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The Diurnal Trend of Water Quality in the Semi-Outdoor Recirculating Aquaculture System for the Red Tilapia (*Oreochromis* sp.) Production

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ABSTRACT

With the increasing global population, ensuring food security has become one of the major global challenges, particularly in relation to protein food sources. Aquaculture activities are generally considered a potential alternative for providing protein-rich food. However, conventional aquaculture production systems require substantial water usage, which is often scarce in certain areas. Recirculating aquaculture systems (RAS) have emerged as a water-efficient method of aquaculture production. However, literature on diurnal trends of water quality within RAS is still limited, making it challenging to establish standard operating procedures (SOPs) for water quality monitoring in RAS. This study aimed to examine the diurnal patterns of several water quality parameters in a semi-outdoor RAS used for the cultivation of the red tilapia (*Oreochromis* sp.). Water quality parameters were measured every three hours over a 24-hour period. The results showed distinct diurnal trends for each observed parameter, including total ammonia nitrogen (TAN), un-ionized ammonia (NH_3), dissolved oxygen, oxygen deficit, pH, and temperature. TAN, dissolved oxygen, and pH exhibited higher values in the morning (6:00–9:00). In contrast, temperature and oxygen deficit were generally higher in the evening (18.00–21.00). Based on these trends, the best times for water quality monitoring in semi-outdoor RAS are predicted to be at 09.00 and 18.00. Further research is needed, especially in locations with different climates and altitudes, to validate and expand these findings.

INTRODUCTION

Aquaculture has emerged as a vital sector for maintaining global food security, offering a sustainable alternative source of protein. Currently, aquaculture fish production has even exceeded capture fisheries **(FAO, 2022)**. Aquaculture has experienced exponential growth, with production increasing from approximately 8 million tonnes in 1985 to around 80 million tonnes in 2016, and up to 122 million tonnes in 2020 **(Bogmans & Soest, 2022; FAO, 2022)**. In contrast, the capture fisheries tend to stagnant due to the overfishing and environmental degradation **(Mackintosh *et al.*, 2023)**. Thus, current aquaculture activities show great potential to become a more sustainable food source. However, conventional aquaculture tends to create environmental issues due to the overuse of water resources and waste generation, which might be released into the natural surroundings **(Martinell, 2024)**.

Public awareness of water availability and usage has recently increased, as evidenced by the Sustainable Development Goals (SDGs) of the United Nations, particularly in SDGs number 6, which emphasize access to clean water and better sanitation **(Mustafa *et al.*, 2022)**. The availability of clean water is currently a global challenge that can be exacerbated by climate change. Climate change has intensified drought events in various regions around the world, leading to a limited supply of freshwater **(Balting *et al.*, 2021; Singh *et al.*, 2021; Wang *et al.*, 2022)**. This water scarcity affects many sectors, including aquaculture, where competition for freshwater resources has escalated **(Jegatheesan *et al.*, 2011)**. In aquaculture, access to a continuous supply of water is essential to ensure the sustainability of this sector **(Hung *et al.*, 2014)**.

To address this problem, several aquaculture production systems have been developed to mitigate the scarcity of water resources. One of the prominent systems that is being adopted is the recirculating aquaculture system (RAS) **(Timmons & Vinci, 2022)**. This closed-loop system, with its efficient use of water, has become a climate-resilient aquaculture production system **(Ahmed & Turchini, 2021; Rajesh *et al.*, 2023)**. This is because RAS requires a smaller amount of water compared to conventional fish production systems. It is estimated that only about 500 liters of water are needed to produce 1kg of fish in RAS, while more than 3600 liters of water are needed per kilogram of fish cultivated with conventional system **(Brune, 2023)**. RAS provides greater flexibility in areas with limited water resources, offering a solution to maintain water quality without requiring extensive water change **(Taufik *et al.*, 2023)**. RAS systems are designed to limit effluent release, thereby conserving water resources and reducing the ecological footprint of aquaculture operations **(Martins *et al.*, 2010)**. This makes RAS particularly appealing in regions where there is a growing public demand for sustainable food production methods and in areas experiencing difficulties in the availability of water.

