COST EFFECTIVENESS OF LOW-DENSITY POLYETHYLENE PELLETS AS BIOFILTRATION MEDIA IN A RECIRCULATING AQUACULTURE SYSTEM

Sushan Mani Shakya

T.U. Regd. No: 5-2-33-321-2009

T.U. Examination Roll No: Zoo 457/073

Batch: 2073/74

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with special paper Fish biology and Aquaculture

Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

22 February 2022

TRIBHUVAN UNIVERSITY
01-433 1896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.

Ref.No.:

RECOMMENDATIONS

This is to recommend that the thesis entitled **"COST EFFECTIVENESS OF LOW-DENSITY POLYETHYLENE PELLETS AS BIOFILTRATION MEDIA IN A RECIRCULATING AQUACULTURE SYSTEM"** has been carried out by Sushan Mani Shakya for the partial fulfillment of Master's Degree of Science in Zoology with special paper Fish and Fisheries. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

.....

Supervisor

Mrs. Santoshi Shrestha

Lecturer

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

Date... 2078/11/13.....

TRIBHUVAN UNIVERSITY
01-433 1896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.

Ref.No.:

LETTER OF APPROVAL

On the recommendation of supervisor Mrs. Santoshi Shrestha, this thesis submitted by Sushan Mani Shakya entitled **"COST EFFECTIVENESS OF LOW-DENSITY POLYETHYLENE PELLETS AS BIOFILTRATION MEDIA IN A RECIRCULATING AQUACULTURE SYSTEM"** is approved for the examination in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Fish biology and Aquaculture.

.....

Prof. Dr. Tej B. Thapa

Head of Department

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

Date..~~2078/12/06~~.....
2078/11/15

TRIBHUVAN UNIVERSITY
01-433 1896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.

Ref.No.:

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Sushan Mani Shakya entitled **"COST EFFECTIVENESS OF LOW-DENSITY POLYETHYLENE PELLETS AS BIOFILTRATION MEDIA IN A RECIRCULATING AQUACULTURE SYSTEM"** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Fish Biology and Aquaculture.

Supervisor	**Head of Department**
Mrs. Santoshi Shrestha	**Dr. Tej Bahadur Thapa**
Lecturer	Professor
Central Department of Zoology	Central Department of Zoology
Tribhuvan University	Tribhuvan University

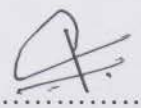
External examiner

Internal Examiner

Date of Examination: 2078/12/06

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).



.....

Sushan Mani Shakya

Date..2078/11/15.....

ACKNOWLEDGEMENT

The Master of Zoology program I had in the Central Department of Zoology, Tribhuvan University was a great chance to learn and develop professional skills. Therefore, I consider myself as a very lucky person as I was provided with an opportunity to be a part of it.

First of all, I would like to express my deepest gratitude to Mrs. Santoshi Shrestha, Central Department of Zoology, Tribhuvan University, for her supervision, guidance and encouragement to carry out this dissertation work.

I would like to acknowledge my sincere gratitude to Prof. Dr. Tej Bahadur Thapa, Head of the Central Department of Zoology for his provision of required departmental facilities to complete my experimental work.

I am also thankful to Prof. Dr. Kumar Sapkota, Central Department of Zoology for his valuable during the course of studying for this degree.

Furthermore, I would like to extend my thanks to my colleagues Genuine Prajapati and Niraj Khadka who helped me throughout my study period.

Last but not the least; I convey my sincere gratitude to every staff of Central Department of Zoology for their immense help and cooperation during study time.

ABSTRACT

This study was carried out at facilities of FreshAcres Agriventure (P) Ltd. in Hattiban, Lalitpur to assess the performance, cost per Kg of ammonia conversion and cost effectiveness of a locally available Low-Density Polyethylene (LDPE) pellets, as an alternative to K1 commercial biomedia in a domestically developed Recirculating Aquaculture system (RAS). Three fiber reenforced plastic tanks T1, T2 and T3 of 1.5m height and 3m diameter with viewing windows were built. Locally available 3000L water tank was used as biofilter chamber. Rotary drum filter with 100micron mesh screen was built for mechanical filtration of suspended solids. 600 common carp fingerlings were purchased from Centre for Aquaculture research and production in Kathar, Chitwan and transported to the study site in oxygenated packaging. Fingerlings were acclimatized, disinfected, quarantined and transferred to the three study tanks equally. Three trial sets TS1, TS 2 and TS3 were consecutively run for 6 months. The fish were fed at 3% body weight. The feeding stages were divided into S1, S2 and S3 according to their growth stages as fingerling, juvenile and adult respectively. Based on the biomass of the fish, feed rate and crude protein percentage of the feed, TAN was calculated for feeding stages S1, S2 and S3. Using this value, the theoretical required volume of LDPE pellet was calculated and used in the system. TAN level was every day and 1mg/L was taken as the base line level. Every time TAN increased by 0.1mg/L, one liter of LDPE pellets was added to the biofilter chamber until the level dropped down to base line level. Then the actual volume of the LDPE pellets required was used to calculate the cost per KG of TAN conversion into Nitrate which was found to be Rs.2208.77/KG. This number, although low compared to the cost per KG TAN conversion to nitrate using commercial biomedia K1, was still very high to be favorable for the Nepalese market. Nevertheless, this study will be a starting point for many more studies on RAS in Nepal. The fact that commercial biomedia is not available in Nepal and very expensive when it is available, makes this study valuable for the Nepalese aquaculture industry. This study also opens a pathway for modernization of the aquaculture industry in Nepal with domestically developed, low-cost technology.

TABLES OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAPHS

LIST OF ABBREVIATIONS

RAS	Recirculation Aquaculture System
MBBR	Moving Bed Bioreactor
LDPE	Low Density Polyethylene
TAN	Total Ammonia Nitrogen
SSA	Specific Surface Area
VTR	Volumetric TAN conversion Rate
FRP	Fiber Reenforced Plastic
TS	Trial Set
NH_3^-	Unionized Ammonia
NH_4^+	Ionized Ammonia
CO_2	Carbon dioxide
HCO_3^-	Bicarbonate
$Ca(OH)_2$	Calcium Hydroxide
No_3	Nitrate
No_2	Nitrite
FAO	Food and Agricultural Organization
HP	Horse Power
DC	Direct Current
RDF	Rotary Drum Filter
PVC	Polyvinyl Chloride
UV	Ultraviolet
CAARP	Centre for Aquaculture Research and Production

CHAPTER-ONE

1. INTRODUCTION

Recirculating aquaculture system (RAS) can be defined as a land based, closed and intensive system of fish farming that repeatedly reuses its water. The water is processed to remove the effluents to a safe level within this system. It uses different equipment to provide accurate control over the different physiochemical parameter of the system hence providing a superior environment for culturing fish at a relatively higher stocking density and higher feeding rate (Summerfelt et a1. 2000). Over the years, RAS has been proving itself in the field of aquaculture as a reliable method to produce large amounts of fish without needing to worry about the external environment and other constraints. RAS was first developed and used in Denmark in the 1980s. Since then, it has been heavily modernized and used all over the world to produce multiple type of sea food, including fresh and salt water fish, crabs, shrimps, prawn, shell fish and many more (Isabella and Hunt 2020).

In Nepal, RAS is close to nonexistent. Few have noncommercial operations and there is no government endorsement. In a country like Nepal, which imports most of the consumed fish from abroad, has little water in city areas and has a voracious appetite for fish, systems like RAS can prove beneficial by becoming a method that requires less land, less water, higher relative yield and low manpower requirements (Warren-Hansen, 2015).

