DEVELOPING A LOW-COST RECIRCULATING AQUACULTURE SYSTEM USING LOCALLY AVAILABLE MEDIA AS BIOFILTERS FOR REMOVING AMMONIA AND NITRITE

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2022

EXTENDED ABSTRACT

Aquaculture is the fastest growing food producing sector in the world and remains a vibrant and important sector for production of high-quality protein food. Success for the aquaculture industry is attributed to growing demand for healthy, tasty and affordable food as well as the sharp decline in wild fish supply due to increasing human population and over exploitation of natural water bodies. To increase production from aquaculture and reduce the widening gap between fish demand and supply, technologies that increase production efficiency and intensity as well as use less water and are environmentally friendly need to be promoted. One of the methods for intensive aquaculture production system is recirculating aquaculture system. Recirculating Aquaculture System (RAS) is a technology designed for holding and growing a wide variety of aquatic species in defined production unit which recycles water by passing it through mechanical and biological filters to remove suspended and dissolved wastes, respectively. This technology (RAS) is widely used in developed countries and the sector is growing tremendously. Yet, developing countries' aquaculture production rarely uses RAS for intensive fish farming. This discrepancy is partially driven by the high costs of the RAS unit and the associated media such plastic beads and Kaldnes (KMT) media, commonly used as biomedia in moving bed bioreactor (MBBR).

Therefore, a study was conducted to develop a low-cost RAS and identify cheap and locally available media that can be used as biofilters in RAS. The use of cheap and locally available media will reduce costs and make RAS affordable to small-scale fish farmers. Development of low-cost RAS unit and availability of cheap, locally available biomedia that can be used in biofilters will encourage local small-scale fish farmers in Tanzania to embark on intensive fish farming using RAS technology. In this study four experiments were conducted.

The first experiment was conducted to establish the suitable water flow velocity to be applied in the development of an ideal RAS for use in developing countries. Three freshwater pilot scale RAS stocked with rainbow trout and fixed bed biofilters were used. Removal of total ammonia-nitrogen (TAN) and nitrite-nitrogen were assessed at four different water velocities in the biofilters (i.e. 1.4, 5.4, 10.8 and 16.2 m/h) under identical conditions (temperature, dissolved oxygen, pH, alkalinity). Results indicated low TAN and nitrite removal rates at water velocities below 10.8 m/h. Five-fold elevated nitrite levels were found in the RAS when biofilters were operated at 1.4 m/h compared to the other velocities, substantiating the significant effect of water velocity on biofilter performance. The best water flow velocity determined in this pilot scale RAS was applied in the second trial.

The second experiment involved developing an ideal low-cost RAS to be used for experiments and production of fish in developing countries. In this study, two pilot RAS units, each with capacity of 900 L of water in circulation were developed and ran for 10 weeks. Synthetic ammonia and nitrite were added in the first four weeks to trigger the development of nitrifying bacteria, after which 20 kg bulk weight of Nile tilapia was stocked in each RAS unit. The average water quality parameters throughout the experimental period were 179.09 ± 85.6 mg $CaCO_3$/L, 6.18 ± 0.8 mg/L, 7.59 ± 0.4, 24.69 ± 1.1 °C, 197.23 ± 92.2 mg/L and 0.20 ± 0.1 ppt for alkalinity, dissolved oxygen, pH, temperature, total dissolved solids and salinity, respectively. The stocked fish biomass increased by 9 kg in each tank for a period of six weeks. Volumetric TAN and nitrite conversion rate exponentially increased from week two and became stable after the 6^{th} week with an average concentration of 450 g/m^3/d and 100 g/m^3/d, respectively. The developed simple low-cost RAS showed performance that is similar to other commercial RASs and, therefore, it is ideal for teaching, research and fish production purposes in developing countries.

The third experiment was done to test locally available materials as biomedia in RAS. In this experiment, six biological filters, of which five media were made from locally available materials (dry cattle horns, dry local ceramic, dry activated charcoal, dry bamboo sticks and dry coconut shells) and one commercial plastic media were evaluated in duplicate in a 1 m^3 tank under pilot scale. Volumetric TAN and Nitrite removal were assessed. The highest VTR recorded in this study was 598.65 ± 15.8 g TAN/m^3/d from coconut shells while the lowest was 343.45 ± 8.93 g TAN/m^3/d from horns. Biofilters containing plastic recorded the highest VNR (704.24 ± 50.30 g NO_2-N/m^3/d) while the horns biofilters recorded the lowest (457.38 ± 46.09 g NO_2-N/m^3/d). This study, therefore, revealed that coconut shells can be used as biomedia in place of plastic materials in recirculation aquaculture system biofiltration.

The fourth experiment was done to further evaluate the ability of coconut shells in comparison with commercially available biomedia (Foam, Leca and plastic beads). Water quality parameters were monitored and the performance of different biofilters were assessed in terms of VTR, VNR and bacterial activity by the use of hydrogen peroxide (H_2O_2) degradation method. Nitrification kinetics for volumetric TAN conversion rates were 4.6 ± 0.3, 3.8 ± 0.3, 3.3 ± 0.3 and 1.7 ± 0.2 g/m^3/d for biofilters containing foam, coconut shells, leca and Plastic beads, respectively. The calculated first order rate constant k_{1a} (1’ order) for volumetric TAN conversion rates were 0.05, 0.04, 0.04 and 0.01 m/d for biofilters containing foam, coconut shells, leca and plastic, respectively. On the other hand, the 0’ order nitrification kinetics for VNR were 5.1 ± 0.8, 4.5 ± 0.6, 3.7 ± 0.2 and 1.4 ± 0.2 g/m^3/d for biofilters containing foam, coconut shells, leca and Plastic beads, respectively. This study found that foam is the best biomedia for RAS, followed by coconut shells and leca. High porosity state of foam makes it easy to clog and, therefore, cannot maintain its nitrification performance for a long time. Therefore, this study recommends the use of coconut shells and leca for biofiltration in RAS.

This study concludes that coconut shell is as good as plastic in serving as a biomedia in RAS. Therefore, it is recommended that coconut shell be used as biomedia in RAS in developing countries. Further research is recommended to determine appropriate sizes of this biomedia during application and its durability during operation.

DECLARATION

I, Mang'era Samwel Mnyoro, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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LIST OF PAPERS AND MANUSCRIPT

Paper I: Mang'era Samwel Mnyoro, Arvin Erik, Renalda N. Munubi, Sebastian W. Chenyambuga, and Lars-Flemming Pedersen. "Effect of water velocity on ammonium and nitrite removal in pilot scale fixed bed biofilters".

Status: Published in *Aquacultural Engineering*. Volume 93, May 2021, 102160. https://doi.org/10.1016/j.aquaeng.2021.102160

Paper II: Mang'era Samwel Mnyoro, Renalda N. Munubi, Sebastian W. Chenyambuga, and Lars-Flemming Pedersen. "Low-cost Recirculating Aquaculture System for Small-scale Farmers in Developing Countries".

Status: Submitted to the *Journal of applied aquaculture*

Paper III: Mang'era Samwel Mnyoro, Renalda N. Munubi, Lars-Flemming Pedersen and Sebastian W. Chenyambuga. " Evaluation of biofilter performance with alternative local biomedia in pilot scale recirculating aquaculture systems".

Status: Published in the *Journal of Cleaner Production.* Volume 633, July 2022, 132929. https://doi.org/10.1016/j.jclepro.2022.132929

Paper IV: Mang'era Samwel Mnyoro, Renalda N. Munubi, Sebastian W. Chenyambuga, and Lars-Flemming Pedersen. "Capacity of Different Biomedia for Removing Ammonia and Nitrite in Recirculating Aquaculture Systems".

Status: To be submitted in the *Journal of Aquaculture research*

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ACKNOWLEDGMENTS

First of all, I would like to thank the Almighty God for granting me life and strength that enabled me to execute this study.

I wish to acknowledge the financial support from Danish International Development Agency (DANIDA) through Building Stronger Universities phase III (BSU III) programme at Sokoine University of Agriculture. Also, I thank my employer, The Mwalimu Julius K. Nyerere University of Agriculture (MJNUAT) for granting study leave, without which this work would never have been completed. Special thanks go to the National Institute of Aquatic Resources, Section for Aquaculture, Technical University of Denmark-DTU, for allowing some of the experiments to be done in their facilities and all the technical support provided by the institution's technical personnel.

I would like to express my sincere gratitude to my supervisors, Professor Sebastian Wilson Chenyambuga, Dr. Renalda Nanziga Munubi and Dr. Lars-Flemming Pedersen who have supervised this Ph.D thesis with invaluable enthusiasm. Their valuable advice and insightful criticism over the whole thesis have been of the utmost significance in an entire period of my study. I would like to extend my deepest sense of both personal and professional gratitude to Ms. Leila Jafari for inspiration and professional help, both when doing the experiments and during data analysis. I am grateful to all MJNUAT colleagues and fellow students who assisted me in one way or another, until I managed to finish this challenging course of action. I will never forget you, my friends!

My heartfelt gratitude goes to my wonderful wife Rhobi Patrick, who allowed and encouraged me to begin Ph.D studies at this age, despite many family responsibilities which I had to handle. Thank you so much! I am deeply grateful to my precious sons Hezron and Hazael for their calmness as they missed my physical presence, love and care during my absence. Lastly but not least, I am indebted to my father Rev. Daniel Munyoro

Mang’era and my mother Elizabeth Boke Bhunyige who laid a foundation for me to adore education. May God bless abundantly all my siblings who helped me in my endeavour to climb an academic ladder!

DEDICATIONS

This thesis is dedicated to my beloved wife Rhobi, my sons Hezron and Hazael. I thank you for your love, support, encouragement and prayers, throughout the entire period of my study. I love you all.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOB	Ammonia Oxidizing Bacteria
BOD	Biological Oxygen Demand
BSU	Building Strong Universities
COD	Chemical Oxygen Demand
CP	Crude Protein
DANIDA	Danish International Development Agency
DBL	Diffusive Boundary Layer
EN	Enhanced Nitrification
FAO	Food and Agricultural Organisation
FBBR	Fixed Bed Bioreactors
FBF's	Floating Bead Filters
LRS	Substrate Loading Rate
LSU	Louisiana State University
MBBR	Moving Bed BioReactors
NOB	Nitrite Oxidizing Bacteria
OTUs	Operational Taxonomic Units
RAS	Recirculating Aquaculture System
TAN	Total Ammonia Nitrogen
USD	United States Dollar
VNR	Volumetric Nitrite Conversion Rate
VNRa	Apparent Volumetric Nitrite Conversion Rate
VOCR	Volumetric Oxygen Conversion Rate
VTR	Volumetric TAN Conversion Rate

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

1.1.1 Aquaculture production

Aquaculture is the fastest growing food producing sector in the world and remains to be a growing, vibrant and important sector for production of high quality protein food. World aquaculture production attained 114.5 million tonnes live weight in 2018, with a total farm gate sale value of 263.6 billion united states dollars (USD) (FAO, 2020). According to FAO (2020) fish production from aquaculture accounts for 46% of the total production. Cultured fish species such as trout, salmon, catfish, tilapia and oysters are highly demanded and, therefore, the profit level for producing these species is very high. The success of aquaculture industry is attributed by the endless growing demand for healthy, tasty and affordable protein foods as well as the sharp decline in wild fish catch due to increasing human population, climate change and over exploitation of natural water bodies.

Tanzania has a substantial potential for development of aquaculture due to availability of water resources which includes rivers, lakes, the Indian Ocean, natural and manmade dams. For many years, aquaculture farmers in Tanzania have practiced extensive fish farming, which refers to fish farming conducted in medium to large sized ponds or natural water bodies relying merely on natural productivity of pond water for feeding fish (Rukanda and Sigurgeirsson, 2018; Mzula *et al*., 2021). Under extensive production system, externally supplied inputs are limited because only fertilizers are applied. This type of fish farming lowers production costs, but the quantity of fish produced per unit area is low (Tidwell, 2012). The only advantage of extensive fish farming is low operation costs. Since human population is increasing and demand for land, water and fish is high,

there is a need to promote intensive fish farming whereby the quantity of fish produced per unit of rearing area is higher. Under intensive culture system, there is steady monitoring of production and the quality of feed, water and fingerlings are controlled to improve production. The need for intensive aquaculture has triggered the rise of different production systems such as Cage, Raceway and Recirculating Aquaculture System (RAS) technologies (Zepeda *et al.*, 2008; Tidwell, 2012; Southgate and Lucas, 2019).

1.1.2 Recirculating Aquaculture Systems

Recirculating Aquaculture System (RAS) is a technology designed for holding and growing a wide variety of aquatic species in defined production units which recycle water by passing it through mechanical and biological filters to remove suspended and dissolved wastes, respectively (Badiola *et al.*, 2012; Boyd and Tucker, 2014). The system provides effective control and treatment of wastes (soluble and particulate) and makes it possible to reuse water. The advantages of RAS include reduced amount of water that needs to be replaced daily to make up for losses due to evaporation, ability to monitor water quality parameters in the rearing units during the life cycle of farmed fish, more stability in culture conditions and reduced oxygen fluctuations (Badiola *et al.*, 2012). Moreover, RAS has the advantage of enabling production of higher fish yields in a relatively small area and year-round production. Recirculating aquaculture systems can be designed to cater for different capacities and efficiencies (Colt *et al*., 2006).