A primary component of the RAS system is its filtration system, which is critical to maintain water quality under optimal conditions. Based on functionality, RAS filters can be classified as mechanical, biological, and chemical filters. Biological filters play an important role in the transformation of ammonia, which is generally toxic to aquatic organisms, into less harmful nitrates **(Ruiz *et al.*, 2020)**. This conversion occurs within the biological filter by providing a habitat for nitrifying bacteria, which is achieved by increasing the surface area, providing oxygen, and ensuring adequate water alkalinity **(Timmons & Vinci, 2022)**.

Research has shown that the different types of biomedia in biofilters can vary in effectiveness in maintaining water quality, not only limited to ammonia level, but can also improve other water quality during the fish farming process **(Wicaksono *et al.*, 2024)**. However, the diurnal patterns of water quality in the RAS remain underexplored. This study examined the diurnal trends in water quality in a RAS designed for the production of red tilapia (*Oreochromis* sp.). The purpose of this research was to evaluate the diurnal trends of the water quality parameters within a semi-outdoor RAS environment. This information will be crucial in developing standard operating procedures (SOPs) for fish husbandry that uses RAS as the main production system, thus enabling a more effective management of RAS in the future.

MATERIALS AND METHODS

1. Research location and facility setup

The research was conducted at the Teaching Farm facility in the Department of Fisheries, Faculty of Agriculture, Gadjah Mada University, D.I. Yogyakarta, Indonesia. This facility is located at 7°46'02"S and 110°22'57"E. The facility is a semi-outdoor structure with open walls that allow natural ventilation. The study period took place during the dry season, with ambient air temperatures ranging from 22 to 31°C. The facility is located at an altitude of 135 meters above sea level (MASL).

2. Fish species and rearing conditions

Red tilapia (*Oreochromis* sp.) were selected as primary test organisms in this study. This fish was selected as the test organism due to its status as a commercially valuable commodity that is widely cultivated for both local and international markets. The samples were obtained from a local aquaculture farm, with an average total length of 7.21±0.267cm and an average weight of 6.80±0.687g. The transportation of fish to the research facility was carried out using plastic bags filled with water and oxygen. On arrival at the research facility, the fish specimens were acclimatized in concrete ponds for 7 days. During this acclimatization phase, fish were fed 5% of the biomass with artificial pellet feed (HI-PRO-VITE 781, CP Prima, Indonesia). Following acclimatization, the fish were transferred and distributed among the fish tanks of the recirculating aquaculture system (RAS) for the experiment. The density of tilapia fish used in the fish tank is 100 fish/m^3. During the experiment, the fish were fed 5% of their biomass. The feeding frequency was divided into 2 times, at 08.00 and 16.00. The experiment was then carried out for 25 days.

3. RAS design and configuration

The recirculating aquaculture system (RAS) used in this study was designed to maintain optimal water quality through a series of filtration stages, specifically tailored for small to medium-scale aquaculture (Fig. 1.). The design is based on the modification of **Wicaksono *et al.* (2024)**. The system included four main fish tanks, each with a capacity of 500 liters, arranged to promote circular water flow for efficient waste removal. The water from the fish tanks went through the central drain outlet and entered a filtration sequence, starting with a radial flow settler prefilter, mechanical filter, biological filter and finally going to the sump tank. In the sump tank, the water was pumped back and distributed into the fish tanks.

The RAS layout was structured to ensure that solid waste was settled, fine debris filtered, and ammonia biologically converted to less toxic substance. The radial flow settler was the first filtration stage, removing large solid particles through gravitational settling and directing the pre-filtered water to the mechanical filter tank. At this stage, the fine particulate matter was captured by fishnet media, which acted as a physical barrier. The water was then moved to the biological filter tank, where nitrifying bacteria facilitated the conversion of ammonia to nitrate with lower toxicity. This research compared the biofilter media as the main treatment, which was divided into 4 types of media: empty tank (as control), pumice, kaldness k1 [with moving bed biofilm reactor (MBBR) configuration] and bioball. Each biofilter chamber was then equipped with an aerator blower to maintain the presence of dissolved oxygen. Each filter tank had a volume of 120 liters and was connected through a 1.5" PVC pipe that enabled a controlled sequential flow of water between filters, maximizing filtration efficiency.