1.1 Working principle of RAS

A basic RAS consists of a closed loop system with fish culture tanks, mechanical filter, biological filter, water disinfection equipment, oxygenation equipment, gas exchange equipment, sump, effluent drainage and temperature control system (Dalsgaard et al., 2013). The system is designed based on the biomass to be cultured and its requirements. A basic flow diagram is presented below:

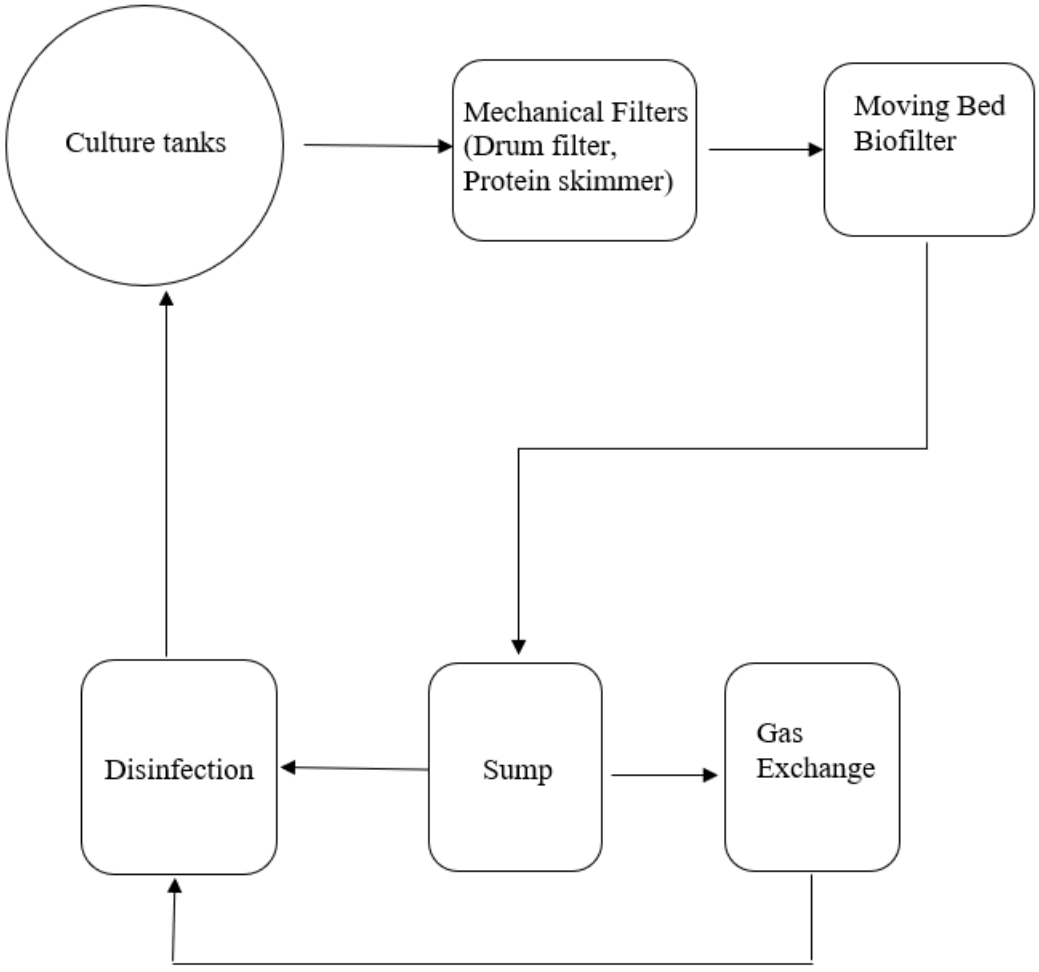


Figure. 1(a): Basic flow diagram of an RAS system

In RAS, fish are usually grown in over ground, water vessels that hold the water and provide space for the fish to grow. These tanks can be circular, rectangular, square, octagonal or hexagonal. Depth varies with type and species to be cultured. These tanks are connected to other equipment in the system with pipes. Fish produce waste that are suspended in water. Uneaten fish feed also contribute to suspended particles in the water. These have to be removed before they can foul the water. For this, mechanical filters are used. These are equipment that filter Total Suspended Solids out of the culture water. The filtrate usually includes fish feces and uneaten feed. Mechanical filtration is mostly carried out using Rotary Drum Filters and Protein Skimmers. The dissolved waste that are

produced by the fish cannot be removed by mechanical filter. It has to be removed using biological process to break them down to safer compounds. Biological filters use bacteria to process and convert toxic nitrogen-based waste into less toxic forms of nitrogen. Bacteria are provided with maximum surface area to grow on different types of media that are housed in bioreactor. As the fish and bacteria grow in biomass, they consume oxygen and give off carbon dioxide. These gasses have to be balanced to give the fish and bacteria an optimum environment to perform. Equipment like speece cones, Low head oxygenators, roots blower etc. are used to oxygenate the water. Whereas, equipment like degasser and carbon dioxide stripper are used to remove carbon dioxide. Either Ultraviolet light or Ozone or both are used to disinfect the water of any free-flowing pathogens. This helps in keeping the system disease free and healthy.

1.2 Biological Filtration

The treatment of wastewater created by the fish rearing tank for reuse in the system is one of the RAS working principles. Water quality management was a critical part of fish productivity in RAS, according to Ridha, Mohammad & Cruz, Emmanuel. (2001). RAS wastewater contains nitrogenous waste, suspended inorganic particles, and a large number of bacterial flocs derived from residual feed, fish feces, dead fish, and bacteria. Biological filtration is the process of removing dissolved, nitrogenous waste from the culture water. Nitrogenous is produced in the form of toxic ammonia. This ammonia is converted into nitrite and then to less toxic nitrate with the help of specific bacteria (Avnimelech Y. 2006). There are different types of biofilters of which Moving Bed Bioreactors (MBBR) are the most popular ones. Despite having many different types, all biofilters have the same working principle. They provide space for bacteria to grow, they provide oxygen to the bacteria and they provide high contact ratio between the circulating water and the bacteria (Abubakar, M., 2022). Below is a diagram of a MBBR that fluidizes biomedia with air bubbles to oxygenate and increase contact ratio between bacteria and the circulating water:

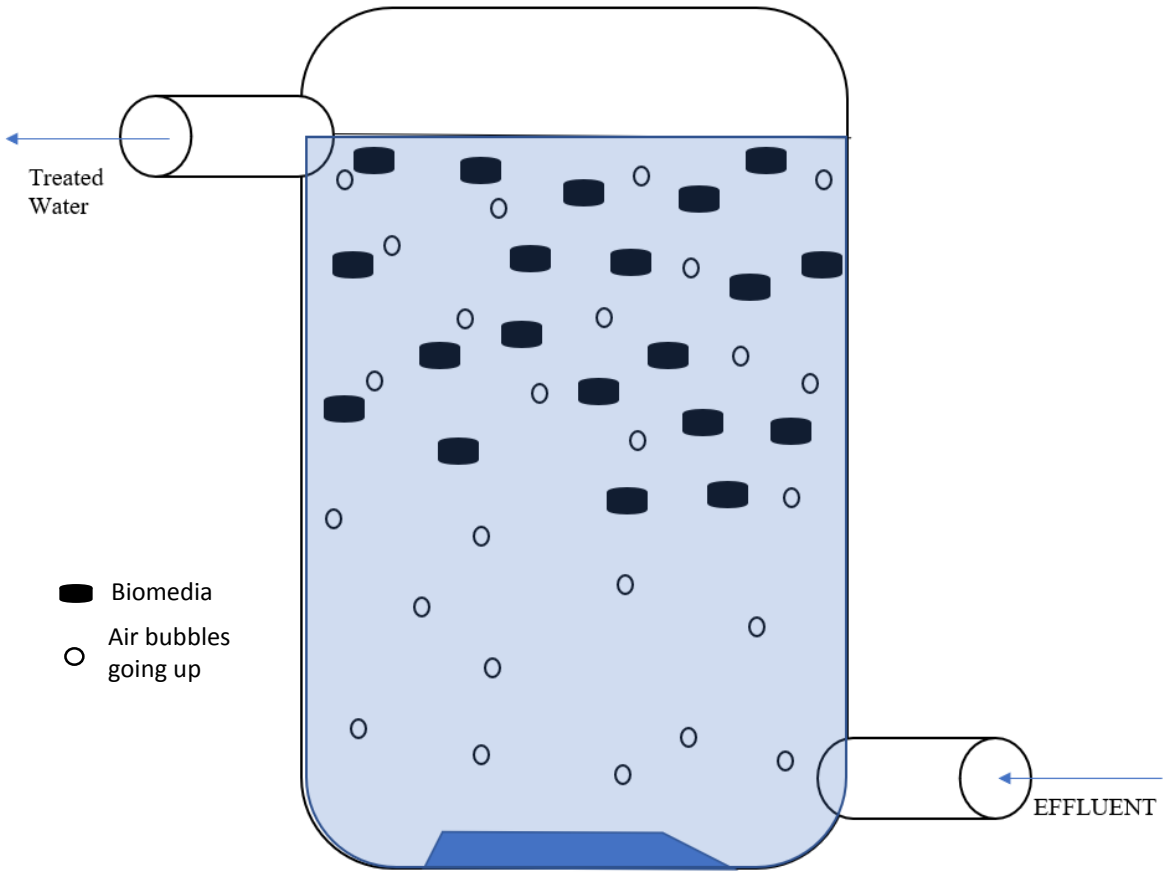


Figure. 1(b): Basic diagram of MBBR

1.2.1 Ammonia in Fish Waste:

As the fish are fed intensively, they catabolize protein in the feed into ammonia. The ammonia released as waste is present in two forms: unionized ammonia (NH_3^-) and ionized ammonia (NH_4^+). The sum of these two forms is referred to simply as "Total Ammonia-Nitrogen" (Anthonisen et al.,1976). Ammonia is toxic to fish. This causes metabolic complications in the fish population (Ridha, Mohammad & Cruz, Emmanuel., 2001). The complications include loss of appetite, lower oxygen affinity of the gills, disease vulnerability etc. To remove this ammonia waste, the most efficient way would be to remove the suspended waste solids mechanically and deal with the dissolved waste using biological degradation. The main source of nitrogenous waste is fish feces and uneaten fish

fed. These degrade to give off ammonia which is highly soluble in water (Bartelme RP, 2017).