Although the productivity is high, the main drawback of RAS is large amount of waste materials produced, especially ammonia (Eding and Kamstra, 2001). The quantity of ammonia increases with increase in stocking density (Dauda and Akinwole, 2014). Ammonia is very toxic to fish, hence, it has to be removed (Ip and Chew, 2010; Randall and Tsui, 2002). RAS relies on biofiltration which converts ammonia and ammonium excreted by the fish and released from decaying feed remains into nitrite and finally

nitrate. In RAS, water from fish production unit is pumped through the filter, and ammonia is utilized by the bacteria for energy to release nitrate which is less toxic than ammonia at levels not greater than 100 mg/L (Chen *et al*., 2006), and can be removed by denitrifying biofilter or by water replacement. Biofilters are the 'central operating systems' in RAS specifically designed for concentrated bacterial attachment required for nitrification. Commonly used biological filters are activated sludge, plant material filters, fluidized bed filters and fixed films (Pedreira *et al*., 2016). Efficiency of a biofilter is measured by its ability to maintain ammonia and nitrite at levels below 0.02 mg/L (Poresky *et al*., 2016).

1.2 Problem Statement and Justification of the Study

In recent years, the demand for fish has increased due to human population increase, thus intensification of fish production by using RAS has been the focus of aquaculture research and developmental efforts in many countries. Biofilters are the most essential component unit in RAS (Bregnballe, 2015), as they are responsible for the removal of excreted toxic ammonia, nitrite and dissolved organic matter (Adamu *et al.,* 2014). Among other factors, the efficiency of a biofilter for removing ammonia and other dissolved organic matters depends on the material and surface area of the media installed in it (Chen *et al.,* 2006). Commonly used commercial biomedia are industrially made of polypropylene or polyethylene plastic. These biomedia are made in different densities and shapes making them submersible, eversible or floating in water. Other industrial biomedia that are used in RAS include; ceramic, silica and aluminium oxide formed into a porous ring, bio foam (sponge), solid pumice and fine glass particles (Lepine *et al*., 2016; Bagaswari and Moersidik, 2018; Boaventura *et al*., 2018; Owatari *et al*., 2018). These materials are expensive and hardly available for local fish farmers in developing countries (Losordo *et al.,* 2000). In order to increase fish production in RAS, developing countries need to identify a bio-media that will be low-cost, durable and readily available. Research shows

that agricultural wastes such as hardwood chips, nut shells, rice husks, horns, seashells, ceramic, coconut shells, bamboo, charcoal, etc. can be shaped and used as biomedia in RAS (Chen *et al*., 2008; Chen and Hoff, 2012). Some of these materials have been used in laboratory nitrification trials (Guerdat *et al*., 2010; Pfeiffer and Wills, 2011). Currently limited information is available on the performance of locally available biomedia in removing ammonia and nitrite in RAS.

Therefore, there is need for evaluating the nitrification performance of cheap and locally available materials that can be used as biomedia in RAS. The use of these materials will minimize costs and make RAS affordable to small-scale farmers. Moreover, development of cheap, locally available and efficient biofilters will encourage local small-scale fish farmers in Tanzania to embark on intensive fish farming using RAS. Therefore, this study aimed at developing a simple and low-cost RAS ideal for use in developing countries and using the same unit for evaluating the efficiency of locally available media for removing ammonia in RAS.

1.3 Objectives

1.3.1 Overall objective

The overall objective was to develop a low-cost pilot RAS using the most appropriate locally available biomedia.

1.3.2 Specific objectives

i. To assess the effect of water velocity on the removal of ammonia and nitrite in recirculating aquaculture system.
ii. To develop a cheap pilot scale recirculation aquaculture system ideal for small scale aquaculture in developing countries.

iii. To determine the performance of locally available materials when used as biomedia to remove ammonia and nitrite in RAS

iv. To compare the performance of selected locally available biomedia with commercial biomedia in terms of removal of ammonia and nitrite in RAS.

1.4 Literature Review

1.4.1 The history of RAS

Recirculation aquaculture was first practiced in Japan in 1950s, where water resource scarcity drove the invention of biofilters designed for carp production (Murray *et al*., 2014). During the same decade, Europe and the United States aquaculture scientists tried to adapt domestic wastewater treatment technologies in order to maximize water reuse within aquaculture projects (Jana *et al*., 2018). Although these early related efforts were key to the development of marine fish production systems, fish farming activities were then introduced in dry areas with limited water availability.

Scientists designed different solutions to maximize water reuse in aquaculture, including highly intensive recirculating systems that include water filtration systems such as drum filters, biological filters, protein skimmers and oxygen injection systems (Badiola *et al*., 2012). Establishment of generally agreed terminologies, units of measurement and reporting standards and formats in 1980 (Colt *et al*., 2006) stabilized the situation. In the mid-1980s, the cycle of water quality parameters was well studied (Weatherley, 1982; Dryden and Weatherley, 1987). It is until this decade when periodically measurement of the concentrations of pH, oxygen, TAN (total ammonia nitrogen), NO_2 (nitrite), BOD (biochemical oxygen demand) and COD (chemical oxygen demand) was made possible. From 1980 to 2000, researches were done accompanied by numerous articles published on the early development of RAS. Rosenthal (1980) as cited by Goddek *et al*. (2019), elaborated on the state of recirculation systems in Western Europe, while Bovendeur *et al*.

(1987) developed a water recirculation system for the culture of high density African catfish. Development of different RAS setups, different biofilter setups, different mechanical filters, use of ozone and ultraviolet radiation for water treatment started in 1990s (Gonçalves and Gagnon, 2011; Van Rijin, 2013).

Optimization of RAS setups in terms of fish carrying capacity, water velocity, pumping speed, aeration, water replacement, feeding regimes etc, started in early 2000 cntinued up to date, with a number of studies done between 2010 and 2020 (Colt, 2006; Badiola *et al*., 2012; Spiliotopoulou *et al*., 2018; Mnyoro *et al*., 2021). Currently, most RAS research are focusing on water quality balance, waste water management (Getting rid of nitrate), energy use and biofilm manipulation (Badiola *et al*., 2018; Xiao *et al*., 2019; Lindholm-Lehto and Vielma, 2019).

Since 2000, fish production from RAS has increased significantly in volume and species diversity. Today, more than 10 species are produced in RAS (i.e. African catfish, eel and trout are the major freshwater species and turbot, seabass and sole are the major marine species) (Dauda *et al*., 2019; Belton *at al*., 2020). Also, in recent years RAS has become a crucial element in the production of larvae and juveniles of diverse species (Hisano *et al*., 2021). While maximum sustainable yields of many aquatic wild stock species have been or will soon be reached, and many species are already overfished, aquaculture production has shifted toward intensive fish farming practices to meet the growing protein demand for the supply of fish for human consumption. RAS is considered a key technology that will help the aquaculture sector to meet the needs for aquatic species over the coming decades (Ebeling and Timmons 2012).

Until 2003, RAS was not adopted by any African country (Dunn, 2004). RAS in Africa was first mentioned in literature in 2004 in Zimbabwe, Namibia, Nigeria, South Africa

and Egypt (Dunn, 2004; Anetekhai *et al*., 2004). Currently, the use of RAS is spreading widely and being adopted by many African countries, with Egypt, Nigeria, Ghana and South Africa leading in adoption of RAS technology (Amponsah and Guilherme, 2021).

1.4.2 Challenges of adopting RAS in developing countries

Recirculating aquaculture system seems to be an expensive production system for small-scale fish farmers. A high initial investment is needed for RAS technology as this technology requires importation of sophisticated units, including drum filter and biofilters. Importation in developing countries involves a lot of paperwork, making it difficult for farmers to manage. Most fish farmers in developing countries do not have sufficient capital (Mzula *et al*., 2021) to support importation and installation of RAS units. Furthermore, RAS is a full-time electricity driven unit and electricity reliability is a challenge in developing countries, especially in the sub-Sahara Africa. A short time electricity failure can result into disaster in RAS. Therefore, as an alternative, investors in RAS are encouraged to use solar energy.

1.4.3 Biofilters designs used in RAS

Fixed bed bioreactors (FBBR), moving bed biological reactors (MBBR's), fluidized bed and floating bead filters are the common aquaculture filtration designs used in RAS (Malone and Pfeiffer, 2006; Sharrer *et al.*, 2010; Ebeling and Timmons, 2012). Moving bed bioreactors or the microbeads filter contain a mix of water and air that move the filtering media through constant motion (Malone and Pfeiffer, 2006). Moving bed bioreactors are often known as three phase reactors. The 3 phases are air, water and bioelements.

RAS filters with static beads (Fixed bed), do not operate in media motion. Water goes through a stationary media bed. Propeller- wash filters, hydraulic filters, and the bubble-

washed are examples of such stationary bioreactor design (Zhang *et al*., 2020). Fluidized beds are suitable under anaerobic conditions. In this design, water is passed through some granular material (beads, sand) at high velocity, forcing the solid to suspended and move with fluid-like properties. Use of hydraulically suspended biomedia is a characteristic feature of fluidized bed reactors (Peirong and Wei, 2013). Fluidized beds are operated to remove soluble components in RAS (Weaver, 2006).

Floating bead filters (FBF's) utilize low-density media like light plastic. The media is prepared for the purpose of floating on the surface of the water column in the reactor. This oval shaped media is intricately carved for crossflow and amplified surface area. This media design enhances an elevated nitrification and, therefore, sometimes it is called Enhanced Nitrification (EN) media (Malone *et al*., 1998; Malone and Beecher, 2000).

Efficiency of biofilter design depends on the intended use in RAS. In comparison trials, MBBR have been found to be better in terms of ammonia and nitrite removal compared to the rest of the biofilters (Choi *et al*., 2012). Fixed bed bioreactors are known to aid in debris capture and removal of finer particles in RAS. Therefore, in RAS containing drum filter and protein skimmers, MBBR are better biofilters to use. Fixed bed bioreactors can be appropriate use in simple RAS set-up without protein skimmers.

1.4.4 Biomedia commonly used in RAS

Different biomedia have been used to provide settlement for bacteria community hence, maximum bacteria attachment. The efficiency of biomedia for nitrification depends on surface area and type of the media used (Pedersen *et al*., 2017). Biomedia can have different shapes, surface areas, and textures.

Most biomedia in the market are made from plastic material, they are made in different shapes and densities to suit the targeted biofilter design. Al-Hafedh *et al.* (2003) evaluating the performance of plastic biofilter media, sliced polyethylene pipes into different shapes and sizes and concluded that the smaller the sizes of the pipe, the higher the nitrification. Also, Al-Hafedh *et al.* (2003) assessed the suitability of sand, rocks, and shells as biofilter media in comparison to plastic and concluded that plastics are good media materials both in terms of TAN and nitrite removal. Polyethylene nets, agrovelo and plastic bottles have been also tested as a way to explore innovative sustainability and recycling options for domestic and agricultural waste products (Viau *et al.,* 2016). In the study by Viau *et al.* (2016) agrovelo generated good water quality, but plastic bottles emerged as the most beneficial substrate because of its reusability. Other biomedia are listed in Table 1.

Table 1: Different types of biomedia used in recirculating aquaculture systems

Media Type	Moving/ fixed bed	Reported TAN removal rate ($g/m^3/d$)	Reference
Kaldnes carrier media of the AnoxKaldnes Company	Fixed/ Moving	267 ± 123	Guerdat *et al*., 2010 Pfeiffer, 2011
Polyethylene dimpled beads	Moving	586 ± 284	Guerdat *et al*., 2010
Silica sand	Moving	667 ± 344	Guerdat *et al*., 2010
Polyethylene 0.32 cm feedstock bead	Moving	752.17 ± 384	
Upflow sand filters	Fixed	150 to 320	Westerman *et al*., 1996
Fluidized bed sand filters	Fixed	250 to 290	Westerman *et al*., 1996
Floating-bead filters	Floating	120 to 160	Westerman *et al*., 1996
Polypropylene aggregate	Moving	300 to 500	Sikora *et al*., 2020
Polyethylene screw caps for PET bottles	Fixed	300 to 400	Sikora *et al*., 2020
Rice hulls	Moving	700 to 1025	Greensword, 2017
Synthetic plastic beads	Moving	300 to 500	Greensword, 2017
Ceramic tubes with finely pitted surface	Fixed	200 to 300	Sajuni, 2011
Japanese filter mat (high quality polyester based material with thick filaments and hard texture)	Fixed	150 to 400	Sajuni, 2011
Filter Wool	Floating	400 to 600	Sajuni, 2011
MB3 media supplied by Waste Management Technology of Baton Rouge, LA, USA,	Moving	250 to 400	Pfeiffer, 2011
AMB media	Moving	300 to 500	Pfeiffer, 2011
Sponge biocarrier	Moving	400 to 700	Zhou *et al*., 2017 Maurya *et al*., 2022 Song *et al*., 2019

Comparative studies on performance of different biomedia have indicated that Kaldness carrier (which is the mostly used biomedia) may not be the most effective media for TAN

removal, bacterial production, and overall nitrification in aquaculture systems (Bellelo *et al*., 2006; Pfeiffer and Riche, 2011), this is because it is not easily affordable (Haandel and Lubbe, 2012), hence, developing countries may not be able to adopt MBBRs in aquaculture industries. Therefore, more research efforts should be put to develop alternative media that are low-cost and environmentally friendly for use in developing countries (Bracino *et al*., 2020).

