Identification and abundance of nitrifying-denitrifying bacteria in malang sand filter based culture environment for mud crabs *Scylla serrata*

Y P Hastuti¹, Y Andina¹, E Supriyono¹, Y S Fatma² and S Tridesianti²

¹Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University (IPB University), Bogor, Indonesia
²Department of Microbiology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB University), Bogor, Indonesia

*E-mail: yuniha@ipb.ac.id*

**Abstract.** Accumulation of organic matter derived from an uneaten feed, faeces, and other metabolic waste of biota can reduce water quality. This issue can be overcome by physical method using filtration systems such as malang sand. The present study aimed to identify the morphology and abundance of nitrifying and denitrifying bacteria in the mud crab *Scylla serrata* cultivation system treated with different weight of malang sand as i.e. 0 kg (control), 5 kg, 10 kg, 15 kg, and 20 kg. Mud crabs with the weight of 73.72±1.05 g and carapace length of 7.28±0.06 cm were reared in a container measuring 60 cm x 30 cm x 30 cm for 30 days fed two times a day using ad satiation method (as satisfied as possible). The results showed that a treatment using 5 kg malang sand was the best treatment with the survival rate of mud crabs reached 77.77±19.2%. At the end of cultivation period, nitrifying and denitrifying bacteria accounted for 1.52x10⁷ CFU mL⁻¹ and 1.06x10⁷ CFU mL⁻¹, respectively. The nitrifying and denitrifying bacteria in the rearing water were classified as *Pseudomonas* sp. and *Acinetobacter* sp., respectively.

**Keywords:** denitrification, filtration, malang sand, nitrification, *Scylla serrata*

1. Introduction

Mud crab *S. serrata* is one of the aquatic resources with high economic value and potential to be cultivated. Consumers favour this crab because of its taste and high nutritional contents (Catacuatan 2002). Proximate analysis showed that mud crab’s flesh contains 44.85-50.58% protein, 10.52-13.08% fat, 3.57-3.72 kcal g⁻¹, and cholesterol 76-78 mg per 100 g (Karim 2005, Syafiq 2008). In Indonesia, mud crab is a major export commodity together with tiger shrimps (KKP 2017). Unfortunately, the increase in demand is not followed by the rise in the mud crab population in natural habitat. The high demand for mud crabs would affect overfishing activities. The research focusing on the optimal cultivation of mud crab is highly needed to overcome this issue (Sentosa and Syam 2011).
FAO (2011) sets water quality standards for maintaining mud crabs, including optimal dissolved oxygen (DO) >5 ppm, temperature 25°C-30°C, pH 7.0-9.0, total ammonia nitrogen (TAN) <3 ppm, alkalinity >80 ppm, and turbidity 20-30 cm. In the mud crab recirculation systems, optimal condition of water quality salinity is 25 ppt (Hastuti et al 2015), pH 7 (Hastuti et al 2016) and temperature 29°C (Hastuti et al 2019a). Mud crabs will grow faster with a better feed conversion ratio, resulting in more resistant to disease. However, water quality parameters fluctuate or even decrease during mud crabs farming. This condition is triggered by a high accumulation of organic matter in rearing water because of metabolic waste released by mud crab and inedible feed. High organic matter content causes the enrichment of inorganic nitrogen compounds, including ammonia, nitrite, and nitrate in the water. Camargo and Alonso (2006) reported that the particular concentration of inorganic nitrogen compounds including ammonia (NH₃), nitrite (NO₂⁻), and nitrate (NO₃⁻), are toxic and could promote mortality for aquatic biota.

Filtration is a physical method to reduce suspended solids in water by passing it through porous media. The ideal material used as a filter should have a porous structure and contains many fine capillaries, so the absorbed substance will be suspended on the sidelines of the capillary (Setyani 2001). Malang sand, which is volcanic particles, maybe potential to be applied as filter media in mud crab cultivation. Malang sand has irregular rough or fine spots texture on the surface. Their pore diameter size varies from 0.5 to 3 mm. Pores texture of malang sand which is similar to pumice pores can be used as a substitute for aquarium filter, such as bio-ball, supporting bacterial growth (Viadolo 2016). The fine cavities in malang sand can trap and absorb dissolved solid particles, including metabolic waste and food which are not consumed, according to the pore size (Hastuti et al 2015). Based on the molecular identification, 16S rRNA sequence in the previous research showed that mud crab culture with the addition of various kinds of filter materials (zeolite, malang sand, bio-ball and dacron) can store several types of nitrifying bacteria like Pantoea calida, Pseudomonas stutzerii and Halomonas sp. (Hastuti et al 2019b). Based on the identification analysis of the mud crab culture in the containers with different light capacities, it was revealed that nitrifying bacteria found during the observation were Pseudomonas sp. groups (Hastuti et al 2018). In the different condition Pseudomonas, sp. and Acinetobacter, sp. have a denitrification activity.

