

Research article

Efficiency of *Chlorella vulgaris* beads in improving water quality and growth of juvenile siamese fighting fish (*Betta splendens*)

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ABSTRACT

Water quality in aquaculture has been considered to be an important factor that influences the growth and survival of fish in nursery to grow-out phase. A microalga, *Chlorella vulgaris* is utilized to filter chemical substances to maintain optimum water quality and growth of fish. The present study was conducted to know the efficiency of *Chlorella vulgaris* in maintaining water quality and growth of juvenile Siamese fighting fish (*Betta splendens*). As nutrient utilization capacity of immobilized microalgae is higher than the free floating microalgae, the experiment was consisted of 4 treatments which were control (blank beads), low (4-5 beads/mL) (T1), medium (10-12 beads/mL) (T2) and high (15-16 beads/mL) (T3) concentration of microalgae beads. It has been found that, in the control treatment, ammonium concentration initially reduced in medium rate (1.5 mg/L to 0.8 mg/L) but become slower (0.8 mg/L to 0.7 mg/L) in later days. But in first four days, reduction of ammonium and nitrite concentration was followed by T3, T2, and T1 and from day five to seven, both were slightly higher than previous day. On the other hand, PO₄³⁺ showed great reduction (<0.30 mg/L) in all treatment where water with blank beads had higher concentration of phosphate ions (>60 mg/L) than the other treatment tanks. Highest SGR found in algal beads containing treatments than control treatment which were recorded as 4.58, 4.61, 4.57 and 3.60 in T1, T2, T3 and control treatment respectively. Results showed that, there was no significant difference (p < 0.05) in TAN, NO₂-N, PO₄-P, pH, DO and temperature among the microalgae beads containing treatments but significant difference (p < 0.05) found among control and microalgae beads containing treatments. It has been recommended to study economic feasibility of using microalgae beads to maintain the water quality in commercially important ornamental and aquaculture species especially in fish and shrimp hatchery for the larval rearing.

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1. INTRODUCTION

In recent years, the depletion of wild fishery resource is leading the fast development of aquaculture worldwide (Ahmed et al., 2019)

whereas in some countries, the farming of aquatic animals has reduced the production of wild fisheries (Cao et al., 2007). However, water pollution has become a serious problem due to

continuous expansion of the scale of aquaculture and the increased production which posing threats to the environmental protection and hindering the sustainable development of aquaculture (Adler et al., 2000;). Aquaculture wastewater is generally high in nutrients and solid waste due to uneaten food and excretory products as cultured aquatic animals stocked at very high density to meet the human demand (Naylor 2001). Because of excessive nutrients like ammonia (NH_4^+), nitrite (NO_2), and phosphate (PO_4) release by aquaculture farms (Zhang et al. 2015) results in water deterioration (Lu et al., 2019) where water deterioration, refers to oxygen depletion (Svobodová et al. 1993), harmful algal bloom, and eutrophication of water body (Paerl and Otten, 2013). For this, environmental conservation is one of the major concerns all over the world especially of the aquatic environment such as rivers, estuaries and the seas as the aquatic environment provides a good source of animal protein and habitat to aquatic lives (Ramachandra et al. 2005).

To overcome the aforementioned problems, from the last decade, freshwater aquaculture is more focused on ornamental fish culture (Naylor 2001), aquaponics (Graber and Junge 2009), bioremediation by microalgae (Cai et al. 2013), multispecies culture and using bio-filter. In a real-world application, the treatment of wastewater by aeration, filtration, and anaerobic-anoxic-oxic (A2O) system (Adler et al., 2000, Altmann et al., 2016) increases energy consumption and the total cost of aquaculture and financial burden of industry (Longo et al., 2016). Moreover in traditional technologies, nutrients, including nitrogen, phosphorus, and carbon, in wastewater could not be completely utilized and recycled as resources (Lu et al., 2019). To overcome environmental and economic barriers in the aquaculture industry, a lot of efforts have been devoted into the application of microalgae for wastewater remediation, biomass production, and water quality control (Han et al., 2019) as microalgae assimilate nutrients in a eutrophic water body and have been proven to be a good way for wastewater remediation (Leng et al., 2018).