1.2.2 Nitrification Process:

In RAS, the principle of nitrification used. Nitrifying bacteria consume the waste and turn ammonia first into nitrite, then the nitrite into nitrate. As ammonia and nitrite are very toxic to fish, they need to be converted into the less toxic nitrate as fast as possible. Nitrification of ammonia is done by autotrophic bacteria which oxidize ammonia in two steps in the presence of oxygen as an oxidizing agent. They utilize CO_2 or HCO_3^- as a source of carbon to grow. *Nitrosomonas* bacteria convert Ammonia (NH_4^+) into Nitrite (NO_2^-). Then, *Nitrobacter* bacteria convert the Nitrite (NO_2^-) into Nitrate (NO_3^-). Nitrite is considered the most toxic to fish, followed by Ammonia and then Nitrate. This process is chemically represented as follows:

$$NH_4^+ + {}^3/_2O_2 \longrightarrow NO_2^- + 2H^+ + H_2O$$

$$NO_2^- + {}^1/_2\, O_2 \longrightarrow NO_3^-$$

1.2.3 Biological Reactors:

Nitrification is carried out in special vessels called bioreactors. These bioreactors provide the required space to hold biomedia, provide oxygen and flow culture water in a manner that allows for higher contact time and area with the bacteria (Balfour, H. and Yoel, P. 1981). The most common type of bioreactor is the Moving Bed Bioreactor (MBBR).

1.2.4 Biomedia:

The bioreactors contain small, specially designed objects called biomedia. The purpose of the biomedia is to provide as much surface area as possible for bacteria to grow on them. The layer of bacteria growing on these surfaces is called biofilm. These biomedia have specially designed curves, ridges and fissure to increase surface area relative to their size. These also have properties to self-clean accumulated sludge and also protect live biofilm present on their surfaces (Davidson, J., Summerfelt, S.T. 2005). Different types of biomedia are used commercially for nitrification. In general, the more surface area there is, the more bacteria can grow and the nitrogen conversion they can do. There are many

different types of biomedia available in the market. All of these fulfill the requirements of a biomedia in one way or the other, but all of them serve to provide as much surface area for biofilm formation as possible. Many of them are made out of sintered ceramics. These can be in the shape of balls, tubes or irregular shapes. The other types are made out of plastics, mostly polypropylene. These are considered best for use in an RAS because of their buoyancy which makes them perfect for fluidization. The most popular commercial biomedia is the K1 biomedia. K1 is a plastic biomedia that has a hollow round shape with multiple partitions and many grooves and fissures. It was developed by a company called Kaldness and is now used worldwide. The key concern for the designer when sizing a biofilter for use in a RAS is to provide enough capacity for the biofilter to manage the total ammonia-nitrogen (TAN) concentration in the culture tanks to a predefined upper limit. The removal rate of a biofilter is proportional to the concentration of ammonia-nitrogen accessible to the biofilter, thus knowing this concentration is crucial.

1.2.5 Biomedia available in Nepal:

Commercially available biomedia are expensive in the Nepalese market. The main reason is that it is not manufactured in Nepal. Import of biomedia is expensive because of multiple reasons. One being Nepal, as a small economy, imports very small volume of biomedia and, that too, very sparsely. This contributes to higher cost at point of origin. Second, biomedia weigh less but occupy larger volume and space. This increases shipping costs. The added shipping cost and low demand are the main reasons biomedia is rarely available in Nepal and when it is available, it's very expensive. Currently, the price of K1 biomedia in Nepal is at around Rs.7000/- per KG. The cost of biomedia has given rise to use of alternative materials that are locally available and do need dedicated manufacturing. Many different materials have been used like bottle caps, plastic straws, corrugated conduit pipes, plastic utensil scrubs, nylon mesh etc. In this study, we look at the performance of Low-Density Polyethylene pellets and investigate its cost effectiveness in the Nepalese market.

1.2.5.1 LDPE Pellets:

LDPE stands for Low-Density Polyethylene. LDPE Pellets are small granular form of LDPE that is melted and injection molded to manufacture different plastic items. It is readily available in Nepal but it is not manufactured in Nepal. It is mostly imported from

India. The smaller size of the pellets gives it more surface is per unit volume and is good for bacterial film formation. It is self-cleaning but has a tendency to lose biofilm faster than other media.

1.2.6 Cost Per KG Ammonia Conversion to Nitrate:

Fish produce a set amount waste and a set amount of ammonia for the feed they eat. The cost of conversion of this ammonia to nitrate can be calculated based on the cost of media and the volume required to convert the ammonia to a safer level of nitrate. The volume of biomedia depends on the Total Ammonia Nitrogen, feed rate, protein percent in feed, nitrogen wastage values and Volumetric TAN Conversion Rate (VTR). Volumetric TAN Conversion Rate (VTR) is the rate of conversion of Total Ammonia-Nitrogen per cubic meter per day.

Mathematically, it is given by

Cost per KG Ammonia conversion to Nitrate = Biofilter Media Volume x cost per liter of media.

Where,

Biofilter media volume (m^3) = TAN production (g TAN/day) ÷ Volumetric TAN Conversion Rate (VTR) (g TAN/m^3/day)

Where,

TAN produced (kg/day) = Feed (KG)/day x Protein % x 50% Nitrogen wasted x 0.16 g Nitrogen/g Protein x 1.2 g TAN/g Nitrogen (N.R. Sajuni., et al., 2010).

Given,

Volumetric TAN Conversion Rate (VTR) = 350 g TAN/m^3/day

And the standard conversion factor is:

$$1m^3 = 1000L$$

The readings in meter cube are converted to liters for ease of measuring volumetrically as it is very practical.

1.3 Objectives:

1.3.1 General Objectives

- To study cost effectiveness of LDPE pellets as biofiltration media in a recirculating aquaculture system.

1.3.2 Specific Objectives

- To study the performance of LDPE pellets as biomedia.
- To calculate cost per kg of conversion of Ammonia to Nitrate using LDPE pellets.
- To evaluate cost effectiveness of LDPE pellets as biomedia.

1.4 Significance of Study

Commercial biomedia is expensive to buy in Nepal and it is very hard to find. This has discouraged many entrepreneurs away from doing aquaculture using RAS. This study will play a significant role in establishing a base for using RAS with locally available component and encourage investment in domestic development of an indigenous RAS.

1.5 Justification

This study is pivotal in the aquaculture arena of Nepal because there is huge void in the development of traditional aquaculture to more advanced, high yielding methods of aquaculture that require less manpower and land. RAS has been proven as the go to method for high density, high yield, low land/water requirement aquaculture in the more developed nations. As Nepal imports a huge number of fish and fish related products from abroad, its high time that we start RAS ventures in Nepal. Despite having the appeal of higher output at lower input, the existing model of RAS in the developed world is not suitable for the Nepalese market where profit margins are low and investing in the high-tech equipment is high. There for this study paves a path for researching adapted domestically developed model of RAS that is more suited to the Nepalese economy. The use of LDPE Pellets is an example of how we can look into using locally available products and locally developed locally.

CHAPTER-TWO

2. LITERATURE REVIEW

In today's world, the aquaculture industry is the fastest growing food-producing sector with annual growth rate of almost 10%, compared with 3% for livestock and 1.6% for capture fisheries production between 1984 and 1995 (FAO, 1997). Recirculating Aquaculture System is an advanced method of aquaculture which is prevalent throughout the world. RAS has gained increased attention in recent years and is believed to be the future of modern-day aquaculture. The major advantage RAS has over other traditional forms of aquaculture is the reusability of 90-99% of water in a closed system. For the reusability of maximum volume of water, filtration system is an inevitable part of RAS for the removal of suspended and dissolved solids, ammonia, carbon dioxide and other harmful chemicals. Controlling the water quality parameters like temperature, pH value and dissolved oxygen concentration is also an integral part of RAS (Dalsgaard et al., 2013). All these factors somehow make RAS a complex system and needs a deeper understanding before it can be managed. Improvement of water quality treatment systems, improvement of monitoring and data assessment system and reducing overall costs has been the major focus of aquaculture engineers. The increasing demand for the aquaculture production cannot be overemphasized. Therefore, it is required to develop systems which will increase fish production with efficient waste management (Piedrahita, 2003; van Rijn, 1996).