Nitrifying bacteria, including Nitrosomonas sp. and Nitrobacter sp, have been frequently reported to have oxidation activity of toxic ammonia and nitrate which are less toxic for mud crabs. Denitrifying bacteria can remove nitrite and nitrate from water ecosystem into the atmosphere. Therefore, those bacteria would be remarkable agents to maintain the concentration of inorganic nitrogen compounds in optimum level and improve water quality. This study aimed to identify the morphology and abundance of nitrifying and denitrifying bacteria in mud crabs S. serrata culture environment with malang sand as filter material.

2. Materials and methods

2.1. Materials
Mud crabs S. serrata were obtained from Pemalang, central Java, Indonesia. Mud crabs S. serrata were reared in Laboratory of Environment, Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Indonesia. Bacterial identification was carried out at Laboratory of Aquatic Organisms Health and Laboratory of Aquatic Environment, Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Indonesia.

2.2. Methods
This study was conducted using a complete randomized design consisting of 5 treatments with three replications, i.e. control (without filter), in addition to using 5 kg, 10 kg, 15 kg, and 20 kg malang sand with their respective thicknesses as follows: 0 cm, 5 cm, 10 cm, 15 cm and 20 cm.
2.2.1. **Preparation of rearing media.** This study used seawater from Ancol, north Jakarta with the salinity of 35 g/L. Seawater was diluted using freshwater to 25 g/L salinity (Hastuti et al 2015).

2.2.2. **Preparation of culture containers.** Fifteen aquariums (60 cm x 30 cm x 30 cm) were filled with seawater with a salinity of 25 g/L to a height of 10 cm. Then, six units of cube-shaped mud crab shelters with a size of 10 cm x 10 cm x 10 cm were put into each aquarium. A total of 15 plastic containers with a dimension of 54.4 cm x 38 cm x 31.9 cm was filled with malang sand, as the filter material, based on the weight of each treatment.

2.2.3. **Experimental fish.** A total of 90 mud crabs with an initial weight of 73.72±1.05 g and a carapace length of 7.28±0.06 cm were used. Six crabs were kept in each aquarium with 6 units of shelters. Mud crabs were acclimatized for 3 days under seawater with a salinity of 25 g/L (Hastuti et al 2015). Mud crabs were reared for 30 days and fed with yellow stripe scad fish pieces measuring 1 cm x 1 cm x 0.1 cm twice a day with ad satiation method. Survival rates of mud crabs were examined at 0 (H0), 15 (H15), and 30 (H30) days of the rearing period, according to Goddard (1996).

2.2.4. **Water quality.** Water quality management was carried out by a recirculation system. Rearing water in a culture container was flowed into a filter container containing malang sand filter material based on each treatment, then pumped into a culture container. Water debit in a culture container was 0.1 L/sec. Freshwater addition was conducted to maintain a water salinity of 25 g/L during mud crabs cultivation. During the experiment, water quality parameter was monitored, comprised of dissolved oxygen, pH, temperature, ammonium, nitrite, nitrate (based on APHA 1989), total organic material (TOM), and biological oxygen demand (BOD), were determined at every 14 days. Data were evaluated using analysis of variance (ANOVA) at a significance level of 95% with SPSS 20.0 software. The significant difference between means was determined using the Duncan test.

2.2.5. **Isolation and identification of nitrifying and denitrifying bacteria.** Mud crabs rearing water samples were serially diluted up to 10⁻⁴ with a physiological solution (NaCl 8 ppt). The diluted water samples were poured into specific nitrification and denitrification agar media, respectively. The bacterial abundance that grows on particular media for ±24 hours was calculated using total plate count (TPC) method at 0 and 30 days of the rearing period. Bacteria was purified to obtain pure culture or single colonies. Each colony was identified by morphological (colour, shape, elevation, edges, and Gram staining), and biochemical characteristics (motility, catalase, oxidase, and oxidative/fermentative (O/F)). Bacterial identification was performed according to Cowan and Steel (2003).