With the concept of bioremediation and the unique characteristics and uses of microalgae *Chlorella vulgaris* in the market, it is used to

recycle nutrients in the water and harvested to be used for supplement in fish feed and also for humans (Priyadarshani and Rath 2012). *Chlorella vulgaris* (Beijerinck, 1890) is a fast growing, green freshwater microalgae with high efficiency in photosynthesis and require low nutrient (Salgueiro et al. 2016). Therefore, in nutrient rich waters, *C. vulgaris* is able to bloom (Khatoon et al. 2016). For this characteristic, *C. vulgaris* is found to be able to grow in lower phosphates and nitrogenous wastewater (Carl et al. 2014). Immobilization technique is introduced to fix microalgae in alginate and to improve harvesting (Huntley et al. 1989) and nutrient absorption rate (Kumar and Saramma 2012). Free floating microalgae in filtration tanks have been reported not efficient in terms of harvesting; obtaining biomass and cost minimization (Gonzalez et al. 2000). The microalgae which are immobilized in the alginate beads can be retrieved by dissolving the microalgae beads in mild acids without harming the microalgae or deteriorate their value (AMSBIO 2013).

Betta splendens is a freshwater ornamental fish commonly known as Siamese fighting fish and categorized under the suborder of Anabantoidei. Fishes under this group are commonly known as labyrinth fishes which mean they are capable of taking oxygen directly from atmosphere (Tate et al. 2017). Very little research has done on the water quality and growth parameters of Siamese fighting fish (*Betta splendens*), one of the most popular ornamental fish. Fish are cultured intensively with high stocking densities to make profits and this will increase the nutrient levels in the water and will affect their growth (Willis 2015). Increased nutrient levels will be toxic for fish and hence affect their health and hormone secretion (Morgan 2010), also affect the growth (Shelton 2010). However in intensive aquaculture, water exchange will increase the chance of releasing nutrients into the aquatic environment with high disease transmission rate (Gormaz et al. 2014) and also increase the cost of aquaculture production.

Therefore, one of the objectives of this study is to maintain the water quality in the culture tanks without changing the water. In addition, to observe the nutrient removal efficiency of *Chlorella vulgaris* beads and the growth of

Siamese fighting fish juvenile in the presence of *C. vulgaris* beads.

2. MATERIALS AND METHODS

Microalgae sample

The pure culture stock of *C. vulgaris* (exponential phase) was obtained from the Institute of Oceanography and Environment (INOS) of University Malaysia Terengganu (UMT) which is isolated from the local area. A small amount of the stock culture was batch cultured using Bold Basal Media (BBM) for 1 month. A cell count was done daily using haemocytometer to observe the growth and the density of *C. vulgaris* to be harvested. The *C. vulgaris* was further sub-cultured to prevent the culture from crashing and to obtain more microalgae cells at the same time (Moretti et al. 1999). The microalgae is grown in a condition with constant and continuous aeration of 1 vvm and 24 hours illumination (Bilanovic et al. 2016) with light intensity of $100\mu\text{ mol m}^{-2}\text{ s}^{-1}$ (Soo et al. 2017). The *C. vulgaris* were harvested when stocking density reached to 1×10^6 cells/mL after 1 month of culturing, using centrifugation method of 6000 rpm for 10 minutes using Hettich EBA 21 centrifuge. The supernatant which is the BBM was poured out leaving the pellet known as the microalgae biomass. The pellet was then washed and re-suspended with distilled water and observed under a light microscope (Optika, B-192) using a haemocytometer to conduct a cell count.

Microalgae culture

BBM is a medium which is enriched with essential nutrients for freshwater microalgal growth. All the chemicals and their concentrations used to make 1 L of BBM was according to Stein (1975) and then the prepared solution were autoclaved at 121°C for 20 minutes (Fraunhofer-Gesellschaft 2016) using HIRAYAMA autoclave (model HVE-50) for sterilization. All the chemicals were measured using an electric balance, cleaned and tarred each time before measuring a new chemical. All the chemicals were dissolved in distilled water, autoclaved, kept in cleaned and sterilized reagent bottles and stored at 4°C in refrigerator.