1.4.5 Biofiltration

Biofiltration is the elimination of dissolved organic waste from the nutrient-rich wastewater via bacterial activity (Swanson and Loehr, 1997). The biofiltration process occurs within biofilters. A biofilter, is the collective environment in which biofiltration process takes place. Biofilters or their components can be artificial or natural. Part or whole of nitrification may take place aerobically or anaerobically (Chaudhary *et al*., 2003). Aerobic and anaerobic processes in filtration membrane reveal comparable Biological Oxygen Demand (BOD) (Bentzon-Tilia *et al*., 2016). However, aerobic biofiltration exhibits higher Chemical Oxygen Demand (COD) and Total Ammonia Nitrogen (TAN) removal efficiencies compared to anaerobic process (Pramanik *et al*., 2012; Permatasari *et al*., 2018). Aerobic bacteria in biofilters have been found to have high capacity to remove carbonous, nitrogenous and phosphorus pollutions at high removal rates with low energy utilization (Rebah *et al.*, 2010). The use of anaerobic filters is, therefore, recommended for less turbid, low COD and BOD water while aerobic biofilters can decontaminate water with high concentrations of organic wastes from fish excretions. Therefore, aerobic filters are principally suitable for aquaculture water filtration because of high TAN loadings.

Aerobic biofiltration in a fixed biomedia includes an organic oxidation process, during which organic wastes are exposed to oxygen. This process provides bacteria enough

oxygen to survive, grow and multiply on the media, thus forming a strong biofilm. Moreover, it is the process by which ammonia-rich excretions from the aquatic system attain its biodegradability (Bracino *et al*., 2020). From the oxidation equation below, the amount of organic carbon produced shows how much oxygen (O_2) is needed for a complete reaction:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad \text{..... (i)}$$

The equation details how heterotrophic bacteria metabolize organic matter ($C_6H_{12}O_6$) with oxygen consumption through a process that produces water (H_2O) and releases gaseous carbon dioxide (CO_2) (Cammack *et al*., 2004). In aerobic biofiltration, nitrogen processing result in an increase in total ammonia nitrogen.

Heterotrophic bacteria are much more prominent and fast growing compared to autotrophic bacteria whose energy for growth is internally produced (Rurangwa and Verdegem, 2015). Autotrophs are the primary nitrifier. These two communities normally compete for substrate, space and for oxygen in the biofilm. This competition leads to the films' layered structure and non-uniform distribution within the biofilm. To support the fast growth of autotrophs, minimal carbon source is supplied into the system while sufficient amount of ammonia substrate is delivered (Bacquet *et al*., 1991). Studies have shown that, nitrification is inhibited when nitrifying bacteria suffer from oxygen shortage, either because of low oxygen supply, high substrate loading or because of competition with heterotrophs (Zhang *et al*., 2019; Zhang *et al*., 2020).

1.4.6 Nitrification in RAS

Nitrification is the process by which ammonia is converted to nitrite (NO_2) and then to nitrate (NO_3) (Ward, 2018). This process naturally occurs in the environment, where it is carried out by specialized bacteria. Organic oxidation helps to facilitate nitrification

because it releases carbon dioxide consumed by these autotrophic bacteria. Nitrification in the bacterial film of a biofilter involves physical, chemical and biological processes controlled by a range of parameters such as substrate concentration, organic matters, dissolved oxygen, alkalinity, pH, temperature, turbulence level and salinity. Oxidized inorganic compounds of nitrogen are the main sources of energy utilized by nitrifying bacteria. Ammonia Oxidizing Bacteria (AOB) obtain their energy by catabolizing un-ionized ammonia to nitrite and include bacteria of the genera *Nitrosomonas* spp., *Nitrosococcus* spp., *Nitrosospira* spp., *Nitrosolobus* spp., and *Nitrosovibrio* spp. Nitrite Oxidizing Bacteria (NOB) oxidize nitrite to nitrate, and include bacteria of the genera *Nitrobacter* spp., *Nitrococcus* spp., *Nitrospira* spp., and *Nitrospina* spp. (Bentzon-Tilia *et al*., 2016).

Nitrification is a two steps process. The two steps are normally carried out sequentially. The first step has a higher kinetic reaction rate than the second step. The overall kinetics is usually controlled by ammonia oxidation and as a result, there should be no appreciable amount of nitrite accumulation (Ward, 2018). During biofilter start/up, ammonia concentration peaks between seven to 14 days, followed by a nitrite peak between 14 and 28 days and nitrate accumulation begins after 21 days as illustrated in Fig. 1. Equations 1.2 shows the overall oxidation reaction occurring during oxidation by *Nitrosomonas* spp. and *Nitrobacter* spp. (Haug and McCarty, 1972). The trend of maturity depends on the type of biomedia used. Pre-seeding a biological filter with both ammonia and nitrite can accelerate this process. For safety, in a new system, a drop in nitrite should be observed as an indication that the biological filtration process is fully activated before stocking fish (Haug and McCarty, 1972).

$NH_4^+ + 1.83\ O_2 + 1.97\ HCO_3^- \rightarrow 0.0244\ C_5H_7O_2N + 0.976\ NO_3^- + 2.90\ H_2O + 1.86\ CO_2$ …….(ii)

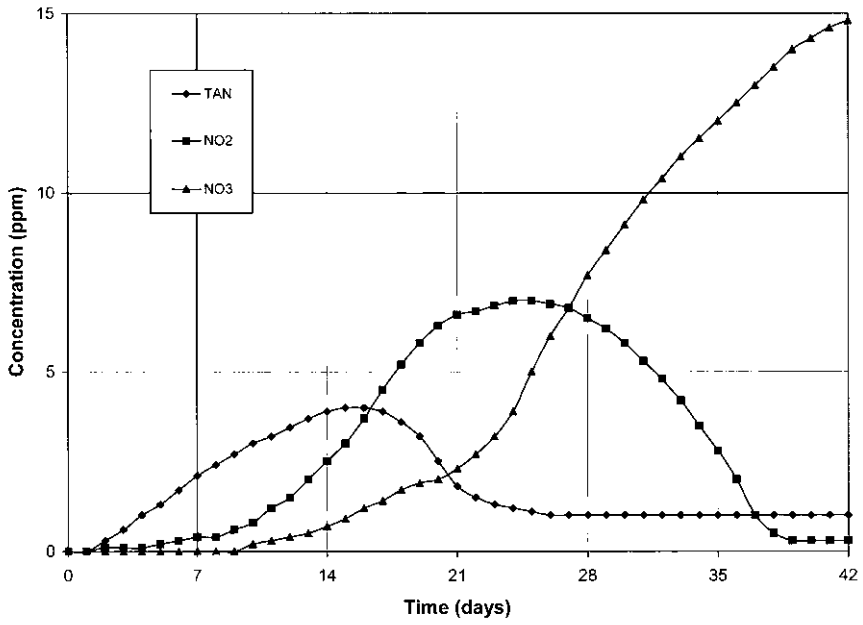

Figure 1: Typical start-up curve for a biological filter (Ebeling *et al.*, 2006)

The movement of nitrogen in recirculation aquaculture system water is facilitated by a diverse group of microorganisms. Researchers have studied and characterized a large number of these nitrifier communities (Chen *et al.*, 2018; Ruiz *et al.*, 2020). Li *et al.* (2018) carried out a phylogenetic identification of nitrogen carrier organisms and identified a cluster of 19 operational taxonomic units (OTUs) from the Proteobacteria and Planctomycetes bacterial library in aquaculture systems. Bacterial biodiversity is vital to guarantee active nitrification. Gao *et al.* (2012) studied the bacterial diversity in recirculation aquaculture systems based on the type of biofilm and found that salty and freshwater affect nitrifying bacterial community composition. Population of nitrifying bacteria in aquatic systems differ according to the cultured species in the production system.

Recently, it has been discovered that there are bacteria species that can perform direct oxidation of ammonia to nitrate (Sobotka *et al.*, 2018). These are bacteria within the genus *Nitrospira* commonly known as Comammox. On the other hand, Comammox have

been found to perform sequential oxidation of ammonia to nitrate via nitrite and this can be performed within a single bacterial cell (Hu and He, 2017). Studies show that comammox *Nitrospira* exhibit a diverse distribution in natural and engineered ecosystems. However, information on the functional properties of comammox *Nitrospira* is limited because of a few numbers of studies (Bentzon-Tilia *et al*., 2016; Hu and He, 2017).

Factors affecting nitrification

Studies on different bioelements used in biofiltration have shown difference in nitrification capacity, even when the bioelements are made of similar materials (Hockenbury *et al*., 1977; Sahrawat, 2008; O'Sullivan *et al*., 2013). A study done by Scott (2002) on nitrification rates of three different types of plastic media based on organic loading revealed that media type is an influential factor on bacterial activity. Nitrifying bacteria feed on nitrogen to facilitate their growth and proliferation. Therefore, the presence of TAN substrate in the system is vital for efficient nitrification. Numerous factors affect nitrification and the nitrifying bacterial activity, including water temperature, alkalinity, salinity, ammonia, dissolved oxygen, pH, turbulence and organics.

Temperature

Water temperature is vital in the nitrification reaction rate and suspended growth systems as it does in all chemical and biological kinetic reactions. Few researches have studied and quantified the effects of temperature on nitrification rates in both fixed and moving bed biofilters (Okey and Albertson, 1989; Zhang *et al*., 2014). Zhu and Chen (2002) studied the impact of temperature on nitrification rates in laboratory experiments, mathematical modeling, and sensitivity analysis. Their studies showed that if there is no oxygen limitation, temperatures from 14 to 27 °C had no significant impact on nitrification rate in a fixed film bioreactor. According to Wang *et al.* (2020), although

originally assumed to be an important factor in biofilter design, temperature is increasingly being viewed as a minor factor in controlling biofilter carrying capacities. This is because there is a wide range of optimum temperatures reported for nitrification (Chen *et al*., 2006; Wang *et al*., 2020), suggesting that nitrifying bacteria are able to adapt to a wide range of environmental temperature, if acclimatized slowly. In practical application, however, the temperature at which the biofilter operates is normally determined by the requirements of the species being cultured, not by the needs of the biofilter bacteria.

Alkalinity

Nitrification process results into acidic condition in the aquatic system. This lowers the water pH and, therefore, buffering is important to ensure that the performance of biofilters is not impaired by the acidic condition. Alkalinity is a measure of the buffering capacity of an aquatic system. Studies have shown that for every gram of ammonia-nitrogen reduced to nitrate-nitrogen, 7.05 gram of alkalinity is consumed (Chen *et al*., 2006). The alkalinity utilized during nitrification reaction in aquatic systems is easily made up by adding sodium bicarbonate ($NaHCO_3$), commonly known as baking soda, or any other available bicarbonate supplement depending on the environment of operation (Wang *et al*., 2020). Research shows that a baking soda supplement of 20 to 25% of the feed provided to the culture system is sufficient to provide the alkalinity needed for total nitrification of the resultant nitrogen (Labib *et al*., 1996).

Salinity

Nitrification takes place in a wide range of salinity. Biofilters can adapt to the salinity in which they are placed to operate. Salinity is similar to both temperature and pH, in that nitrifying bacteria can acclimatize to almost any salinity range, given sufficient time to

acclimatize. Research has shown that it takes significantly longer time to fully acclimate a biofilter in salt water than in fresh water. Buhmann *et al.* (2015) reported that in commercial fish farms operating at a salinity of 21 - 24 ppt, the nitrification rate is approximately 60% of what would be expected in a freshwater system for Moving Bed Bio-Reactors (MBBR). Research has revealed that, an increase in salinity during biofilter performance can significantly affect nitrification negatively (Kinyage, 2019). Abrupt variation in salinity of greater than 5 g/L, will shock nitrifying bacteria and decrease the reaction rate for both ammonia-nitrogen and nitrite-nitrogen removal (Chen *et al*., 2006).

Ammonia

Ammonia, as a substrate of the nitrification process, directly affects nitrification rate (Chen *et al*., 2006). Nitrification performance of any biofilter increases proportionally with increase of ammonia concentration. This linear, proportional relationship exists from very low ammonia concentrations to the concentration between 2 and 3 mg/L (Rutting *et al*., 2021). At elevated ammonia concentration, research shows that the proportional relationship decreases and eventually constant ammonia removal rate is attained. There is some evidence in the literature that at extremely high concentrations of ammonia and nitrite and much above any expected concentrations that will be seen in aquaculture applications, accumulating ammonia will become inhibiting to nitrification (Kim *et al*., 2008).