3. **Results and discussion**

3.1. **Isolation and identification of nitrifying and denitrifying bacteria**

The abundance of nitrifying bacteria was determined at the beginning (H0) and the end of the rearing period (H30). The abundance of nitrifying bacteria in each treatment increased from H0 to H30 (table 1). At day 30th of the rearing period, malang sand 5 kg treatment showed the highest abundance of nitrifying bacteria accounting for 15.2 x 10⁶ CFU/mL. In contrast, malang sand at 0 kg treatment (control) had the lowest bacterial abundance making up 2.4 x 10⁶ CFU/mL. Meanwhile, at the end of the rearing period (H30), denitrifying bacteria in malang sand at 5 kg treatment showed the most abundant number (106 x 10⁵ CFU/mL) compared to other treatments. In contrast, malang sand at 0 kg had the lowest abundance of denitrifying bacteria (6 x 10⁵ CFU/mL). This is presumably due to the number of mud crabs in the control treatment that was fewer than other treatments, leading to relatively low organic matter compound derived from their metabolic waste. Microorganisms need organic matter in waters as a source of energy, hormones, and vitamins for their growth and development, including nitrifying and denitrifying bacteria. This result is in agreement with Kristiawan et al (2014) who
reported that there is a relationship between total bacteria and total organic matter in the water environment. Bacterial abundance in aquatic ecosystems is a productivity indicator as it plays critical roles in nutrient availability. In addition, in the control treatment with no malang sand, bacteria were not able to grow properly.

**Table 1.** The abundance of nitrifying and denitrifying bacteria (CFU mL⁻¹) in mud crabs-rearing water with malang sand as filter material.

| Treatments | Nitrifying bacteria | Denitrifying bacteria |
|------------|---------------------|-----------------------|
|            | H0                  | H30                   | H0         | H30         |
| 0 kg (C)   | 84×10⁴              | 2.4×10⁶               | 22×10⁴    | 6×10⁵       |
| 5 kg       | 42×10⁴              | 15.2×10⁶              | 40×10⁴    | 106×10⁵     |
| 10 kg      | 26×10⁴              | 11×10⁶                | 38×10⁴    | 92×10⁵      |
| 15 kg      | 24×10⁴              | 8.2×10⁶               | 26×10⁴    | 78×10⁵      |
| 20 kg      | 34×10⁴              | 7×10⁶                 | 36×10⁴    | 54×10⁵      |

Two bacterial isolates were selected from each treatment. We obtained a total of 10 isolates of nitrifying bacteria from all procedures, then their morphological and biochemical characteristics were determined (table 2 and table 3). Morphological characteristics of bacterial colony and cell are shown in table 2. All of the nitrifying bacterial colonies were in a milky white irregular shape, undulating edges, and flat surface. Nitrifying bacterial cells were bacil and classified as Gram-negative. On the other hand, denitrifying bacteria formed circular and milky white colonies, entire edges and elevated surface. Obtained denitrifying bacterial cells were confirmed as Gram-negative coccus.

**Table 2.** Morphological characteristics of the nitrifying and denitrifying bacterial colony and cell in the mud crabs-rearing water.

