DESIGN OF AZOLLA PINNATA CULTURE SYSTEM FOR REMEDIATION OF AQUACULTURE WASTEWATER

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PROJECT APPROVAL SHEET

This project report attached here, entitled of Design of *Azolla pinnata* Culture System for Remediation of Aquaculture Wastewater prepared by Ahmad Nurfazly bin Ahmad Termizi in partial fulfilment of the requirement for the degree of Bachelor of Engineering (Agricultural and Biosystems) with Honours is hereby accepted.

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ABSTRACT

A recirculating aquaculture system (RAS) is developed to reduce water usage and to improve waste management and nutrient recycling. The first objective of this study was to evaluate the ammonia removal by *Azolla pinnata in water*. The second objective of this study was to design a RAS which integrated the *A. pinnata* for the removal of ammonia in the RAS. In RAS, the nitrification process is the main process to reduce ammonia concentration but the process is less sustainable if compared to ammonia assimilation by plant. Therefore, *Azolla pinnata* is expected to be suitable to be used for ammonia removal in RAS. For the first experiment, the experiment was conducted using two treatments to measure and to compare the removal of ammonia by *A. pinnata*. The two treatments were: 1) pond water with added ammonia concentration (pond water + ammonia), 2) pond water with added NPK fertilizer as control treatment (pond water + NPK). The concentration of ammonia was measured by using salicylate method. Ammonia removal per day in each treatment was summed up until the last day of the study. At the end of the experiment, *A. pinnata* growth between the two treatments was compared. Through independent sample t-test, it was found that ammonia removal was significantly higher in treatment pond water + ammonia than treatment pond water + NPK. Growth of *A. pinnata* was not significantly different between the two treatments. The uptake rate of ammonia was found at 0.31 mg per g of *A. pinnata* per day for treatment ammonia and 0.12 mg per g of *A. pinnata* per day for treatment NPK. Integration of *A. pinnata* culture system in RAS was to improve ammonia purification level. From the proposed design of RAS, the *A. pinnata* was placed in the sump which would cover the total surface area of the sump and the *A. pinnata* would be able to remove all of ammonia produced in the RAS at hydraulic retention time of 160 min. The study has shown that *A. pinnata* could be used as water purifier i.e., to remove the ammonia released by fish.

ABSTRAK

Sistem akuakultur kitaran semula (RAS) dilakukan untuk mengurangkan penggunaan air dan meningkatkan pengurusan sisa dan kitar semula nutrien. Objektif pertama kajian ini adalah untuk menilai amonia yang disingkirkan oleh *Azolla pinnata* dalam air. Objektif kedua kajian ini adalah untuk merancang aplikasi *A. pinnata* untuk menyingkir amonia dalam RAS. Dalam RAS, proses nitrifikasi adalah proses utama untuk mengurangkan kepekatan amonia tetapi prosesnya kurang lestari jika dibandingkan dengan asimilasi amonia oleh tanaman. Oleh itu, *Azolla pinnata* dijangkakan sesuai digunakan untuk menyingkirkan amonia di RAS. Untuk eksperimen pertama, eksperimen dijalankan dengan menggunakan dua rawatan untuk mengukur dan membandingkan penyingkiran amonia oleh *A. pinnata*. Dua rawatan tersebut adalah: 1) air kolam dengan kepekatan amonia (air kolam + amonia), 2) air kolam dengan penambahan baja NPK sebagai rawatan pengendalian (air kolam + NPK). Kepekatan amonia diukur dengan menggunakan kaedah salisilat. Penyingkiran amonia setiap hari dalam setiap rawatan dijumlahkan sehingga hari terakhir kajian. Pada akhir eksperimen, pertumbuhan *A. pinnata* antara kedua rawatan dibandingkan. Melalui ujian t sampel bebas, penyingkiran ammonia jauh lebih tinggi pada air kolam + rawatan amonia daripada air kolam + rawatan NPK. Pertumbuhan *A. pinnata* tidak berbeza antara air kolam + rawatan amonia dan air kolam + rawatan NPK. Pengambilan amonia didapati pada kadar 0.31 mg per g *A. pinnata* per hari untuk air kolam + rawatan ammonia dan 0.12 mg per g *A. pinnata* sehari untuk air kolam + rawatan NPK. Integrasi sistem tanaman *A. pinnata* dalam RAS adalah untuk meningkatkan tahap pembersihan amonia. Dari reka bentuk RAS yang dicadangkan, *A. pinnata* diletakkan di dalam takungan yang akan meliputi keseluruhan luas permukaan takungan dan *A. pinnata* dapat mengeluarkan semua amonia yang dihasilkan dalam RAS pada masa penahan hidraulik 160 min. Kajian telah menunjukkan bahawa *A. pinnata* dapat digunakan sebagai pembersih air, untuk menghilangkan amonia yang dikeluarkan oleh ikan.

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CHAPTER 1 INTRODUCTION

1.1. Background

World fish supply is depending on fisheries and aquaculture activities. Fisheries is defined as the capture of aquatic organisms in marine, coastal and inland areas, while aquaculture is defined as activities of farming aquatic organisms, including fish, molluscs, crustaceans, and aquatic plants (FAO, 1988). Worldwide, fisheries are threatened by the issue of fish stock depletion which is led by super-exploitation and inefficient management. (Fathi, S et al., 2018). Around 33% of fish stocks are over-fished, while over 60% fully fished and 7% resource remained worldwide (FAO, 2018). The impact of climate change also contributes to the depletion of fish stock (Yusoff, A., 2015). The invasion of Malaysian waters by foreign vessels including prohibited areas intruded by local fishing vessels has led to the depletion of national wild fish (DOF, 2015). Due to these reasons, aquaculture has become more important to secure the fish supply.

Aquaculture systems can be categorised into open and closed systems. Open systems are fish farming which is placed within a large body of water, for example, marine and oceans. Cage system is one of the examples of an open system. Meanwhile, closed systems are those in which water is reconditioned and recirculated to the culture unit, thus allowing a minimum exchange of water during the culture period. Recirculating aquaculture system (RAS) is one of the examples of a closed system. In this study, the focus will be on RAS.

A RAS can be placed indoor and outdoor. Indoor fish production is more sustainable compared to outdoor due to significant environmental issues. There are many advantages in using RAS such as possibilities to decrease water use which is about 2 or 3% system discharge per day (Timmons M.B, Ebeling J. M., 2010). With a small value of discharge water, RAS is the most environmentally friendly aquaculture system which can minimize pollution to water bodies. Furthermore, RAS can improve system hygiene and gives better management of diseases (Summerfelt et al., 2009). In several RAS systems, for instance, ozonation and UV processes were used at the reservoir to treat the water in RAS before water flow back to the fish tanks.

Figure 1:An indoor recirculation system

Source: (Bregnballe, J., 2015)

Figure 2: An outdoor recirculation system

Source: (Bregnballe, J., 2015)

Waste excreted by fish in RAS will generate ammonia (NH_3), which in aquaculture systems is one of the most harmful wastes. Ammonia exists in two forms: un-ionized NH_3, and ionized NH_4^+. In RAS, ammonia concentration is reduced by the nitrification process. In this process, ammonia is converted to nitrite under an oxidized condition and then, nitrite to nitrate also under an oxidized condition. However, nitrate will accumulate in the RAS. High nitrate concentrations in the water may affect the growth of fish and thus nitrate must be eliminated from RAS.

1.2. Problem statement

Recirculating aquaculture systems (RAS) becomes an essential system in aquaculture production due to limitations in land and water supply. RAS gives benefits to aquaculture by giving a low impact on the environment. Waste excreted by fish in RAS will generate ammonia, which is one of the most harmful wastes in aquaculture systems. High concentrations of ammonia may affect the growth of aquatic organisms that are commercially grown, and it may decrease the organisms' survival rate (Ray,2016). In RAS, the nitrification process is the main process to reduce ammonia concentration. However, nitrification is less sustainable if compared to ammonia assimilation by plant. Therefore, in this study examined the potential of *Azolla pinnata* to be used for ammonia removal in RAS. It was hypothesized that the integration of *Azolla pinnata* in a RAS would be able to eliminate NH_3-N and could improve ammonia purification level in RAS. The hypothesis was based on the study by Mohamed Ramli et al., (2018) which uses photosynthetic organisms (which was microalgae) to improve ammonia purification level in RAS as well as act as a water purifier (D. C. Roy, M.C.Pakhira and S.Bera, 2016).

1.3. Study objective

This study embarks on the following specific objectives:

1. To evaluate the ammonia removal by *Azolla pinnata*.

2. To propose the design of RAS using tanks which are available in the hatchery and to calculate the capacity of *Azolla pinnata* to remove ammonia in the RAS design, based on the result achieved in Objective 1.

CHAPTER 2 LITERATURE REVIEW

2.1. Current situation of aquaculture industry in Malaysia.

Aquaculture has been recognized as an important potential export earner after oil palm and rubber. Compared with other agricultural sectors such as paddy, aquaculture is one of the most productive in terms of income per hectare per annum and return to investment. Statistics from Department of Fisheries Malaysia (DOF,2017) stated that brackish water aquaculture production was 324.3 thousand tonnes, increase by 6.7 percent in the previous year. For marine fish landing and production, freshwater aquaculture was dropped in 2017 by 7.5 percent and 0.8 percent respectively. It shows that the sector contributes to national food security and provide adequate food access to Malaysian. Aquaculture can be a threat to society, for example, environmental pollution, especially in water. However, we can control the threats by practicing Good Aquaculture Practices(GAP) and Environmental Impact Assessment(EIA) before any aquaculture project is undertaken.

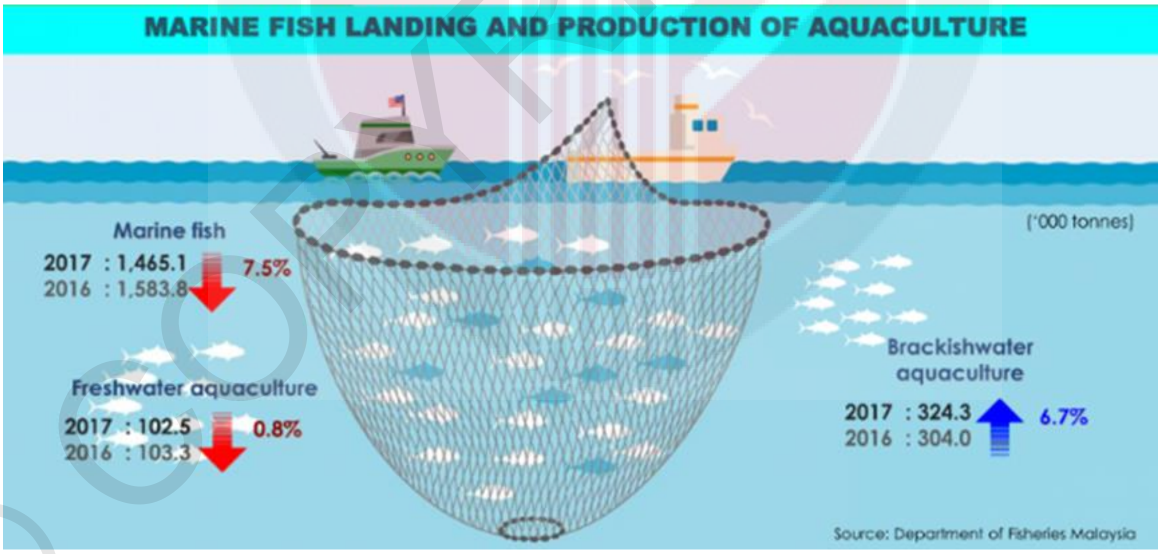


Figure 3: Statistics of marine fish landing and production of aquaculture in 2016 and 2017

Source: (Department of Fisheries Malaysia, 2017)

To develop aquaculture projects, areas such as coastal waters, seas, lakes including land have been explored. By year 2020, aquaculture production is estimated to grow by almost 8.6% per annum. Department of Fisheries has created Aquaculture Industrial Zones (AIZ) which is a zone for lands and bodies of water which are given by the state government for aquaculture projects to overcome the land issues. About 40 000 ha are given to aquaculture development by the states (FAO,2019). One of the objectives that stated by Department of Fisheries Malaysia is to increase fish production in line with the goals of the Balance of Trade Plan.