Dissolved Oxygen

Oxygen is likely to limit any biochemical reaction. Likewise, in recirculating aquaculture systems, dissolved oxygen is a rate-limiting factor in certain biofilters (Chen *et al*., 2006; Wang *et al*., 2020). In some instances, it has been reported that a dissolved oxygen concentration in excess of 4.0 mg l^{-1} is required to achieve maximum nitrification rates

(Yorkor and Momoh, 2019). Research has shown that, for every gram of ammonia-nitrogen oxidized to nitrate-nitrogen, 4.57 g of oxygen is required (Ebeling *et al*., 2006; Yorkor and Momoh, 2019). Cui *et al*. (2020) studied the effect of dissolved oxygen on a mixed bio elements reactor and found a non-significant effect on growth rate of *Nitrosomonas* spp. at dissolved oxygen levels above 2.0 mg/L. *Nitrobacter* bacteria were found to exhibit a significantly reduced growth rate at dissolved oxygen levels less than 4 mg/L.

Turbulence

Turbulence is an irregular motion of water resulting from variation in water flow velocity and pressure. Turbulence affects the thickness of the stagnant water film covering the bacteria and thus, the transfer rate of the nutrients from the bulk liquid into the biofilm for effective nitrification. Ammonia removal rate, therefore, increases with increase in turbulence (water flow and velocity) through the filter (Kugaprasatham *et al*., 1992; Mnyoro *et al*., 2021; Zhu *et al*., 2021). Prehn *et al*. 2012 exposed active nitrifying biofilter units from a RAS to a range of hydraulic flow velocities and quantified the corresponding nitrification rates. The study proved that nitrification performance of biofilters could be significantly increased by increasing the hydraulic flow velocity in the filter. Excessive shear (high water velocity) or abrasion (sand particles) would be assumed to have a negative impact on biofilm growth and film thickness.

Organics

Normally, the effluent from RAS have a considerable amount of both dissolved and suspended particulate matter. Organic matter provides food for heterotrophic bacteria, which, in turn, show a higher growth rate and may out compete the autotrophic bacteria and consequently affect nitrification. Chen *et al*. (2006) observed an exponential decrease

in nitrification rate with the increase in Chemical Oxygen Demand and Nitrogen (COD/N) ratio in a laboratory study using a chemically fed reactor series system with a floating bead filter, a fluidized sand filter, and a submerged biocube filter. In recirculating aquaculture systems, organic matter has to be removed immediately and continuously by use of drum filtration or fixed bed filtration (Schumann *et al*., 2017).

1.4.7 Denitrification

Nitrate removal in aquaculture systems is mainly accomplished by bacterial denitrification and water exchange (Van Rijn, 2013; Moisescu *et al*., 2018). Denitrification is a process whereby microorganisms are directly involved in reducing nitrate (NO_3^-) to molecular nitrogen (N_2) through a series of intermediate gaseous nitrogen oxide products (Golterman, 2013). This process is performed by facultative anaerobic bacteria by reducing oxidized forms of nitrogen. Although nitrate has a mild effect on fish development, efforts are made to reduce over accumulation by partly replacing system water up to 10% daily or installation of denitrification plant within the RAS. The most common reported condition associated with high accumulation of nitrite concentrations in RAS is the formation of nitrite resulting from incomplete reduction of nitrate under oxygen deficient conditions (Seitzinger, 2018).

Nitrate pollution is known to have a negative effect on human health. Reports show that high nitrate concentrations in drinking water pose a high risk to human, especially infants (Bednarek *et al.,* 2014; Parvizishad *et al*., 2017; Rodgers and De Boeck, 2019). Chamandoost *et al.* (2016) reported abortion in pregnant women exposed to drinking water with high nitrate levels. Unrestrained release of nitrogen compounds into the water ecosystem impairs nutrient balance in the environment, leading to eutrophication, which is currently a global pandemic. Eutrophication leads to surface water hypoxia resulting in

the death of coral reefs and various other aquatic animals (Scavia *et al*., 2014). These ecological and health related impacts of nitrate drives the world into strong regulations on nitrate discharge. In many countries, the acceptable nitrate levels in effluent water are set below 11.6 mg NO_3-N/l (Kumar and Puri, 2012; Mohseni-Bandpi *et al*., 2013).

Denitrifying bacteria

Denitrification is mainly facilitated by bacteria from the genera Pseudomonas, Alcaligenes, Paracoccus and Bacillus (Herreros and Letelier-Gordo, 2017). Addition of carbon sources in denitrification reactors control the composition of bacteria. The complexity of the added carbon compounds determines the diversity of denitrifying bacteria colonizing the reactors (Adouani *et al*., 2010). In view of the large diversity of denitrifiers, denitrification takes place at a wide range of environmental conditions (temperature, salinity, etc.). Unlike nitrification, where the species diversity is narrow, single environmental determinants do not have a significant effect on denitrification (Adouani *et al*., 2010). Nitrite accumulation by denitrification is extremely toxic to fish. It is therefore, important to understand the factors underlying nitrite accumulation during this process. Hypoxia condition during denitrification leads to nitrite accumulation resulting from differential repression of nitrite reductase synthesis activity, compared to nitrate reductase. pH fluctuations during denitrification have been found to affect nitrite accumulation (Pan *et al*., 2012; Cao *et al*., 2013).

1.5 General Methodology

1.5.1 Study area

This thesis contains four experiments, which were conducted in two different countries. Experiment one and four were done at the Section for Aquaculture, The North Sea Research Centre, Technical University of Denmark (DTU Aqua), Hirtshals, Denmark.

This institute is situated at the top of the Jutland peninsula, Hjørring municipality in Nordjylland region, northern Denmark, Europe. Experiment one and four were conducted under climate-controlled conditions and, therefore, were not affected by the external conditions. Experiment two and three were conducted at Magadu fish research station (Fish farm), Department of Animal, Aquaculture and Range Sciences, Sokoine University of Agriculture, Morogoro, Tanzania. The climatic condition of Morogoro is characterized by bimodal rainfall pattern, with short rains received from November to December and long rains starting in March and ending in June. Magadu area receives 767 mm rainfall per annum. Relative humidity and temperature ranges from 30 to 96 % and 26 to 35.5^{o}C, respectively, (TMA, 2020).

1.5.2 Study design

A completely randomised experimental design was applied in all the experiments. Throughout this study, four experiments were conducted. Before developing a simple RAS, which is comprised of the main fish tank, mechanical filter and an upward biofilter, there was a need to identify working conditions for the simple RAS. Therefore, experiment one was conducted to find out the optimal biofilter flow velocity. In this experiment, three replicates were used to test different flow velocities. The findings from experiment one were used as the baseline for water circulation in the pilot scale RAS that was developed in trial two. The pilot scale RAS was developed following the standards set for an ideal RAS (Colt, 2006). This experiment tested the performance of the developed pilot scale RAS in terms of ammonia and nitrite removal as well as tilapia growth performance in the RAS culture unit. The developed RAS units were used in experiment three whereby, different locally available materials were tested as biomedia for removing ammonia and nitrite in the RAS unit. The third experiment led to the identification of coconut shell as a better locally available biomedia with similar

performance as commercial plastic biomedia. The coconut shells were then used in the fourth experiment in which they were compared with other commercial biomedia in terms of ammonia and nitrite removal. In this experiment more parameters like COD, BOD and H_2O_2 degradation were also assessed.

1.5.3 Organization of the Thesis

This thesis is prepared according to "Publishable Manuscripts" format of the Sokoine University of Agriculture. It is organized into seven chapters precede by an extended abstract that summarizes the objectives, materials and methods, principal research findings and conclusion of this study. Chapter one consists of the general introduction that covers background information on aquaculture, Recirculating Aquaculture Systems (RAS), the history of RAS, challenges of adopting RAS in developing countries, biofilters designs used in RAS, biomedia (Bio-elements) commonly used in RAS, problem statement and justification of the study and objectives. Chapter one also covers Literature review on biofiltration, nitrification in RAS, factors affecting nitrification, denitrification and denitrifying bacteria. The last part of chapter one contains general methodology, which describes; study area, study design and organization of the thesis.

Chapter two, three, four and five presents the results obtained from each specific objective which are synthesized into either published papers (paper I and III), or publishable manuscripts (Paper II and IV) submitted for publication in peer reviewed scientific journals. Chapter six covers general discussion of the results. Chapter seven covers key findings, general conclusions and recommendations. The format and writing style of published papers were according to the requirements of respective journals.

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CHAPTER TWO

PAPER ONE

Aquacultural Engineering 93 (2021) 102160

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Aquacultural Engineering

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https://doi.org/10.1016/j.aquaeng.2021.102160

Received 16 July 2020; Received in revised form 1 March 2021; Accepted 19 March 2021. Available online 27 March 2021.

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ARTICLE INFO

Keywords:
Nitrification
Biofiltration
Water velocity
Hydraulics

ABSTRACT

The effect of water velocity on nitrification rates in fixed bed biofilters was investigated in three freshwater pilot scale RAS with rainbow trout. Removal of total ammonia nitrogen (TAN) and nitrite-nitrogen were assessed by NH_4Cl spikes and tested at four different water velocities in the biofilters (1.4, 5.4, 10.8 and 16.2 m h^{-1}) under identical conditions. Water velocities below 10.8 m h^{-1} significantly reduced TAN- and nitrite removal rates. The surface specific TAN removal rates correlated with the TAN concentrations at the water velocities 10.8 and 16.2 m h^{-1}, and the first order surface removal rate constant was estimated at 0.45 m h^{-1}. However, no correlations between TAN removal and TAN concentrations were found at the lowest velocities. Up to five-fold elevated nitrite levels were found in the RAS when biofilters were operated at 1.4 m h^{-1} compared to the trials at other water velocities, substantiating the significant effect of water velocity on both nitrification processes. The importance of biofilter hydraulics documented in this pilot scale RAS probably have implications for design and operation in larger scale RAS.

1. Introduction

Recirculation aquaculture technology allows rearing of fish at high densities under controlled conditions with reduced water consumption (Losordo et al., 1998; Piedrahita, 2003; Lekang, 2020). Conversion of excreted ammonium is crucial for optimal water quality in recirculation aquaculture systems (RAS) and takes place in nitrifying biofilters (Colt et al., 2006; Badiola et al., 2012). Here microbiological processes are performed by autotrophic nitrifying bacteria (Hagopian and Riley, 1998; Espinal, and Matulić, 2019) that colonizes surfaces on carrier materials inside biofilter units Biofiltration sets high demands for volume, dimensioning and management to achieve optimal performance and hence stable water quality (Drennan et al., 2006; Pedersen et al., 2015; Xiao et al., 2019). There is an ongoing need to investigate factors affecting nitrification under real conditions to optimize design and prevent accumulation of TAN and nitrite (Emparanza, 2009; Gutierrez-Wing et al., 2014).Various factors affect the nitrification performance (Chen et al., 2006; Malone and Pfeiffer, 2006; Rusten et al., 2006; Kinyage et al., 2019), in particular the concentration of the directly involved compounds: total ammonium nitrogen (TAN), bicarbonate and oxygen. The nitrification process takes place in the biofilm, requiring mass transport of these three substances from the water bulk phase via the diffusive boundary layer into the biofilm (Szwerinski et al., 1986; Guimerà et al., 2016). This process is highly affected by the water flow and turbulence (Chen et al., 2006; Kamstra et al., 2017). Despite the significance of hydraulic on biofilter performance, relatively few studies have been made. Prehn et al. (2012) showed a significant positive effect of water velocity up to 40 m h^{-1}on TAN removal under TAN limiting conditions in a bench scale setup. Some commercial RAS operate with elevation speeds in biofilters above 30 m h^{-1} (*Personal obs.*) while pilot scale and lab biofilters typically have much lower flow rates. The aim of this study was to evaluate the effect of water velocity on TAN and nitrite removal at TAN limiting conditions in pilot scale RAS. This was investigated under controlled conditions using fixed bed biofilters with colonized carrier elements connected to a pilot scale freshwater RAS with rainbow trout.

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https://doi.org/10.1016/j.aquaeng.2021.102160
Received 16 July 2020; Received in revised form 1 March 2021; Accepted 19 March 2021
Available online 27 March 2021

Table 2
Mean water quality parameters in the triplicate RAS after 25 days of initial acclimatization under constant conditions.

	Temp. °C	Dissolved oxygen		pH	Alkalinity (mg/L $CaCO_3$)	NH_4 –N (mg N/L)	NO_2 -N (mg N/l)	NO_3 -N (mg N/L)
		(%) sat.	mg/L					
RAS 1	19.6 ± 0.6	110 ± 6	10.1 ± 0.3	7.8 ± 0.6	185 ± 40	0.35 ± 0.2	0.8 ± 0.4	132 ± 12
RAS 2	19.3 ± 0.7	103 ± 8	9.5 ± 0.4	7.9 ± 0.4	219 ± 34	0.27 ± 0.1	0.7 ± 0.3	126 ± 21
RAS 3	19.1 ± 0.7	100 ± 9	9.3 ± 0.5	7.9 ± 0.4	222 ± 28	0.22 ± 0.1	0.8 ± 0.3	123 ± 22

$$STr = (([TAN]_{in} - [TAN]_{out}) * Q)/A_m, \quad (1)$$

Where [TAN] is the Total Ammonium Nitrogen concentration (g N m^{-3}) from in- and outlet of the specific biofilter; Q is the flow (m^3 d^{-1}) and A_m is the nominal surface area of the carrier elements (m^2).