In Recirculating Aquaculture System, fish are not the only living things existing. Besides fish, bacteria thrive and needs to be taken into account. These bacteria are integral part of the system which is a part of biological filtration. The solid wastes are removed from mechanical filtration. Drum filter is the mainly used mechanical filtration. After mechanical filtration comes biological filtration in which the existing bacteria converts harmful ammonia into nitrite and less toxic nitrate through the process of nitrification by nitrifying bacteria. The biofilter is contained with materials such as plastic, sand or gravel for the residence of bacteria. These materials are called bio-media.

Biological filter is one of the waste water treatment technologies which has the advantages including reduced size of the treatment plant and excellent performance at high organic loads compared with conventional biological process (Mann et al. 1998). This system converts high ammonia and is more efficient in removal of suspended solids in a single unit (Stephenson et al. 1993; Fdz Planco et al. 2000). Different alternative media have been researched and tested as bio-media alternative. Bottle caps are also considered a better alternative of bio-media. The community of nitrifying bacteria in bottle caps system showed an average removal of chemical oxygen demand equal to 76%. (Oliveira et al. 2014).

The aquaculture sector is rapidly expanding. Aquaculture is divided into two types: intensive and extensive aquaculture. Intensive aquaculture refers to systems in which the majority of environmental parameters are regulated, whereas extensive aquaculture relies primarily on natural circumstances with little or no input and low production levels (Ladon, 1992). The quality of the feeding water has a considerable impact on the productivity of aquaculture.

The physical and chemical features of water, such as suspended particulates, temperature, dissolved gas, pH, and potential hazardous substrates, might affect the production of an aquaculture system (Laden, 1993). Chen et al. (1994) showed that the suspended particles on the recirculation system can damage fish gills, mechanical clogging of filter, mineralization to produce ammonia and increasing oxygen demand when the particles decay. In order to assess the quality of water in the fish farms, many parameters are employed. Many types of biofilter have been applied in recirculation aquaculture system. The filter's efficiency is determined by the retention time and the number of times it is used.Smith (2003) proposed that the A submerged filter's flow channel should be as long as possible with the ideal option being a long thin raceway. Trickling filter is effective in treating nutrient. This type of biofilter is effective at eliminating biodegradable particles, consumes less energy, and may be placed in the culture tank to conserve space (Smith, 2003). Bead filters are a relatively new and widely used form of biofilter that use small plastic beads (polyethylene or polypropylene) as filter material. (Wheaton et al., 1994) The beads float in wastewater that is forced upward by air, water jets, or mechanical means. A

potential drawback of the bead filter is that the presence of significant volumes of carbonaceous materials can promote heterotrophic development at the expense of nitrobacteria (Smith, 2003). The filter media of a fluidized bed filter is sand, which is kept suspended in the influent due to the force of water loading upward. The particle's shape, size, and density affect the filter velocity (Smith, 2003). It's critical to forecast system parameters including fine and dissolved particles, total ammonia nitrogen (TAN), dissolved oxygen, and pH when designing a biofilter for a recirculation aquaculture system. The size and kind of biological filter are determined by the TAN design parameter (Wheaton et al., 1994).

In a two-step process, the nitrifying bacteria in the biofilter convert the deadly ammonia released by the fish to non-toxic nitrate. The oxidation of ammonia to nitrite is mainly carried out by Nitrosomonas species in marine RAS, whereas the oxidation of nitrite to nitrate is usually carried out by Nitrospira species (Foesel et al., 2008). Because cultured examples of these nitrifying bacteria have limited growth rates (Spieck et al., 2011) activation of new RAS biofilters can take a long period. In general, nitrite-oxidizing bacteria have slow growth rates (Abeliovich, 2006; Spieck & Bock, 2015a), and nitrite-oxidizing bacteria in marine RAS appear to be more vulnerable to stress and altering growth circumstances (Blancheton et al., 2013; Graham et al., 2007). High concentrations of nitrite can cause hypoxia in fish, as it oxidizes haemoglobin to methaemoglobin that reduces the blood cells ability to bind oxygen (Jensen, 2003; Kroupova, Machova, & Svobodova, 2005).

CHAPTER-THREE

3. MATERIALS AND METHOD

3.1 Materials

The following materials were used to perform this study:

- FRP fish tanks (10ft diameter, 5 ft depth)
- MBBR
- Rotary Drum filter
- LDPE pellets
- 5HP circulation pump
- 12v DC air compressor
- Fish Nets
- pH/Temperature Meter (Hanna Inc.)
- API^{Tm} Master test kit for Freshwater
- Measuring cylinder (250ml, 500ml, 1000L)
- Weighing balance
- Vernier calipers
- Canon 60D camera
- Quarantine tanks
- UV Water sterilizer

3.2 Study Site and Period:

The study of conducted Hattiban, Lalitpur. The site was a 90ft x 25ft plot of land divided into three vertical steps. The bottom most step was designated for filtration while the remaining two were for the culture tanks. The middle step with four tank was used for this study. The study was performed over a period of 18 months April 2020 to Nov 2021. Three replications were conducted in which six months experiment was done for each replication. Six month was chosen because that time is necessary to grow to plate size (500g) for common carp.

Experimental Design:

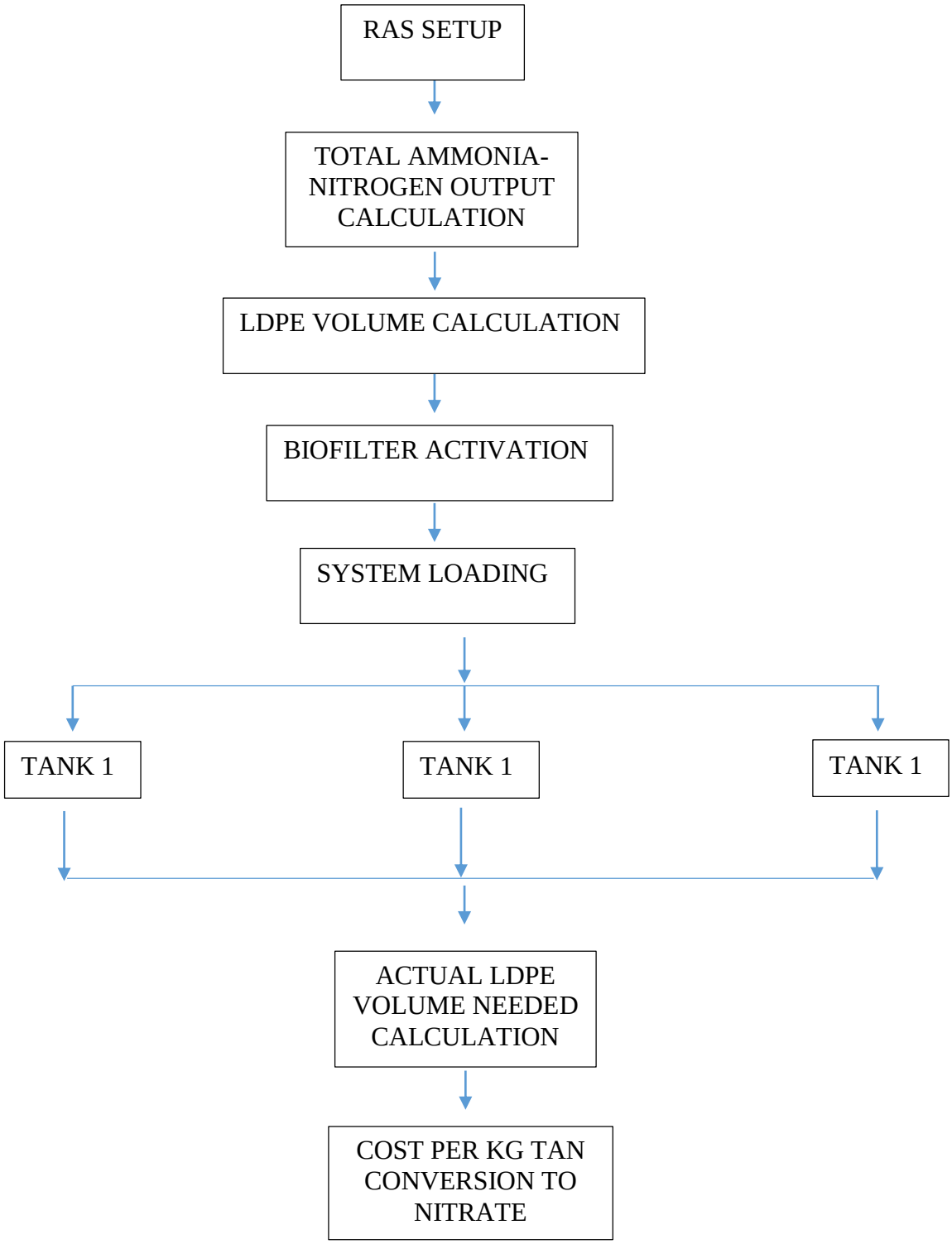


Figure. 3(a): Experimental design

3.4 Recirculatory Aquaculture System Setup

3.4.1 Culture Tanks:

Three circular fish culture tanks, T1, T2 and T3, were constructed for the study. The tanks were made of composite fiber reenforced plastic (FRP). The tanks had a conical base so as to make the tank five feet deep in the center and four feed deep on the edges. The tanks were centrally drained from the deepest point of the tank. Each tank had a polycarbonate observation window for easier supervision of the fish inside the tank. Total water volume of each tank was $8m^3$ making the total volume $24m^3$. (Photo

Photo 3 (a): RAS set up

3.4.2 Mechanical Filter:

A rotary drum filter (RDF) was fabricated for removal of suspend solids. The mesh size of the filter mesh was 100 microns with a total mesh surface area of $2.28m^2$. The RDF was set to backwash automatically using a float switch.

3.4.3 Biological Filter:

A commercial water storage tank of 3000liters capacity was converted into a flow through, aerated, moving bed biofilter.

3.4.4 Biomedia:

300 kg of virgin, LDPE pellets were procured from the local market 25kg bags. Each of the 12 bags cost It was washed thoroughly to remove residual chemicals and unwanted particles from the manufacturing, packaging and storage processes. Required volume of the LDPE pellets was calculated and then loosely put in the biofilter tank.

Photo 3 (b): LDPE pellets

3.4.5 Sump:

A commercial 5000liter water storage tank was modified into a sump.

3.4.6 Plumbing:

PVC pipes, gate valves and FRP flanges were used to connect the system between each other in a linear closed loop.

3.4.7 Circulation Pump:

Two 5 HP monoblock, self-priming water pumps were connected to the closed loop system. These pumps were wired to operate alternatively at every 6 hours to improve longevity of the pumps.

3.4.8 Disinfection:

Two flow through UV-C lamp system of 50watts each and one flow through UV-C lamp system of 200 watts was used to disinfect the water of pathogens. These were wired to operate alternatively every six hours to prolong lamp life.

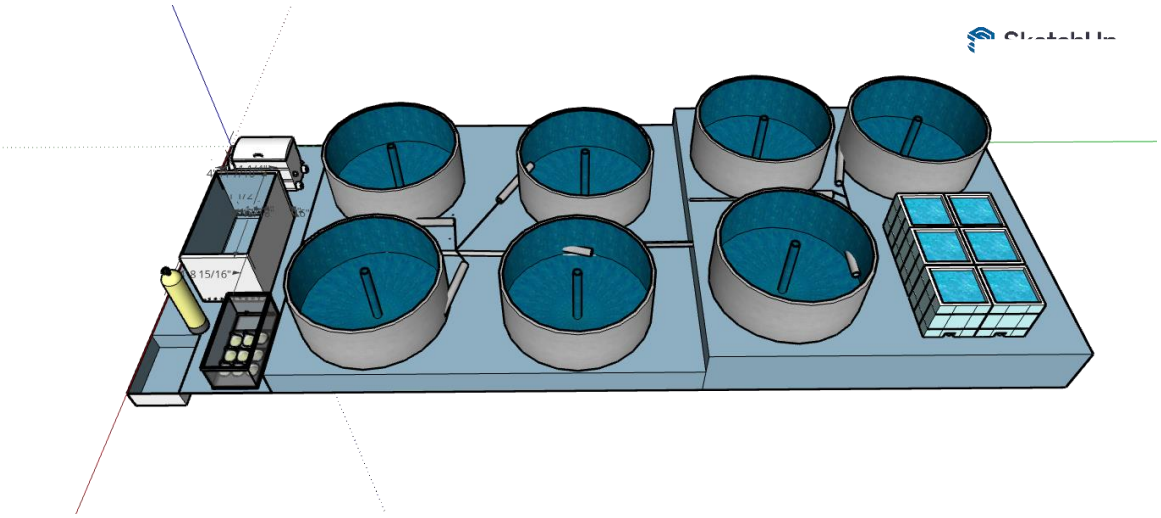


Figure. 3(b): RAS set up

3.5 System Loading:

The following series of actions were performed to load the system:

3.5.1 Bioreactor activation:

For the nitrifying process to begin, an optimal environment must be created to promote bacterial development in the biomedia before the fish are inducted into the system. For this, five liters of water from a running, well established biofilter was used to inoculate the new system. Then, 100ml ammonium hydroxide was added everyday to the system to provide a nitrogen source. This was done until the concentration of ammonia dropped down to less than 2mg/l. only then, fish were added and addition of ammonium hydroxide was stopped.

3.5.2 Fish Procurement and Transport:

Three species were selected for this study based on availability and cost. 200 Common Carp (*Cyprinus carpio* Linnaeus, 1758) fries were procured from CAARP farm in Kathar, Chitwan. The fries were three grams on average in weight. The fish were packed in a

plastic pouch and sealed with oxygen inside (Mandal et al. 2010). They were transported to the study area in a car. The time in transport was six hours.

3.5.3 Disinfection and Quarantine:

The fish transport bags were acclimatized to the water temperature of the quarantine tanks by keeping the bags suspended in the quarantine tank for half an hour. Then they were opened and dipped in a 2 mg/l potassium permanganate solution for 20 seconds and released into the quarantine tank. They were kept in quarantine for one week.

3.5.4 Prophylaxis Induction into System:

The fish were given feed infused with 0.5% tetracycline for one week to kill any bacterial pathogens present in them from the farm of origin. After this period, common carp was released into the tanks T1, T2 and T3.

3.6 Feed Management:

The fish were fed with fish feed containing 35% crude protein at 3% of body weight. The size of the fish feed pellet was increased from 1mm to 2mm and 3 mm for fries, juveniles and adults and termed S1, S2 and S3 respectively as the fish grew. The fish feed was purchased from the local market.

3.7 Water Quality Parameters:

The basic water parameters were measured using the API Freshwater Master Test Kit and Hanna handheld pH meter and temperature meter and Hanna handheld TDS meter**.** The parameters were maintained constant for whole experimental period (Table 1).

Table 1: Parameters Maintained in the tanks

SN	Parameter	Set Value	Remarks
1	Temperature	24^0C-26^0C	Set using a solar water heater and a thermostat.
2	pH	6-8	Checked daily and adjusted using calcium hydroxide ($Ca(OH)_2$)
3	Dissolved Oxygen	5mg/l-8mg/l	Using aerators
4	Ammonia	0.01mg/l-1mg/l	Using the biofilter

3.8 Specific Surface Area and Porosity of LDPE Pellet:

The surface area for LDPE pellet was provided by the manufacture of the pellet AVH Polychem (P) Ltd. the porosity was calculated using the following formula:

$$\text{Porosity (\%)} = \frac{\text{water volume to fill a cube box with pellets}}{\text{Water volume to fill cube box without pellets}} \times 100$$

The Specific Surface Area (SSA) was calculated using the following formula:

$$\text{Specific Surface Area (SSA)} = \frac{(\text{Number of pellets to fill cube}) \times (\text{surface area of pellet})}{\text{Cube volume}}$$

3.9 Total Ammonia Nitrogen (TAN) Calculation:

The Total Ammonia-Nitrogen (TAN) of the system at three different stages of feeding was calculated using the following formula:

TAN produced (kg/day) =

Feed (KG)/day x Protein % x 50% Nitrogen wasted x 0.16g Nitrogen/g Protein x 1.2 g TAN/g Nitrogen

TAN was calculated for fry stage (S1), Juvenile stage (S2) and adult stage (S3).