2.2. What is a RAS?

Recirculation aquaculture system (RAS) is a technique used to recycle the water use to farm fish or other aquatic organisms. Fish are raised in tanks and placed inside an enclosed building in the safest setting to also control the aerial environment. Water flows throughout the system, and water released daily in a small percentage. A high utilization level of the feed is advantageous in a recirculation process as the number of excretion products will decrease and the impact will reduce on the water treatment system.

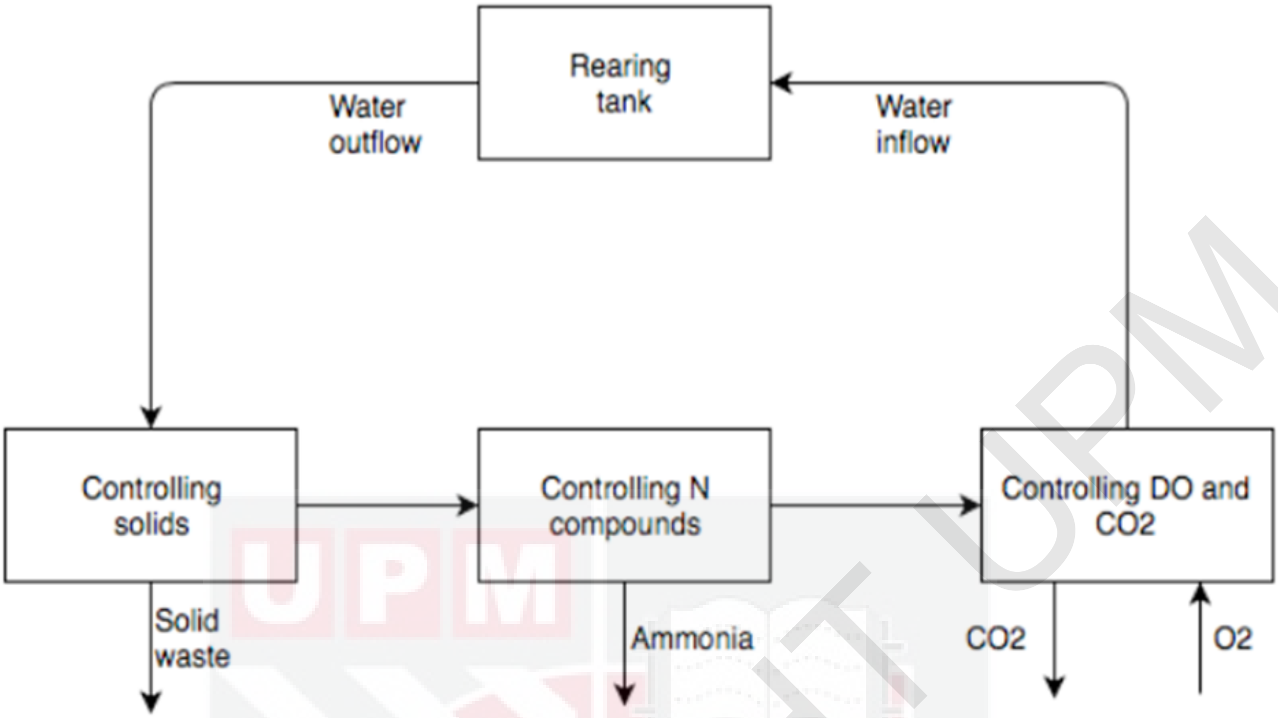


Figure 4: Principle Concept of Recirculating Aquaculture System

Source: (Tryggvason, 2016)

The concept of RAS begins with water from rearing tank flow through a filter to excrete the solid waste. The water moves towards the bottom centre drain due to rotation of water and a small percentage of total flow is removed. Then the water flows through biofilter where bacteria like Nitrosomonas grow and it will transform harmful ammonia to less harmful nitrate under an oxidized condition. The final is step controlling dissolved oxygen by adding O_2 from water and controlling carbon dioxide by removing CO_2 from water. Timmons and Ebeling (2010) stated that indoor RAS offers advantages of raising fish in a controlled environment, permitting controlled product growth rates and predictable harvesting schedule. RAS conserve heat and water through water reuse after reconditioning by biological filtration using biofilter. RAS is environmentally sustainable; they use 90-99% less water than conventional aquaculture systems and provide for environmentally safe water management treatment.

Component of RAS

2.2.1. Fish tank

Fish in the tank require feeding a few times per day. The feed is eaten and digested by fish to furnish the energy for them, give good growth behaviour and other physiological processes. Energy will produce when oxygen enters through the gills and breakdown protein where ammonia and carbon dioxide produced as products of waste.

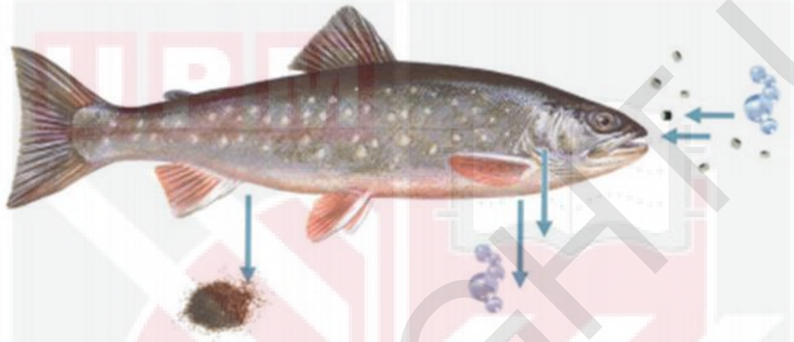


Figure 5: Feeding and respiration result excretion of waste products

Source: (Bregnballe, J., 2015)

Waste solids accumulate in an aquaculture process are derived from uneaten food, feeding fines, fish fecal matter, algae, and cell mass of bacteria sloughed from biological filters. Fish contain total suspended solids (TSS) between 0.3 and 0.4 kg per 1 kg of feed (Bregnballe, J., 2015). The solids of waste give the most influence on the efficiency of the process in recirculating aquaculture systems. They are a major source of demand for carbonaceous oxygen and nutrient input into the water, and by destroying fish gills and propagating pathogens they can directly affect fish safety within recirculating systems.

2.2.2. Waste solids filtration

Waste solids, such as faeces, feed fines, untreated feed, sloughed biofilm and ammonia, must be collected from the water tanks daily to prevent toxicity. The way solid waste is collected depends on the type of waste. Settling solids sink and can be extracted continuously by gravity and stream and periodically by siphoning.

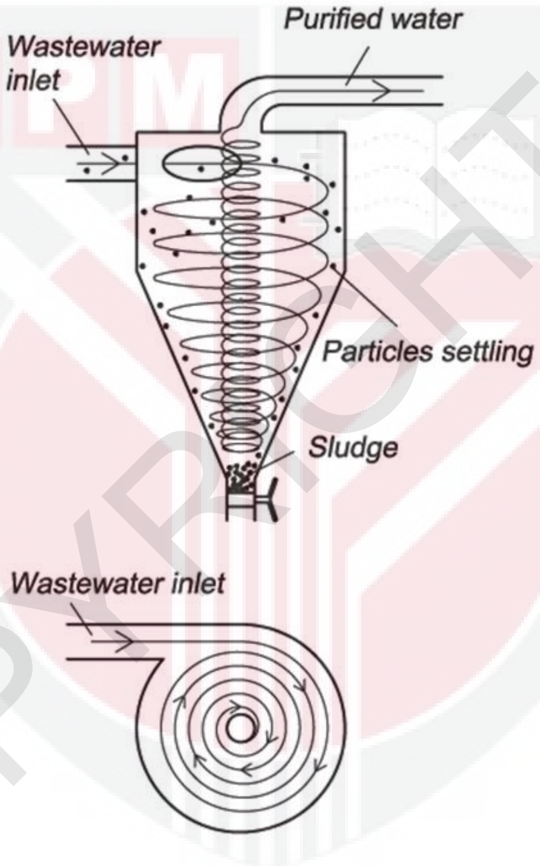


Figure 6: Example of waste solids filtration

Source: (Lekang, 2007)

2.2.3. Bio-filtration

Biological filtration can be a good way to reduce ammonia. Biofilter contain media with large surface areas for the growth of nitrifying bacteria. RAS applications have effectively used ‘bead’ filters, which use a floating plastic media provide a medium to which nitrifying bacteria attach. Bead filters are operated to capture solids and provide biological filtration. The filtration undergoes nitrification process which is a two-step process where ammonia is converted to nitrite under an oxidized condition and then, nitrite to nitrate also under an oxidized condition.

1st step: $NH_4^+ + 3/2\ O_2 \longrightarrow NO_2^- + H_2O + 2H^+$

2nd step: $NO_2^- + ½\ O_2 \longrightarrow NO_3^-$

2.2.4. Reservoir

The reservoir or sumps acts as a treatment water tank. After filtration, the water becomes purify and any treatment such as UV light, ozonation and temperature can occur. This is because the water has been treated will recirculate to the fish tank and used by fish.

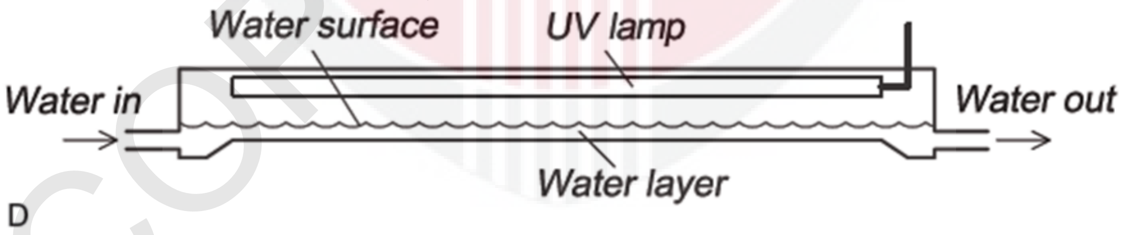


Figure 7: Treatment water by UV light

Source: (Lekang, 2007)

2.3. Water quality in RAS

2.3.1. Dissolved Oxygen (DO)

Of all the water quality parameters, DO is the most important and most critical parameter, requiring continuous monitoring for intensive production systems. The saturation concentration of DO would be higher at higher temperatures and lower at lower temperatures. In addition, the solubility of oxygen decreases as salinity increase. The amount of DO in the water also dependent on temperature where cooler water having more than DO than warmer water. Aquatic plants will give extra oxygen to water but over abundance can lead to super saturation of oxygen during the day and consequently very low levels at night causing stress aquatic communities. A common cause of low oxygen water is where organic materials (from wastewater treatment works) added to water. Bacteria break down organic material and in so doing use up the available oxygen in the water to the extent that waters can become severely deplete of oxygen. Fish required optimum dissolved oxygen concentration to grow and stay alive which is 5.0 mg L^{-1} to 7.0 mg L^{-1}(Pillay, T.V.R., Kutty, M.N., 2005). If not get enough oxygen, fishes in the tank will be gulping. Therefore, aeration is applied to ensure fish get sufficient oxygen.