The total surface NO_2^--N removal rate (SNr) taking the de facto oxidized TAN contribution into account was be calculated as:

$$SNr = ([NO_2^-\text{-N}]_{in} + ([TAN]in - [TAN]out) - [NO_2^-\text{-N}]out) * Q/A_m \quad (2)$$

Analysis of variance (one-way ANOVA) was done for both STr and SNr results from all treatments by using R statistical programme (Chambers, 2008). Differences between treatment means were considered significant at $P \leq 0.05$.

3. Results and discussion

3.1. TAN removal rates at different water velocities

The TAN concentrations in the tanks dropped within a 12 h. period following NH_4Cl spiking, and the water velocity in the biofilters significantly affected the TAN removal (Fig. 2). The TAN concentrations measured were a result of two processes: 1) the removal of TAN in the biofilters and 2) the excretion of TAN from the fish. The TAN contribution is considered being equal in all trials as the fish were fed similar fixed amounts in a fixed period of time (Dalsgaard et al., 2015). The calculated TAN removal rates ranged from 0.11 ± 0.03 g N m^{-2} d^{-1} to 0.30 ± 0.02 g N m^{-2} d^{-1}, and water velocities below 10.8 m h^{-1}significantly reduced the TAN removal (Fig. 3).

A significant positive correlation between TAN concentration and corresponding STr was found at the highest water velocities tested, 10.8 and 16.2 m h^{-1} ($p < 0.01$; Fig. 4). However, no correlation or even a negative correlation was found at 5.4 and 1.4 m h^{-1}, respectively (data not shown). The regression coefficient at 0.45 m h^{-1} (Fig. 4) reflects the first order surface removal rate constant for TAN, which is an important process parameter when operating aquaculture biofilters at low, TAN limited conditions. The study by Von Ahnen et al. (2015) showed significant effects of N-loading on the zero order TAN removal rate in biofilters connected to flow through systems, but that was not observed under TAN limited conditions. There, the first-order rate constants for TAN were similar and not affected by N-loading levels (0.13 to 0.17 m d^{-1}) and calculated based on spiking trials with a constant flow (0.05 m^3

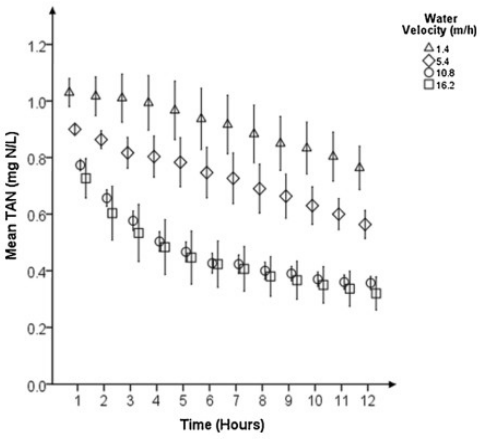

Fig. 2. Total ammonia-nitrogen (TAN) concentration after spiking with NH_4Cl (expected nominal TAN concentration of 1 mg TAN/L) at different water velocities in the biofilter. Each symbol represents mean ± SD; n = 3.

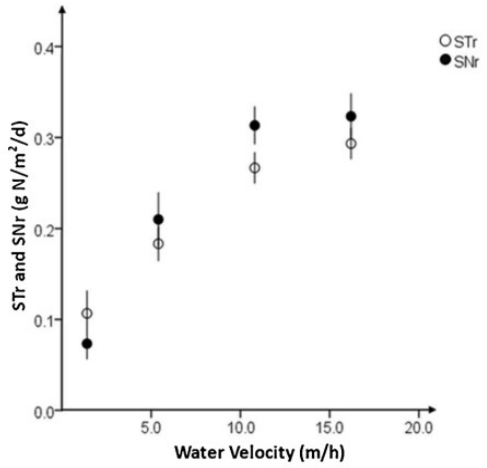

Fig. 3. Average surface TAN (STr) and nitrite removal (SNr) rates (g N/m^2/d) at different water velocities (m/h) during the 12 h spiking trial (mean ± SD; n = 3).

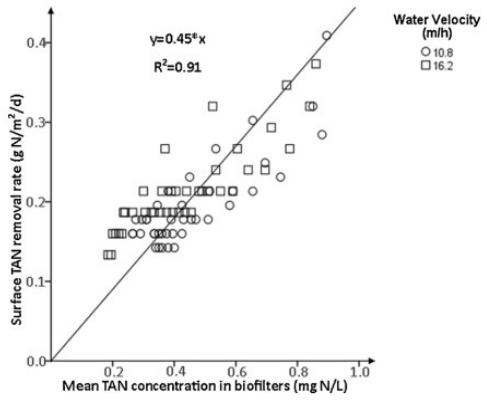

Fig. 4. Linear regression of the calculated surface TAN removal rates (STr) vs. mean TAN concentration ((TAN_{Inlet}+TAN_{outlet})/2) in the biofilters based on data from trials with 10.8 m h^{-1} and 16.2 m h^{-1} water velocity (n = 72). The slope of the line, 0.45 m d^{-1}, is the first order removal rate constant.

h^{-1}). The pore velocity was not measured but estimated to be below 4 m h^{-1} based on the specifications provided. Our present study included random order of the flows tested, but as it did not include subsequent repeated measurements of the flows, we cannot rule out any temporal or independent effect. Ideally, this should be considered in future studies

Previous studies have described the positive effect of increased mixing, flow and turbulence on nitrification rates (Stoodley et al., 1997; Zhu and Chen, 2003; Prehn et al., 2012; Lopato et al., 2013; Nogueira et al., 2015), but rarely under real aquaculture operational conditions. Kumar et al. (2011) reported significant effects of water flow on TAN- and nitrite removal in a marine RAS, however, information of biofilter pore velocities was not provided. Kamstra et al. (2017) observed a significant positive effect of superficial air velocity on TAN removal rate in moving bed biofilm reactors (MBBR) in RAS. An air velocity below a threshold of 5 m h^{-1} decreased TAN removal at both small and medium scale. These findings emphasizes that mixing and thereby a reduction of the boundary layer increases diffusion of TAN, oxygen and bicarbonate into the biofilm which results in improved TAN oxidation (Szwerinski et al., 1986; Suhr and Pedersen, 2010).

The significant positive effect of water velocity on nitrification in the biofilters is a consequence of the boundary layer which becomes thinner and thereby eases mass transport from the water phase into the biofilm (Prehn et al., 2012). A potential additional parameter which were not quantified in our study is channeling; where only parts of the biofilter is active (Ozis et al., 2007). The lower the velocity, the more pronounced is the channeling and seems to be negligible above 10.8 m h^{-1} in the present setup. Further studies are needed to test hydraulics and optimal water velocities in commercial scale fixed bed biofilter designs (Gutierrez-Wing et al., 2014). Provided that water flows can increase TAN removal to a certain threshold, smaller biofilter units with powerful pumps could be considered as part of the ongoing development of RAS technology. Oxygen and alkalinity will be transferred more efficiently than during suboptimal, heterogeneous hydraulic conditions and the risk of anaerobic zones will be reduced. The drawbacks of increased flow include reduced particle removal and the liberation of biofilm formed.

3.2. Nitrite accumulation and removal rates at different biofilter inflow rates

Average surface nitrite removal rates (SNr) increased with increased water velocity from 0.11 g NO_2-N m^{-2} d^{-1} at the velocity 1.4 m h^{-1} to 0.33 g NO_2-N m^{-2} d^{-1} at the water velocity 16.2 m h^{-1} as shown on Fig. 3. Between water velocities 10.8 m h^{-1} and 16.2 m h^{-1}, nitrite removal rate (SNr) was not significantly different.

Despite the experimental systems being sufficiently supplied with bicarbonate and oxygen (Table 2, Prehn et al., 2012), changing the water velocity from 5.4 m h^{-1} to 1.4 m h^{-1} demonstrated gradual, significant increase in nitrite concentration in the rearing tank from 0.8 ± 0.2 g NO_2-N m^{-3} on the first day to an average of 3.4 ± 0.7 g NO_2-N m^{-3} on the third day (spiking day) (Fig. 5). All three replicates at 1.4 m h^{-1} were found to have constant nitrite levels during the 12 h spike but significant differences in the nitrite background levels. Sufficient alkalinity and oxygen saturation above 80 % saturation in the effluents from the biofilter were measured, suggesting differences in hydraulics (channeling or preferential flow) to be involved in the results obtained.

The observed nitrite accumulation in the present setup is in accordance with the general perception that nitrite oxidation is more sensitive and thus easier affected by environmental disturbances than ammonium oxidation; an effect that has been reported previously (Schwartz et al., 2000 and Timmons et al., 2002; Pedersen et al., 2009).

At the water velocities 5.4–16.2 m h^{-1}, the nitrite concentrations in the rearing tanks at the beginning of each trial were 0.65 ± 0.16 g NO_2-N m^{-3}. These concentrations were significantly lower ($p < 0.05$) than the 3.47 ± 0.18 g NO_2-N m^{-3} measured at lowest velocity (1.4 m h^{-1}, Fig. 5). The cause of the substantial difference in nitrite background concentrations within the three pilot scale RAS at 1.4 m h^{-1} remains unknown.

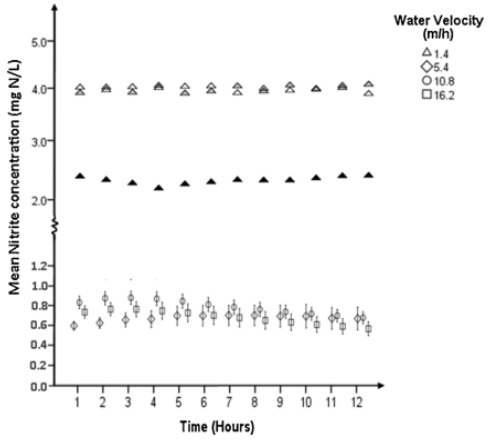

Fig. 5. Mean concentration ± std. dev. of nitrite-N in the rearing tanks during TAN spiking at different water velocities at 5.4, 10.8 and 16.2 m h^{-1} in triplicates. Nitrite values are depicted from each of the three trials at 1.4 m h^{-1}.

Despite these severely elevated nitrite levels (Svobodova et al., 2005; Ciji and Akhtar, 2020) during that particular trial, fish behavior or appetite remained unchanged and no fish mortality was observed during the entire study. Elevated chloride concentrations, in the tap municipal water used, is assumed to have had a protective effect (Gutiérrez et al., 2019).

4. Conclusion

This study has verified under near-practice conditions that water velocity strongly affects the TAN removal rate in pilot scale fixed bed reactors. An increase in biofilter water velocity from 1.4 m h^{-1} to 16.2 m h^{-1} tripled the nitrification rate from an average of 0.1 to 0.3 g N $m^{-2}d^{-1}$. At the low velocities, 1.4 m h^{-1} and 5.4 m h^{-1}, the TAN concentration surprisingly had very limited effect on the nitrification rate. At 10.8 m h^{-1} and 16.2 m h^{-1}, a first order nitrification rate was observed with a surface removal rate coefficient of 0.45 m d^{-1}.

The nitrite concentrations in the pilot scale RASs increased from approx. 0.8 up to 4.0 g NO_2-N m^{-3} as water velocity was reduced from 5.4 m h^{-1} to 1.4 m h^{-1}. These elevated nitrite levels and the associated variation between RAS ceased again when the water velocities were increased to 10.8 and 16.2 m h^{-1}.

Author contributions

L.F.P. and M.S.M. conceived the idea for the research. M.S.M. and L. F.P. designed the trial. S.W.C. ensured funding for the research and exchange. M.S.M. conducted the experiments, collected the samples and performed the laboratory analyses. M.S.M., EA, L.F.P. and R.N.M. processed and analyzed the data and wrote the draft. All authors reviewed the final version of the manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

The technical and analytical support by Jens H. Nedergaard, Ole Madvig Larsen, Brian Møller, Ulla Sproegel and Carina Bebe Jensen, all at DTU Aqua Section for Aquaculture, Hirtshals, is highly appreciated. The study was funded by Danida: "Building Stronger Universities Phase

III (BSUIII).

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CHAPTER THREE

PAPER TWO

Low-cost Recirculating Aquaculture System for Small-scale farmers in Developing Countries

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STATUS: submitted to the Journal of Applied Aquaculture

Abstract

Recirculation aquaculture system (RAS) is a method of fish farming, whereby fish are reared at high densities. In RAS, water is continuously cleaned and reused and fish are produced under controlled environment. Recirculation aquaculture system has a big potential for increasing fish production and meet the ever-increasing demand for seafoods. However, the costs associated with construction of RAS is exorbitantly high, and small-scale farmers in developing countries cannot afford. With the focus of establishing simple and low-cost RAS, this study designed and constructed a pilot scale RAS ideal for small-scale farmers of the developing countries.