| Isolates | Colony | Cell | Colony | Cell |
|----------|--------|------|--------|------|
| K.1      | Milky white, irregular, flat, undulating | Gram -, bacil | Milky white, circular, raised, entire | Gram -, coccus |
| K.2      | Milky white, irregular, flat, undulating | Gram -, bacil | Milky white, circular, raised, entire | Gram -, coccus |
| 5.1      | Milky white, irregular, flat, undulating | Gram -, bacil | Milky white, circular, raised, entire | Gram -, coccus |
| 5.2      | Milky white, irregular, flat, undulating | Gram -, bacil | Milky white, circular, raised, entire | Gram -, coccus |
| 10.1     | Milky white, irregular, flat, undulating | Gram -, bacil | Milky white, circular, raised, entire | Gram -, coccus |
| 10.2     | Milky white, irregular, flat, undulating | Gram -, bacil | Milky white, circular, raised, entire | Gram -, coccus |
| 15.1     | Milky white, irregular, flat, undulating | Gram -, bacil | Milky white, circular, raised, entire | Gram -, coccus |
| 15.2     | Milky white, irregular, flat, undulating | Gram -, bacil | Milky white, circular, raised, entire | Gram -, coccus |
| 20.1     | Milky white, irregular, flat, undulating | Gram -, bacil | Milky white, circular, raised, entire | Gram -, coccus |
| 20.2     | Milky white, irregular, flat, undulating | Gram -, bacil | Milky white, circular, raised, entire | Gram -, coccus |
Biochemical characteristics of obtained bacteria can be seen in Table 3. Nitrifying bacterial isolation has no mobility with catalase and oxidase-positive and was categorized as oxidative bacteria. Denitrifying bacteria obtained in this study have no mobility with catalase-positive and considered as oxidase negative as well as fermentative bacteria. According to Cowan and Steel (2003), all the nitrifying and denitrifying bacteria were suspected as *Pseudomonas* sp. and *Acinetobacter* sp., respectively. In general, nitrifying bacteria are Gram-negative bacteria and can colonize the aquarium filter (Cheatham 2009). A previous investigation reports that nitrifying bacteria inhabiting the mud crabs-rearing water cultured in containers with different light were *Pseudomonas* sp. and *Bacillus* sp. (Hastuti et al. 2018). Denitrifying bacteria obtained from rearing water were classified as *Acinetobacter* sp. Su et al. (2015) reported that *Acinetobacter* sp. had been applied to groundwater treatment. In the natural environment, *Pseudomonas* sp. and *Acinetobacter* sp. contributes to oil degradation from industrial waste in the sea (Safitriani et al. 2017) and phenanthrene degradation which is one of the polyaromatic hydrocarbons (PAHs) frequently found in polluted soils, estuary areas, and other water ecosystems (Murniasih et al. 2009).

### Table 3. Biochemical characteristics of nitrifying and denitrifying bacteria in the mud crabs-rearing water.

| Isolates | Motility | Catalase | Oxidase | O/F<sup>a</sup> | Genus          |
|----------|----------|----------|---------|-----------------|----------------|
|          |          |          |         |                 | Nitrifying bacteria |
| K.1      | -        | +        | +       | O               | *Pseudomonas* sp. |
| K.2      | -        | +        | +       | O               | *Pseudomonas* sp. |
| 5.1      | -        | +        | +       | O               | *Pseudomonas* sp. |
| 5.2      | -        | +        | +       | O               | *Pseudomonas* sp. |
| 10.1     | -        | +        | +       | O               | *Pseudomonas* sp. |
| 10.2     | -        | +        | +       | O               | *Pseudomonas* sp. |
| 15.1     | -        | +        | +       | O               | *Pseudomonas* sp. |
| 15.2     | -        | +        | +       | O               | *Pseudomonas* sp. |
| 20.1     | -        | +        | +       | O               | *Pseudomonas* sp. |
| 20.2     | -        | +        | +       | O               | *Pseudomonas* sp. |
|          |          |          |         |                 | Denitrifying bacteria |
| K.1      | -        | +        | -       | F               | *Acinetobacter* sp. |
| K.2      | -        | +        | -       | F               | *Acinetobacter* sp. |
| 5.1      | -        | +        | -       | F               | *Acinetobacter* sp. |
| 5.2      | -        | +        | -       | F               | *Acinetobacter* sp. |
| 10.1     | -        | +        | -       | F               | *Acinetobacter* sp. |
| 10.2     | -        | +        | -       | F               | *Acinetobacter* sp. |
| 15.1     | -        | +        | -       | F               | *Acinetobacter* sp. |
| 15.2     | -        | +        | -       | F               | *Acinetobacter* sp. |
| 20.1     | -        | +        | -       | F               | *Acinetobacter* sp. |
| 20.2     | -        | +        | -       | F               | *Acinetobacter* sp. |