2.3.2. Temperature

Temperature is the second important part after dissolved oxygen in the aquaculture system. This is because the temperature can affect the growth of fish and the respiration rate. Each species of fish has its optimum temperature range that maximizes growth and also has lower and upper limits for them to stay alive. Within the species tolerable temperature range, growth rates increase as the water temperature increases, until the optimum temperature is reached. In addition, at higher than optimum temperatures, the fish food conversion ratios are lower. Optimum temperature range for tilapia should contain from 28-32°C (Aston,1981). There are many factors influencing water temperature. But there are other influences, as well such as inflows and outflows and colour of the water. Water temperature change slowly compare to air temperature due to the energy from the sun. moreover, the aquatic environment is a stable place to live for many organisms.

2.3.3. pH

The pH value expresses the intensity of the acidic or basic characteristics of water. The optimum pH for the growth and health of most freshwater aquatic animals is in the range of 6.5 to 9.0. Exposure to extreme pH can be stressful or lethal, but it is indirect effects resulting from the interactions of pH with other variables that are more important in aquaculture (Timmons and Ebeling, 2010). The nitrifying process produces acid (H+) and the pH level falls. To stabilize the pH, a base must be added. For this purpose, lime or sodium hydroxide (NaOH) or another base needs to be added to the water. Sodium hydroxide is a strong alkaline that can burn eyes and skin heavily. Safety precautions must be taken and when handling this and other heavy acids and bases, glasses and gloves must be worn.

2.3.4. Pathogens

Continuous disinfection of water in a recirculating tank system can help to limit disease spread and the production of pathogenic bacteria. Ultraviolet (UV) light and ozonation are two methods of continuous disinfection used in RAS. UV light is electromagnetic radiation with a wavelength of 1-400 nanometre located at the lower end of the visible spectrum and beyond (Figure 8). UV disinfection works by applying light in wavelengths that destroy DNA in biological organisms

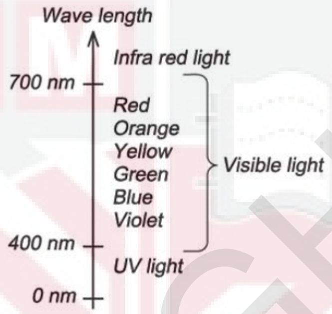


Figure 8: Different wavelength of light

Source: (Lekang, 2007)

The use of ozone (O_3) in fish farming has been criticised because the effect of overdosing can cause severe injury to the fish and people working in farms inside the building. So, correct loading dosage and monitoring along with proper ventilation are indeed crucial to achieving a positive and safe result. Ozone treatment by heavy oxidation of organic matter to biological organisms is an efficient way to destroy unwanted organisms. Microparticles are broken into molecular structures in ozone treatment systems that will bind together again and form larger particles.

2.4. Azolla pinnata

Azolla pinnata is a species of fern that is floating in the water. *Azolla* was recommended by FAO (2009) as feed in small-scale aquaculture and had been used as a main component in food for Tilapia (Fiogbé et al. 2014). The blue-green algae grow in symbiotic association with this fern can fix atmospheric nitrogen, carry out photosynthesis and uptake nutrients from its surrounding environment through its root system. When it comes to nutrient value, *A. pinnata* possesses high protein content, amino acids, vitamins and minerals like phosphorus. It is easily grown in a wild environment and even can be grown under a controlled environment like a greenhouse.

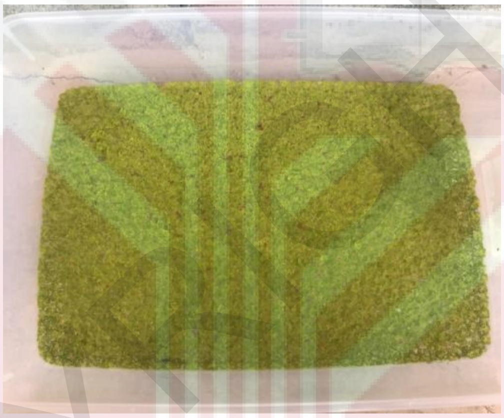


Figure 9: Azolla pinnata

2.4.1. Function of *A. pinnata* in RAS

Azolla pinnata is known as “green gold mine” as it has many utilizations, for instance, water purifiers and a nutritional supplement for livestock (Roy, 2016). Uptake of ammonia by *A. pinnata* can purify the water in RAS. Accumulation of nitrate with high concentration from the ammonia can be toxic to fish and affect their growth. Therefore, *A. pinnata* will be used instead of discharging large of high nitrate water to the environment.

CHAPTER 3 MATERIAL & METHODS

3.1. Evaluation of the ammonia removal by *Azolla pinnata*.

Azolla pinnata will be tested for its effectiveness in removing NH_3-N in water. For this experiment, *A. pinnata* will be cultured in a plastic basin (34.5 cm x 29 cm) using pond water. The pond water was collected from the pond of faculty of engineering. Five litres of pond water were filled up in the plastic basin, which resulted 5.0 cm depth of water for the culture of *A. pinnata.* This experiment was conducted for two weeks and due to Recovery Movement Control Order (RMCO) data could not be retrieved during weekend. The experiment had two treatments i.e., ammonia concentration (pond water +ammonia) which is 10 mg/L and 2g of NPK fertilizer as control treatment (pond water + NPK) which was equal to 40 mg/L as shown in Figure 11. The amount of 2 g of NPK in 5 litres of water used in the experiment was based on the commercial application of hydroponic system (Quarters, 2017). Each treatment had three replicates. In each plastic basin, 10 g of *A. pinnata* and 5 litre pond water were added. The pond water was sterilized using UV light for an hour. This is a disinfection method that uses 254 nm wavelength ultraviolet to kill bacteria or microorganisms (as indicated by manufacturer). The ammonia removal by *A. pinnata* and water quality parameter such as dissolved oxygen, temperature and pH were monitored during the experiment. The growth of *A. pinnata* were monitored during this experiment through determination of net wet weight. The growth was calculated by taking the value of wet weight during harvest (final weight) minus the initial wet weight which was 10g. When the concentration of ammonia in the culture water was approximately 3.0mg/L, the concentration was increased to 10mg/L to avoid the concentration of ammonia reaching zero during weekend.

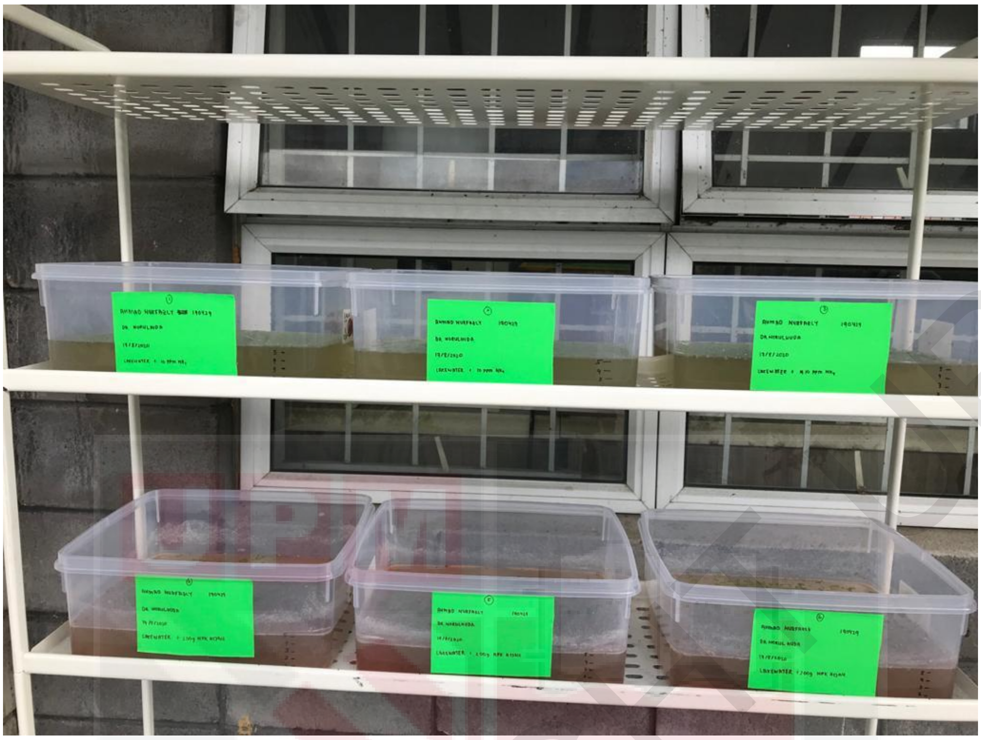


Figure 10: Top level for ammonia treatment while bottom level for fertilizer treatment

3.1.1. Parameters to Monitor

3.1.1.1. Ammonia Measurement

Salicylate method using HACH Spectrophotometer DR 4000 which is method 10031 to measure ammonia nitrogen (NH_3-N) concentration. In this test reagents that are used consist sodium nitroferricyanide. Firstly, choose program 343N, ammonia HR TNT and push START to start the program. Then prepare the blank and the sample. For the blank, add 0.1ml of distilled water to one reagent test tube for High Range Ammonia Nitrogen. For the sample add 0.1ml of treatment sample to another reagent test tube for High Range Ammonia Nitrogen. Next, add one sachet of Ammonia Salicylate Reagent Powder Pillow and Ammonia

Cyanurate Reagent Powder Pillow in each vial. After that shake thoroughly the vials to dissolve the powder. The reaction of the solution was set for in 20 minutes. The blank vial must be cleaned using tissue paper before it was inserted it into 16 mm cell holder for reading. Push ZERO button and it will show 0.0 mg/L NH_3-N. For the sample also clean the vial first before inserting the sample vial into 16mm cell holder. Push READ and it will show in the ammonia concentration in mg/L NH_3-N.

Figure 11: HACH Spectrophotometer DR 4000

3.1.1.2. Dissolved Oxygen, pH and Temperature Measurement

Parameter of water quality such as dissolved oxygen, temperature and pH were measured using YSI Multiparameter. When using the YSI Multiparameter, it has to be calibrated properly to get accurate readings. Otherwise, the measurements will be meaningless, or worse, inaccurate readings can lead to wrong conclusions.