3.10 Biofilter Media Volume Calculation:

The total required volume of biomedia for each feeding stages S1, S2 and S3 was calculated using the following formula:

Biofilter media volume (m^3) = TAN production (g TAN/day) ÷ Volumetric TAN Conversion Rate (VTR) (g TAN/m^3/day)

Given,

Volumetric TAN Conversion Rate (VTR) = 350 g TAN/m^3/day

And the standard conversion factor is:

$1m^3 = 1000L$

The volume of biomedia was adjusted according to the ammonia readings. Whenever the ammonia readings were more than 1mg/L, 1 liter of LDPE pellet by volume was added per 0.1mg/L

3.11 Cost per KG Ammonia Conversion to Nitrate:

The cost per Kg of ammonia conversion to nitrate was calculated using the following formula:

Cost per KG Ammonia conversion to Nitrate =

$$\frac{\text{Total Volume of LDPE pellets used (L)}}{\text{Total ammonia converted (KG)}} \times \text{Price Per liter of LDPE pellets}$$

3.12 Growth Cycles and Trial Sets:

Each growth cycle was six months long. The growth cycles were replicated three times TS1, TS2 and TS3. Total trial set duration was 18 months.

CHAPTER-FOUR

4. RESULTS

4.1 Surface Area of LDPE Pellet:

As it was not possible for us to calculate the surface are of the pellets on our own, the manufacturer was contacted in India. Total surface area of each individual LDPE pellet as provided by the manufacturer was $0.000028m^2$.

4.2 Porosity of LDPE Pellets:

Porosity was measured using a cube of volume $0.00001m^3$. It was filled with pellets to the brim and the volume of water taken to fill the cube with those pellets were measured. Using these measurements, the porosity was calculated to be 50%.

$$\text{Porosity (\%)} = \frac{\text{water volume to fill a cube box with pellets}}{\text{Water volume to fill cube box without pellets}} \times 100$$

$$= 50\%$$

4.3 Specific Surface Area of LDPE pellets:

To measure the SSA, a cube of volume $0.0000118m^3$ was taken. It was filled to the brim with LDPE pellets. The number of pellets taken to fill the cube was counted which was 186. This number was then used along with the manufacturer provided surface area and the volume of cube to get an SSA of $411.36m^2/m^3$.

$$\text{Specific Surface Area (SSA)} = \frac{\text{(Number of pellets to fill cube) x (surface area of pellet)}}{\text{Cube volume}}$$

$$= 441.36m^2/m^3$$

4.4 Total Ammonia Nitrogen (TAN) Calculation:

Based on the requirements of the fish at different age and the number of fish in the system, the feed rate was calculated to be 3% of the total biomass of fish each day. Protein percentage in the feed given was 35%. The Total Ammonia-Nitrogen (TAN) was

calculated as shown in table 2. The TAN output in S1, S2 and S3 was calculated to be 0.003kg/day, 0.09kg/day and 0.303kg/day respectively.

Table 2: Total Ammonia-Nitrogen Production at different feeding stages in all three trial sets:

	Feeding Stage S1 (kg/day)	**Feeding Stage S2 (kg/day)**	**Feeding Stage S3 (kg/day)**
Trial Set TS1	0.003	0.09	0.303
Trial Set TS2	0.003	0.09	0.303
Trial Set TS3	0.003	0.09	0.303

4.5 Biofilter Media Volume Calculation:

Using the formula, required volume of biomedia was calculated. As the concentration of ammonia crossed 1mg/L at times, some biomedia were added to the biofilter. the calculated volume of biomedia and actual consumed volume of biomedia according to the feeding stage and trial set is shown in table 3 below:

Table 3: Calculated and Total Consumed Biomedia Volume

	S1			**S2**			**S3**		
	Increased ammonia above 1mg/L (mg/L)	Calculated volume of LDPE pellets required (L)	Actual consumed LDPE pellets (L)	Increased ammonia above 1mg/L (mg/L)	Calculated volume of LDPE pellets required (L)	Actual consumed LDPE pellets (L)	Increased ammonia above 1mg/L (mg/L)	Calculated volume of LDPE pellets required (L)	Actual consumed LDPE pellets (L)
TS1	0.2	8.6	10.6	0.3	260	263	0.6	870	876
TS2	0.1	8.6	9.6	0.3	260	263	0.4	870	874
TS3	0.1	8.6	9.6	0.2	260	262	0.4	870	874

4.6 Cost per KG TAN Conversion to Nitrate:

Total TAN output in each of the tanks T1, T2 and T3 were added at the end of each feeding stage S1, S2 and S3. Then the total volume of LDPE pellets that was used to keep the ammonia concentration below 1mg/l was taken. The cost per liter of LDPE pellets was Rs.60/-. These values were then used to calculate the mean cost per KG conversion of TAN nitrate was calculated to be 2208.77/KG.

Total ammonia produced and cost of conversion of ammonia is given by the following table 4:

Table 4: Total ammonia produced and cost of conversion of ammonia

	Ammonia produced during S1 (KG TAN/day x60days)	Ammonia produced during S2 (KG TAN/day x60days)	Ammonia produced during S3 (KG TAN/day x60days)	Total Ammonia produced S1+S2+ S3 (KG)	Total Volume of LDPE pellets used (L)	Cost per KG ammonia conversion to Nitrate (Rate of LDPE Pellets at Nrs60/L) Rs/KG	Mean Cost per KG ammonia conversion to Nitrate (Rs/KG)
TS 1	0.18	5.4	18.18	23.76	876	2212.12	2208.77
TS 2	0.18	5.4	18.18	23.76	874	2207.1	
TS 3	0.18	5.4	18.18	23.76	874	2207.1	

CHAPTER-FIVE

5. DISCUSSION

5.1 Surface Area, Porosity and Specific Surface Area of LDPE Pellet

Surface are of biomedia is very critical in the sense that more surface area is more real estate for bacteria to grow and more bacteria means more ammonia conversion. LDPE pellets are biconvex granules of LDPE plastic that is about 4.5mm in diameter and about 3mm in thickness at the highest point in its center. The manufacturer was contacted to obtain the exact surface area of each pellet which was $0.000028m^2$. The porosity was measured to 50%. This means, in a perfect cube, when the pellets are filled completely to the top of the cube and water is poured in to the top as well, the volume occupied by both water and the pellets is 50% each. The Specific Surface Area was calculated to be $441.36m^2/m^3$. This means that in a $1m^3$ cube, when completely filled by the LDPE pellets, the total surface of the pellets would be $441.36m^3$. A commercial K1 biomedia has a porosity of 75%. This means that 75% of the volume is occupied by water and 25% the media itself. K1 biomedia has a specific surface area of 500m2/m3. Comparing these values to that of the LDPE pellets, it is better to use the K1 biomedia as less volume is required to perform the same task. The individual unit of the K1 biomedia itself is larger, measuring 10mm x 7mm and has fissures and ridges on its surface along with multiple holes. The holes aid in self-cleaning as sludge becomes lodged over time. On the other hand, pellets are small, biconvex grains with smooth surface and no holes or grooves. Pellets make up the surface area by means of sheer weight of numbers. Kikuchi et al, (1994) discovered that effective ammonia removal was proportional to the particular surface area of the medium in nitrifying biological filters. They came to the conclusion that medium surface texture had an impact on nitrification. The bigger the accessible surface area of biological filter medium, the higher the rate of nitrification per volume of media, as long as the interstices between the media do not become clogged with particles. According to the research by (Dalakrishnan and EKckentelder, 1969), raising the specific surface area of plastic media increased nitrification efficiency.

5.2 Total Ammonia Nitrogen (TAN) Calculation

The sum of all forms of nitrogen-based ammonia released in the system is Total Ammonia-Nitrogen. As TAN is highly soluble in water, it cannot be removed by mechanical filtration. Organic matter is a limiting parameter for controlling nitrification because autotrophic nitrifying bacteria are sensitive to this substrate (Avnimelech Y. 2006). According to Pujol et al. (1992), Fdz Polanco et al. (2000), and Ling & Chen (2005), organic matter removal and ammonia conversion can be done in a single unit, and this behavior was also found in this work. As TAN depends on the feeding rate and protein percentage in the feed, the value of TAN release was found to be different for different stages of the growth of the fish. The designer's principal concern when sizing a biofilter for use in a RAS is to provide enough biofilter capacity to keep the total ammonia-nitrogen (TAN) concentration in the culture tanks under a predefined upper limit. The removal rate of a biofilter is proportional to the concentration of ammonia-nitrogen accessible to the bacteria in the biofilter, hence knowing this concentration is crucial. The lower the designer's TAN concentration limit, the lower the biofilter's removal rate. Thus, TAN was sampled at three different growth period divided into three periods of 60 days each, S1, S2 and S3. TAN values of each sample period were calculated to be 0.003kg/day, 0.09kg/day and 0.303kg/day respectively at 35% protein in the feed. This incremental difference is attributed to the increase in biomass of fish as they grow in the system and the increased feeding rate there forth.