<sup>a</sup>O: oxidative; F: fermentative

### 3.2. Water quality

During the mud crabs-rearing period, water quality parameters were examined, i.e. ammonium, nitrite, nitrate, TOM, and BOD. Several factors affecting the oxidation process of organic matter in water are temperature, pH, DO, and nitrogen (Boyd 1998). In this study, water temperature during mud crabs cultivation ranged from 26.5 °C to 28.3 °C, which are included in the optimal temperature range for *S. serrata* cultivation (25 °C-35 °C) (FAO 2011). If the water temperature is outside of the optimal range, it can result in the slow growth of mud crabs and affect other water quality parameters, such as DO. During mud crabs cultivations, DO concentrations ranged from 4.2 to 6.8 mg/L and were still in the optimum number >5 mg/L according to FAO (2011). The result is in line with a previous study reported
that the optimum DO for mud crab cultivation is 5.11-5.77 (Faturrohman et al 2017). We found that DO concentration of all treatments during the cultivation period decreased (data not shown). This condition is likely due to the accumulation of organic matter, including uneaten feed and metabolic waste of mud crabs. In addition, the bacterial abundance increasing along the cultivation period presumably resulted in high oxygen consumption and organic matter degradation. The final DO concentration in the control treatment tends to be higher than that of the malang sand at 5 kg, 10 kg, 15 kg, and 20 kg treatment (data not shown). Control treatment at day 15th and 30th had the least number of mud crabs compared to other treatments, which resulted in low organic matter and high DO concentration.

At a specific environment with pH range of 6.5-7.5, it is found the optimal amount of organic matters. Bacteria grow optimally within that pH range and actively decompose organic matter compound (Syarief and Halid 1993). Our study showed that water pH during mud crab cultivation ranged from 5.6 to 7.8. The optimal pH for the mud crabs cultivation is 7.5-8.5 (FAO 2011) and pH 7 (Hastuti et al 2016). Some treatments had no optimal pH (data not shown) which were resulted from organic matter accumulation. Mud crabs could still tolerate fluctuating pH value. However, the pH range should be maintained because it is related to ammonia toxicity, in which the increased pH promotes ammonia toxicity enhancement (van Wyk and Scarpa 1999).

During mud crabs cultivation, water salinity remained stable at 25 g/L which is optimum salinity for mud crabs growth (Hastuti et al 2015). More salinity value can cause mud crab into stress and interfere feed energy consumption resulting in stunted mud crab growth. Stressed mud crabs will lose their appetite leading to a decrease in the immune system and body weight or even dies (Fujaya 2011). Nitrifying and denitrifying bacteria play an essential role in the organic matter decomposition in the waters. Nitrifying bacteria transform ammonia compounds that are harmful to aquatic biota into nitrites, then nitrites to nitrates which are less toxic.

3.2.1. Ammonium. Ammonium concentration in mud crabs cultivation with malang sand as filter material showed significant differences in day 15th and 30th of the rearing period (figure 1), which were in the optimal range for mud crab growth (<3 mg/L) (FAO 2011). Ammonium concentration ranged from 0.162 to 0.535 mg/L. Some treatments showed the enhancement of ammonium concentration which might be due to an influence of bacterial activity (Fang et al 2009). The highest and lowest ammonium concentration after 15 days of rearing was the control and the treatment with 15 kg malang sand accounting for 0.21±0.02 mg/L and 0.21±0.02 mg/L, respectively. After 30 days of the cultivation, malang sand at 10 kg treatment showed the highest ammonium concentration (0.45±0.02 mg/L), while at 20 kg showed the lowest concentration (0.21±0.01 mg/L).

3.2.2. Nitrite. Results demonstrated that the nitrite level in the rearing water was different between treatments in the 0th (H0), 15th (H15), and 30th (H30) days of rearing process (figure 2). Nitrite concentration in the H0 was having insignificant differences between treatments, yet it statistically showed a significant difference between H15 and H30. In the H15, nitrite concentration in the control treatment was the highest (0.33±0.004 mg/L), and the lowest was found in the 5 kg treatment (24±0.008 mg/L). At the end of the cultivation period (H30), malang sand at 5 kg and 20 kg had the greatest and lowest nitrite content constituting 0.23±0.02 mg/L and 0.11±0.02 mg/L, respectively. Generally, nitrite concentrations during mud crabs cultivation ranged from 0.11 to 0.38 mg/L. According to FAO (2011), this value was still in the optimal range for the mud crabs growth, i.e. <10 mg/L.
Figure 1. Ammonium concentration in the mud crabs-rearing water at 0, 15, and 30 days of the rearing period. Different letters in the same day denote significant differences between treatments \((P<0.05)\). (\(\square = 0\) kg), (\(\blacksquare = 5\) kg), (\(\blacklozenge = 10\) kg), (\(\blacktriangle = 15\) kg), (\(\blacklozenge = 20\) kg).