Growth analyses

Cell count was conducted daily to check and estimate the growth of the microalgae cells. The cell count was conducted using a Neubauer haemocytometer which was cleaned every time before and after use (Graham 2015). The cells in the Neubauer counting chamber was counted and calculated using the formula (Bastidas 2008) as shown:

$$d\left(\frac{\text{cells}}{\text{volume,ml}}\right) = \frac{\text{Total number of cells counted}}{10 \times 4 \times 10^{-6}} \text{cells/ml}$$

The cell count was done till the day of harvesting to make sure that the cultures consist of the concentration of microalgae biomass that is needed to make the microalgae beads.

Harvesting microalgae

After 1 month of culturing the microalgae and having the concentration needed, aeration was removed from the microalgae culture to allow the microalgae biomass to undergo sedimentation for about 2 hours. The suspended biomass was then centrifuged at 6000rpm for 10 minutes (Ndikubwimana et al. 2010) using Hettich Universal 320 Benchtop Centrifuge to obtain a concentrated microalgae pellet which is the biomass. The supernatant which is the BBM was then poured out and then re-suspended with distilled water. Cell count was done again to the re-suspended concentrated *C. vulgaris* solution. After knowing the concentration, calculation was done to ensure the microalgae cells in the sodium alginate solution consist of 1×10^6 cells/mL.

Immobilizing the microalgae

Sodium alginate was used as the matrix to immobilize *C. vulgaris*. The immobilization method was according to Moreno-Garrido (2008) where a 4% of sodium alginate solution was made by mixing the sodium alginate powder into distilled water and the mixture was stirred using a magnetic stirrer for about an hour to allow the sodium alginate powder to be fully dissolved into the distilled water. 1 ml of concentrated microalgae were added every 50ml of 4% sodium alginate solution, stirred using a magnetic stirrer until even and titrated using a burette with a pipette tip of orifice 1.5mm and a

retort stand with clamp to hold the burette upright. The mixture was titrated and form small sphere shapes which are identical to beads. The collecting distance between the tip of the burette and the surface of the stabilizing solution was approximately 5 - 9 cm to allow the microalgae alginate solution to form the sphere shape when dropped into a continuous stirring stabilizing solution which was calcium chloride solution (CaCl₂). The stabilizing solution allowed the alginate undergoes cross-linking which makes them denser that is not so easily crushed (Lee et al. 2013). The beads were left in the continuous stirring SrCl₂ overnight to allow the beads to be hardened. The beads were drained off from the SrCl₂ solution and washed with distilled water for 3 times. The microalgae beads were then put into the canister filter to start the filtering of water and any excess microalgae beads are stored in BBM until further use. The beads were changed in every 4 days and the quantity were the same as mentioned.

Tank setup

There were a total of 4 types of treatments including 1 control and 3 different variable treatments with different concentrations in number of microalgae beads. The control was a negative control which consisted only of blank alginate beads. The 3 different variable sets were low, medium and high number of microalgae beads which was having 4-5 beads/mL, 10-12 beads/mL and 14-15 beads/mL respectively (Tam and Wong 2000). All the 4 treatments had duplicates and therefore this study had a total of 8 tanks (45×30×30 cm). The beads were put into a net bag and aeration stone connected to the aeration system was inserted together inside to allow filtration happen so that water was able to move throughout the tank and between the microalgae beads to be recirculate.

Feeding

The experimental fish was in juvenile stage of 7 weeks old and each tank contains 20 pieces of fish. Fries were usually fed to excess with high protein diet as protein is needed for growth (Craig and Helfrich 2009). The diet was commercial pellets with high protein content. Fishes of all tanks were fed with the same type of pellet with the same content and the amount

of feed was 3% of total body weight of all fish in a tank for twice in a day, daily for the optimum growth of the fish fry (Sipaúba-Tavares et al. 2016). The same feeding regime was given to all fishes throughout the entire experiment for 14 days.

Physico-chemical parameters of water

Temperature, pH, and dissolved oxygen level in the culture tanks were measured daily using a YSI 556 MPS (YSI, New Jersey, USA). Total ammonia nitrogen, nitrite nitrogen and phosphorus were also analyses daily following the method of Parsons et al. (1984).