Two pilot RAS units, each with 900 L of water in circulation were constructed and operated for 10 weeks. Six types of biomedia made from different locally available biomedia were installed in each RAS. Synthetic ammonia and nitrite were added in the first four weeks, after which 20 000 g bulk weight of Nile tilapia was stocked in each RAS unit.

Water quality parameters were measured, fish growth rate assessed, volumetric TAN and nitrite conversion rate determined. Each RAS unit was found to cost TZS 2 125 200.00 (~ $ 1000). The average water quality parameters throughout the study were 179.09 ± 85.6 mg $CaCO_3$/L, 6.18 ± 0.8 mg/L, 7.59 ± 0.4, 24.69 ± 1.1 °C, 197.23 ± 92.2 mg/L and 0.20 ± 0.1 ppt for alkalinity, dissolved oxygen, pH, temperature, total dissolved solids and salinity, respectively. The stocked fish biomass increased by nine kilogram in each tank for a period of six weeks. Fish mortality was very low (1.25%). At the start of the experiment (week one to three), ammonia and nitrite accumulated in the systems to a maximum concentration of 3.8 mg/L and 3.7 mg/L, respectively. TAN and nitrite concentrations in the system later dropped and stabilized at 1.2 mg/L and 0.8 mg/L, respectively. Volumetric TAN and nitrite conversion rate exponentially increased from the second week and became stable after the 6^{th} week of the experiment with average concentrations of 450 g/m^3/d and 100 g/m^3/d, respectively. The performance of these pilot RAS units was found to be similar to other standard RASs in terms of ammonia and nitrite removal. Therefore, it is ideal for intensive fish culture in developing countries.

Keywords; Aquaculture intensification, Local biomedia, water quality parameters, volumetric TAN conversion rate

3.0 INTRODUCTION

In recent years, aquaculture has expanded and developed rapidly in response to increased global demand for fish products for food and research purposes (Stentiford *et al*., 2020; Suantika *et al*., 2020; FAO, 2020). Because of the need to meet the demand of animal protein, aquaculture producers choose to intensify production using high stocking densities, many times above the carrying capacity of their systems in order to increase productivity. In recent years, aquaculture practice has been greatly transformed from semi intensive production to intensive production systems (Pillay and Kutty, 2005; Hai *et al*., 2018).

Intensive aquaculture systems require sustainable availability of high degree of technical and management skill (Ebeling and Timmons, 2012) in order to enable fish to be produced on a fixed input budget and thus, leading to predictable output volume which corresponds with the market needs and production target (Kazmierczak and Caffey, 1995). Intensive systems apply high stocking levels and high feeding rates so as to maximize the production. Stocking density in RAS is dictated by the efficiency of the biofilter to be used (Ebeling and Timmons, 2012). Under intensive systems water need to be treated in order to maintain the quality of the water by controlling the levels of oxygen, carbon dioxide, organic and inorganic solids and dissolved compounds. Disease prevention and control are as well vital in intensive systems (Oddsson, 2020).

The most common intensive aquaculture production systems adopted in Africa include the cage system technology, raceway technology and recirculating system (Maulu *et al*., 2019; Adeleke *et al*., 2021). Unlike intensive earthen ponds, these systems operates to strike a balance between high productivity, water quality parameters, reduced water

exchange and greater biosecurity measures (Aalimahmoudi *et al*., 2017). Among the intensive aquaculture production systems, recirculating aquaculture system (RAS) is the most promising aquaculture production system. Recirculating aquaculture system can be practiced in a range of scales from small-scale to large-scale and from closed systems to open systems (Piedrahita, 2003; Ahmed *et al*., 2019; Angel *et al*., 2019; Bergman *et al*., 2020; Stentiford *et al*., 2020).

Recirculating aquaculture systems have the advantage of reducing the amount of water and physical space used, improving waste management, increasing fish production per cubic meter and is considered to be environmentally friendly and sustainable (Zhang *et al*., 2011; Pulkkinen *et al*., 2018; Calone *et al*., 2019). Recirculating aquaculture system has emerged to be a reliable production system in establishing stable water quality parameters such as temperature, pH, dissolved oxygen and alkalinity (Pulkkinen *et al*., 2018; Holan *et al*., 2020). Compared to other aquaculture production systems, recirculating aquaculture system is considered to be more bio-secure because it allows greater control of water quality, avoiding possible environmental contamination through aquaculture effluent water discharges (Yanong and Erlacher-Reid, 2012; Rurangwa and Verdegem, 2015; Muthu *et al*., 2020). In addition, recirculating aquaculture system greatly prevents escape of exotic species to natural aquatic environments. This minimizes the risk of interfering with the natural biodiversity and prevents the spread of pathogens and consequently diseases (Yanong and Erlacher-Reid, 2012).

Recirculating aquaculture system (RAS) technology have gained popularity as it is highly productive and environmentally friendly land based closed fish farming systems (Martins *et al.*, 2010; Bregnballe, 2015). The supremacy of RAS to other aquaculture production system such as ponds, flow through and cage is because of treatment of nitrogenous

wastes using biofilter component (Lekang, 2013; van Rijn, 2013). Biofilters transform harmful form of nitrogen; ammonia and nitrite to less harmful form through nitrification process using bacteria; *Nitrosomonas* spp. and *Nitrobactor* spp. as nitrifying agents (Ødegaard, 2006; Suhr and Pedersen, 2010; Timmons and Ebeling, 2010).

Aquaculture production in Tanzania is practiced in numerous small earthen ponds all over the country (Rukanda and Sigurgeirsson, 2018; Mulokozi *et al*., 2020). Freshwater aquaculture dominates the subsector and at the moment there are about 26,445 earthen ponds producing over 18,075.6 metric tons of fish yearly (URT, 2020). Despite all the effort in aquaculture development, fish production from inland aquaculture is still very low (FAO, 2020). Therefore, aquaculture needs to be improved in such a way that it can contribute significantly to the national economy and food security. Adoption of low-cost and simple RAS technologies can result into increased fish production from aquaculture. However, intensification by using recirculating aquaculture system is not widely practiced in Tanzania. This is because information on RAS practices in Tanzania is quite marginal and limited to research institutes (Matondo and Mtalika, 2018; Senff *et al*., 2020). The high cost of investment and availability of RAS components including biomedia as it has been mentioned in other developing countries (Helfrich and Libey, 1991; Losordo and Westerman, 1994), are suspected to be the major reasons for the slow development of RASs in Tanzania.

This study developed a recirculating aquaculture system ideal for Tanzanian environment, and other developing countries, that can be used for indoor fish production by small-scale farmers. The developed pilot scale RAS uses locally available biomedia selected based on easy of availability and being not easily degradable, taking into account the basic requirements and standards set for an ideal RAS (Colt *et al*., 2006; Ebeling and Timmons,

2012; Badiola *et al.*, 2012). The efficiency of the low-cost RAS in maintaining water quality and removing nitrogenous compounds was evaluated. In addition, the growth performance of Nile tilapia reared in the low-cost RAS was assessed. Ammonia and nitrite removed by individual locally available media used in this study were not evaluated and therefore this would be done in prospective studies.

3.1 Materials and Methods

3.1.1 Study area

The experiment was conducted at Magadu Aquaculture unit, Sokoine University of Agriculture, Morogoro, Tanzania. The aquaculture unit is well equipped with necessary aquaculture research supportive facilities for aquaculture related research. The said research station is located between latitude 6° 48’S and longitude 37° 42’E at an altitude of about 500 - 600 m above sea level. The climatic condition of Morogoro is characterized by bimodal rainfall pattern, with short rains received from November to December and long rains starting in March and ending in May. Magadu area receives 767 mm rainfall per annum. Relative humidity and temperature ranges from 30 to 96 % and 26 to 35.5^{o}C, respectively, (TMA, 2020).

3.1.2 Construction of a pilot scale RAS unit

Two pilot experimental RASs were designed; each was constructed as shown in Figure 1. A 1000 L plastic pellet tank was used as a fish rearing tank in each experimental RAS. The pellet tank was well graduated for controlling the water volume. Aluminum bars for enhancement (Fig. 1) supported the bottom and sides of the pellet tank. The upper side of the tank was cut open to allow ease management and installation of other internal parts. The bottom of the tank was modified by cutting an aluminum sheet in circular shape, making it easy for the tank to form a trough with concave shape when filled with water

(Fig. 1). The biofilters were anchored on the sides of the pellet tank and supported by the extended metal stand (Fig. 1 and 2 E). The bottom of the tank was directly linked to a total drainage system, which had a pipe to serve as sediment collector. The trough like structures supported swilling of sediments to the center of the troughs. Plastic sieves were included to prevent fish escape through the total drainage pipe. Two inches elbow pipes with valve gates were fitted at the center of the troughs to serve as the sediment collectors as well as drainage outlets. The said pipes also directed effluent water into a collecting trench with a dimension of 0.3 by 0.3 by 5 m for depth, height and length, respectively. This trench directed effluent water into a treatment pond out of the research facility.

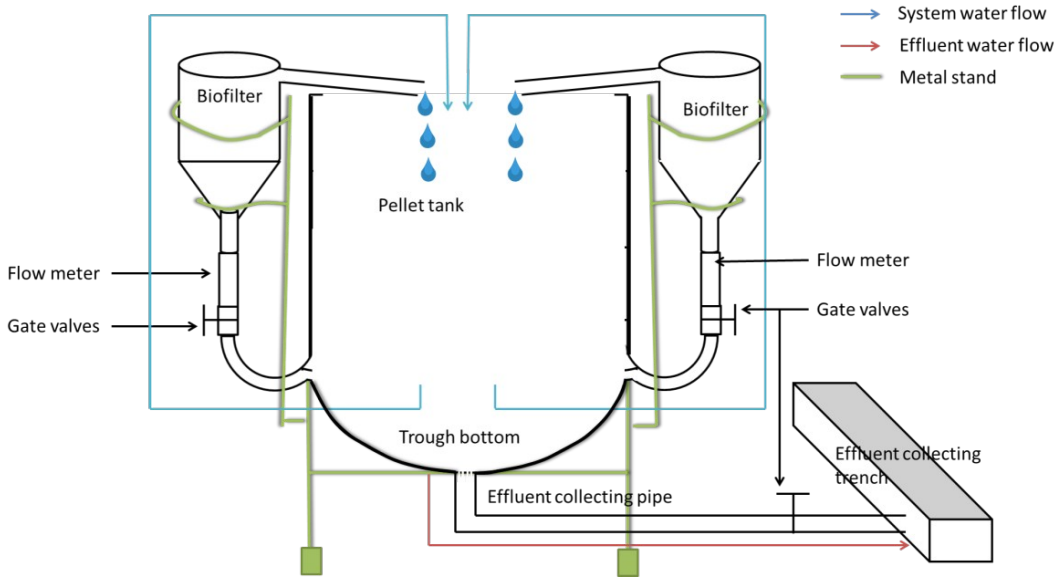

Figure 1: Schematic drawing showing the front lateral view of the experimental pilot RAS constructed

3.1.3 Biofilter and Pumps

Twelve 10 L cylindri-conical shaped plastic tanks were designed to serve as biofilter containers. Each biofilter was attached to a water flow meter, valve regulator, tap outlet (sampling pint) and a silencer pump installed inside the rearing tank (Fig. 2 E). A one-inch hole was drilled at the cone bottom of each biofilter container and flat top tank connectors (IPS) were used to make the inlet of the biofilters (Fig. 2 A, C). One inch IPS

pipe with sampling tap were fastened on the tank connector to form the biofilter inlet. Two insulated metal rings (with mesh fixed on both ends) connected by a central rod were placed inside the biofilter container to ensure that the biomedia are not directly suspended to the inlet opening and are also held below the upper water level and, therefore, making all the biofilters to be fixed bed (Fig. 2 B, C, D). Six similar biofilters were placed on two sides of each pellet tank, three on each side, leaving the other two sides free for management practices as shown in Fig. 2 E.

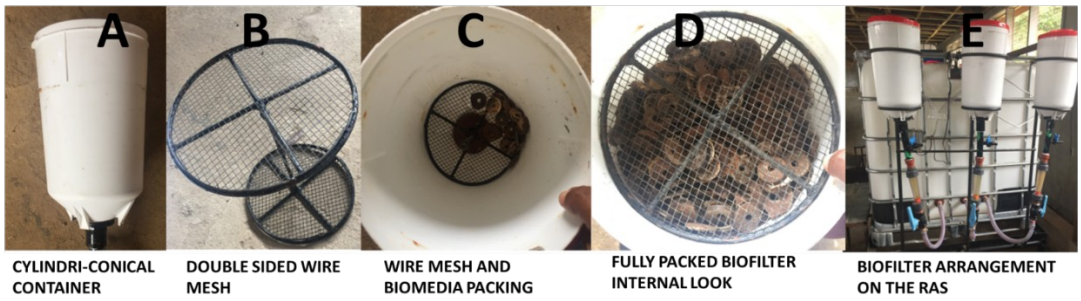

Figure 2: A RAS unit and biofilter external and internal look

The biomedia used for biofiltration in this RAS were; commercial biomedia (Kaldnes plastic rings in the form of pipes with a diameter of 9.1 mm and a length of 7.2 mm across the inside and fins on the outside), dry cattle horns, locally made ceramic from clay, dry activated charcoal, dry bamboo sticks and dry coconut shell. An electronic hand drill (INGCO Impact Drill, Shanghai, China) with 1 inch hole saw (INGCO hole saw kit, Shanghai, China) was used to shape the locally available biomedia into similar 3 cm circular discs. The biomedia were then packed into the biofilter containers and randomly placed on the sides of the rearing tanks in duplicate.