Figure 2. Nitrite level in the mud crabs-rearing water at day 0, 15, and 30. Different letters in the same day denote significant differences between treatments \((P<0.05)\). (\(\square = 0\) kg), (\(\blacksquare = 5\) kg), (\(\blacklozenge = 10\) kg), (\(\blacktriangle = 15\) kg), (\(\blacklozenge = 20\) kg).

3.2.3. Nitrate. Nitrate concentration (figure 3) showed insignificant differences between treatments along the same rearing period as the other concentration (\(H_0, H_{15}, \text{and } H_{30}\)). The lowest nitrate concentration at \(H_{30}\) was found in the 5 kg treatment (0.61\pm 0.09 mg/L) and the highest was in 20 kg treatment (0.79\pm 0.11 mg/L). Nitrate concentrations during cultivation ranged from 0.51 to 1.262 mg/L, which fit the optimal range of <80 mg/L (Kwong and Choudhury 2016).

Fluctuating ammonium, nitrite, and nitrate concentrations is caused by the accumulation of organic matter along with the mud crabs cultivation. It is found that the concentration of inorganic nitrogen decreased during the rearing period (figure 1-3). The decrease might be caused by the increase in the abundance of nitrifying and denitrifying bacteria which is actively degrade organic matter in the rearing water (table 1). Organic matter concentration will influence the survival rate and growth of aquatic biota (Badjoeri and Widiyanto 2008).
Figure 3. Nitrate concentration in the mud crabs-rearing water at 0, 15, and 30 days of the rearing period. Different letters in the same day indicate significant differences between treatments ($P<0.05$).

- $0$ kg
- $5$ kg
- $10$ kg
- $15$ kg
- $20$ kg

3.2.4. Total organic matter (TOM). TOM level in the rearing water is depicted in figure 4. After 15 days of the rearing period, malang sands in the 5 kg treatment showed the highest TOM level of $72.89\pm6.67$ mg/L, while malang sand in the control treatment had the lowest with $42.03\pm5.10$ mg/L. Until the end of cultivation (H30), both malang sand in the 5 kg and control treatments showed the highest and lowest TOM concentration accounting for $74.15\pm8.96$ mg/L and $40.45\pm4.55$ mg/L, respectively. In addition, according to Hastuti et al (2019c), the TOM concentration of the original habitat of mud crabs, which is in Pemalang Regency, Central Java was found 72.82%. There are differences in the profile of organic matter in farms according to the different systems and technologies. The increase in TOM level during mud crabs farming result from the rise in the level of metabolic waste, such as faeces and remaining feed. In the natural ecosystem, organic matter in water can be derived from plants, animal, microorganisms and the results of their excretion/secretion.

Figure 4. TOM in the mud crabs-rearing water at the sampling time point (0, 15, and 30 days of the rearing period). Different letters above the bars in the same day denote significant differences between treatments ($P<0.05$).

- $0$ kg
- $5$ kg
- $10$ kg
- $15$ kg
- $20$ kg

3.2.5. Biological oxygen demand (BOD). Same as other concentration, the BOD level was also examined at day $0^{th}$ (H0), $15^{th}$ (H15), and $30^{th}$ (H30) (figure 5). In the H15, the highest BOD level was found in the 5 kg malang sand treatment ($5.06\pm0.05$ mg/L), while the lowest was in the control treatment.
(3.46±0.15 mg/L). At the end of the cultivation period, the control treatment showed the lowest BOD compared to other treatments. This condition was likely due to the number of mud crabs in the control treatment was fewer than other treatments at the end of cultivation period which reduce the accumulation of organic material and bacterial abundance. Results showed that BOD ranged from 3.01 to 5.06 mg/L, while optimal BOD level for mud crabs growth based on Mocuba (2010) is <5 mg/L. High organic matter promotes the competition for obtaining oxygen between aquatic biota, bacteria, and other aquatic microorganisms.