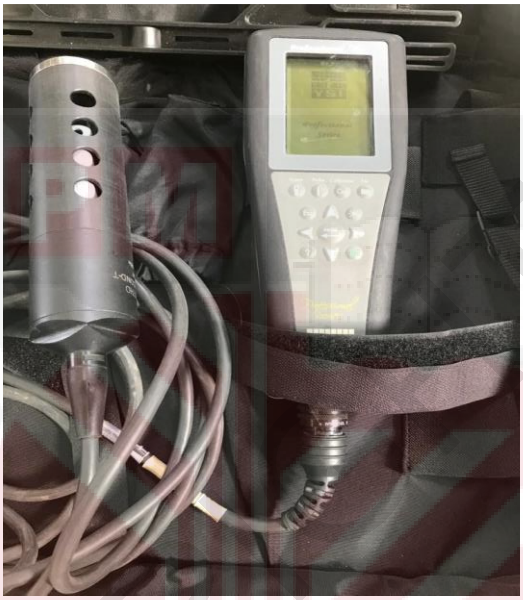


Figure 12: YSI Multiparameter

3.2. Design of RAS using tanks for removal of ammonia in the RAS

3.2.1. Site location

Study on RAS system was conducted Aquaculture Research Station UPM, Puchong. It is located between latitude 2°59’22” N and between longitude 101°38’48” E.

Figure 13: Site location for RAS system 2°59’22” N 101°38’48” E

3.2.2. To propose the design of RAS

The proposed design of RAS was based on the schematic diagram shown in Figure 14. Based on the functions of all tanks, the RAS was built based on available tanks in the hatchery. The functions of fish tank, biofilter tank, *Azolla pinnata* culture tank and reservoir or sump can be referred to Chapter 2 (Literature Review). From the resources available at the hatchery, tanks which were suitable for each function were selected.

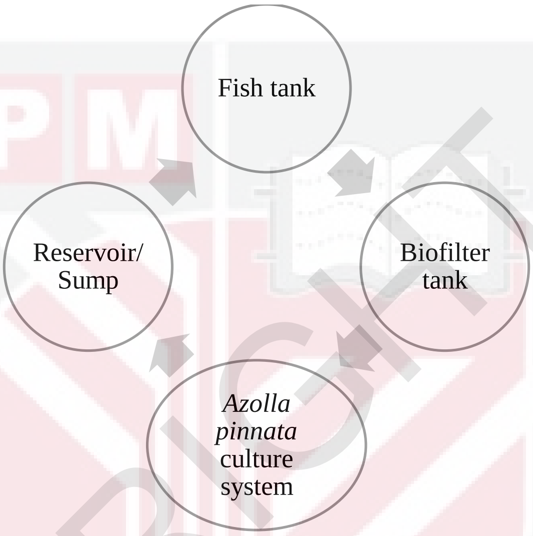
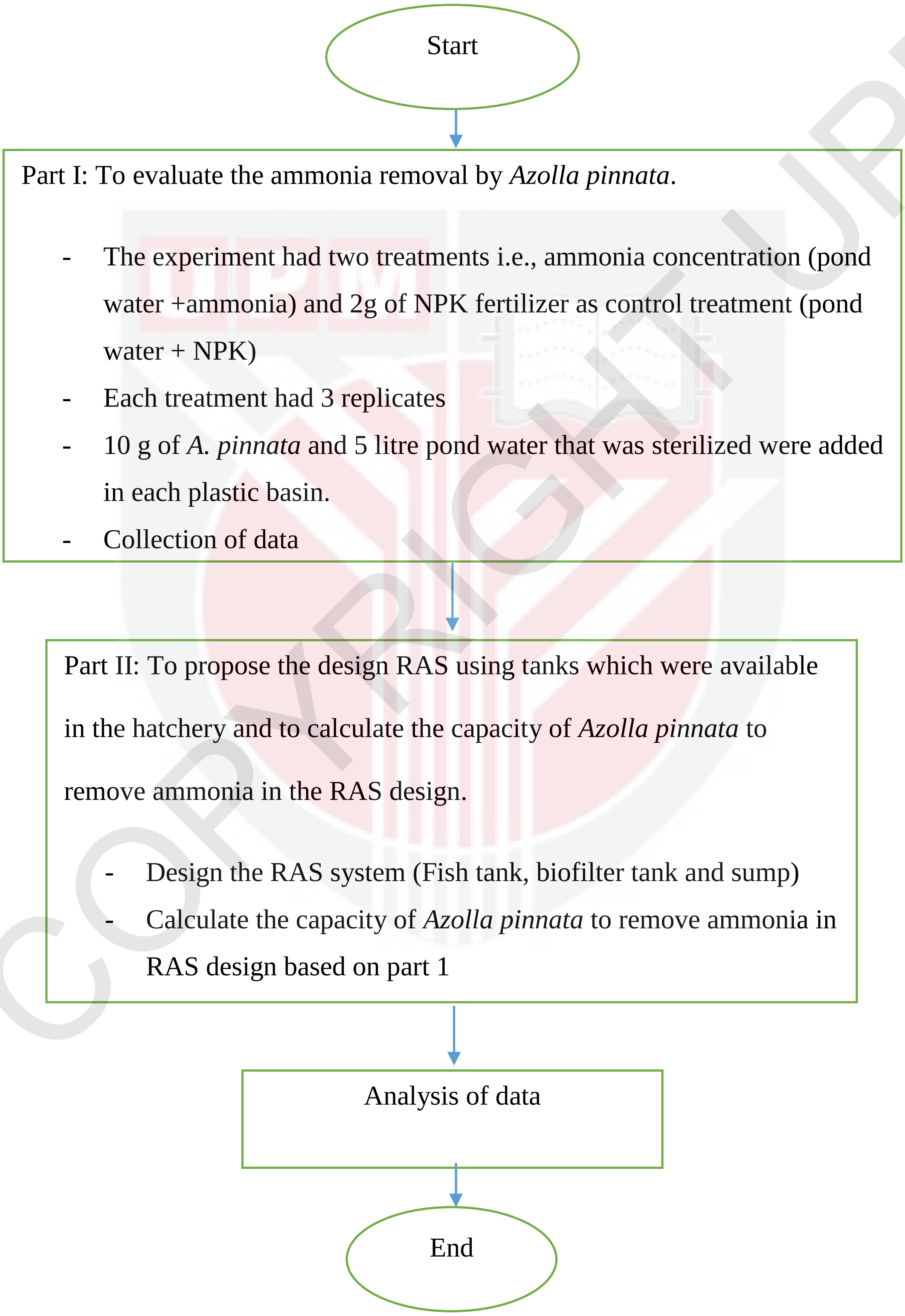


Figure 14: The propose schematic diagram of of RAS system with the integration of Azolla pinnata.

3.3. Flowchart of Methodology

The flowchart shows the flow of the study from the beginning to the end. The flowchart is constructed to achieve the result of the study.



Start

Part I: To evaluate the ammonia removal by *Azolla pinnata*.

- The experiment had two treatments i.e., ammonia concentration (pond water +ammonia) and 2g of NPK fertilizer as control treatment (pond water + NPK)
- Each treatment had 3 replicates
- 10 g of *A. pinnata* and 5 litre pond water that was sterilized were added in each plastic basin.
- Collection of data

Part II: To propose the design RAS using tanks which were available in the hatchery and to calculate the capacity of *Azolla pinnata* to remove ammonia in the RAS design.

- Design the RAS system (Fish tank, biofilter tank and sump)
- Calculate the capacity of *Azolla pinnata* to remove ammonia in RAS design based on part 1

Analysis of data

End

3.4. Statistical Analysis

The judgement on the two treatments are subjected to statistical analysis T-test, mean comparison and SPSS was the software used to determine the significance of mean value for ammonia removal, wet weight and parameter of water quality such as dissolved oxygen concentration, temperature and pH.

CHAPTER 4 RESULTS AND DISCUSSION

4.1. Evaluating the ammonia removal by *Azolla pinnata*.

4.1.1. Ammonia removal

Results of total ammonia removal are shown in Figure 15 and Table 1. *A. pinnata* was used to assimilate ammonia in each treatment. Ammonia removal was significantly higher (P-value < 0.05, t-test analysis) in treatment 1 (pond water + ammonia) than treatment 2 (pond water + NPK). The cumulative ammonia removal at the end of the experiment was 8.54 mg/L/12 day for treatment 1 (pond water + ammonia) and 2.93 mg/L/12 day for treatment 2 (pond water + NPK). This is equal to 0.71 mg/L/day for treatment 1, and 0.24 mg/L/day for treatment 2. Percentage of ammonia removal per day for treatment 1 was higher than treatment 2 which indicated that *A. pinnata* was more suitable in treatment 1. Detail of statistical analysis can be referred to (Appendix I). When the concentration of ammonia decreased to approximately 3.0mg/L on day 4, the concentration was increased to 10mg/L to avoid the concentration of ammonia reaching zero during weekend, where the measurement could not be done to MCO.

Removal of total ammonia nitrogen (TAN) (which is the sum of the two unionized NH_3 and ionized NH_4^+) is one of the most important processes in a RAS because ammonia highly toxic to fish. Ammonia appears to have a direct effect on the growth of aquatic animals. Ammonia removal by *A. pinnata* to improve RAS stability as well as act as a water purifier.

Table 1: Average ± standard deviation of ammonia removal per day(mg/L/day), cumulative ammonia removal per day(mg/L/day) and percentage of ammonia removal per day(%). Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK) which was control treatment.

Day	Treatment 1 (pond water + ammonia)			Treatment 2 (pond water + NPK)		
	Ammonia removal per day(average ± standard deviation)	Cumulative ammonia removal per day (mg/L/day)	Percentage of ammonia removal per day (%)	Ammonia removal per day (average ± standard deviation)	Cumulative ammonia removal per day (mg/L/day)	Percentage of ammonia removal per day (%)
0	0	0	0	0	0	0
1	1.77±0.06	1.77	18.4	0.73±0.49	0.73	1.66
2	2.34±0.40	4.11	24	0.47±0.21	1.20	1.07
4	2±0.2	6.11	20	0.77±0.15	1.97	1.75
8	1.1±0.17	7.21	11.7	0.23±0.06	2.20	0.52
10	0.8±0.17	8.01	8.5	0.4±0.10	2.60	0.91
12	0.53±0.15	8.54	5.7	0.33±0.12	2.93	0.75

