aquaculture system (RAS) (Davis and Arnold, 1998; Badiola *et al*., 2018; Sun *et al*., 2023) and zero water discharge (ZWD) or biofloc system (Crab *et al*., 2012; Hussain *et al*., 2021; Yu *et al*., 2023). Both cultivation techniques are controlled and environmentally friendly systems that offer excellent water quality management, water efficiency as well as site and culture dimension flexibility (Suantika *et al*., 2018). In principle, RAS is a cultivation technology of aquatic organisms in which the cultivation water is treated externally and the treated water is returned to the cultivation ponds. The external water treatment is intended to maintain the quality of the cultivation water. A recirculating aquaculture system usually consists of a series water treatment unit to control cultivation water quality, including nitrogen compounds, organic matter, bacteria, suspended solids, and viruses (Jegatheesan *et al*., 2009). This process can be carried out by administering ozone or irradiating it with UV light. However, these two things have no drawbacks. Providing ozone can trigger the formation of toxic substances when applied to sea water (Holan *et al*., 2020). Meanwhile, irradiation with UV light will not be efficient if there are still fine solids present (Bregnballe, 2015). Membrane application can help processing in the

RAS. Membrane options that can be used are microfiltration, ultrafiltration and nanofiltration.

de Jesus Gregersen *et al*. (2020) have researched the use of microfiltration in RAS systems. The use of microfiltration is quite useful for RAS systems because it reduces microscopic particles in the system. However, because the pore size is still too large, harmful microorganisms can still be filtered out, so additional tools are needed for the disinfectant process. The addition of this tool can increase production costs. In nanofiltration viruses and ammonia can be filtered. The weakness of the nanofiltration application is that it blocks the membrane and requires high costs. Apart from that, with nanofiltration some of the salt content in the water can be filtered out so that brackish water cultivation is not suitable (Boffa *et al*., 2022). The use of ultrafiltration in RAS has been carried out by Fossmark *et al*. 2020. This research shows that the use of ultrafiltration makes the water quality in the pond better compared to the conventional RAS system used on salmon. Apart from that, ultrafiltration also has pores that can filter pathogens (Ng *et al*., 2018).

Apart from the disinfectant process, there are other problems with the biofiltration process. In the biofiltration process, there is a denitrification stage. This denitrification process aims to convert the nitrate content into nitrogen gas. This process requires anoxic operating conditions to run optimally so that after the biofiltration process an oxygenation process is required (van Rijn *et al*., 2006). Oxygenation is needed so that DO in the pond is maintained. Nevertheless, with the addition of oxygen, production costs will increase. In most biofiltration systems, the nitrate that is formed cannot be completely converted into nitrogen gas (Deng *et al*., 2020; Lindholm-lehto *et al*., 2020). The remaining nitrate will recirculate into the system so that the feed water entering the WWTP contains nitrate. With the presence of nitrate in the feed, the denitrification process can be carried out at the beginning of the process. Oxygen levels in water can also be maintained by aeration during the nitrification process.

The RAS system which uses an ultrafiltration membrane and a different biofiltration process setup is a system that is rarely applied so further research is needed before it can be applied on an industrial scale.

2. Material and methods

2.1 Materials

Evergreen Vanname Feed 922 series was used for feeding shrimp in the ponds. The bacteria in the waste water treatment were applied with Super NB (PT. Marindolab Pratama) and EM4 (PT. Songgolangit Persada). Sugar was added to enhance the process in the waste water treatment. For analysis the cultivation pond water used reagents from ammonium test kit (Merck test kit no. 1.11117.001), nitrite test kit (Merck test kit no. 1.14774.0001), nitrate test kit (Merck test kit no. 1.11170. 0001), alkalinity test kit (Merck test kit no. 1.11109.0001).

2.2 Methods

The RAS system for cultivating *L. vannamei* shrimp was carried out in one cluster containing four ponds. The four cultivation ponds was connected to the same water treatment system. The cultivation ponds was supplied with sea water that has been filtered with an ultrafilter (ultrafiltration membranes). Overflow water of the cultivation ponds was treated by an external biological treatment (WWTP) prior to be returned back to cultivation ponds. The scheme of the RAS system can be seen in the Figure 1.

The cultivation process began when *L. vannamei* shrimp was stocked. Shrimp stocking was carried out according to the stocking number of 400 ind/m^2. Shrimp was given feed based on procedure from feed manufacture, average body weight (ABW), survival rate (SR), and the presence of residual feed in anco. Stabilization of the pH value of water is carried out by adding CaO periodically. To handle ammonia spike, bacteria and molasses were added to the cultivation ponds.Shrimp cultivation finished at 100 days for one cycle. Every day the water quality parameters were measured such as dissolved oxygen (morning) (YSI Pro20), temperature (morning) (YSI Pro20), salinity (morning) (ATC Salinity Refractometer), brightness (morning & noon) (secchi disk), pH (morning & noon) (Smart Sensor PH818). Analysis of total ammonia, nitrite, nitrate, and alkalinity was also carried out once a week using a test kit. In the middle of the cycle, partial harvests were carried out to reduce shrimp density. After all the shrimp were harvested, the survival rate was calculated. Survival rate (SR) = (number of shrimp harvested)/(total number of shrimp)×100%

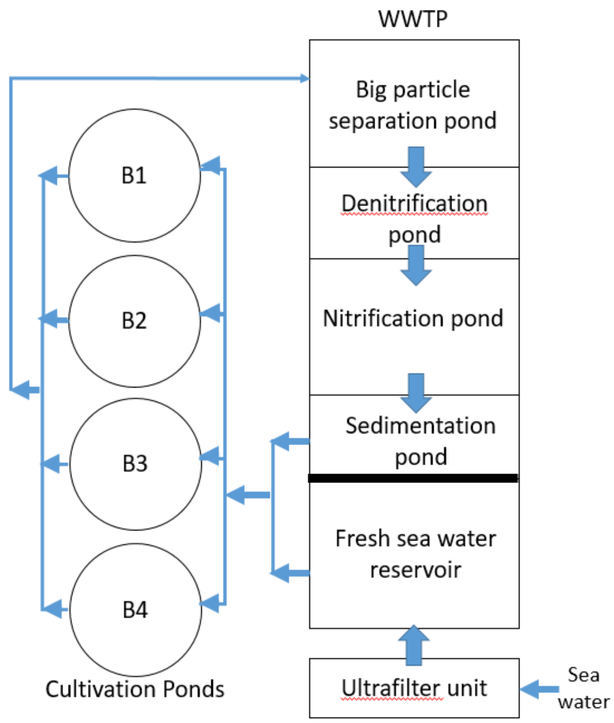

Figure 1. Cultivation Ponds with RAS Scheme

3. Results

3.1. Dissolved oxygen (DO)

Figure 2 shows the DO dynamics of cultivation ponds. At the beginning DOC was in the range of 5-6 mg/L. As time goes on, the DO decreased until DOC 22 then increased again. This increase was caused by quite a lot of water replacement due to a large increase in ammonia levels. After that, there was a fairly low decline again at DOC 49. This could possibly happen because the shrimp were large enough and ready for partial harvest. Therefore, cluster B was then harvested at DOC 50. At DOC above 60, DO levels become more fluctuating because the water in the pond was over saturated. DO levels can be maintained at levels above 4 mg/L with several partial harvests and water change.