![Figure 5. BOD in the rearing water of mud crabs at 0, 15, and 30 days of rearing process. Different letters in the same days denote significant differences between treatments (P<0.05).](image)

**Figure 5.** BOD in the rearing water of mud crabs at 0, 15, and 30 days of rearing process. Different letters in the same days denote significant differences between treatments (P<0.05). (☐=0 kg), (☐=5 kg), (☐=10 kg), (☐=15 kg), (☐=20 kg).

3.3. **Survival rates (SR)**

Optimal water quality can enhance the abundance of nitrifying and denitrifying bacteria in the rearing waters, which can break down nitrogen compounds that are toxic to mud crabs. Water quality affects the survival rate of mud crabs. Results revealed that the survival rate of mud crabs at day 15th was the lowest in the control treatment (44.44%±9.6%), and the survival rates at 5 and 15 kg were still 100% (figure 6). Furthermore, at the end of experiment, the survival rate of mud crabs in the 5 kg treatment was the highest rate (77.77%±19.2%), followed by the 10 kg treatment (66.66%±16.6%), 15 kg (61.11%±19.2%), 20 kg (55.55%±9.6%), and control (16.66%±0%). Different survival rates were caused by differences in the weight of the malang sand filter material. The results confirmed that malang sand filter could increase the survival rate of mud crabs for 30 days. The 5 kg treatment showed the greatest survival rate of mud crabs, which indicates this treatment was the most favoured by nitrifying and denitrifying bacteria (table 1). As with the bacteria can highly contribute to water quality maintenance, this condition is potential to reduce the stress level and to improve the efficiency of feed energy utilization by mud crab, which promotes their growth.

In conclusion, the filter using 5 kg malang sand had the most abundant nitrifying and denitrifying bacteria and the highest survival rate of mud crabs reaching up to 77.77±19.2% in comparison to other treatments. During the mud crabs cultivation, water quality parameters fluctuated along with increasing of maintenance, but it was within the optimal range. According to morphological and biochemical characteristics, nitrifying and denitrifying bacteria inhabiting the rearing water of all treatments were classified as *Pseudomonas* sp. and *Acinetobacter* sp., respectively.
Figure 6. Survival rates in the rearing water of mud crabs at day 0, 15, and 30. Different letters in the same days denote significant differences between treatments ($P<0.05$). ($\square = 0$ kg), ($\Box = 5$ kg), ($\blacksquare = 10$ kg), ($\equiv = 15$ kg), ($\downarrow = 20$ kg).

References

APHA 1989 *Standards Methods for the Examination of Water and Waste Water* (Washington: APHA) p 1193

Badjoeri M and Widiyanto T 2008 Usage of denitrifying bacteria for bioremediation and its influence to concentration of ammonia and nitrite in prawn pond *OLDI* 34 261-278

Boyd C E 1998 *Water Quality for Pond Aquaculture* (Alabama: Auburn University) p 37

Camargo J A and Alonso A 2006 Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment *Environ. Int.* 32 831-849.

Catacutan M R 2002 Growth and body composition of juvenile mud crab, *S. serrata*, fed different dietary protein and lipid levels and protein to energy ratios *Aquaculture* 208 113-123

Cheatham A K 2009 Responses of nitrifying bacteria to aquaculture chemotherapeutic agents (Dissertation) (Virginia: Virginia Polytechnic Institute and State University) p 248

Cowan I S T and Steel K J 2003 *Cowan and Steel’s Manual for the Identification of Medical Bacteria*, ed Barrow G I and Feltham R K A (Cambridge: Cambridge University Press) P 353

Fang F, Liu X, Xu J, YU HQ and Li Y 2009 Formation of aerobic granules and their PHB production at various substrate and ammonium concentration *Biosenresour Technol* 34 421-428

FAO 2011 *Mud Crab Aquaculture: a Practical Manual* (Rome: FAO) p 78

Faturrohman K, Nirmala K, Djokosetyanto D and Hastuti Y P 2017 The concentration of optimum dissolved oxygen levels for growth of mangrove crab *S. serrata* seed in recirculation system *JAI* 16 107-115

Fujaya Y 2011 Growth and molting of mud crab administered by different doses of vitomolt *JAI* 10 24-28

Goddard S 1996 *Feed Management in Intensive Aquaculture* (New York: Chapman and Hall)