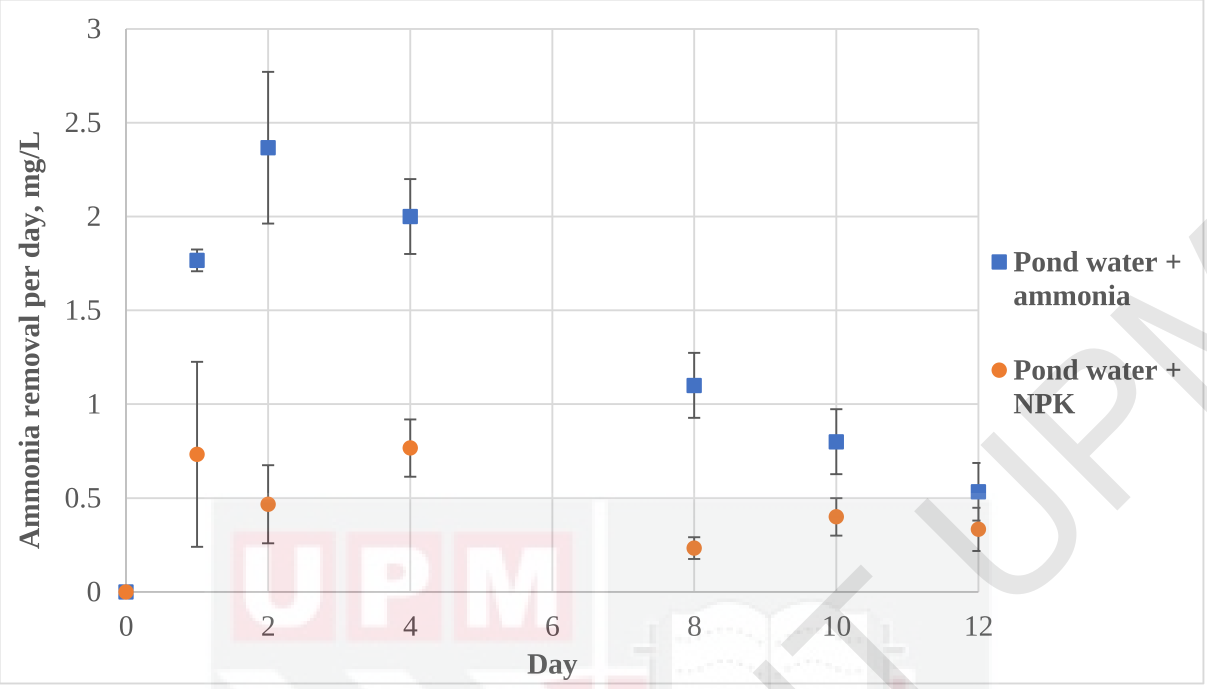


Figure 15:Ammonia removal(mg/L) by Azolla pinnata in two treatments. Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK) which was control treatment.

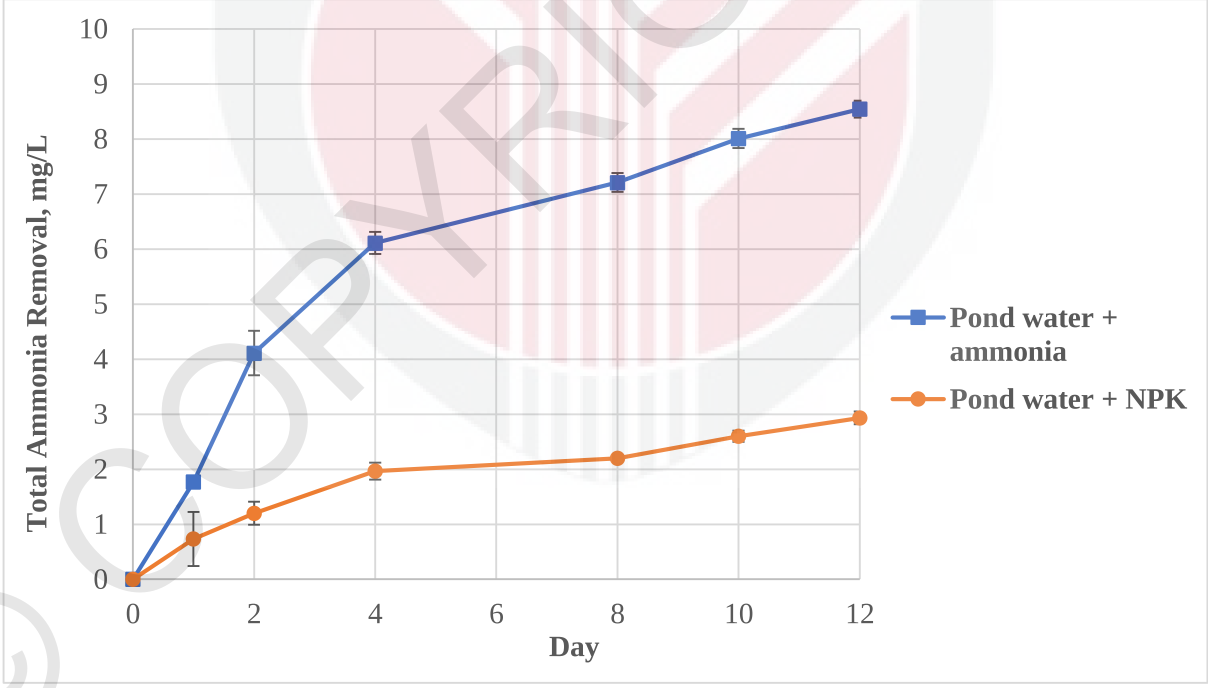


Figure 16: Cumulative of ammonia removal(mg/L) by Azolla pinnata in two treatments. Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK) which was control treatment.

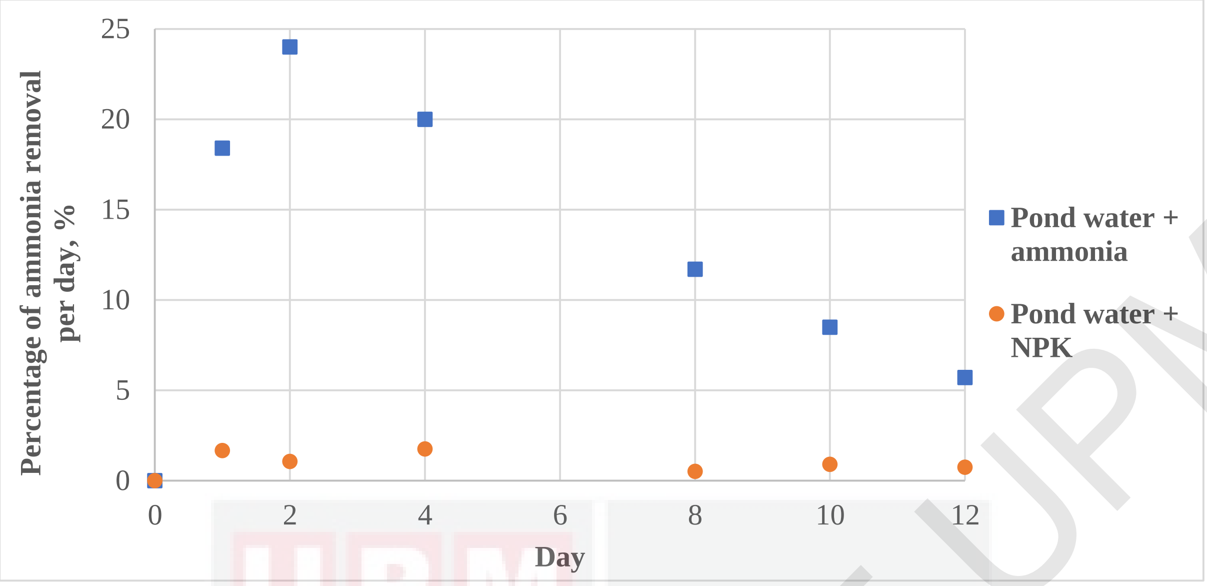


Figure 17: Percentage of ammonia removal(%) by Azolla pinnata in two treatments. Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK) which was control treatment.

4.1.2. Wet weight

The wet weight was calculated by subtracting the final wet weight with the initial wet weight. During experiment, *A. pinnata* was harvested two time which was on day 8 and day 12. The results of wet weight are shown in Figure 16 and Table 2. The cumulative of wet weight for treatment 1 is 27.5g while treatment 2 is 24.43g. Using the wet weight values, the growth of *A. pinnata* per day can be calculated and for treatment 1 the growth was 2.29 g *A. pinnata*/day while for treatment 2 was 2.03 g *A. pinnata*/day. An independent sample t-test was performed to see either the parameters were statistically significant. P-values of the test between two treatments for the growth was 0.953 which confirmed that it was not significant. Details can be referred to (Appendix II).

Table 2: Average ± standard deviation of wet weight(g) and cumulative wet weight(g). Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK which was control treatment.

Day	Treatment 1 (pond water + ammonia)		Treatment 2 (pond water + NPK)	
	Wet weight(average ± standard deviation)	Cumulative wet weight(g)	Wet weight(average ± standard deviation)	Cumulative wet weight(g)
0	10±0	10	10±0	10
8	15.27±0.38	25.27	13.33±0.51	23.33
12	2.23±0.21	27.5	1.1±0.3	24.43

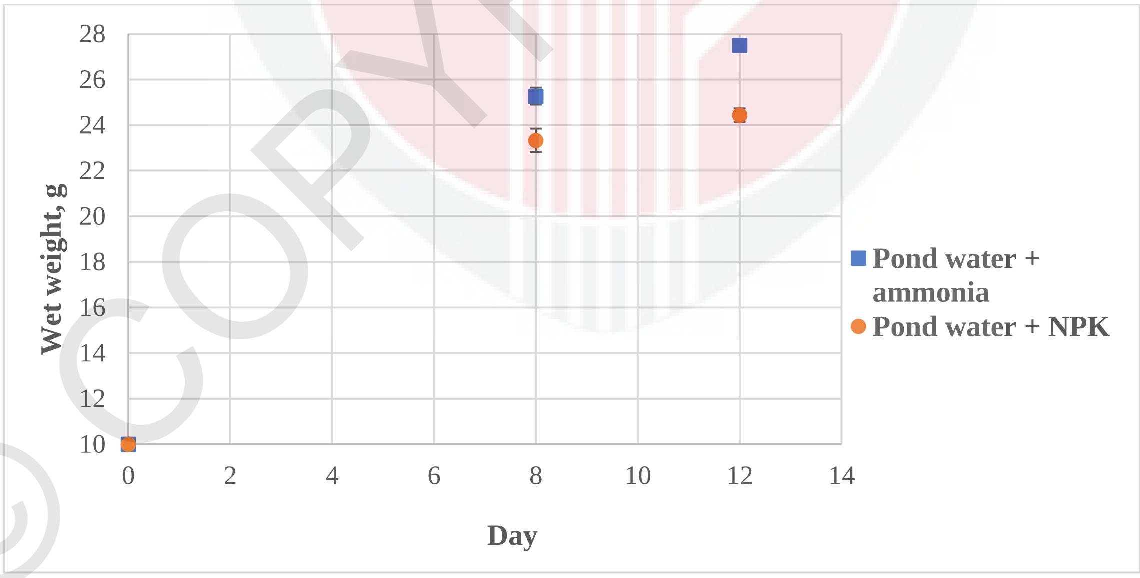


Figure 18: Cumulative uptake of wet weight(g) by Azolla pinnata in two treatments. Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK) which was control treatment.