3.2. Temperature

At the beginning of the DOC (Figure 3) it can be seen that the cultivation pond water temperature was very low. After DOC 5, temperature increase gradually until 28.2

ºC. In general, temperature dynamic was in the range of 26.7 – 28.2 ºC.

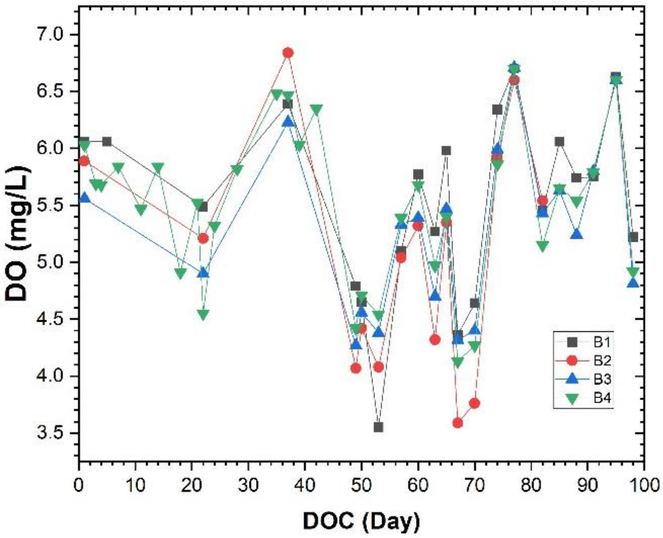

Figure 2. DO Dynamic

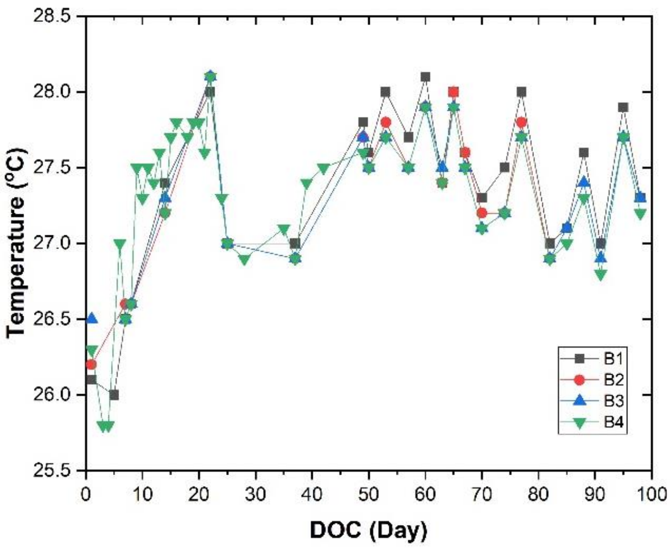

Figure 3. Temperature Dynamic

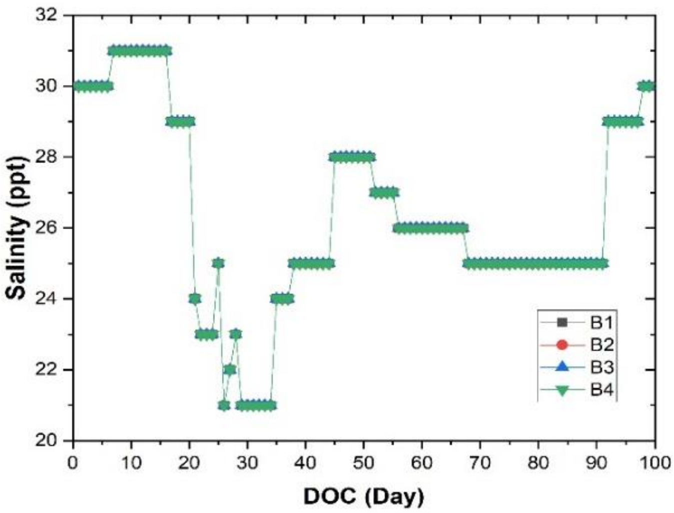

Figure 4. Salinity Dynamic

3.3. Salinity

In Figure 4, it can be seen that the four ponds have salinity that was not much different. At the beginning of DOC, the cultivation pond salinity was around 30-31 ppt for 2 weeks then slowly decreased. The next decrease in salinity occurred at DOC 53 to DOC 69.

3.4. Brightness

Figure 5 shows that there was a slight difference in brightness in morning and afternoon measurements. At the beginning of cultivation, the water in shrimp ponds still looks transparent. As time goes by, the water transparency of the shrimp pond decreases in which the lowest value was aproximately 30 cm.

3.5. pH

In the daily pH graph for these four cultivation ponds (Figure 6) it can be seen that the pH decreased during the day. A significant decrease in pH occurred at DOC 16 . After DOC 86, the pond pH tends to be high. Apart from that, the pH change after the DOC 86 was not too high. Overall, the pH level of cultivation water maintained at 7.5 – 8.5 and daily pH fluctuation less than 0.5. Controlling of pH was conducted by CaO addition as shown in Figure 7.

3.6. Ammonia, nitrite and nitrate content

Figure 8 shows total ammonia levels from cultivation pond and external biological treatment (WWTP). At DOC 13 – 22, the total ammonia levels were quite high, namely 5 mg/L, and then continued to decrease to a level of 0.5 mg/L. The ammount of sugar to control the ammonia level is shown in Figure 9. Moreover, Figure 10 and Figure 11 show the dynamics of nitrite and nitrate, respectively. The highest level of nitrite was 32 mg/L at DOC 63, whereas the highest level of nitrate was 100 mg/L at DOC 70.