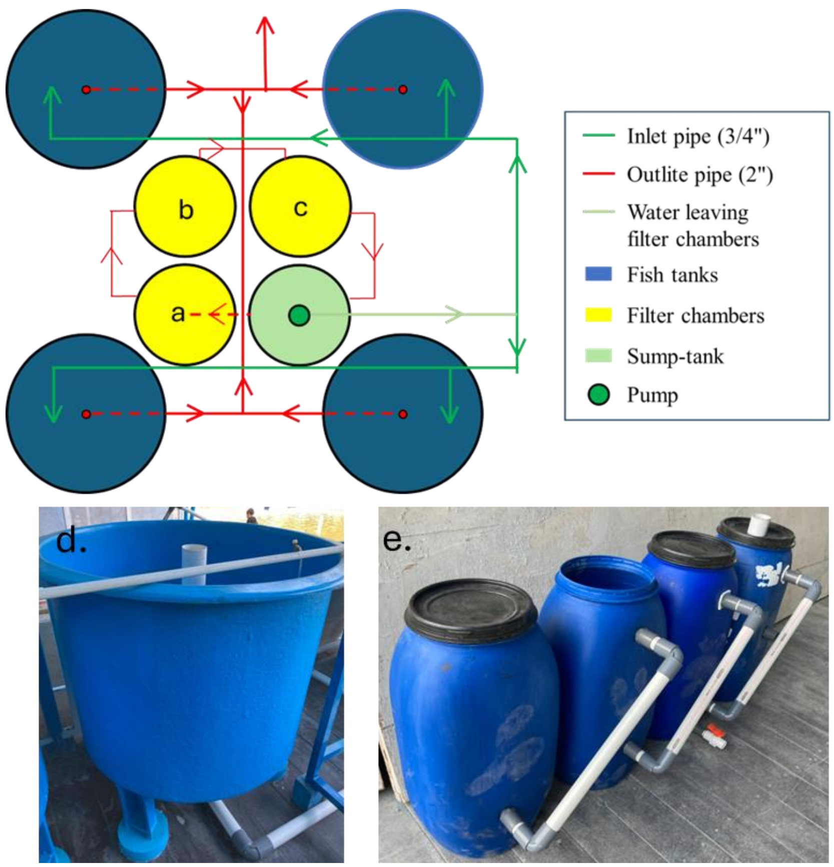

Fig. 1. Top view of the RAS design used in this study. (a) Radial flow settler, **(b)** mechanical filter, **(c)** biological filter, **(d)** fish tank design (500L) and **(e)** filter chamber system (120L, each) and design of the piping system

4. Data collection

This study focused on the diurnal pattern of water quality in the system, which was measured every 3 hours for a full 24 hours. This measurement of water quality was carried out on the 25th day of culture (DOC). Water quality measurements in each treatment were carried out three times at each data point. The parameters measured *in situ* were total ammonia nitrogen (TAN), dissolved oxygen, pH, and temperature. Measurements were made using an Aquatroll 500 multiparameter sonde (*In situ*, United States). The oxygen parameters were measured optically (luminescence), with TAN using the ion-selective electrode, temperature using a thermistor, and pH using the electrode method.