4.1.3. Dissolved oxygen

Results of dissolved oxygen (DO) measurement are shown in Figure 17 and Table 3. In the treatment 1 (pond water + ammonia), average concentration dissolved oxygen ranged from 5.5 to 8.16 mg/L whereas the treatment 2 (pond water + NPK), dissolved oxygen ranged from 5.3 to 8 mg/L. An independent sample t-test was performed and the result showed that DO level in treatment 1 was significantly than treatment 2 (P-value < 0.05, details may be referred to Appendix III). This could be due to higher photosynthesis occurred in treatment 1, as demonstrated by higher growth rate of *A. pinnata* in treatment 1 than treatment 2. Higher dissolved oxygen concentrations are generally more desirable than lower concentrations for maintaining water quality to support aquatic organisms. Numerous scientific studies suggest that 4-5 mg/L of DO is the minimum amount that will support a large, diverse fish population. Adequate dissolved oxygen is necessary element to all forms of life. If dissolved oxygen levels in water drop below 5.0 mg/L, aquatic life will be under stress. Oxygen levels that remain below 1-2 mg/L for a few hours can result in large fish kills.

If the water is too warm, there may be not enough oxygen. When there are too much bacteria or aquatic animal in the area, they may overpopulate, using DO in great amounts. Quantifying of DO an aquatic organism need depends upon its species, water temperature and pollutants present.

Table 3: Average ± standard deviation of dissolved oxygen(mg/L) in the culture of Azolla pinnata. Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK)] which was control treatment.

Average ± standard deviation of dissolved oxygen level (mg/L)		
Day	Treatment 1 (pond water + ammonia)	Treatment 2 (pond water + NPK)
0	6.39±0.44	5.39±0.06
1	7.21±0.28	5.53±0.24
2	5.53±0.24	5.48±0.28
4	6.80±0.01	6.80±0.06
8	7.54±0.24	7.20±0.40
10	7.83±0.34	7.88±0.10
12	8.16±0.16	8.00±0.10

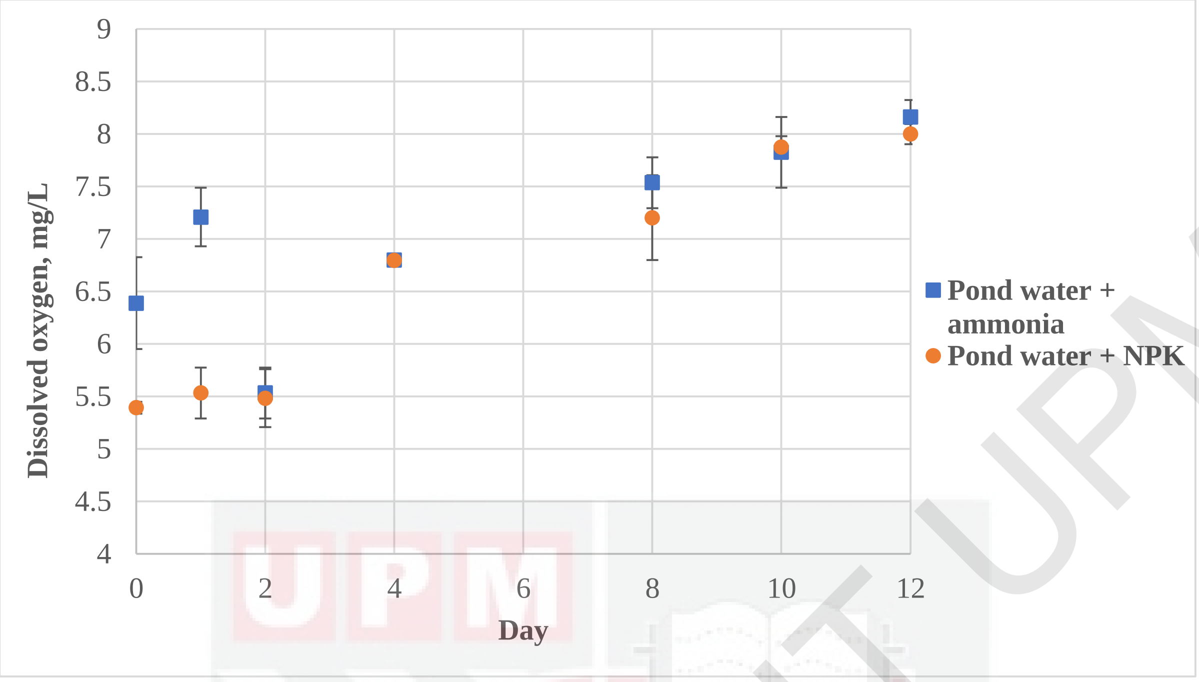


Figure 19: Dissolved oxygen(mg/L) in the culture of Azolla pinnata in two treatments. Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK) which was control treatment.

4.1.4. Temperature

Results for the temperature are shown in Figure 18 and Table 4. The ammonia treatment is recorded minimum values of 25.57°C and maximum 31.6°C. The temperature value for the second treatment is between 25.3°C to 30.9°C. There was not significant between the temperature for both treatments during the experiment because both treatments were placed at same place (t-test, P-value > 0.05, details may be referred to Appendix IV). The temperature at day 1 is the highest for both treatment as the weather is too hot at that time. The optimum temperature gives a good effect to survival of aquatic animals and plants.

Table 4: Average ± standard deviation of temperature(°C) in the culture of Azolla pinnata. Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK) which was control treatment

Average ± standard deviation of temperature(°C)		
Day	Treatment 1 (pond water + ammonia)	Treatment 2 (pond water + NPK)
0	30.33±0.31	29.47±0.40
1	31.6±0.20	30.9±0.30
2	31.17±0.31	30.47±0.32
4	27.83±0.32	27.9±0.17
8	28.07±0.15	27.97±0.21
10	25.57±0.29	25.73±0.06
12	25.7±0.26	25.3±0.17

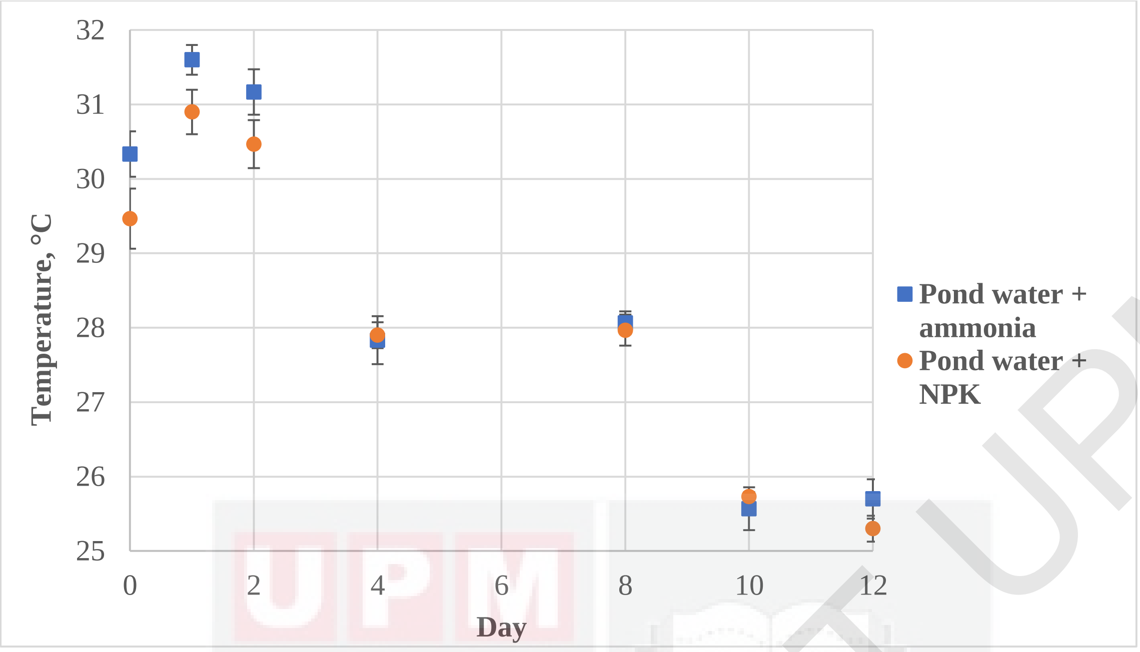


Figure 20: Temperature(°C) in the culture of Azolla pinnata in two treatments. Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK) which was control treatment.

4.1.5. pH

Hydrogen activity of water is measured by pH. Values for pH greater than 7.0 standard units are indicative of alkaline water and values less than 7.0 standard units are indicative of acidic water. Results of pH measurement are shown in Figure 19 and Table 5. In the treatment 1 (pond water + ammonia), average pH ranged from 7.01 to 8.55 whereas the treatment 2 (pond water + NPK), average pH ranged from 6.5 to 8.42. In day 4, average pH for both treatments was the highest which might be due to the highest photosynthesis as the result of the highest biomass of *A. pinnata* on that day when compared to other days of the experiment. Photosynthesis can drive pH to high levels. Peak pH levels normally coincide with peak oxygen levels during mid-afternoon when plant photosynthesis is at highest and similarly the lowest levels are typically recorded at night when photosynthesis is at its minimum and when plant respiration is high.

Low pH levels (below optimal) can result in fish kills and dead plants by stressing their systems causing physical damage, which in turn can make them more vulnerable to disease, similarly high pH particularly in combination with high water temperature, can increase the amount of unionized ammonia which is highly toxic for fish. A high or low pH can also adversely affect the availability of nutrients in the water.

An independent sample t-test was performed to see either the parameters are statistically significant. P-values was 0.933 which showed that the pH between the two treatments was not significant. Details may be referred to (Appendix V)

Table 5: Average ± standard deviation of pH in the culture of Azolla pinnata. Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK) which was control treatment

Average ± standard deviation of pH		
Day	Treatment 1 (pond water + ammonia)	Treatment 2 (pond water + NPK)
0	7.61±0.23	6.50±0.21
1	7.01±0.54	7.20±0.16
2	7.06±0.26	7.54±0.28
4	8.55±0.04	8.42±0.14
8	7.45±0.05	7.53±0.06
10	7.49±0.09	7.43±0.12
12	8.16±0.16	8.00±0.10

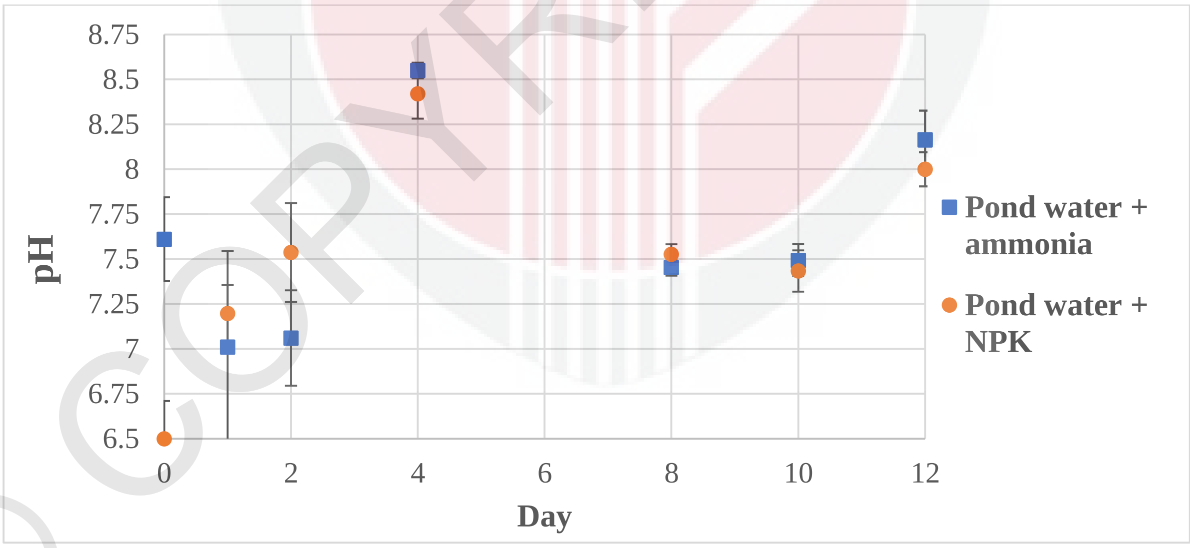


Figure 21: pH in the culture of Azolla pinnata in two treatments. Treatment 1 is pond water with added ammonia concentration (pond water + ammonia), treatment 2 is pond water with added NPK fertilizer (pond water + NPK) which was control treatment.