Growth measurement

Length and weight was measured daily using ruler with accuracy of 0.1cm and electronic balance of 0.1 g. Both the fishes from the entire experimental tank were measured before and after the experiment end which was day 0 and day 14. The growth was measured in weight gain and specific growth rate (SGR) where the formulas (Nyina-Wamwiza et al. 2016) were as follows:

$$\text{Weight gain (\%)} = 100 \times \frac{W_f - W_i}{W_i}$$

$$\text{Specific growth rate (SGR, \% per day)} = 100 \times \frac{\ln(W_f) - \ln(W_i)}{\Delta t}$$

Where W_i , W_f , and Δt are initial body weight in mass (g), final body weight in mass (g) and, duration of experiment respectively.

Statistical analysis

The collected data was analyzed using a statistical program to conduct one-way ANOVA by using SPSS 20 and MINITAB 14. All the data had underwent normality test and test of homogeneity of variance which were Anderson-Darling test and Levene's test respectively before conducting the one way ANOVA to make sure to use parametric or non-parametric one-way ANOVA (Nayak and Hazra 2011). After the data had went through both the preliminary test, the results of normality test and test of homogeneity of variance showed that the data were not suitable for parametric one-way ANOVA test, therefore non-parametric one-way

ANOVA test was used which was Kruskal-Wallis test. After running Kruskal-Wallis test there were several parameters showed significant difference between treatments. These parameters needed to undergo a Post HOC test where Bonferroni test were used due to the significance used was an adjusted p-value in non-parametric analysis (Shingala and Rajyaguru 2015).

3. RESULTS

Reduction of total ammonia nitrogen (TAN)

The total ammonia nitrogen (TAN) concentration in all the treatments have shown to reduce significantly ($p > 0.05$) than the control treatment with blank beads. All the treatments dropped to less than 0.200mg/L within 24 hours and maintained until day 14. The daily concentration graph of TAN of all treatments is shown in Figure 1 (a). In first four days, reduction of ammonium concentration was followed by T3, T2, T1 and control. From day five to seven, the NH_4^+ concentration was slightly higher in T2 than T1 and T3. In the control treatment, ammonium concentration initially reduced in medium rate (1.5 mg/L to 0.8 mg/L) but become slower (0.8 mg/L to 0.7 mg/L) in later days. The concentration cycle of ammonium reduction repeated after changing beads in seventh day to day fourteenth.

Reduction of nitrite-nitrogen ($\text{NO}_2\text{-N}$)

For nitrite-nitrogen ($\text{NO}_2 - \text{N}$), there were significant difference in all the treatments but was maintained between 0.002 and 0.028 mg/L throughout the experiment. The concentrations of $\text{NO}_2\text{-N}$ of all treatments reduced in day 5 and also comparatively higher reduction have been seen in day 12, after the second loading of microalgae beads. Figure 1(b) shows slight increment in day 6 and day 13; whether control shows significantly higher concentration of nitrite nitrogen than other treatment.

Reduction of phosphate phosphorous ($\text{PO}_4\text{-P}$)

For the concentrations of phosphate phosphorous ($\text{PO}_4\text{-P}$), there was a significant difference ($P>0.05$) between control with algal beads treatment (T1, T2, T3) where the control treatment remained in higher concentration

while T2 and T1 and T3 remained in lower concentration throughout the experiment. Generally, $\text{PO}_4\text{-P}$ in all the treatments was reduced in a parallel pattern with the NH_4^+ concentration. The daily concentrations were as shown in Figure 1(c). The $\text{PO}_4\text{-P}$ showed great reduction (<0.30 mg/L) in all treatment where water with blank beads had higher concentration of phosphate ions (>60 mg/L) than the other treatment tanks.