The un-ionized ammonia (NH_3) and oxygen deficit parameters were measured using equation calculation. Un-ionized ammonia was measured using the Henderson-Hasselbalch equation based on the TAN and pH values in the water **(Ip *et al.*, 2001)**. The equation used to calculate un-ionized ammonia was as follows:

$$[NH_3] = TAN \times \left(\frac{1}{1 + 10^{pKa - pH}}\right)$$

NH_3 = concentration of un-ionized ammonia (mg/L)
TAN = concentration of total ammonia nitrogen (mg/L)
pKa = is the acid dissociation constant of ammonia
pH = the pH of water

The acid dissociation constant of ammonia was calculated using this formula:

$$pKa = 0.09018 + \left(\frac{2729.92}{T°C + 273.15}\right)$$

Meanwhile, oxygen deficit value was known from the difference between the theoretical oxygen saturation value in water and the measured dissolved oxygen value in water **(Boyd, 1998)**. The oxygen deficit value in water was determined using the following formula:

$$OD = C_s - C_m$$

OD = Oxygen deficit (mg/L)
C_s = DO concentration at saturation (mg/L)
C_m = DO concentration that measured on the water (mg/L)

The dissolve oxygen at saturation was calculated using the following formula:

$$C_s = C_{tab} \times (\frac{BP}{760})$$

Where:

C_{tab} = theoretical oxygen saturation (mg/L) at a water temperature of 25°C and a pressure of 1 atm (760 mm Hg).

BP = barometric pressure

In addition to water quality parameters, fish growth parameters in the form of length and weight specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR) were measured to provide an overview of the performance of the tilapia being cultured.

5. Data analysis

The data obtained were presented in the form of tables and graphics. The analysis and interpretation of the water quality data were carried out descriptively, primarily by looking at the dynamics of changes in the water quality values based on time trends in 24 hours. Furthermore, analysis of water quality data was viewed based on parameters that can influence each other and a review of the optimal values needed by tilapia in general. The analysis was associated with the growth parameters of tilapia. The normality and homogeneity of the data were checked for fish growth parameters (SGR, FCR, and SR). When both criteria were met, the ANOVA inferential statistical test was used.

RESULTS

Based on the results, the diurnal pattern of each water quality parameter showed their own trends (Fig. 2). For the TAN parameter, it was generally observed that the control treatment (0.82 – 0.89mg/ L) and the pumice treatment (0.84 – 0.92mg/ L) had higher values compared to the MBBR (0.73-0.79mg/ L) and bioball treatments (0.76 – 0.81mg/ L). Overall, the trend for TAN values tends to be lower in the afternoon-evening compared to the morning-afternoon values. On the contrary, the highest values of unionized ammonia were shown by bioball treatment (0.11 – 0.12mg/ L). Meanwhile, MBBR treatment showed the lowest unionized ammonia values among the treatments (0.08 - 0.10mg/ L).

In terms of dissolved oxygen levels, all treatments showed similar trends, with higher values in the morning and lower values in the evening. Dissolved oxygen levels between 18:00 and 24:00 showed the most instability, as indicated by a larger standard deviation compared to other time points. The opposite pattern was observed for the oxygen deficit values. In general, all treatments showed a pattern in which the oxygen deficiency was greater late afternoon to evening (1.1 – 1.7mg/ L) compared to morning and midday (1.07 – 1.53mg/ L). The oxygen deficiency values also tended to have a larger deviation in the evening (between 18:00 and 21:00).

Based on pH values, it can generally be observed that biofilter treatment using bioball has a higher pH range (8.40 – 8.49) compared to other treatments. According to the diurnal trend, the overall pH values in all treatments showed a similar trend, with a tendency to be higher from morning to midday, followed by a decrease in the afternoon and evening. At 18:00, there was a significant variation in pH, indicated by a relatively higher standard deviation compared to other time points. Regarding temperature trends, bioball treatment also showed higher values compared to other treatments (26.88 – 25.45°C). The temperatures in all treatments were lower in the morning and started to rise between 09:00 and 18:00, gradually decreasing until around 09:00 the next day. Generally, the highest temperature range was observed at 18:00, while the lowest was observed at 09:00.