4.2. To propose the design of RAS using tanks which were available in the hatchery and to calculate the capacity of *Azolla pinnata* to remove ammonia in the RAS design, based on the result achieved in Objective 1.

Based on the tanks available in the hatchery, these tanks were selected. Two trials were conducted for the design. In the first trial, four components were selected which were fish tank, solid removal tank, biofilter tank, and sump. The design is shown as in Figure 20. The RAS was undergone a trial to ensure the water flow was stable and no overflowed occurred. For this design the weakness was identified on the solid removal tank selected. It turned out that the tank was not suitable because the tank was meant for culturing artemia (baby shrimp), and no modification was allowed by the hatchery manager. Therefore, the pipe connection which was made at the bottom of the solid removal tank didn't meet the purpose of the solid removal function. Therefore, this design was modified to the second design.

Figure 22: First design of recirculating aquaculture system(RAS)

In the second design, the solids removal tank was omitted, which left the RAS with only three components, fish tank, biofilter tank, and sump. The *A. pinnata* would be cultured in the sump to optimize the function of sump, and to reduce the use of tank and water which would save the cost of building the RAS in future. Each of the component is shown in Figure 21 (fish tank), Figure 22 (biofilter tank) and Figure 23 (sump). In Figure 24, the complete assemble of the RAS is shown. The real RAS was managed to be built, unfortunately, suddenly the Movement Control Order was given by the Malaysian government, and thus I wasn't able to continue the experiment in the hatchery.

During the operation of RAS, the flowrate for inlet and outlet in each tank was approximately 2L/min. The flow rate must be equal for inlet and outlet to prevent water overflows. For the small scale of RAS, this flowrate is considered enough as to provide sufficient oxygen for the fish. In addition, a suitable hydraulic retention time (HRT) is important: for the fish tank, it is important to ensure the wastes is transported to the other tank as fast as possible, in biofiltration tank, the HRT must allow the nitrification bacteria to grow and not be sloughed away by too quick HRT, thus the nitrification process to perform efficiently, for the sump, to allow sufficient time for *A. pinnata* to absorb ammonia. If the HRT is too short, *A. pinnata* cannot get enough time to complete ammonia removal while if too long *A. pinnata* will be starved.

The HRT in each tank depends on the volume of tank and the flowrate of water. As the flowrate for each tank is the same, so HRT depends only on the volume of the tank. The bigger the tank, the more time for the HRT. The HRT for fish tank was 175 minutes while sump require 160 min HRT and lastly biofilter is the smallest tank, require 30 minutes for HRT. Details may be referred to Appendix VI.