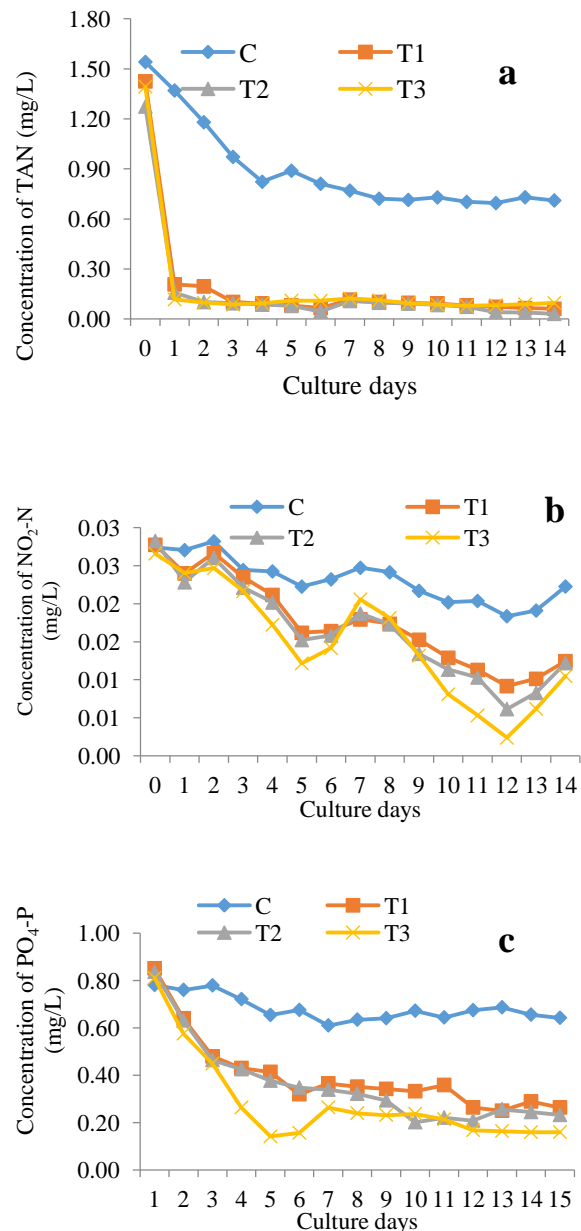


Figure 1. Daily concentration of (a) TAN, (b) $\text{NO}_2\text{-N}$ and (c) $\text{PO}_4\text{-P}$ (mg/L) in all treatments (values are mean \pm standard error)

Fluctuations of pH

In this experiment, the average pH levels were recorded and observed showing that the pH of the water samples were slightly alkaline more than 7 (7.1 to 7.8) from day 1 to 7. The daily average pH readings of all treatments are shown in the Figure 2 (a).

Fluctuations of dissolved oxygen (DO)

The daily concentration of dissolved oxygen (DO) was observed throughout the experiment. The DO concentration remained within a range which was in between 5.00mg/L to 8.00mg/L. The daily dissolved oxygen level is shown in Figure 2 (b).