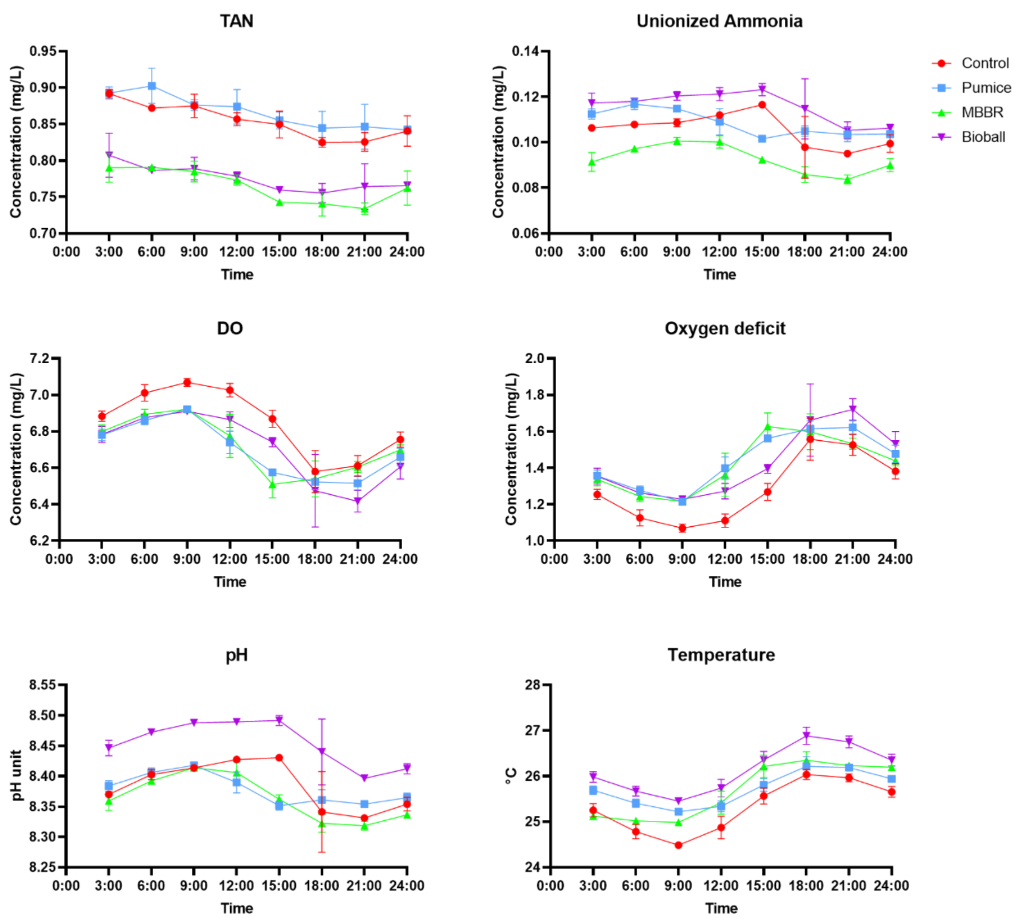

Fig. 2. Diurnal trend in water quality parameters measured every 3 hours over a 24-hour period in RAS using four different bio media

Table 1. Specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) of red tilapia cultured in a recirculating aquaculture system (RAS) using different types of biofilter media

Parameter	Control	Pumice	MBBR	Bioball
Weight SGR (%/d)	4.97±0.788	5.53±1.184	5.48±0.448	5.36±0.994
Length SGR (%/d)	1.32±0.233	1.69±0.339	1.54±0.147	1.51±0.170
FCR	0.75±0.19	0.70±0.22	0.65±0.10	0.75±0.21
SR	100	100	100	100

Based on the SGR weight and length parameters (Table 1), there were no significant differences as indicated by the ANOVA tests (P=0.81, P=0.21; respectively). Generally, the SGR weight showed a daily weight gain range of 3.9 – 6.7% per day, while the SGR length ranged from 1.0 to 2.0% per day. Regarding the FCR, all treatments demonstrated an FCR below 1, ranging from 0.5 to 1.0. However, statistically, there was no evidence of significant differences in FCR between treatments (P=0.88). Furthermore, in all treatments, the survival rate in DOC 25 remained 100%.