Hastuti Y P, Affandi R, Safrina M D, Faturrohman K and Nurussalam W 2015 Optimum salinity for growth of mangrove crab *S. serrata* seed in recirculation systems *JAI* 14 50-57

Hastuti YP, Nadeak H, Affandi R and Faturrohman K 2016 Optimum pH determination for mangrove crab *S. serrata* growth in controlled containers *JAI* 15 171-179

Hastuti Y P, Nirmala K, Merani D and Tridesianti S 2018 Actual activity of nitrifying bacteria in culture of mud crab *S. serrata* under recirculating system with various light treatments *AAACL Bioflux* 11 1476-1485
Hastuti YP, Affandi R, Millaty R, and Nurussalam W 2019a Suhu terbaik untuk meningkatkan pertumbuhan dan kelangsungan hidup benih kepiting bakau *S. serrata* di sistem resirkulasi JITKT. *Biodiversitas* 20:1339-1343

Hastuti YP, Rusmana I, Nirmala K, Affandi R, and Tridesianti S 2019b Short communication: Identification and characterization of nitrifying bacteria in mud crab *S. serrata* recirculation aquaculture system by 16S rRNA sequencing. *Biodiversitas* 20:1339-1343

Hastuti YP, Nirmala K, Suryani I, and Prasetyo SL 2019c Environmental characteristics of mangrove forest as a reference for development of mud crab *S. serrata* cultivation: A case study in Mojo Village, Ulujami, Pemalang. *IOP conference Series Earth and Environmental Science*. 278:012035

Karim M Y 2005 Growth performance of female mud crab (*S. serrata* Forsskal) at various medium salinity and evaluation its optimum salinity at various of feeding protein levels [Dissertasion] (Bogor: IPB University) p 30-37

KKP 2017 *Indonesia’s Fisheries Productivity (in Indonesia)* Available at https://kkp.go.id/wp-content/uploads/2018/01/KKP-Dirjen-PDSPKP-FMB-Kominfo-19-Januari-2018.pdf

Kristiawan D, Widyorini N, and Haeruddin 2014 The relationship of total bacteria with total organic matter content in Muara Kali Wiso, Jepara (in Indonesia) *Maquares*. 3:24-33

Kwong K O and Chowdhury A 2016 The beneficial effect of multispecies *Basilus* as probiotics in enhancing culture performance for mud crab *S. Aquacul. Int.* 1 1-19

Mocuba J J 2010 Dissolved oxygen and biochemical oxygen demand in the waters close to the quilimane sewage discharge (Thesis) (Bergen: University of Bergen)

Murniasih T, Yopi and Budiawan 2009 The biodegradation of phenanthren by marine bacteria *Pseudomonas* sp KalP3b22 from Kumai Central Borneo *MAKARA SAINS* 13 77-80

Safitriani, Thontowi A, Yetti E, Suryani and Yopi 2017 The optimal growth of marine *Pseudomonas aeruginosa* LBF-1-0132 in pyrene *J. Biologi Indonesia*. 13 107-116

Sentosa A A and Syam A R 2011 Temporal distribution of condition factor of muddy crab (*S. serrata*) in Mayangan coastal waters, Subang regency, West Java. *Jurnal Perikanan (J. Fish. Sci.)* 13 35-43

Setyani D 2001 *Water Quality for Freshwater Ornamental Fishes* (Jakarta: Penebar Swadaya).

Su J F, Zheng S C, Huang T L, Ma F, Shao S C, Yang S F and Zhang L N 2015 Characterization of the anaerobic denitrification bacterium *Acinetobacter* sp. SZ28 and its application for groundwater treatment *Bioresour. Technol.* 192 654-659

Syafiq A 2008 *Indonesian Food Composition Table* (Jakarta: Elex Media Komptindo).

Syarief R and Halid 1993 Food Storage Technology (in Indonesia) (Jakarta: Arean).

van Wyk P and Scarpa J 1999 *Water Quality Requirement and Management* (Fort Pierce: FAU Harbor Branch Oceanographic Institute).

Viadolo N R L 2016 Effect of Malang sand usage as filter in batik wastewater media on the survival of koi fish (*Cyprinus Carpio* Linn) (in Indonesia) *PENA Akuatika* 14 1-9