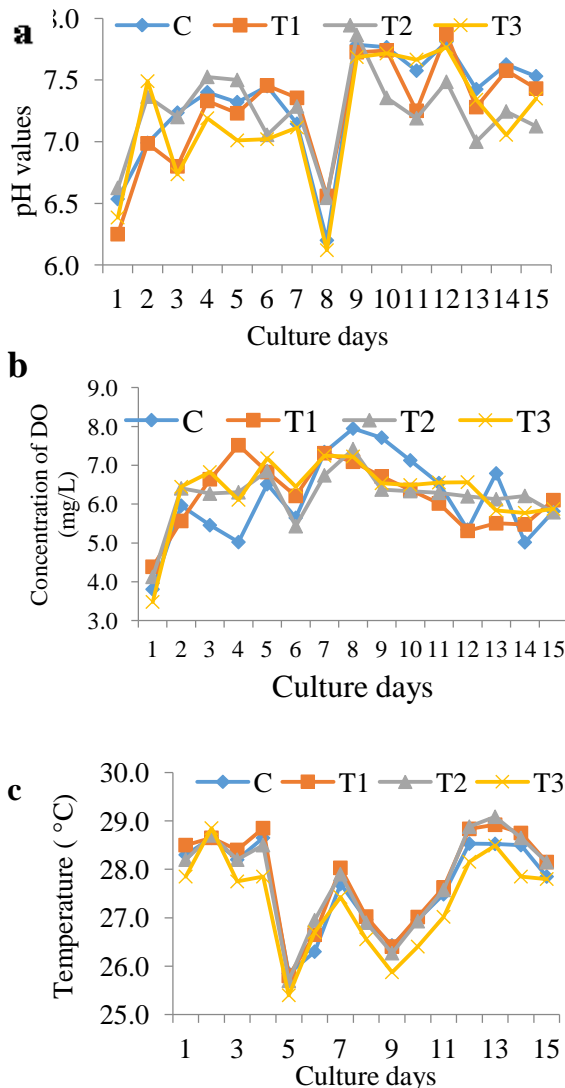


Figure 2 Daily (a) pH, (b) dissolved oxygen (mg/L) and (c) temperature (°C) reading in all treatments throughout the experiment

Temperature fluctuations were observed throughout the experiment as well. There was a sudden drop on day 4 which was almost 2.0 °C and a drop on day 8 which was 1.5 °C. The daily average temperature readings are tabulated as in Figure 2 (c).

Specific growth rate (SGR)

Specific growth rate (SGR) results showed a significant difference $p = 0.035 (<0.05)$ between control and treatments. Figure 3 shows the SGR of all treatment. There was no recorded mortality during this 14 days nursery experiment of the Siamese fighting fish. Highest SGR found in algal beads containing treatments than the control one which were recorded as 4.58, 4.61, 4.57 and 3.60 in T1, T2, T3 and control treatment respectively. But, among the algal beads containing treatments there was no significant difference (<0.05) in SGR.

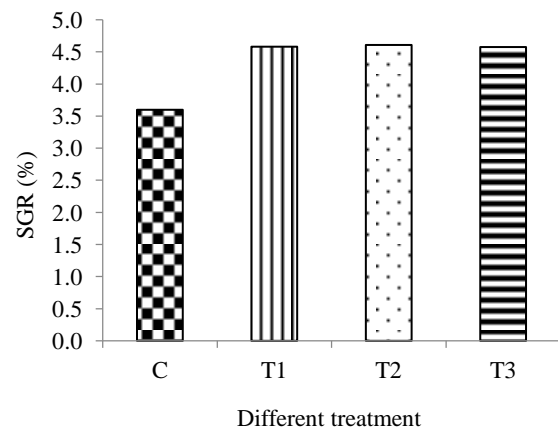


Figure 3. specific growth rates (SGR %/day) of *B. splendens* in all treatments (values are mean \pm standard error).

4. DISCUSSION

Ramos and Gonçalves (2019) found common genetic and physiological mechanisms of aggression in both male and female Siamese fighting fish except desired pair. Furthermore, males shouldn't stock together unless there are separators (Sharpe, 2020) and female-female aggression occurs naturally. That's why, in this study, each tank consisted of 500 mL of water and only a pair of juvenile Siamese fighting fish (Overstreet et al. 2000).

Water quality consists of three parameters which are chemical, biological and physical

parameters (Bartram and Ballance 1996). Chemical parameters are such as total ammonia nitrogen (TAN), nitrite-nitrogen ($\text{NO}_2 - \text{N}$), soluble reactive phosphates (SRP), pH and dissolved oxygen (DO). However, TAN, $\text{NO}_2\text{-N}$ and SRP are especially focused as these three water quality parameters affects the health and growth of fish specially when fish are kept in a high stocking density, confined space and limited water volume with high feeding rate (Furtado et al. 2014). Fishes those are not able to withstand the chemical stress may grow weaker and eventually die (Kumar 1992). Moreover, pathogens will take advantage of the situation to reproduce and spread disease to other individuals in the same habitat causing disease outbreak which will cause losses to aquaculture farm owners.