DISCUSSION

In aquaculture activities, ensuring the quantity and quality of water is one of the essential factors to maintain its sustainability. Currently, with increasing awareness of water use efficiency, various alternative aquaculture production systems are being developed. Among these systems, biofloc technology (BFT) and RAS show promising potential for further development. The primary concept of BFT is to utilize heterotrophic bacteria to maintain water quality, primarily by reducing inorganic nitrogen levels **(Dauda *et al.*, 2019)**. However, this system has several limitations, such as the high level of suspended solid that at some level can lead to instability of inorganic nitrogen, and no effectiveness for all aquaculture species **(Betanzo-Torres *et al.*, 2020; Sin *et al.*, 2024)**. On the other hand, RAS generally employs a filtration system, with one of its key components being the biological filter, which uses chemoautotrophic bacteria to reduce ammonia levels in the culture tanks **(Timmons & Vinci, 2022)**. This condition will lead to more precise water quality management and will create a more controlled fish farming environment **(Hisano *et al.*, 2019; Lindholm-Lehto & Vielma, 2019)**. Therefore, RAS can be considered superior to biofloc systems in terms of production consistency and operational reliability.

Various studies have demonstrated that biofilters in RAS can effectively control ammonia levels, preventing ammonia spikes during aquaculture operations. Ammonia is one of the substances in water that, at a certain level, can be toxic to organisms. Ammonia is typically expressed as total ammonia nitrogen (TAN), which is the sum of two forms of ammonia: unionized ammonia (NH_3) and ionized ammonia (NH_4). Of these two forms, NH_3 has significantly higher toxicity to aquatic organisms compared to NH_4 **(Ip & Chew, 2010)**. The presence of NH_3 can reduce oxygen uptake in the gills of fish, potentially leading to hypoxia, even when dissolved oxygen is high in water. The proportion of these two forms of ammonia is influenced by the pH levels. Higher water pH tends to increase the proportion of NH_3 relative to NH_4 **(Ip *et al.*, 2001)**. This means that the toxicity of ammonia increases under more alkaline water conditions.

In this study, the diurnal pattern of TAN concentration tended to decrease between all treatments from morning to night time. This indicates the effectiveness of the biofilter in reducing the levels of TAN in the system. In general, TAN values in the control and pumice treatments were higher compared to those in the MBBR and Bioball treatments. This may be influenced by the value of surface specific area (SSA) in biofilter media, which serve as sites for the growth of nitrifying bacteria responsible for reducing TAN levels in water. A higher SSA value contributes to a greater reduction in the TAN level in the system. Control and pumice treatments generally had lower SSA values compared to Bioball and MBBR treatments, resulting in higher TAN levels in control and pumice treatment **(Wicaksono *et al.*, 2024)**.

Although the control and pumice treatments showed higher levels of TAN, the Bioball treatment exhibited the highest NH_3 values among all treatments. This could be attributed to the pH values in the Bioball treatment, which were the highest compared to the other treatments. As a result, although the TAN levels in Bioball treatment were relatively lower, the proportion of NH_3 was higher due to alkaline conditions. When examining the diurnal trend, it was observed that NH_3 levels in all treatments tended to increase in the early morning, around 03:00 to 09:00, and then decreased between 15:00 and 21.00. This indicates that, according to NH_3 levels, the critical period for cultured organisms tends to occur in the early morning. This is likely influenced by the TAN values, which also showed a tendency to be higher during morning observations.

The diurnal trend of dissolved oxygen showed differences in oxygen concentration between the morning-to-afternoon and afternoon-to-evening periods. Dissolved oxygen levels tended to be higher during the morning-to-afternoon period compared to the afternoon-to-evening period. This pattern may be closely related to water temperature fluctuations. Generally, water temperature in the morning-to-afternoon period is lower compared to the afternoon-to-evening period. When temperatures are lower, gas solubility in water, including oxygen, tends to increase.