5.3 Biofilter Media Volume Calculation

The surface area available for bacteria to grow on a biomedia is directly proportional to the amount of TAN they can convert into relatively harmless nitrate. So, when designing a system, the sizing of the biofilter is very critical. The volume of biomedia is dependent the amount of TAN produced by the system and the volumetric TAN conversion rate (VTR) of the system. The VTR is the rate at which TAN is converted to nitrate by a meter cube of biomedia in a day. For a Moving Bed Bioreactor (MBBR), at 25^0C and pH 7, the VTR is given at 350 g TAN/m^3/day. With this value, the volume of the required biomedia was calculated to be 8.6L, 260L and 870L for feeding stages S1, S2 and S3 respectively. This means that these respective volumes of biomedia are required to convert the TAN safely to nitrate in each of the respective feeding stages. As the TAN increased in the different

feeding stages, the required volume of biomedia also increased accordingly. In the present study, in each feeding stage, some amount of LDPE pellets needed to be added to maintain the ammonia concentration below 1mg/L (See table 3). Even though the exact volume of the LDPE pellets was calculated, somehow, it was not enough. This increase in the required volume of LDPE pellets can be credited to the lack of grooves and holes in the pellets. The pellets were rubbing against each other which led to the scraping off of the biofilm of bacteria growing on them. This reduced the amount bacteria in the effective surface area of the pellet. This, in turn, degraded performance. This fact emphasized the importance of grooves and holes in the biomedia which prevent premature shedding of the biofilm.

5.4 Cost per KG Ammonia Conversion to Nitrate

In today's capitalist economy, money matters. Wherever one can save money or make more money is an added advantage. RAS is a high density, high risk and high reward methos of farming fish. So, cost reduction at all available avenues must be explored as long as it remains within the safety bracket. Specially in Nepalese economy, where the investing capacity is low and the profit margins are small, low cost, domestically available and locally serviceable system is important. Locally available biomedia is one of the many aspects that can reduce cost of the system and hence the cost of the fish produced. The cost of the biomedia and its properties are directly linked to how much it would cost to keep the ammonia concentration in an RAS system in a safe level. In this study, LDPE pellet was chosen because it is available in almost every major city of Nepal. It is used to make plastic bags, mineral water bottles etc. It is in small granular form that gives it more effective surface area. It is also almost neutrally buoyant with makes it easier for the air bubbles in the MBBR to fluidize. The cost of the LDPE pellets purchased from the local market was for this study was Rs.60 per L. The mean cost for converting a kg of TAN was calculated to be Rs.2208.77/- over the three trial sets. This amount is expensive considering that if we had access to cheaper commercial biomedia, the cost per kg would have far less. This revelation also points focus to the fact that there is a lot more to be done to find other suitable alternatives for use as biomedia that have significantly lower cost per kg conversion rate. There has been work on using PET bottle caps. Studies have shown that half cut bottle caps are a good alternative to commercial biomedia.

CHAPTER-SIX

6. CONCLUSION

This study recorded the performance of LDPE pellets in a $24m^3$ system growing 600 individuals of common carp to market size at 25^0C over a period of 18months in three replicated sets of six months each. The study revealed that LDPE pellets were suitable for use in a RAS as an alternative to commercial biomedia as it was cheaper. The average cost per kg of ammonia conversion to nitrate was Rs.2208.77/-. This rate is still expensive from a Nepalese market perspective. But there were two main factors that affected the performance of the LDPE pellets compared to the commercial biomedia. The performance of the LDPE pellets was reduced by premature shedding of biofilm due to friction amongst the pellets. The lack of holes and grooves make the pellets unable to hold a healthy biofilm layer. If we can find an alternative that is locally available, cheaper and with physical characteristics that provide increased surface area with grooves and fissures, that would lower the cost per KG conversion of TAN to nitrate. Thus, the study indicates that there is need of further research into finding other cheaper alternatives to the expensive commercial biomedia if RAS is to succeed in Nepal.

REFERENCES

1. Abubakar, M., Zhang, Y., Muhammad, A. I., Liu, G., Shitu, A., Qi, W., et al. 2022. Recent advances in application of moving bed bioreactors for wastewater treatment from recirculating aquaculture systems: A review, Aquaculture and Fisheries, 7 (3): 244-258
2. Akeem Babatunde Dauda, Abdullateef Ajadi, Adenike Susan Tola-Fabunmi, Ayoola Olusegun Akinwole, Waste production in aquaculture: Sources, components and managements in different culture systems, Aquaculture and Fisheries, Volume 4, Issue 3, 2019, Pages 81-88
3. Anthonisen, A.C., Loehr, R.C., Prakasm, T. B. S., Srinath, E.G., 1976. Inhibition of nitrification of ammonia and nitrous acid. J. Water Pollut. Control Fed. 48(5): 853-852.
4. Aquaculture Systems. Aquaculture Water Reuse Systems: Engineering Design and Management. 3rd ed. Elsevier
5. Avnimelech Y. 2006. Bio-filters: the need for a new comprehensive approach - Aquacultural Engineering, 34: 172-178.
6. Balami, Sujita. (2021). Recirculation Aquaculture Systems: Components, Advantages, And Drawbacks. 2. 104-109. 10.26480/taec.02.2021.104.109.
7. Balfour, H. and Yoel, P. (1981): Commercial fish farming. A Wiley-Interscience Publication. John Wiley and Sons, New York. Pp 259.
8. Bartelme RP, McLellan SL and Newton RJ. 2017. Freshwater Recirculating Aquaculture System Operations Drive Biofilter Bacterial Community Shifts around a Stable Nitrifying Consortium of Ammonia-Oxidizing Archaea and Comammox Nitrospira. Front. Microbiol. 8:101
9. Boyd, C. E., 1979. Water quality in warmwater fish ponds. Agric. Exp. Stn., Auburn, AL. 359p.
10. B. Singh, K. Al-Haddad and A. Chandra, "A review of active filters for power quality improvement," in IEEE Transactions on Industrial Electronics, vol. 46, no. 5, pp. 960-971, Oct. 1999, doi: 10.1109/41.793345.
11. Davidson, J., Summerfelt, S.T. 2005. Aquacultural Engineering, 33:47–61

12. Damasceno de Oliveira, L., et al. 2014. Bottle caps as submerged aerated filter medium Water Science & Technology. 69 (7): 201
13. FAO. 2000 Fishery Statistics (Aquaculture Production), Food and Agriculture Organization of the United Nations, Rome, Italy 90: 13-22
14. John, Ed-Idoko. (2022). Relevance of Water Recirculating System in Modern Aquaculture. 15. 107-113. 10.9734/AJFAR/2021/v15i630355.
15. LaDon, S.(1992). A Basic Overview of Aquaculture History - Water Quality - Types of aquaculture - Production Methods. Purdue University. West Lafaette. IN
16. Lugert V, Georg T, Jen T, Carsten S, Joachim K. 2016. A review on fish growth calculation: multiple functions in fish production and their specific application. Reviews in aquaculture, 8: 30-42.
17. Montorio, L. 2004. Assesment of a novel filter system for recirculating aquaculture. P.hD thesis. Institute of Aquaculture, University of Stirling, Scotland.
18. N.R. Sajuni, A.L. Ahmad and V.M. Vadivelu, 2010. Effect of Filter Media Characteristics, pH and Temperature on the Ammonia Removal in the Wastewater. Journal of Applied Sciences, 10: 1146-1150.
19. Paul, D. 2021. Hall, S.G. 2021. Biochar and Zeolite as Alternative Biofilter Media for Denitrification of Aquaculture Effluents. Water 2021, 13, 2703.
20. Ridha, Mohammad & Cruz, Emmanuel. (2001). Effect of biofilter media on water quality and biological performance of the Nile Tilapia Oreochromis niloticus L. reared in a simple recirculation system. Aquacultural Engineering. 24. 157-166. 10.1016/S0144-8609(01)00060-7.
21. Smith, M. (2003). Biological filters for Aquaculture, L.S. Enterprises.
22. Steven C., 2001. Understanding Fish Nutrition, Feeds and Feeding. 3rd ed. pp. 181-257. Academic Press, San Diego, CA, USA.
23. Sultana T, Haque MM, Salam MA, Alam MM (2016) Effect of aeration on growth and production of fish in intensive aquaculture system in earthen ponds. MS Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh.
24. Wagner, S. C. 2011 Biological Nitrogen Fixation. Nature Education Knowledge 3(10):15.