Biological treatment for water quality management with bacteria is the most common exercise, accomplished with mechanical aeration (Muñoz et al. 2005) whereas microalgae can be used as either solo or in consortia along with other micro-organisms (mainly bacteria) as potential source for supplying dissolved oxygen (DO) for bacteria in the wastewater treatment process which increase degradative efficiency of bacteria by 37% in the presence of microalgae (Karya et al. 2013). One of the benefits of cultivating microalgae in alginate beads is to prevent it from being grazed upon by zooplankton (Faafeng et al. 1994). By immobilizing the microalgae, it keeps the cells in place, thus solving the harvest problem (Chevalier and de la Noue 1985). It also helps to facilitate easier operation and separation (Mallick and Rai 1993) during culture of any aquatic organisms. Hameed (2002) reported that the immobilized algae are more efficient in removing nutrients than the freely suspended cells of the same algal species. At present, the most universally used material for microalgal immobilization is alginate. Therefore, alginate beads show excellent permeability, low or null toxicity and high transparency of alginate matrix produce a suitable environment for immobilized microalgae (Zhang et al. 2012). Microalgae beads can be harvested effortlessly and further processed to obtain the microalgae inside by dissolving the beads. Furthermore, it can be processed into powdered form for fish feed. Besides that, the supernatant obtained from the

microalgae beads can reuse in land base agriculture as fertilizers.

Total ammonia nitrogen (TAN) is important to be accessed as it affects the growth of fish (da Silva et al. 2013). Results from this experiment showed that beads with algae was very efficient in removing $\text{NH}_4^+ - \text{N}$ from the culture tanks within 24 hours ($<0.2\text{mg/L}$), while *Scenedesmus sp.* remove ammonium (NH_4^+), up to 90%- 93% within four hours (Kaparapu and Geddada 2016; Pham and Bui 2020) from waste water. On the other hand, reduction of ammonium in the control treatment was slower than other treatments. Reduction of ammonium concentration was followed by high (15-16 beads/mL), medium (10-12 beads/mL), low (4-5 beads/mL) and control (blank beads) in first four days. From day five to seven, the NH_4^+ concentration was slightly lower in treatment beads with medium algal biomass than the high one followed by low and control treatments as ammonium is among the most preferable chemical forms of nitrogen that can be readily absorbed by almost all microalga species (Perez-Garcia et al. 2011). This result suggests that beads with too few algal concentrations would reduce the N removal efficiency of beads. On the other hand, too high algal biomass was also not very effective as their utilization rate decreases rapidly because of concentrated biomass within the limited bead's space, because increased density in beads would reduce the amount of light penetrating and space utilization which can be the limiting factors for growth and metabolic activities of the algal cells (Tam and Wong 1999). In blank beads the ammonium ions are adsorbed onto the bead's surface in a certain level lowering the concentration in addition with air stripping of ammonia, as ammonium ions are adsorbed and rapidly bound to the carboxyl groups of alginate gel (Tam et al., 1994; Lau et al., 1997). The adsorption rates become slower after the fourth day because of saturated concentration in alginate surface. Cellular uptake and utilization with algal beads resulted in high ammonium reduction in the treatment tanks together with adsorption and volatilization as ammonium is absorbed directly into the cells and then transformed into amino acids, less energy is required for its uptake than for nitrate or urea (McCarthy et al., 1977).

Nitrite-nitrogen is the intermediate of nitrate and ammonia which is very deadly and has acute effects on fish health. Even though $\text{NO}_2\text{-N}$ does not affect fish growth directly, but it can cause mortality to fish when exists in high concentration (Davidson et al. 2014). In this experiment, there was significant difference among all the treatments along with control throughout the experiment wherever the $\text{NO}_2\text{-N}$ level was within the optimum range for fish. Since there was only bacterial activity for nitrification in control unit, as well as having greater surface area for nitrifying bacterial load, and blank alginate beads provided as proper culture medium for bacteria (Nikolajeva and Zommere 2017). Therefore, the results showed that $\text{NO}_2\text{-N}$ were unstable within desired range for Siamese fighting fish, where typical concentrations of nitrite-N in pond water range from 0.005 to 0.5 mg/L (Stone et al., 2013). But in microalgae beads containing treatments $\text{NO}_2\text{-N}$ concentrations were lower whereas air-stripping of ammonia is a possible mechanism for N removal intensively aerated microalgal system with alkaline pH resulting from photosynthetic activity and aeration (Lau et al. 1998). Along with this, Immobilized *Chlorella vulgaris* alone removed 92% of nitrogen (Mujtaba et al. 2018). The alginate immobilised *C. sorokiniana* represents a promising tool for the removal of nitrogen from drinking water sources (Petrović and Simonic 2015).