3.7. Alkalinity

Figure 12 shows an increase in alkalinity levels up to DOC 77 and then decrease due to several water changes. The alkalinity level was higher than 150 mg/L since the initial of cultivation, and then and continues to increase gradually. The highest level was 361 mg/L at DOC 56 – 77.

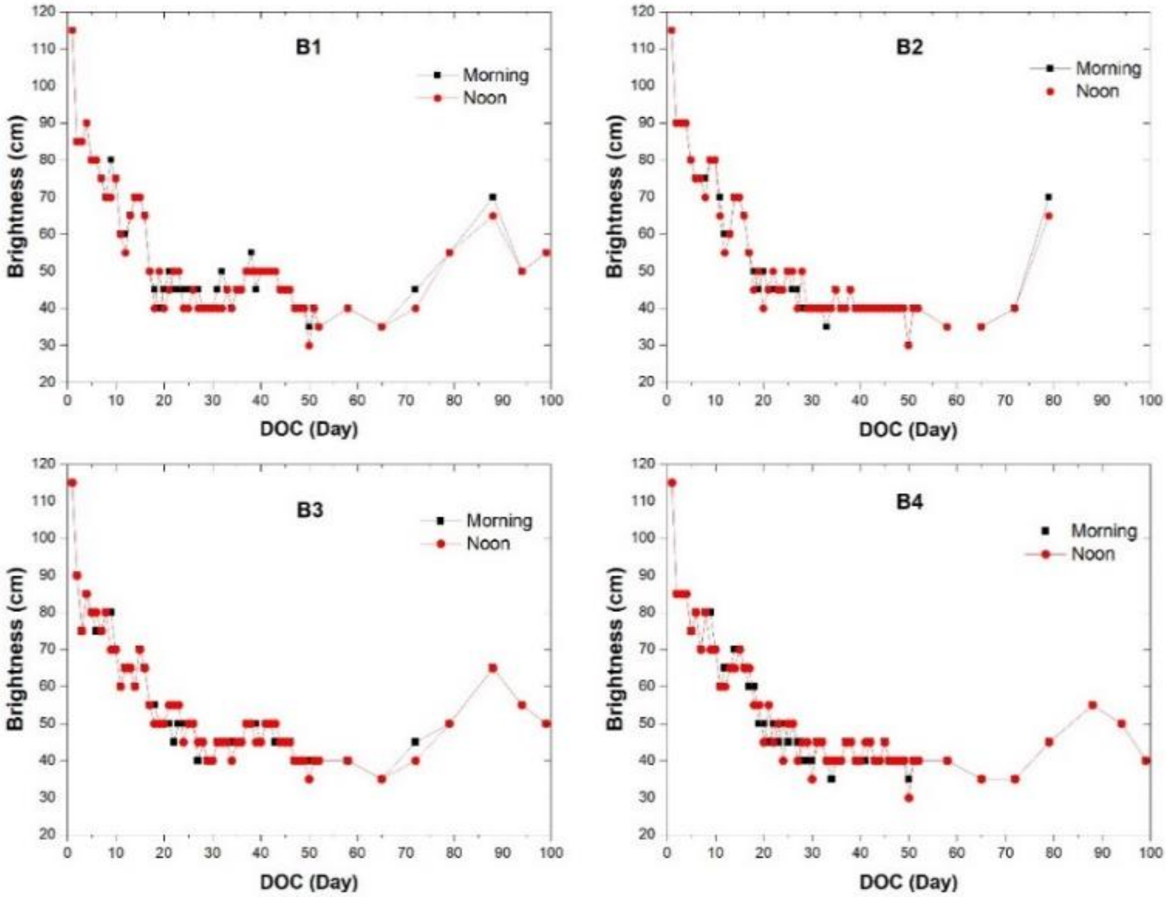

Figure 5. Brightness Dynamic

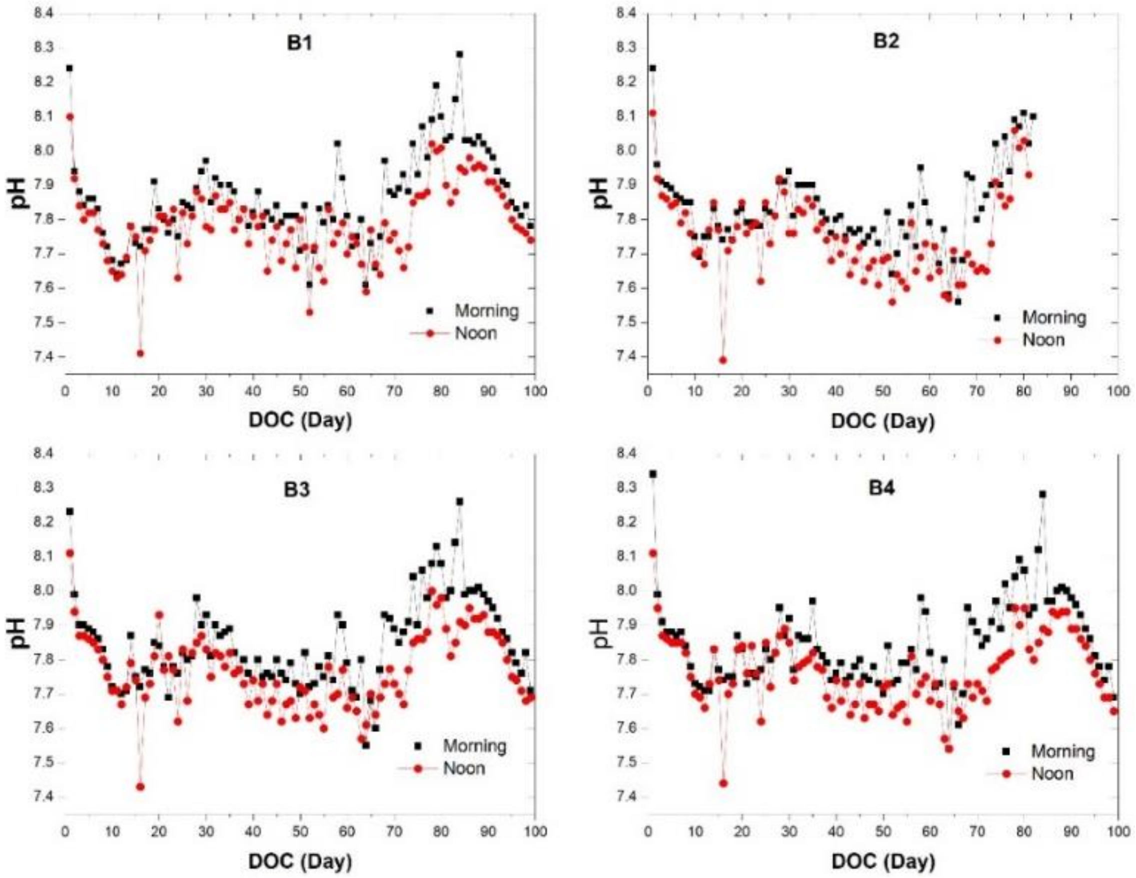

Figure 6. pH Dynamic

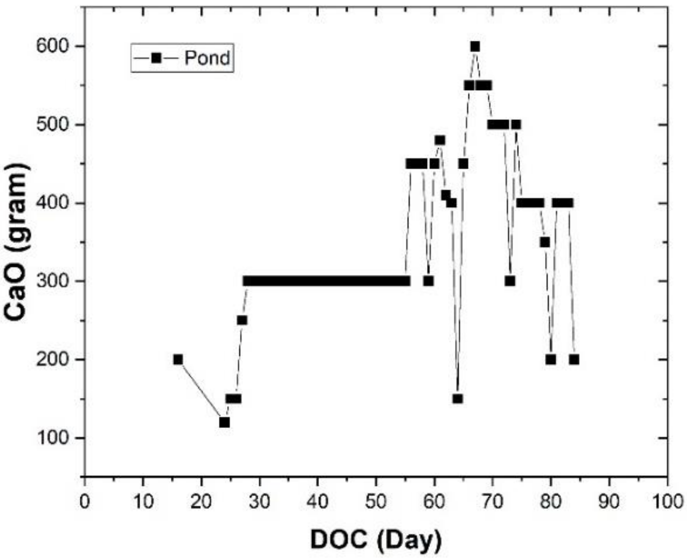

Figure 7. Addition of CaO to Cultivation Ponds

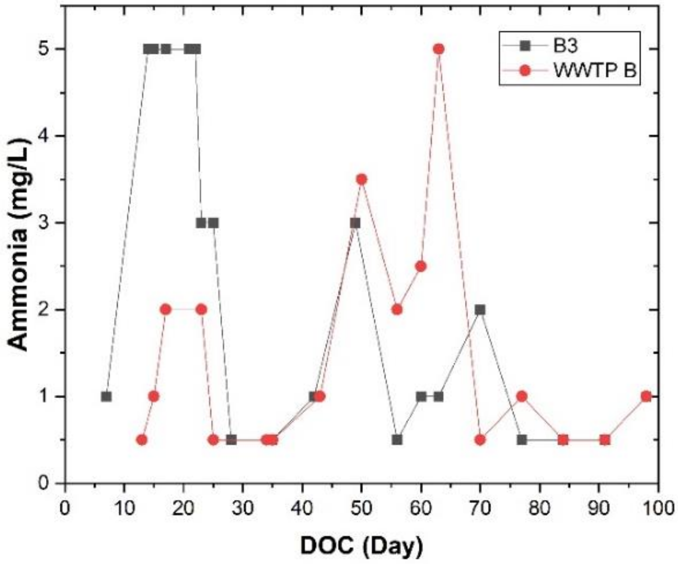

Figure 8. Total Ammonia Dynamic

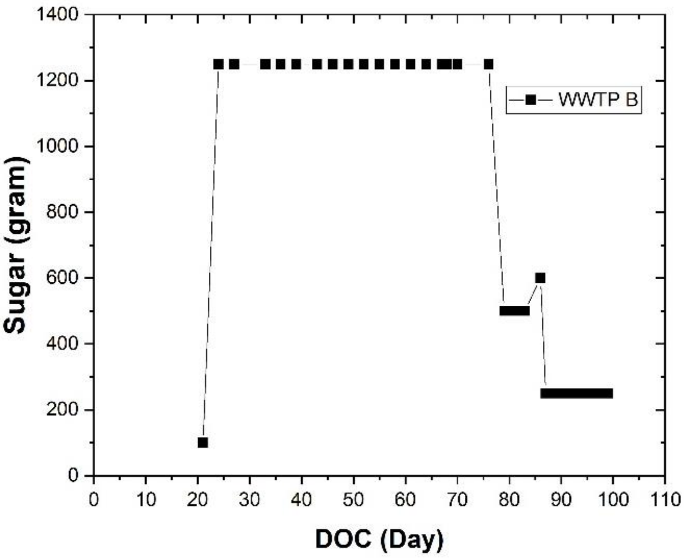

Figure 9. Sugar Addition to WWTP B

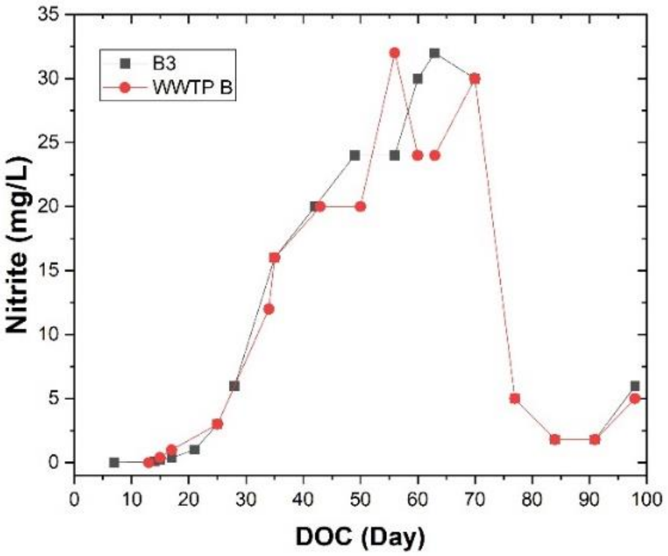

Figure 10. Total Nitrite Dynamic

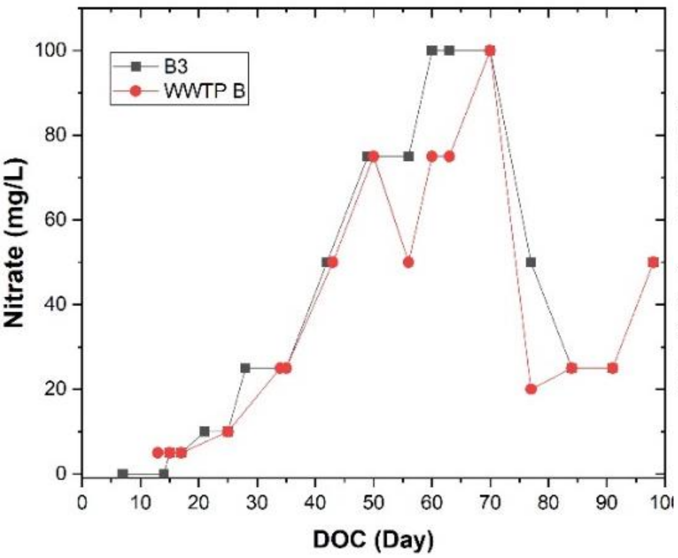

Figure 11. Total Nitrate Dynamic

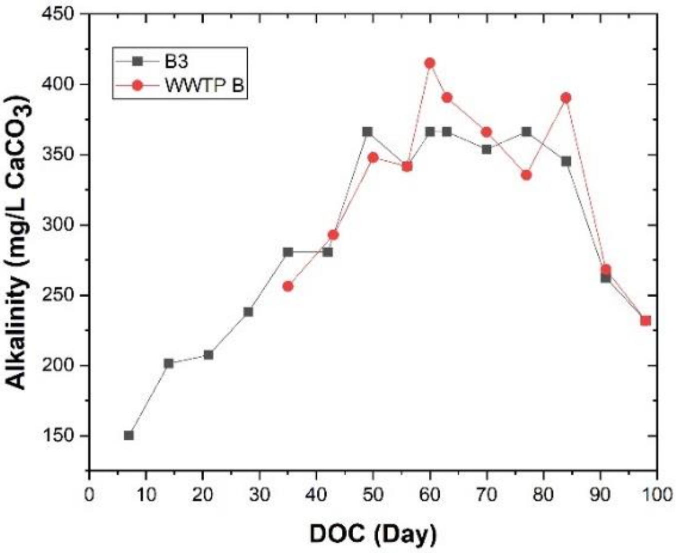

Figure 12. Total Alkalinity Dynamic

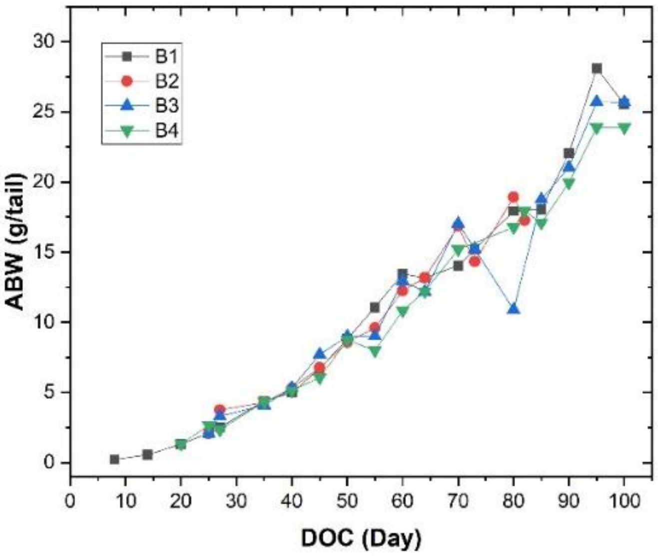

Figure 13. ABW during 100 days of cultivation

4. Discussion

The low temperature at the beginning of the DOC was caused by bad weather at the start of DOC. it rains every day, covering the sun's rays. After a few days, the weather improved and the intensity of the rain was not too high. There was some temperature decline but it was not