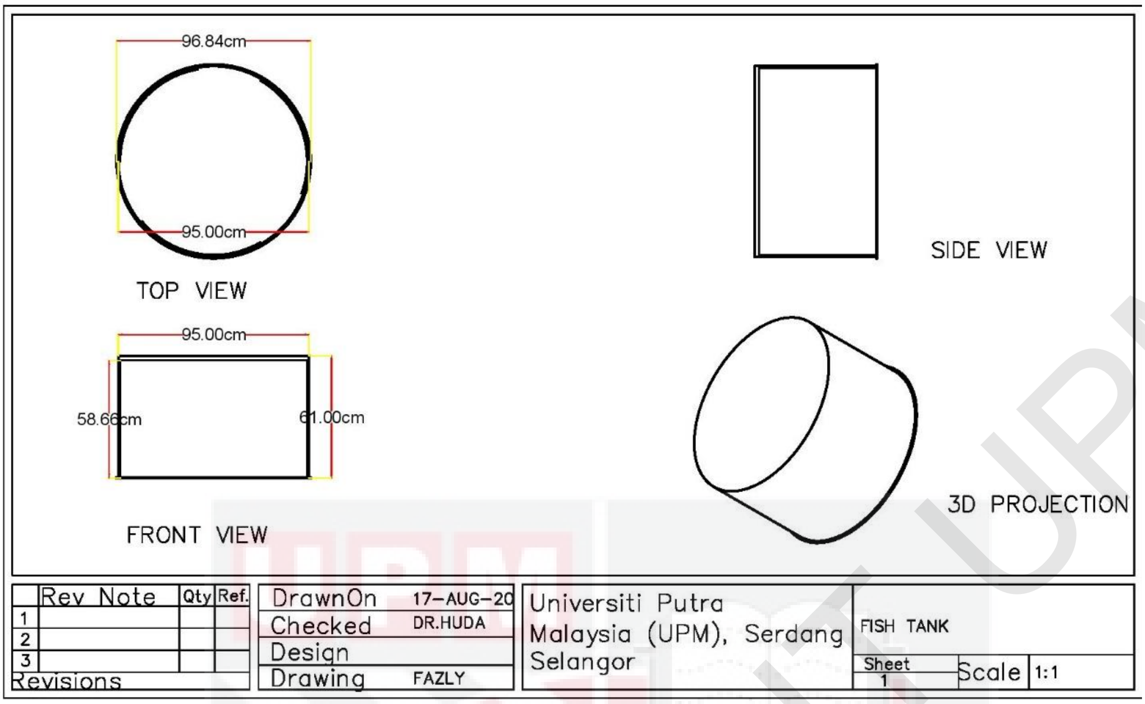


Figure 23: 350L of Fish Tank

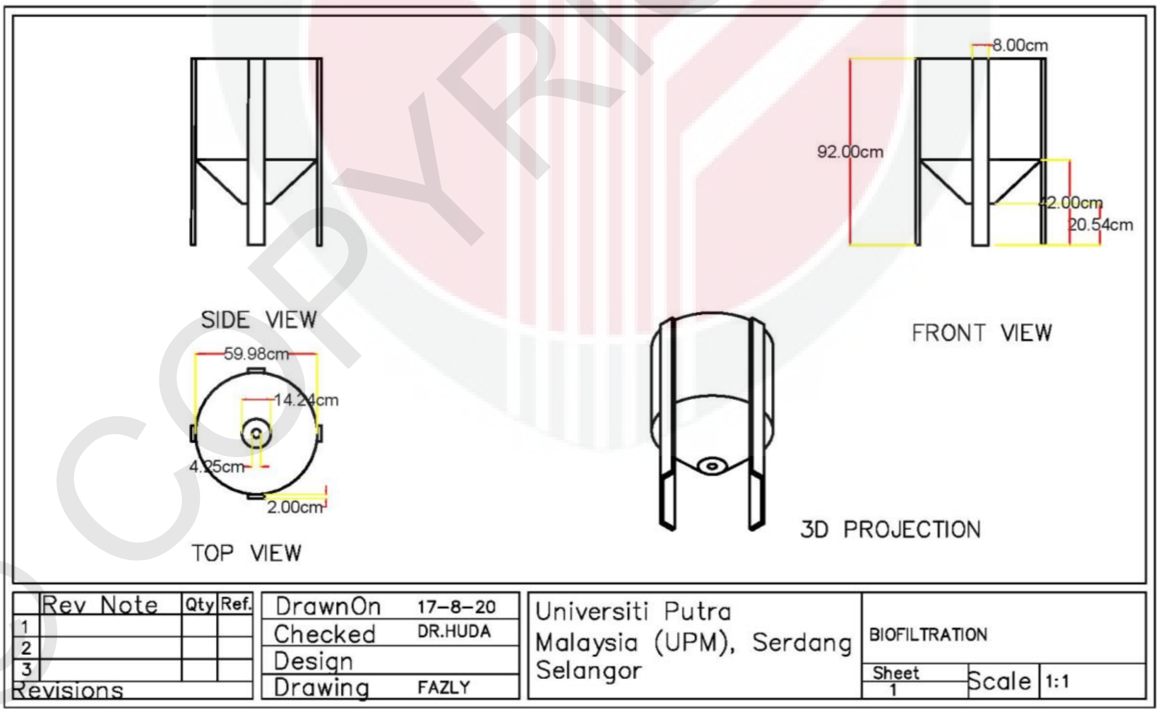


Figure 24: 60L of biofilter tank

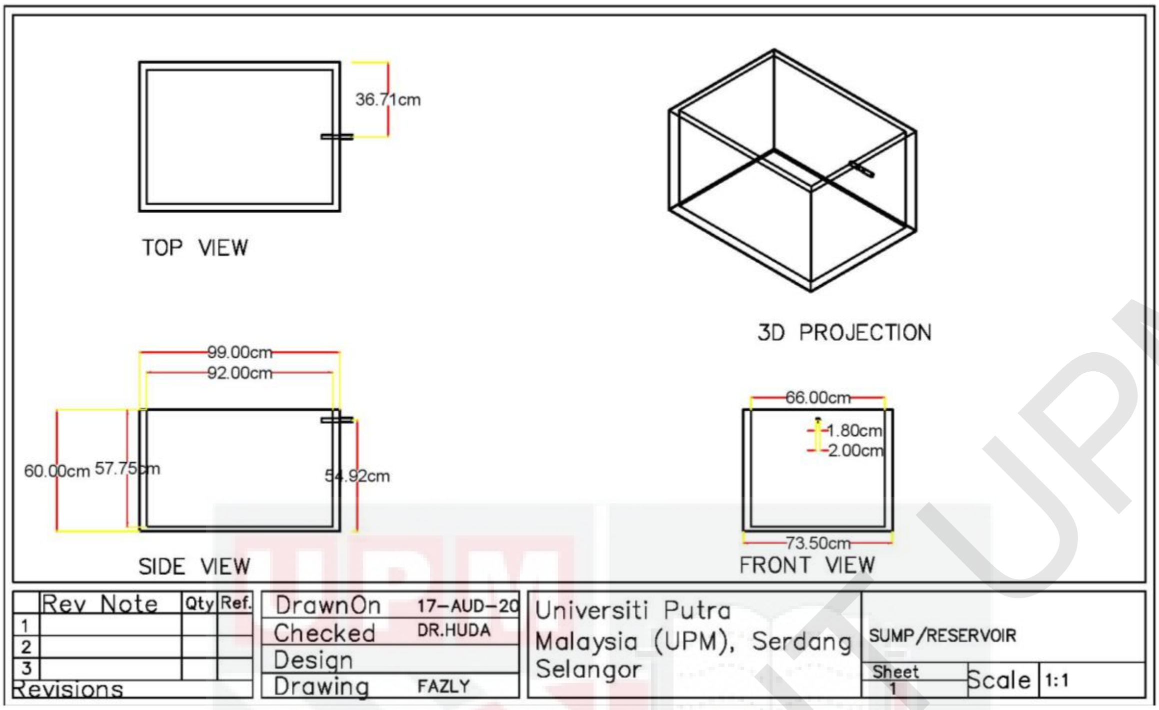


Figure 25: 320L of sump

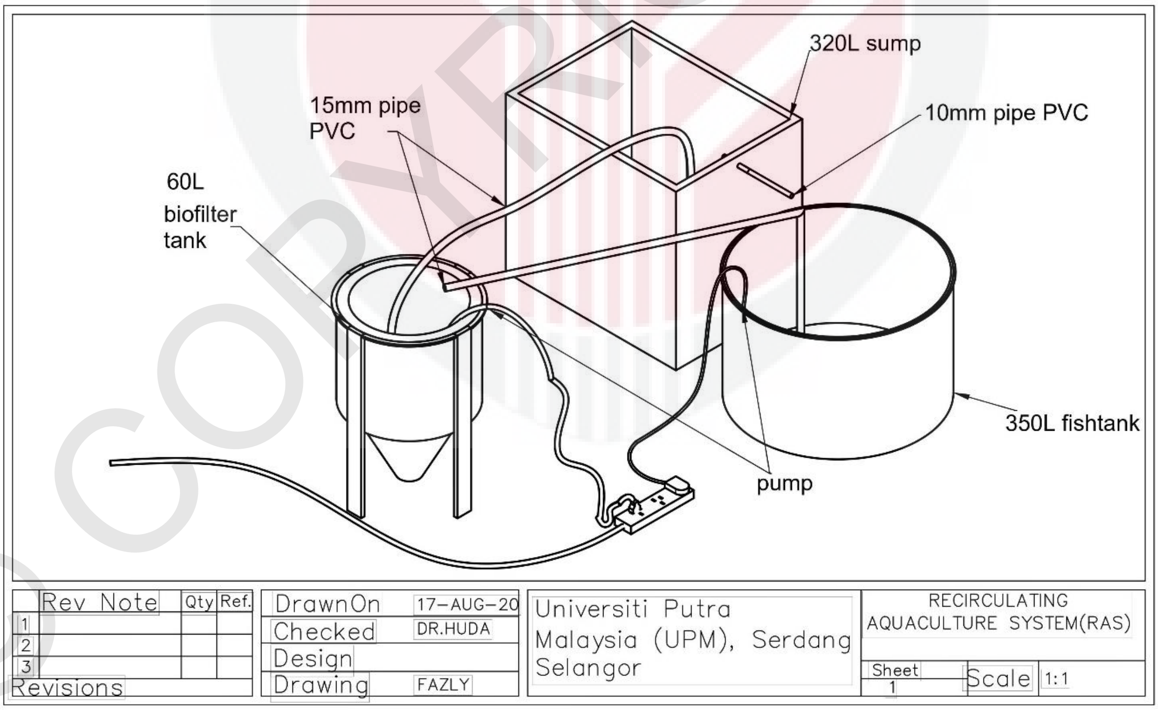


Figure 26: Design of Recirculating Aquaculture System

4.2.1. Determining the capacity of *Azolla pinnata* to remove ammonia in the RAS design

From the RAS built for this study as shown in previous section, the stocking density that is suitable for the RAS is 10 kg/m^3. This value is based on commercial practice of RAS. Given the fish stocking density mentioned, the amount of feed per day for the fish is 2% of the biomass, which means 200g feed per day feed design. According to (Ebeling et al., 2006), if 1 kg of feed that consists of 32% crude protein in a 1m^3 RAS, 30g will be produced in the system. Then, the ammonia concentration in water will be 30mg/L.

Based on the study in Part 1, to evaluate the ammonia removal by *A. pinnata*, the rate of ammonia removal is 0.71 mg of ammonia/ g *A. pinnata*/ day. From this information, it is estimated that 252.7 g *A. pinnata* will cover 1m^2 area. Therefore, since the sump will be used to culture *A. pinnata*, 153 g of *A. pinnata* will occupy on the surface water in sump. As a result, from the 153 g of *A. pinnata* that will be able to grow in the sump, the *A. pinnata* will be able to remove the ammonia in RAS at the rate of 47.43 mg/L of ammonia/day. The result explained that the removal of ammonia will be excellently done by the *A. pinnata* since it could remove more than the amount of ammonia produced by fish. Details may refer to (Appendix VII)

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

An experiment was conducted to evaluate ammonia removal by *A. pinnata* between two treatments; pond water + ammonia and pond water + NPK fertilizer. The study had demonstrated that the amount of ammonia removal by the *A. pinnata* under pond water with added ammonia concentration (pond water + ammonia) was significantly higher than pond water with added NPK fertilizer (pond water + NPK). The uptake rate of ammonia was found at 0.31 mg per g of *A. pinnata* per day for treatment pond water + ammonia. Integration of *A. pinnata* culture system in RAS was to improve the stability of system and to calculate the capacity of *Azolla pinnata* to remove the ammonia. From the proposed design of RAS, the total surface area of the sump is expected to be covered by the *A. pinnata*. With the covered surface area by the *A. pinnata*, ammonia produced in the RAS will be able to be removed by the *A. pinnata* of at hydraulic retention time of 160 min. The ratio of the fish tank and the sump tank is 1:1 as both tanks have the same surface area which is 0.6 m^2. The study has shown that *A. pinnata* could be used as water purifier i.e., to remove the ammonia released by fish in aquaculture system.

5.2. RECOMMENDATIONS

Some recommendations as follows:

1. In future, an *A. pinnata* culture system can be designed for the removal of not only ammonia, but also nitrate and ammonia in aquaculture wastewater.
2. The experiment should run for a long period where the effect of *A. pinnata* to the removal of ammonia can be known accurately.

APPENDINCES

Appendix I: Independent Samples t-test of Ammonia (mg/L)

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
NH3	Equal variances assumed	23.101	.000	5.237	34	.000	.93889	.17927	.57457	1.30320
	Equal variances not assumed			5.237	22.396	.000	.93889	.17927	.56749	1.31029

Appendix II: Independent Samples t-test of Wet weight (g)

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
wet_weight	Equal variances assumed	.004	.953	.388	16	.703	1.02222	2.63255	-4.55853	6.60297
	Equal variances not assumed			.388	15.980	.703	1.02222	2.63255	-4.55910	6.60354

Appendix III: Independent Samples t-test of Dissolved Oxygen (mg/L)

<table>
<tr><th colspan="11">Independent Samples Test</th></tr>
<tr><th colspan="2" rowspan="3"></th><th colspan="2">Levene's Test for Equality of Variances</th><th colspan="7">t-test for Equality of Means</th></tr>
<tr><th rowspan="2">F</th><th rowspan="2">Sig.</th><th rowspan="2">t</th><th rowspan="2">df</th><th rowspan="2">Sig. (2-tailed)</th><th rowspan="2">Mean Difference</th><th rowspan="2">Std. Error Difference</th><th colspan="2">95% Confidence Interval of the Difference</th></tr>
<tr><th>Lower</th><th>Upper</th></tr>
<tr><td rowspan="2">DO</td><td>Equal variances assumed</td><td>34.859</td><td>.000</td><td>2.729</td><td>10</td><td>.021</td><td>.96333</td><td>.35303</td><td>.17673</td><td>1.74994</td></tr>
<tr><td>Equal variances not assumed</td><td></td><td></td><td>2.729</td><td>5.781</td><td>.036</td><td>.96333</td><td>.35303</td><td>.09148</td><td>1.83518</td></tr>
</table>

Appendix IV: Independent Samples t-test of Temperature (°C)

<table>
<tr><th colspan="11">Independent Samples Test</th></tr>
<tr><th colspan="2" rowspan="3"></th><th colspan="2">Levene's Test for Equality of Variances</th><th colspan="7">t-test for Equality of Means</th></tr>
<tr><th rowspan="2">F</th><th rowspan="2">Sig.</th><th rowspan="2">t</th><th rowspan="2">df</th><th rowspan="2">Sig. (2-tailed)</th><th rowspan="2">Mean Difference</th><th rowspan="2">Std. Error Difference</th><th colspan="2">95% Confidence Interval of the Difference</th></tr>
<tr><th>Lower</th><th>Upper</th></tr>
<tr><td rowspan="2">temp</td><td>Equal variances assumed</td><td>.000</td><td>1.000</td><td>1.629</td><td>10</td><td>.134</td><td>.4333</td><td>.26604</td><td>-.15944</td><td>1.02611</td></tr>
<tr><td>Equal variances not assumed</td><td></td><td></td><td>1.629</td><td>9.961</td><td>.135</td><td>.4333</td><td>.26604</td><td>-.15976</td><td>1.02643</td></tr>
</table>

Appendix V: Independent Samples t-test of pH

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
pH	Equal variances assumed	.007	.933	-.934	10	.372	-.19500	.20876	-.66015	.27015
	Equal variances not assumed			-.934	9.989	.372	-.19500	.20876	-.66021	.27021

Appendix VI

$$HRT = Volume\ of\ tank \div flowrate\ through\ the\ tank$$

$$HRT\ Fish = 350L \div 2L/\text{min} = 175\ min$$

$$HRT\ Biofilter = 320L \div 2L/\text{min} = 160\ min$$

$$HRT\ Sump = 60L \div 2L/\text{min} = 30\ min$$

Appendix VII

$$\frac{mg/L\ ammonia}{g\ Azolla}\Big/_{day} = \frac{0.71mg/L}{2.29g}\Big/_{day} = 0.31\ \frac{mg/L\ ammonia}{g\ Azolla}\Big/_{day}$$

$$surface\ area\ for\ basin, m^2\ = length\ of\ basin\ \times width\ of\ basin$$

$$= 0.345m\ \times 0.29m\ = 0.1m^2$$

$$surface\ area\ for\ sump, m^2\ = length\ of\ sump\ \times width\ of\ sump$$

$$= 0.92m\ \times 0.66m\ = 0.607m^2$$

$$average\ wet\ weight\ of\ A. Pinnata\ that\ fulfill\ surface\ area\ of\ basin, g$$

$$= (25.0 + 25.7 + 25.1)g \div 3 = 25.27g$$

$$average\ wet\ weight\ A. Pinnata\ per\ m^2, \frac{g}{m^2} = 25.27g\ \div 0.1m^2 = 252.7\frac{g}{m^2}$$

$$average\ wet\ weight\ can\ fulfill\ surface\ area\ of\ sump$$

$$=\ average\ wet\ weight\ A. Pinnata\ per\ m^2 \times surface\ area\ for\ sump$$

$$= 252.7\frac{g}{m^2} \times 0.607\ m^2 = 153g$$

$$\frac{g\ ammonia}{day} = \frac{mg/L\ ammonia}{g\ Azolla}\Big/_{day} \times g\ azolla$$

$$= 0.31\ \frac{mg/L\ ammonia}{g\ Azolla}\Big/_{day} \times 153g\ = 47.43\ \frac{mg/L\ ammonia}{day}$$

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