Six silencer aquarium pumps (SOBO® WP-3300C, Shanghai, China) were submerged inside the pellet tank and connected to each biofilter. Each pump supplied water out into one biofilter through water flow meters (LZS 15 1inch 300-100L/h Shanghai, China) and

regulator valves. Water inflow rate of 360 L/h was set using valve inlets gates in all biofilters and was maintained throughout the study period.

3.1.4 Aeration

Two electronic air compressors (HAILEA ACO-308, 30W, Shanghai, China), each connected to six air stones were used to supply air into the water in the pellet tank.

3.1.5 Cost of the simple RAS unit

The cost for the RAS unit is as shown in Table 1. The estimated cost for a complete low-cost pilot RAS was TZS 2 125 200.00 which is equivalent to approximatly1000 USD.

Table 1: Components of a single RAS unit and their prices

S/n	Component	Size	Quantity/size used	Unit Price (TZS)	Total price (TZS)
1	Cubic Pellet tank	1000 L	1	250 000	250 000
2	IPS Tank connectors	2 inches	1	5 000	5 000
		1 inch	6	3 000	3 000
3	PVC pipe class B	2 inches	10 feet	1 000	10 000
4	PVC elbow	2 inches	1	2 000	2 000
5	Gate valves	2 inches	1	18 000	18 000
		1 inch	6	10 000	60 000
6	Horse pipe	1 inch	12 feet	2 000	24 000
7	Water flow meter	1 inch	6	70 000	420 000
8	IPS pipe	1 inch	18 feet	2 000	36 000
9	Silicon pipe	5mm	50 feet	300	15 000
10	Silencer pumps	12w	6	80 000	480 000
11	Air stones	50mm	6	4 000	24 000
12	Air compressor	30w	1	300 000	300 000
13	Chicken drinkers	10L	6	10 000	60 000
14	Media enhancer	-	6	12 000	72 000
15	Charcoal	-	7 liters (2.57 kg)	400	2 800
16	Coconut shells	-	7 liters (2.69 kg)	200	2 100
17	Bamboo sticks	-	7 liters (2.11 kg)	200	2 100
18	Cattle horns	-	7 liters (2.21 kg)	200	2 100
19	Ceramic	-	7 liters (6.54 kg)	300	2 100
20	Commercial biomedia	-	7 liters (1.23 kg)	5 000	35 000
21	Metal stand and stairs	-	1	300 000	300 000
22	Labor charges	-	3 people	200 000	600 000
Total					0

3.1.6 Operation and collection of samples

Initially, the biofilter inflow valves were closed to prevent water from entering the biofilters, then water was filled up to 900 L in each pellet tank. Valves were then opened

to allow water to move into the biofilters and the level of water in the pellet tank dropped to 840 L. On average, 10% of the water was drained out weekly to remove waste materials (feaces and left-over feeds). Water was refilled back to 840 L to compensate for the 10% of the removed water and losses due to evaporation. Each biofilter inflow was set at 360 L/h (Mnyoro *et al*., 2021) and, therefore, the turn-over time for each tank was 2.4 times per hour (Fernandes *et al*., 2014).

This experiment was run for 10 weeks. At the start of the experiment, 13.3 g of ammonium chloride (NH_4Cl) and 2.3 g of $NaNO_2$ were added to each tank to make 5 mg/L and 2 mg/L of ammonia (TAN) and nitrite-N concentrations, respectively, in the water (Pulkkinen *et al*., 2018). Fifty gram of pelleted commercial fish feed (Koudijs Tilapia grower feed, 3.0 mm) with approximately 30% crude protein, 5.5% crude fat, 5.0% crude fiber, 14.0% ash and 11.0% moisture content was added into each rearing tank on day one of the experiment to raise the organic matter content within the system (Jiang *et al*., 2019). Sodium bicarbonate ($NaHCO_3$) was added into the system to raise the alkalinity, and was maintained at 120 mg/L $CaCO_3$ throughout the experimental period (Pedersen *et al*., 2012).

Ammonia and nitrite spiking was done every day to bring the concentrations close to 5 mg/L and 2 mg/L, respectively. Spiking was done after determination of background concentrations by using rapid calorimetric tests (HC879811. MColortest^{tm}. Germany for ammonia and 1.08024.0001. MQuant^{tm}. Germany for nitrite). A total of 15 mL water samples were taken 15 minutes after spiking from the rearing tank (inflow) and from the outlet of the biofilters (outflow) for analysis of ammonia, nitrite and nitrate removal. Spiking was continuously done for four weeks, followed by stocking of Nile Tilapia (*Oreochromis niloticus*) at a stocking density of 20 kg/m^3 (Wanja *et al*., 2020) in order to

ensure a steady and continuous supply of ammonia to the biofilters to enhance their full maturity. The same commercial feed used during spiking of organic matter (Koudijs, 2021) was hand fed two times a day at 2% body weight of the fish at 9:00 am and 4:00 pm. Sampling of fish for body weight measurements was done two times a week in the morning before feeding.

Water samples were sterile filtered (by use of a 0.22 µm Sartorius filter) and kept refrigerated until analysis. Total Ammonium Nitrogen (TAN) and nitrite nitrogen (NO_2−N) were analyzed spectrophotometrically (JENWAY 7310. Bibby Scientific. Stone, Staffs, UK) at 680 nm and 545 nm, respectively (ISO 7150-1: 1984; ISO 13395: 1996). Nitrate nitrogen (NO_3-N) was analyzed using water quality parameter test stripes for nitrate (Aquacheck. HACH. Germany). Alkalinity was measured by an end point titration to pH 4.5 manually. Water pH, dissolved oxygen, temperature, total dissolved solids and salinity were measured using Multimeter tool (HANNA HI 98194 PH/EC/DO. Düsseldorf, Germany) with an HI-7698194 probe which contains HI-7698194-1 pH and platinum ORP Sensor, HI-7698194-3 Four ring, stainless steel conductivity sensor and HI-7698194-2 Galvanic dissolved oxygen sensor.

3.1.7 Calculations of nitrogenous compound removal and fish growth performance

The volumetric TAN conversion rate (VTR), the volumetric nitrite conversion rate (VNR), and the volumetric oxygen consumption rate of the biofilter (VOCR) can be used as principal parameters for evaluation and comparison of biofilter performance (Malone and Beecher, 2000). Nitrification kinetics in this research was, therefore, determined by calculating the volumetric TAN conversion rate (VTR) and volumetric nitrite conversion rate (VNR) as described in the following formulas.

$VTR = 1.44(Q_f)\{(TAN_{in} – TAN_{out})/V_m\}$ in g TAN/m^3/d …… (i)

Where; [VTR] reflects corresponding volumetric ammonium N concentrations (g N/m^3) from in- and outlet of the biofilters; Q_f is the water flow into the media (m^3/d) and V_m is the available volume of the carrier elements (m^3) (Malone and Beecher, 2000; Guerdat *et al*., 2010).

The apparent volumetric nitrite conversion rate (VNRa) was calculated as follows:

$VNRa = 1.44(Q_f)\{(NO_2^-\text{-}N_{in} – NO_2^-\text{-}N_{out})/V_m\}$ in g NO_2^--$N/m^3/d$ ……. (ii)

Where, [NO_2^--N] reflects corresponding volumetric nitrite concentrations (g N/m^3) from in- and outlet of the biofilters; Q_f is the water flow into the media (m^3/d) and V_m is the available volume of the carrier elements (Malone and Beecher, 2000).

The actual volumetric nitrite conversion rate (VNR) taking the de facto oxidized TAN contribution into account was calculated as follows:

VNR = VTR + VNRa ……..……. (iii)

Growth performance of fish was assessed by computing daily weight gain and specific growth rate as shown below:-

Daily Weight gain was calculated as;

DWG = (Wf – Wi)/Time ……(iv)

Where, *DWG* indicates the weight gain in gram per day (g/d), *Wf* is the final weight (g) and *Wi* is the initial weight (g). Time is defined as the number of the experimental days (Pauly, 1983).

Specific growth rate was calculated as;

$SGR = (\ln W_f - \ln W_i) /t \times 100$…… (v)

Where; SGR = specific growth rate, $\ln W_f$ = the natural logarithm of the final weight, $\ln W_i$ = the natural logarithm of the initial weight and t = time (days) interval between $\ln W_f$ and $\ln W_i$ (Lugert *et al*., 2016).

Statistical analysis

Data was normalized and normality was assessed by Shapiro–Wilk test. Analysis of variance (ANOVA) was done to assess the influence of time and background TAN concentration, on water quality parameters and nitrogen conversion rates (VTR, VNRa and VNR), over time by using R statistical programme version 3.9.9. Tukey post-hoc test was carried out for multiple comparisons, as appropriate. Differences between treatment means were considered significant at $p < 0.05$.

3.2 Results

All the findings in this study are presented as a mean from two identical developed low-cost RASs.

3.2.1 Water quality parameters

Water quality parameters were measured throughout the experimental period and the results are presented in Table 2. Alkalinity gradually increased from 57.0 mg $CaCO_3$/L in week one to 280.0 mg $CaCO_3$/L in week eight. The last two weeks of this study demonstrated a stable alkalinity of 240 mg $CaCO_3$/L. Dissolved oxygen level increased from week one to week six, followed by a slight gradual drop from week seven to ten. The pH values showed a small variation with a mean of 7.59 ± 0.4 throughout the study period. The trend of water temperature was not consistent; there was an increase in temperature during the following weeks; two – three, four – six and eight – ten. The rest of the weeks demonstrated a drop in temperatures. Total dissolved solids increased with time from week one to week ten. Salinity also increased with time from week one to week seven, followed by a slight decrease and then constant level between week eight and ten.

Table 2: Weekly water quality parameters (Mean ± SD) for the whole experimental period

Weeks	Alkalinity (mg $CaCO_3$/L)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Total dissolved Solids (mg/L)	Salinity (ppt)
Week 1	73.3± 16.3	5.3 ± 0.4	7.7 ± 0.2	24.3 ± 0.3	59.9 ± 8.8	0.1 ± 0.0
Week 2	66.7 ± 20.7	5.4 ± 0.2	7.8 ± 0.7	24.8 ± 0.8	75.7 ± 8.9	0.1 ± 0.0
Week 3	120.0 ± 0.0	5.2 ± 0.3	8.1 ± 0.5	25.7 ± 0.0	138.8 ± 21.2	0.1 ± 0.1
Week 4	120.0 ± 0.0	6.6 ± 0.2	7.4 ± 0.2	23.1 ± 0.5	197.0 ± 11.7	0.3 ± 0.1
Week 5	240.0 ± 0.0	7.0 ± 0.5	7.4 ± 0.2	23.4 ± 0.2	220.0 ± 4.4	0.3 ± 0.0
Week 6	240.0 ± 0.0	7.5 ± 0.3	7.3 ± 0.1	25.7 ± 0.0	272.8 ± 11.4	0.3 ± 0.0
Week 7	280.0 ± 0.0	6.7 ± 0.2	7.6 ± 0.2	25.1 ± 0.0	283.3 ± 5.1	0.3 ± 0.0
Week 8	280.0 ± 0.0	6.7 ± 0.1	8.0 ± 0.5	23.5 ± 0.0	283.0 ± 6.1	0.2 ± 0.0
Week 9	240.0 ± 0.0	6.3 ± 0.1	7.3 ± 0.4	25.2 ± 0.6	284.3 ± 1.9	0.2 ± 0.0
Week 10	240.0 ± 0.0	6.1 ± 0.1	7.3 ± 0.0	26.6 ± 0.0	287.3 ± 1.7	0.2 ± 0.0
Overall Mean	**179.09 ± 85.6**	**6.18 ± 0.8**	**7.59 ± 0.4**	**24.69 ± 1.1**	**197.23 ± 92.2**	**0.20 ± 0.1**

3.2.2 Fish growth performance

A total of 120 fish with approximately 20.0 kg (bulk weight) were stocked into each pilot RAS unit and cultured for six weeks. After six weeks of culture, the mean bulk weight increased by 9.1 ± 0.4 kg and feed conversion ratio was 0.94 ± 0.1. The daily weight gain was 1.8 ± 0.1 g/d throughout the experimental period (six weeks). The specific growth rate (SGR) was found to be 0.4 % g/d. Three fish died during the first week. However, the survival rate at the end of the experiment was 98.7%.