In addition, water temperature affects the metabolism of aquatic organisms. Lower water temperatures reduce the metabolic rate of fish, which leads to lower oxygen consumption, allowing dissolved oxygen levels in the water to remain higher **(Enders *et al.*, 2006)**. On the other hand, higher temperatures reduce gas solubility in water and increase the metabolic rate of fish, resulting in lower dissolved oxygen levels during the warmer afternoon and evening periods. The water temperature is also influenced by the ambient air temperature around the RAS facility. In this study, the RAS design was set up in a semi-outdoor environment. Although the setup was protected from direct sunlight, the air temperature remained influenced by the external heat during the day. Additionally, since water has a higher heat capacity compared to other substances, it tends to retains its temperature for longer periods. Even after the heat source (sunlight) disappears in the afternoon (around 18:00), the water temperature in the system takes approximately 15 hours to reach its lowest point (around 09.00). After this point, the temperature begins to rise due to the warming of the air, driven by sunlight from the outside of the RAS facility.

The observed trend for oxygen deficit tends to be the inverse of the dissolved oxygen trend, with lower oxygen deficit values in the morning to afternoon period and higher deficits in the afternoon to evening period. These trends might be influenced by the respiration activities in the system. In the evening, where there is no sunlight, all organisms in the system tend to do the respiration, which at some level will increase the oxygen deficit value in the water. The increase in respiration activity is also shown by the pH trend, which tends to decrease from afternoon to evening. Respiration involves the consumption of oxygen by organisms, with the by-product of CO_2 release. When CO_2 dissolves in water, it leads to the formation of carbonic acid (H_2CO_3), which could lower the pH of the water **(Boyd, 2020)**. If the system lacks sufficient alkalinity, this can result in a significant pH drop. These patterns suggest that even in the semi-outdoor environment, the photoautotrophic organism might exist in the system.

Based on the diurnal trend of water quality, the optimal times for water quality monitoring in RAS are 09.00 and 18.00. At those times, these correspond to the extreme states of water quality parameters. At 09.00, the pH and dissolved oxygen levels peak. Monitoring during this time is crucial, especially to assess the potential toxicity of ammonia in water, since elevated pH levels can increase ammonia toxicity **(Ip *et al.*, 2001)**. The second optimal time to monitor water quality is at 18.00. This period is associated with the most significant fluctuations in water quality parameters, including the highest levels of oxygen deficit, which, at certain thresholds, can lead to hypoxic conditions. This timing is further influenced by relatively warm water temperatures, which accelerate the metabolic rates of fish and bacteria in the system, leading to faster biochemical processes and reduces dissolve oxygen level in the system. Monitoring at these times helps prevent problems from occurring due to fluctuation in water quality parameters and can support better management decisions during the fish rearing period.

Based on fish growth parameters, fish reared in RAS using biofilter media such as pumice and MBBR tend to show better growth performance compared to other filter media treatments. This is evidenced by the highest specific growth rate (SGR) values among all treatments, indicating faster daily growth rates. In terms of feed conversion rate (FCR), these two treatments also exhibited the lowest values. A lower FCR indicates a more efficient feed utilization, which is beneficial for overall system performance. In all treatments, the survival rate (SR) remained 100%, indicating that there was no mortality in any of the treatments. This emphasizes the effectiveness of the systems in maintaining optimal conditions for the survival and growth of fish.

To obtain more comprehensive information, further studies should be conducted in various locations with differing weather, climate, and altitude conditions. Variations in weather and climate typically influence air temperature and the duration of sunlight exposure in a given location. To some extent, water temperature can become a key parameter that affects several other water quality parameters. Additionally, altitude can influence barometric pressure at the study site. As a result, locations with different altitudes will exhibit variations in gas solubility in water, which can impact several water quality parameters. These differences may lead to outcomes that significantly differ from the findings of this study, emphasizing the need for broader research in diverse environmental settings.

CONCLUSION

In conclusion, the semi-outdoor RAS system developed in this study shows that the optimal times for monitoring water quality in the semi-outdoor RAS system are 09:00 and 18:00, as these times capture the critical points of each water quality parameter. While different biological filter media can influence water quality values, they do not alter the observed diurnal trends. Based on growth parameters, fish cultured in RAS systems with pumice and MBBR filter media demonstrated better performance compared to other types of biomedia filter, highlighting its effectiveness in supporting fish growth and the overall efficiency of RAS.

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