Soluble reactive phosphate (SRP) need to be analyzed as the presence of phosphorus in the water helps promote algae growth as it is a source of nutrient for algae to grow and multiply where in excess will cause eutrophication (Zeng et al. 2012). The reduction pattern of phosphate concentrations in culture water was similar to the N uptake and the utilization of phosphate by algae is related to algal biomass and the optimal algae concentration in beads. The phosphate concentration declined rapidly in all algal beads treatment with a significant difference compared to the control one. In algal beads treatments, phosphate removal were achieved by both algal uptake and chemical precipitation (Tam and Wong 1999), whereas, precipitation was the only reason of phosphate reduction in control tank as phosphate phosphorous ($\text{PO}_4\text{-P}$) were able to bind with the divalent calcium ions (Ca^{2+}) released into the waters from the calcium

alginate beads and precipitated as calcium phosphate (Lee and Mooney 2012). It is a common occurrence at pH values around 8 and higher (Megharaj et al., 1992; Moutin et al., 1992). Due to the higher pH of the waters, calcium alginate tends to be less stable (Dainty et al. 1986). Even though the Ca^{2+} released into the water but that alginate beads did not lose their integrity or dissolved and no cell leakage was observed in this study. This can be explained by the fact that, the initial phosphate concentration was much lower (<0.8 mg/L) than the minimum concentration (950 mg/L) requires to dissolve the algal beads according to the experimental results of Robinson et al. (1985). Nevertheless, pH is also a very important parameter as TAN and $\text{NO}_2\text{-N}$ concentrations are related to the pH. Some researchers have found out that pH increases as TAN and $\text{NO}_2\text{-N}$ increases and vice versa (Lawson 1995). The water showed to be slightly alkaline (Figure 2a) in pH around throughout the whole experiment. Increase in pH values resulted from the photosynthetic activity and aeration supplied in the culture water (McCarthy et al., 1977; Lau et al. 1998). On the other hand, the presence of calcium ions in alginate matrix and waste in water together elevated pH values (Tam and Wong 2000; Lee and Mooney 2012). The pH value was higher in treatment tanks compared to the control one as aquatic plants remove carbon dioxide from the water during photosynthesis and respiration resulting in elevated pH level (The Fish Site, 2013).

Different species of fish have their own different optimal temperature range where their behavior such as activeness, reproduction, growth and metabolic rate can progress at an optimum level (Dodson 2005). According to Shapley (2012), gases in water tend to achieve equilibrium with factors such as temperature. The equilibrium of oxygen follows Henry's Law where equilibrium is achieved with the atmospheric temperature. In this study, the temperature was not controlled which resulted in some fluctuations (Figure 2c) because of the unstable weather condition throughout the experimental period. On day 6, the sudden reduction of temperature was due to the rain which lowered the temperature. Although there was no rain on day 8, but the weather was cloudy causing temperature fall from other days. This inconsistent daily

temperature could affect the concentration and equilibrium of dissolve oxygen as well as the appetite of the fishes.

In this study, *Chlorella vulgaris* beads maintained the water quality parameters within optimum range through reduction of TAN, NO₂-N and PO₄-P resulting better specific growth rate of cultured fish species compared to the control and according to Makori et al., optimum level of Ammonia, DO and pH increases the growth rate of Tilapia. Further, along with the beads efficacy in improved water quality and the hardy nature of Siamese fighting juveniles as they have an accessory organ in the breathing mechanism (Tate et al. 2017) ensured zero mortality during this experiment.

5. CONCLUSION

Microalgae immobilized in alginate beads have been proved to perform an efficient wastewater tertiary treatment. In this experiment, due to the application of *Chlorella vulgaris* beads which maintained the water quality parameters within optimum range through reduction of TAN, NO₂-N and PO₄-P resultant in better specific growth rate in compare to the control. The result showed that immobilized *Chlorella vulgaris* beads are appeared to be more effective in removing N and P from wastewater than blank alginate beads. Removal of nutrients from wastewater was also facilitated by the calcium alginate (immobilization matrix), the alkaline pH that prevailed in the experiment and the constant aeration of provided together with the algal cells in beads. Moreover, future investigations should be conducted to work on the drawbacks of this treatment process and establish an ideal method based on the research outcomes.

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