as low as at the start of DOC where the temperature was below 26ºC. Indirectly, temperature can affect the quality of pond water. Low temperatures can reduce the shrimp's appetite so that a lot of shrimp food remains. The remaining shrimp feed can increase the water treatment burden. Throughout this cycle the temperature of the four cultivation ponds cannot reach 29ºC. This temperature was still relatively low because the optimal temperature that can maximize shrimp appetite is above 30ºC (Sukenda, 2021).

The optimal brightness for shrimp cultivation is 30-50 cm (KKP, 2017). At the beginning of DOC the water transparency was still high and as time goes by the water will become more concentrated. Changes in brightness levels are influenced by the growth of microorganisms, shrimp feed and shrimp feces. It is important that the brightness should be maintained at no less than 30 cm. The control of cultivation water brightness can be done by replacing the water and siphoning (cleaning the sediment at the bottom of the pond). Sediments which are leftover feed can become nutrients for the growth of toxic microorganisms and can also decompose into toxic H_2S (Rakhmanda *et al*., 2021). The growth of microorganisms during the day resilted in a slight brightness difference in morning and afternoon.

The good DO level in cultivation pond keeps the shrimp in a strong immune condition where the optimum DO condition in the pond is more than 4 mg/L (Sukenda, 2021). This is clarified by the research results of Nonwachai *et al*. (2011) who stated that vannamei shrimp treated with different dissolved oxygen produced different immune systems. Treatment with dissolved oxygen concentration greater than 4 mg/L produced better immune parameters as seen from hemocyte count, phagocytosis percentage, bactericidal activity, phenoloxidase activity, superoxide dismutase activity than treatment with an oxygen concentration of less than 2 mg/L. Lehmann *et al*. (2016) elaborated that the hypoxia condition increased the susceptability of shrimp to the infection of white spot syndrome virus. Also another resercher, Chang *et al*. (2022) found that 6 hours of hypoxic stress caused a significant reduction in total hemocyte number in L. vannamei. However, as the DOC progresses, the pond's DO decreased. Several ways to maintain pond DO are by enlarging the aeration tap opening and changing the water. If DO of the pond continues to deteriorate after various treatments, it is necessary to carry out a partial harvest of the shrimp. In this cycle five harvests are carried out.