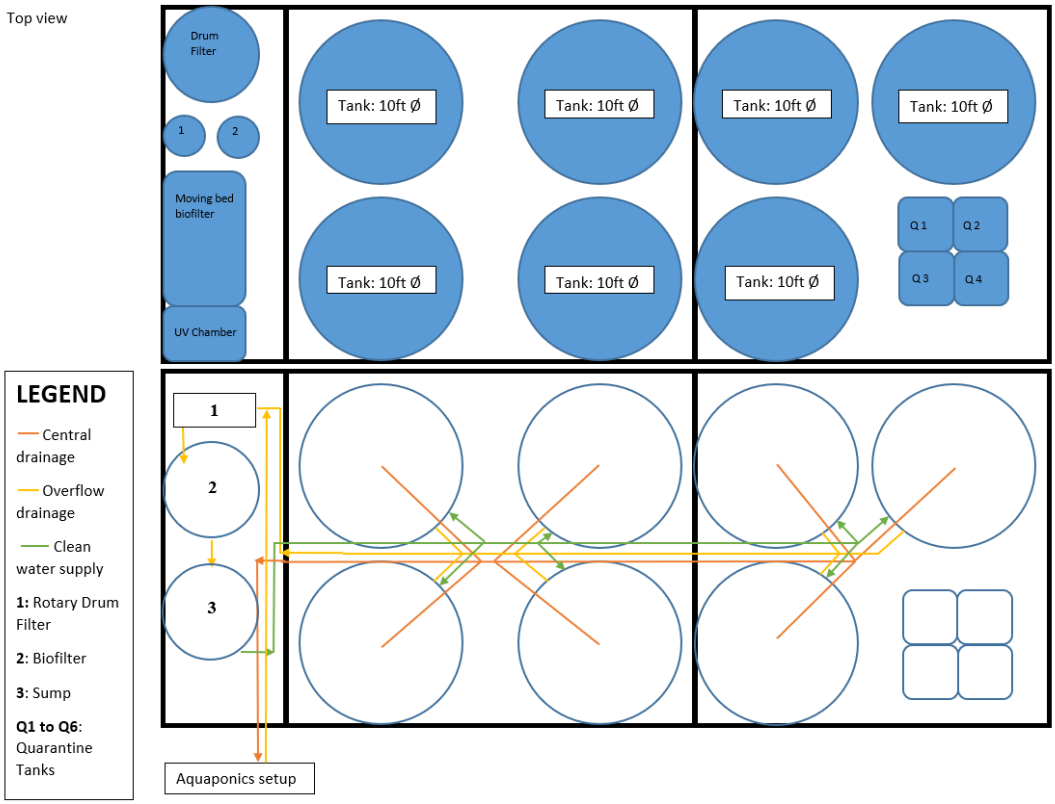
25. Wheaton F.W., Hochheimer J. n. Kiser G.E., Malone R. F., Krones M. J., Libey G. S. and Easter C.C. (1994). Nitrification Filter Design Methods. Aquaculture water reuse systems: Engineering Design and Management, 3rd ed. Elsevier
26. Warrer-Hansen, I., 2015. A brief history of RAS: recirculating aquaculture systems have made steady progress, from early farmers to today's potential for salmon grow-out. *Fish Farmer*, *38*(5), pp.22-25.

.

PHOTOPLATES

PLATE 1

Experimental System Layout Diagram



Cross-sectional view

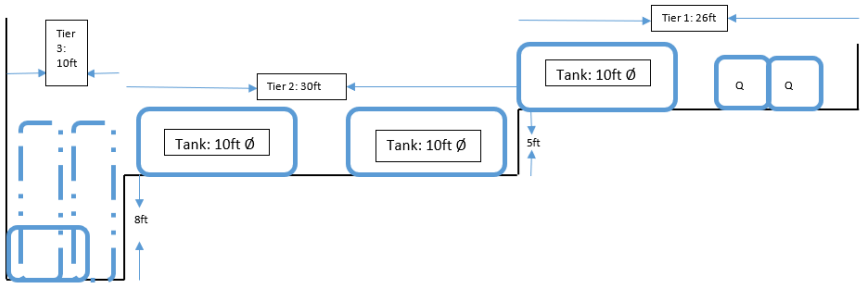


PLATE 2

Water Flow Diagram

PLATE 3

Water Distribution and Disinfection System

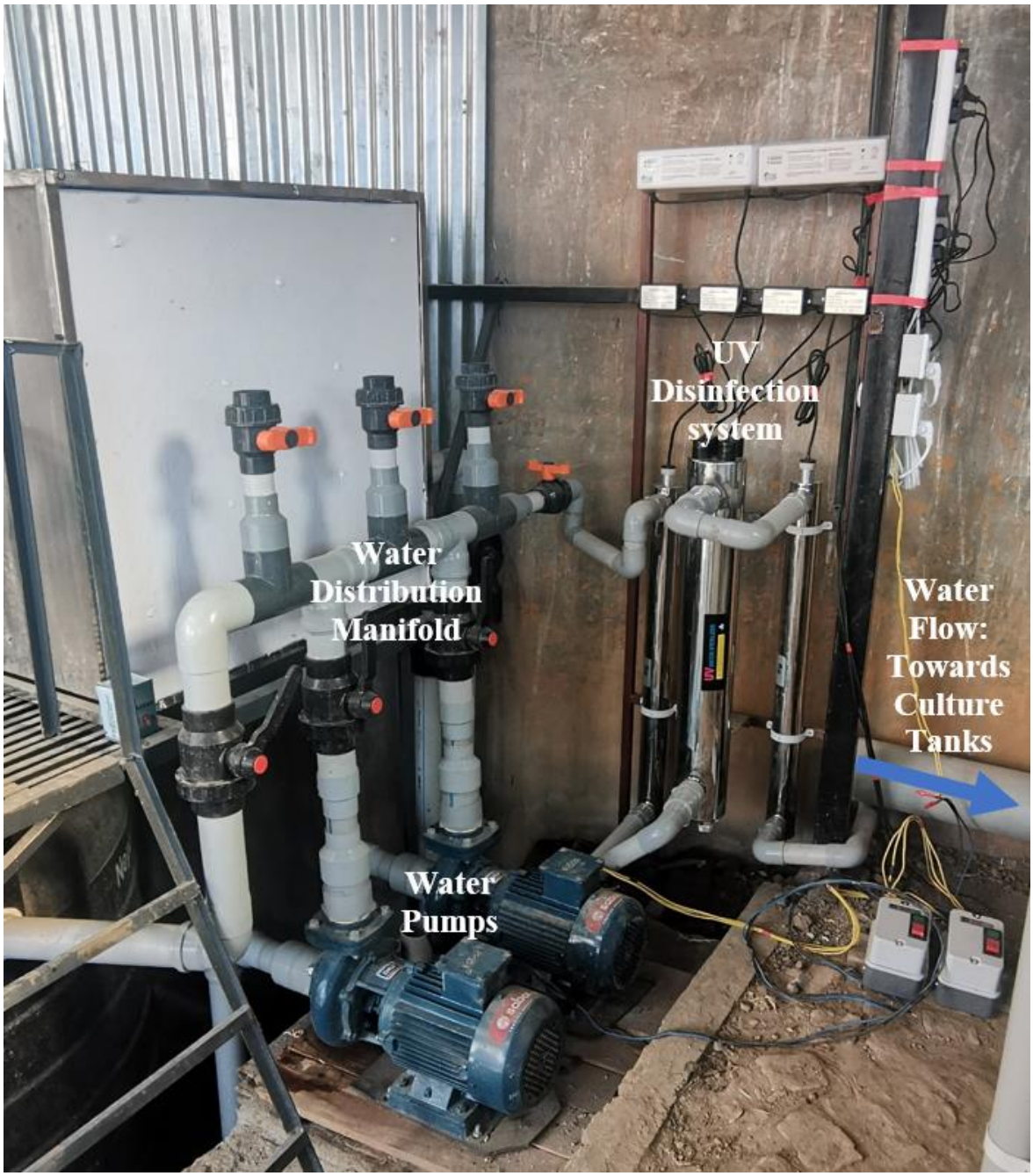


PLATE 4

Commercial K1 Media

PLATE 5

Low Density Polyethylene Pellets