During the first 13 days of cultivation, the cultivation water salinity was around 30-31 ppt. Water replacement resulted ini the decrease of salinity because rainfall was high. The increase in salinity occured when there was no water replacement. This can be caused by evaporation of water from the cultivation pond, thereby concentrating the salt content in the pond. The next decrease in salinity occurred at DOC 53 to DOC 69 which was caused by replacing water in the pond. Indonesia standards for shrimp culture water quality recomends the salinity are 5 – 40 ppt, 10 – 35 ppt, and 26 – 32 ppt for extensive, semi-intensive, and intensive/super-intensif, respectively (KKP, 2017)

The pH level greatly affects other water quality parameters, the performance of bacteria and farmed shrimps. The pH of water affects the appetite of shrimp and chemical reaction in the water (Darmawan and Maizar, 2023). Chemical reaction in water, especially the ammonia toxicity to shrimp is affected by the daily change of pH value. Due to CO_2 produced by respiration, pH may be significantly decreased in a closed culture system. The dense concentration of algae can results in a large variation in pH, where the pH is higher during the day when photosynthesis occurs and the pH is lower at night. The pH level of cultivation water was maintained at 7.5 – 8.5 and daily pH less than 0.5 (KKP, 2017). Wasielesky *et al*. (2015) suggest to maintain a pH level between 7.4 and 8.2. Reddy and Mounika, 2018) stated that the optimal pH value for vannamei shrimp culture is 7.5-8.5 with range of 0.5. Boyd (2010) elaborated that the average pH difference in the morning and evening was 0.65 ± 0.19.

Too low pH (<7) can negatively impact on shrimp and bacteria (Ebeling *et al*., 2006). Growth and survival in the biofloc system was lower at pH 7 than at pH 8 (Wasielesky *et al*., 2015), and the function of nitrifying bacteria decreased below pH 6.8 (DeLong *et al*., 2009). Chen *et al*. (2015) reported that long-term exposure to low pH (6.8) decreased the juvenile immune response of Pacific White Shrimp and its resistance to *Vibrio alginolyticus*. A significant decrease in pH usually occured when molasses and bacteria are added. This was clarified by Hartutik *et al*. (2021) who stated that the use of 10% molasses for making maize stover silage reduces pH. Other researchers Sagir *et al*. (2017) also stated that the growth of purple non sulfur bacteria in sucrose media to produce biohydrogen can result changes in pH. Therefore, the addition of CaO was continued to anticipate a decrease in pH due to the addition of molasses. In the contratry, the proportion of toxic ammonia increases at higher pH.

Dissolved nitrogen compounds that need attention in shrimp grow-out ponds include ammonia, nitrite, and nitrate. The existence of these three compounds is closely related to the density of cultivated shrimps, feed management, and biological degradation processes (nitrification-denitrification processes and autrophic processes). When a culture system is managed properly, ammonia and nitrite concentrations are below 2 mg/L and can even be close to zero. Nitrates will accumulate when the denitrification process is not inadequate and can cause algae blooms. Shrimp cultured in a limited exchange biofloc system may tolerate high levels of ammonia and nitrite (Krummenauer *et al*., 2014), but their toxicity is greater at low salinity.

Among the water quality parameters that affect shrimp especially in intensive systems, dissolved ammonia nitrogen is the most important after dissolved oxygen. In water, dissolved ammonia nitrogen occurs in two forms, ionized ammonia (NH_4^+) and unionized ammonia (NH_3), which together are called total ammonia nitrogen (TAN). This difference form is important to know that NH_3 is more toxic to fish than NH_4^+ due to its non-polar property and readily soluble in the lipids of biological membranes (Eddy, 2005). Valencia-Castañeda *et al*. (2019) proposed safety levels of single exposure to TAN for L. vannamei was 1.45 mg/L. However, this study shows that a TAN level of 5 mg/L for a short duration is still safe for the life of vannamei shrimp as long as the pH can be maintained at less than 8.0, where TAN is almost entirely in the form of ionized ammonia (NH_4^+).

The alkalinity component mainly consists of bicarbonate ions (HCO_3^-), carbonate (CO_3^{2-}), and hydroxide (OH^-). Alkalinity functions as a buffer system that maintains the pH of the water. The increase of alkalinity levels up to DOC 77 was mainly due to the addition of CaO lime (See Figure 7). González-Vera and Brown (2017) stated that total hardness and alkalinity of the water are important considerations in the culture of crustacean. The concentrations of cations (calcium-magnesium) and anion (carbonates) are necessitated for the mineralization in hardenning of the exoskeleton when moulting. Shrimps body are protected and supported by calcareous exoskeleton, and growth is only possible by moulting. The alkalinity level correlates with the amount of Ca and Mg which can be useful for the formation of shrimp shells when molting occurs. Moulting is a high energy consumption process and the newly-soft exoskeleton exposes shrimps to a high risk of mortality. Experts suggest an ideal pH of 7.8-8.2 for molting. pH stability for optimally moulting in crustacean is apropriatelly on alkalinity of 100-120 mg/L.

Timmons and Ebeling (2013) stated that alkalinity is important for keeping the pH optimal in the recirculating system and supplying bicarbonate ions to the reactions of nitrification. Summerfelt and Sharrer (2004) explained that nitrification in a recirculation system has an impact on reducing the pH value of the system. The alkalinity level plays an important role in adjusting the pH needed in the biofiltration system. Alkalinity of water or a solution is the quantitative capacity of that solution to buffer or neutralize an acid. Seawater typically has levels between 100-125 mg/L. The alkalinity dynamics that occur in the shrimp rearing ponds still show good conditions for shrimp life and biological process.

The continued increase of the average body weight (ABW) of shrimps during 100 days of cultivation shows that the physico-chemical dynamics of cultivation water was quite good for the growth of vannamei shrimps. ABW of 25.70 g/ind at the end of harvest means that the daily increase in shrimp weight was 0.25 g/day. This result is believed to be closely related to the partial harvest that was carried out at the 50th, 64th, 73rd, and 82nd day of cultivation (DOC). The survival rate was in the range 87.64%–91.45%. The shrimp growth achieved in this study is comparable to the results of studies published in various journals (Suantika *et al*. 2018; Novriadi *et al*., 2023).

.

5. Conclusions

The dynamics of water quality parameters in ponds tend to vary, in DO parameters pond oxygen becomes smaller as the shrimp grow, salinity increases during the cultivation process if there is no water replacement, brightness decreases every day to a certain point and is maintained at that point with siphon treatment and will increase if there is significant water replacement. Temperatures tend to be low due to weather factors. Ammonia levels tend to decrease but nitrite and nitrate levels tend to increase due to the slower rate of change in the decomposition of nitrite and nitrate. The operating system for this process is carried out by adding CaO and sugar. The addition of CaO ranges from 100-600 g/day. The effective addition of sugar if there is a spike in ammonia levels is 1.25 kg/day. The continued increase of the average body weight (ABW) of shrimps during 100 days of cultivation shows that the physico-chemical dynamics of cultivation water was quite good for the growth of vannamei shrimps.

Ethics approval

No need permit to *Litopenaeus vannamei*

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Credit authorship contribution statement

S., E.Y. and I.N.W.: Conceptualization, methodology, validation, investigation, resources, writing original draft preparation, writing review and editing, visualization, supervision, project administration, funding acquisition. A. R: formal analysis, writing original draft preparation, visualization. All authors have read and agreed to the published version of the manuscript.

Funding

This work was funded by the Indonesia Endowment Fund for Education (LPDP) - the Ministry of Finance Republic Indonesia under the contract number of PRJ-034/LPDP/2021 and Diponegoro University under contract number 185-101/UN7.6.1/PP/2021.

Acknowledgments

I would like to state my special thanks of gratefulness to Diponegoro University and CV Riz Samudera.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Referrences

Badiola, M. O.C. Basurko, R. Piedrahita, P. Hundley, D. Mendiola. 2018. Energy use in Recirculating Aquaculture Systems (RAS): A review. *Aquacult. Eng*. 81: 57–70.

Boffa, V., Fabbri, D., Calza, P., Revelli, D., & Christensen, P. V. 2022. Potential of nanofiltration technology in recirculating aquaculture systems in a context of circular economy. *Chemical Engineering Journal Advances*. 10: 100269. https://doi.org/10.1016/j.ceja.2022.100269.

Boyd, C. E., & Hanson, T. 2010. Dissolved-oxygen concentrations in pond aquaculture. *Glob Aquac Advocate*.

Bregnballe, J. 2015. A Guide to Recirculation Aquaculture : An Introduction to the New Environmentally

Friendly and Highly Productive Closed Fish Farming Systems. *FAO and EUROFISH International Organisation*.

Chang, F., Li, N., Shi, X., Olga, V., Wang, X., Diao, X., . Zhou, A., and Tang, X. 2022. Physiological and muscle tissue responses in *Litopenaeus vannamei* under hypoxic stress via iTRAQ. *Frontiers in Physiology*. 13: 979472. https://doi.org/10.3389/fphys.2022.979472.

Chen, S., Yu, J., Wang, H., Yu, H., and Quan, X. 2015. A pilot-scale coupling catalytic ozonation–membrane filtration system for recirculating aquaculture wastewater treatment. *Desalination*. *363*: 37-43.

Crab, R., Defoirdt, T., Bossier, P., and Verstraete, W. 2012. Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture*. *356*: 351-356.

Darmawan, A., & Maizar, A. 2023. Pathway analysis of pH in whiteleg shrimp, *Litopenaeus vannamei* concrete pond intensifies in Banyuwangi East Java. In *IOP Conference Series: Earth and Environmental Science*. 1191(1): 012015.

Davis, D. A., and Arnold, C. R. 1998. The design, management and production of a recirculating raceway system for the production of marine shrimp. *Aquacultural Engineering*. 17(3): 193-211.

de Jesus Gregersen, K. J., Pedersen, P. B., Pedersen, L. F., Liu, D., and Dalsgaard, J. 2020. UV irradiation and micro filtration effects on micro particle development and microbial water quality in recirculation aquaculture systems. *Aquaculture*. 518: 734785.

DeLong, D.P., Losordo, T.M., Rakocy, J.E., 2009. Tank culture of Tilapia. Southern Regional Aquaculture Center Publication No. 282.

Deng, M., Dai, Z., Senbati, Y., Li, L., Song, K., and He, X. 2020. Aerobic denitrification microbial community and function in zero-discharge recirculating aquaculture system using a single biofloc-based suspended growth reactor: Influence of the carbon to-nitrogen ratio. *Frontiers in Microbiology*. 11: 1760.

Ebeling, J.M., Timmons, M.B., and Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture*. 257: 346–358.

Eddy, F. B. 2005. Review Paper—Ammonia in estuaries and effects on fish. *J. Fish Biol*. 67: 1495–1513.

Emeria, Damiana Cut. 2022. “Top! Udang Indonesia Cetak Sukses Saat Pandemi, Ini Buktinya.” (https://www.cnbcindonesia.com/news/20220221 62842-4-316828/top-udang-indonesia-cetak sukses-saat-pandemi-ini-buktinya) (accessed 17 September 2022).

Fossmark, R. O., Vadstein, O., Rosten, T. W., Bakke, I., Košeto, D., Bugten, A. V., Helberg, G. A., Nesje, J., Jørgensen, N. O. G., Raspati, G., Azrague, K., Østerhus, S. W., and Attramadal, K. J. K. 2020. Effects of reduced organic matter loading through membrane filtration on the microbial community dynamics in recirculating aquaculture systems (RAS) with Atlantic salmon parr (*Salmo salar*). *Aquaculture*. 524: 735268. https://doi.org/10.1016/j.aquaculture.2020.735268

González-Vera, C., and Brown, J. H. 2017. Effects of alkalinity and total hardness on growth and survival of postlarvae freshwater prawns, *Macrobrachium rosenbergii* (De Man 1879). *Aquaculture*. 473: 521 527.

Hartutik, Marjuki, Huda, A.N., Ndaru, P.H., and Yuanita Nur Arsianty,Y.N., and Binti Nur Rohmah,B.N. 2021. The use of molasses as additive with different ensiling time and physical quality, pH and nutritive of value maize stover silage. *Jurnal Nutrisi Ternak Tropis*. 4(2):88-92.

Holan, A. B., Good, C., and Powell, M. D. 2020. Health management in recirculating aquaculture systems (RAS). In *Aquaculture Health Management*. Academic Press.

Hussain, A. S., Mohammad, D. A., Sallam, W. S., Shoukry, N. M., and Davis, D. A. 2021. Effects of culturing the Pacific white shrimp *Penaeus vannamei* in “biofloc” vs “synbiotic” systems on the growth and immune system. *Aquaculture*, 542: 736905.

Jegatheesan, V., Senaratne, N., Steicke, C., Kim, S.-H. 2009. Powdered activated carbon for fouling reduction of a membrane in a pilot-scale recirculating aquaculture system. *Desalnation Water Treat*. 5: 1-5. https://doi.org/10.5004/dwt.2009.556.

Kementerian Kelautan dan Perikanan Republik Indonesia, 2017. Peraturan Menteri Kelautan dan Perikanan Republik Indonesia Nomor 75/Permen-Kp/2016 Tentang Pedoman Umum Pembesaran Udang Windu (*Penaeus Monodon*) dan Udang Vaname (*Litopenaeus Vannamei*). Jakarta.

Kementerian Kelautan dan Perikanan Republik Indonesia. 2021. Statistik Ekspor Hasil Perikanan Tahun 2016 2020. Ditjen PDSPKP Kementerian Kelautan dan Perikanan.

Krummenauer, D., Samocha, T., Poersch, T.L., Lara, G., and Wasielesky Jr., W. 2014. The reuse of water on the culture of Pacific white shrimp, *Litopenaeus vannamei*, in BFT system. *J. World Aquacult. Soc*. 45(1): 3–14.

Lehmann, M., Schleder, D.D., Guertler, C., Perazzolo, L.M., and Vinatea, L. 2016. Hypoxia increases susceptibility of pacific white shrimp to white spot syndrome virus (WSSV). *Arq. Bras. Med. Vet. Zootec.* 68(2): 397-403. http://doi.org/10.1590/1678-4162-7942.

Lindholm-Lehto, P., Pulkkinen, J., Kiuru, T., Koskela, J., and Vielma, J. 2020. Water quality in recirculating aquaculture system using woodchip denitrification and slow sand filtration. *Environmental Science and Pollution Research*. 27: 17314-17328.

Ng, L. Y., Ng, C. Y., Mahmoudi, E., Ong, C. B., and Mohammad, A. W. 2018. A review of the management of inflow water, wastewater and water reuse by membrane technology for a sustainable production in shrimp farming. *Journal of Water Process Engineering.* 23: 27-44. https://doi.org/10.1016/j.jwpe.2018.02.020.

Nonwachai, T., Purivirojku,W., Chuchid, N., and Limsuwan, C. 2011. Effects of dissolved oxygen levels on growth, survival rate and immune response of juvenile pacific white shrimp *Litopenaeus vannamei*. *Journal of Fisheries and Environment*. 35(3):1-10.

Novriadi, R., Istiqomah, I., Isnansetyo, A., Balk, D., Jolly Breithaupt, M., and Davies, S. 2023. Corn fermented protein in production diets for pacific white legged shrimp *Litopenaeus vannamei*: Improved growth performance, health status and resistance to infection. *Aquaculture Reports*, 30: 101571.

Rakhmanda, A., Pribadi, A., Parjiyo, P., and Wibisono, B. I. G. 2021. Production performance of white shrimp *Litopenaeus vannamei* with super-intensive culture on different rearing densities. *Jurnal Akuakultur Indonesia*. 20(1): 56-64.

Reddy,M.H., and Mounika, K. 2018. Determination and comparative study of water quality parameters in shrimp culture ponds. *International Journal for Research in Applied Scence and Engineering Technology*. 6(9): 216-221.

Rubel, H., Woods, W., Pérez, D., Unnikrishnan, S., Felde, A. Z., Zielcke, S, Charlotte, L., and Carolin, L. 2019. A strategic approach to sustainable shrimp production in Thailand: The case for improved economics and sustainability. *Boston: Boston Consulting Group*.

Sagir, E., Özgür, E., Gunduz, U., Eroglu,I., and Yücel, M. 2017. Single-stage photofermentative biohydrogen production from sugar beet molasses by different purple non-sulfur bacteria. *Bioprocess and Biosystem Engineering*, 40(11): 1589-1601. https://doi.org/10.1007/s00449-017-1815-x.

Suantika, G., Situmorang, M. L., Kurniawan, J. B., Pratiwi, S. A., Aditiawati, P., Astuti, D. I., Azizah, F. F. N., Djohan, Y. A., Zuhri, U., and Simatupang, T. M. 2018. Development of a zero water discharge (ZWD)-recirculating aquaculture system (RAS) hybrid system for super intensive white shrimp (*Litopenaeus vannamei*) culture under low salinity conditions and its industrial trial in commercial shrimp urban farming in G. *Aquac. Eng.* 82: 12–24. https://doi.org/10.1016/j.aquaeng.2018.04.002.

Sukenda. 2021. Prosedur Operasional Budidaya Udang Vaname (*Litopenaeus Vannamei*).

Summerfelt, S. T., Mark, J., and Sharrer, M. J. 2004. Design implication of carbon dioxide productionwithin biofilters contained in recirculatingsalmonid culture systems. *Aquacult. Eng.* 32: 171–182

Sun, Y., Hou, H., Dong, D., Zhang, J., Yang, X., Li, X., Song, X. 2023. Comparative life cycle assessment of whiteleg shrimp (*Penaeus vannamei*) cultured in recirculating aquaculture systems (RAS), biofloc technology (BFT) and higher-place ponds (HPP) farming systems in China. *Aquaculture*. 574: 739625. https://doi.org/10.1016/j.aquaculture.2023.739625

Timmons. B., and Ebeling J. M. 2013. Recirculating Aquaculture, 3rd Edition. *Ithaca Publishing Company*, LLC.

Valencia-Castañeda, G., Frías-Espericueta, M. G., Vanegas Pérez, R. C., Chávez-Sánchez, M. C., and Páez Osuna, F. 2019. Toxicity of ammonia, nitrite and nitrate to *Litopenaeus vannamei* juveniles in low salinity water in single and ternary exposure experiments and their environmental implications. *Environmental toxicology and pharmacology*. 70: 103193.

Van Rijn, J., Tal, Y., and Schreier, H. J. 2006. Denitrification in recirculating systems: theory and applications. *Aquacultural Engineering* 34(3): 364–76. https://doi.org/10.1016/j.aquaeng.2005.04.004.

Wasielesky, W., Furtado, P., Poersch, L., Gaona, C., and Browdy, C. 2015. Alkalinity, pH and CO_2: Effects and tolerance limits for *Litopenaeus vannamei* superintensive biofloc culture system.

Yu, Y.-B., Choi, J.-H., Lee, J.-H., Lee, J.-H., Jo, A-H., Lee, J.-W., Choi, H.J., Kang, Y.J., Choi, C.Y., Kang, J. K., Lee, K.M., Kim, J.-H. 2023. The use, application and efficacy of biofloc technology (BFT) in shrimp aquaculture industry: A review. *Environmental Technology & Innovation*. 103345. https://doi.org/10.1016/j.eti.2023.103345.