3.2.3 Total Ammonia Nitrogen, nitrite and nitrate concentrations changes with time

TAN and nitrite were measured and the results are presented in Fig. 3. At the beginning of the experiment, ammonia and nitrite were spiked into the system. Nitrite concentration was 1.5 ± 0.3 mg/L in week one, followed by elevated concentrations (3.8 ± 0.6 mg/L) between week three and four and then gradually dropped down to 0.6 ± 0.01 mg/L in week 10. On the other hand, TAN concentration was found to be above 4.7 ± 0.3 mg/L in week one and two, then the concentration dropped gradually to 0.8 ± 0.1 mg/L in week 10. The mean nitrate concentration level in the pilot RAS increased lineally from 0.0 mg/L during the first week to 55 ± 3.2 mg/L in the fifth week, then substantially dropped

to 25.2 ± 2.1 mg/L between week eight and ten. There was no significant difference in TAN, nitrite and nitrate between the two pilot RASs used in this study.

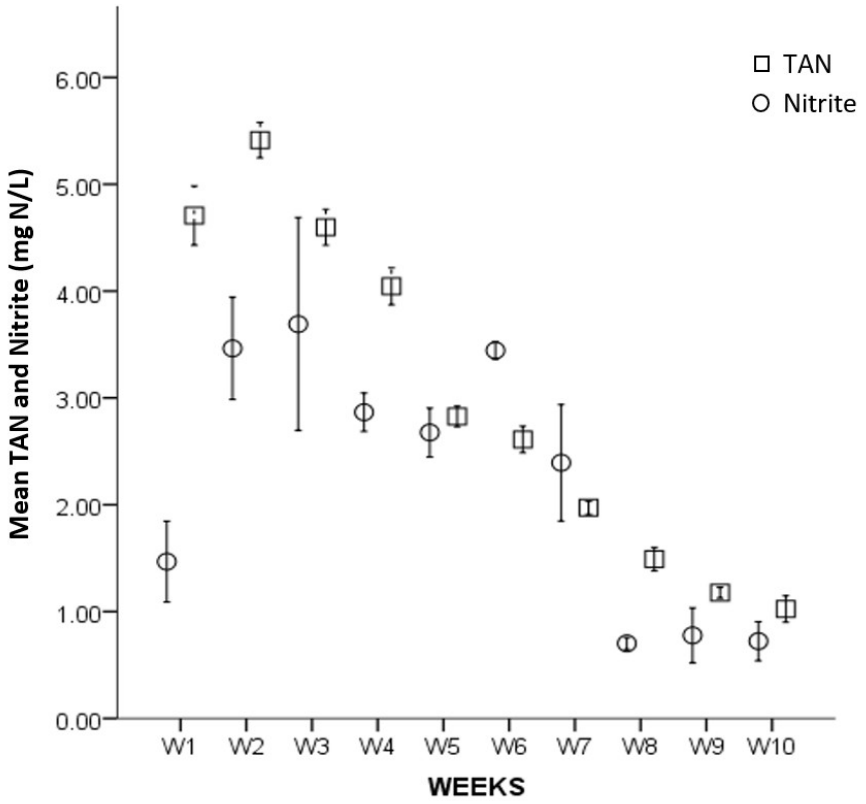

Figure 3: The amount of TAN and Nitrite (mean ± SD) present in the system during the experimental period

3.2.4 TAN and Nitrite removal

TAN and nitrite conversion ratios were computed according to equation number 1, 2 and 3 and the results are shown in Fig. 4. Volumetric TAN conversion rate (VTR) exponentially picked from 25 ± 2.1 g $N/m^3/d$ at the end of week two to 450 ± 21.4 g $N/m^3/d$ in week six. After the six week it was found to be stable through week 10. The apparent volumetric nitrite conversion rate (aVNR) was higher compared to VTR while the volumetric nitrite conversion rate (VNR) was low starting at 10 ± 1.2 g $N/m^3/d$ in week three and increased to 100 ± 5.2 g $N/m^3/d$ in week 10 (Fig. 4). The TAN and nitrite conversion rates differed significantly among weeks as shown in Table 3.

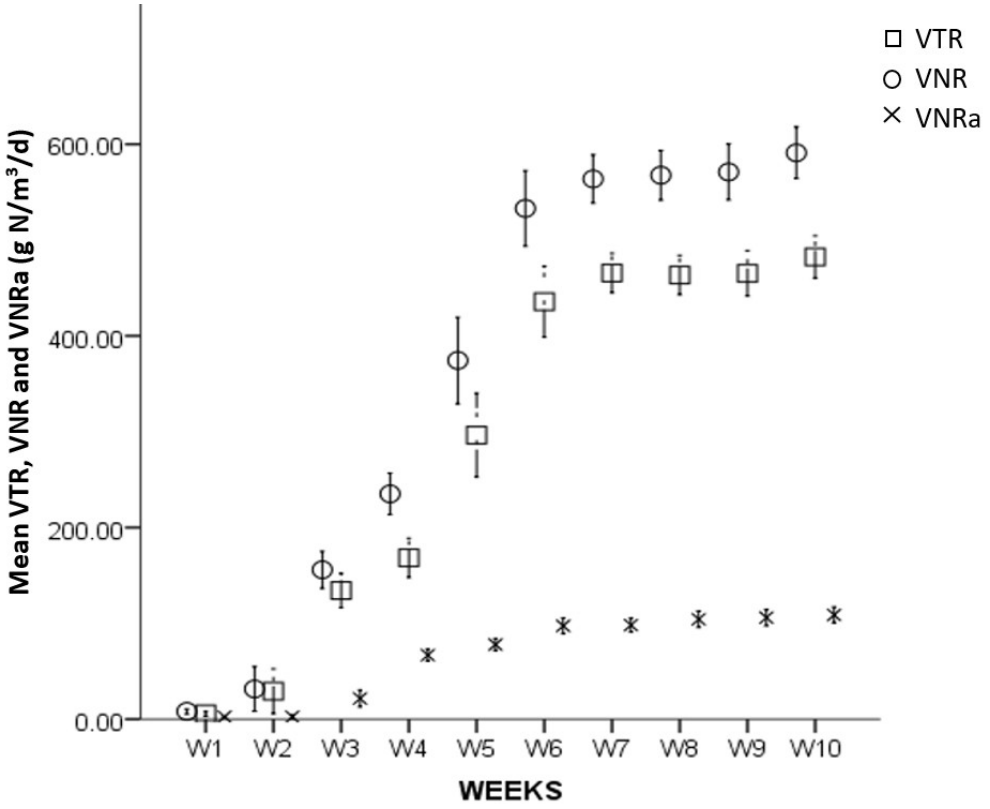

Figure 4: Mean ± SD Volumetric TAN conversion Rate (VNR), Volumetric Nitrite conversion Rate (VTR) and apparent Volumetric Nitrite conversion Rate (VNRa) trends in g N/m^3/d for all used biofilters during the experimental period of 10 weeks

Table 3: Weekly Volumetric TAN conversion Rate, apparent Volumetric Nitrite conversion Rate and Volumetric Nitrite conversion Rate (g N/m^3/d) [a,b,c] means with different superscript letters within columns differ significantly at $p \leq 0.05$

	Volumetric TAN conversion rate (g N/m^3/d)	Apparent Volumetric Nitrite conversion rate (g N/m^3/d)	Volumetric Nitrite conversion rate (g N/m^3/d)
Week 1	5.5 ± 10.1^a	2.5 ± 2.6^a	8.0 ± 12.4^a
Week 2	29.1 ± 68.1^a	2.4 ± 4.9^a	31.5 ± 66.1^a
Week 3	134.2 ± 63.3^a	21.6 ± 6.4^b	155.8 ± 67.1^a
Week 4	168.3 ± 51.7^a	66.8 ± 5.3^c	235.1 ± 56.0^a
Week 5	296.5 ± 69.5^b	77.9 ± 4.6^c	374.3 ± 72.2^b
Week 6	435.7 ± 46.4^c	97.4 ± 3.3^d	533.1 ± 48.4^c
Week 7	465.6 ± 24.0^c	98.2 ± 1.5^d	563.8 ± 25.5^c
Week 8	463.5 ± 24.1^c	104.3 ± 4.6^d	567.7 ± 28.1^c
Week 9	465.2 ± 25.7^c	105.9 ± 3.3^d	571.1 ± 25.6^c
Week 10	482.4 ± 9.2^c	108.7 ± 2.2^d	591.2 ± 9.2^c

3.3 Discussion

3.3.1 Water quality parameters

Water quality parameters were ascertained on every sampling day before spiking ammonium chloride or feeding the fish in the system. Among the parameters, temperature plays a significant role in the nitrification reaction rate as it does in all chemical and biological kinetic reactions. The temperature observed in this study was with the wide range of ideal temperature for nitrification. Studies have shown that the optimal temperature for ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) is between 15 and 30 °C (Kinyage and Pedersen, 2016; Ouyang *et al*., 2017; Young *et al*., 2017; Zhang *et al*., 2019).

The mean oxygen concentration was within the range acceptable for tilapia culture as well as nitrification (Shammas, 1986; Jiang *et al*., 2018). Based on the nitrification chemical processes; 4.57 g of oxygen is needed for the complete oxidation of one gram of ammonia-nitrogen (Hargreaves, 1998). Research has revealed that at least 2 mg/L of oxygen is adequate to maintain maximum nitrification through biofilters (Wezernak and Gannon, 1967). This study observed higher dissolved oxygen values compared to the results reported by Wezernak and Gannon, (1967) as well as González-Cabaleiro *et al*. (2019).

Both the rate of nitrification and the association between the ionized and unionized forms of ammonia-nitrogen affect nitrification (Shammas, 1986). Studies on the effect of pH on the nitrification rate for biofilters have revealed that nitrifying biofilters operate over a much broader pH range from 6 to 9, due to the adaptation of the bacteria in a filter under actual operating conditions (Teutscherova *et al*., 2017; Le *et al*., 2019). The model RAS

in the current study was therefore operated within the pH ranges recommended by other studies (Xiao *et al*., 2017; Jiang *et al*., 2018).

This study recorded levels of alkalinity which is acceptable for fish culture and efficient nitrification. Biesterfeld *et al.* (2003) studied the effect of alkalinity on nitrifying biofilm activity and found that nitrification is not limited when alkalinity is above 45 mg $CaCO_3$/L. Another study by Shanahan and Semmens (2015) on nitrification also indicated that sodium bi-carbonate is a good alkalinity booster in nitrification with the highest nitrification rates occurring at alkalinity between 120 and 300 mg $CaCO_3$/L.

The results of this study showed that the level of salinity gradually increased from week one to week 10. The gradual increase in salinity was caused by mixed effects of partial degradation of the biomedia used and sodium bi-carbonate spiked (Ahmad *et al*., 2014) into the RAS at the beginning of the experiment. Research has revealed that nitrifying bacteria can acclimatize to almost any salinity range, given sufficient time (Holan *et al*., 2020). Abrupt changes in salinity of greater than 5 g/L, will shock nitrifying bacteria and decrease the reaction rate for both ammonia-nitrogen and nitrite-nitrogen removal (Sudarno *et al*., 2011). Studies have proved that salinity levels between 0 and 10 ppt does not cause significance difference in nitrification rates (Kinyage *et al*., 2019; Mehzabin, 2019). Therefore, the reported salinity levels in the current study are within the acceptable ranges.

3.3.2 Fish survival and biomass gained over six weeks

The results on growth performance indicated that fish biomass increased. The daily fish mass increase observed in the present study is similar to what was reported by Wanja *et al*. (2020) who did a comparable experiment and found that tilapia grows at 2.32 g/d with

feed conversion ratio of 1.1 in RAS. It has been reported that, juvenile tilapia cultured in RAS can gain as much as 50 % of body weight daily at a temperature between 20 and 30 °C (Gullian-Klanian and Aramburu-Adame, 2013). The high growth performance obtained in the present study demonstrates the suitability of the low-cost RAS for tilapia farming.

3.3.3 Volumetric TAN and nitrite conversion rates

At the beginning of this trial, TAN was supplied to the system to accelerate initiation of the nitrification process (Hagopian and Riley, 1998) and was kept above the concentration of 3.0 mg N/L between week one and two. Influent TAN concentration then dropped sequentially from this level to below 1.0 mg N/L in week ten. This drop was due to volumetric TAN and nitrite conversion rates, which increased from week two, followed by exponential increase between week three and six and then stabilized from week seven to ten. The volumetric TAN and nitrite conversion rates remained low in the first two weeks (< 200 g $N/m^3/d$) and then significantly increased to > 400 g $N/m^3/d$ at week six. From week six onwards, VTR and VNR plateaued and remained stable indicating mature nitrifying biofilters (Sikora *et al.*, 2020). The findings observed in this study concur with the results obtained by Wimberly (1990) who assessed the performance of low density biofilters and found that the typical values for volumetric TAN conversion rate under conditions derived from operational filters ranged from 140 to 350 g $N/m^3/d$ for grow out fish systems in warm temperatures. Peng and Jo (2003) also tested performance of different media in fixed bed biofiltration and reported volumetric nitrite conversion rates ranging from 150 to 400 g NO_2-$N/m^3/d$, which is similar to the results of the present study.

3.3.5 Nitrate accumulation

Nitrate accumulates as an end-product of nitrification (Chen *et al.*, 2006; Pedersen *et al*., 2012). In the current study, nitrate accumulated exponentially from slightly above 0.0 mg NO_3-N/L at the beginning to 55 mg NO_3-N/L in week five. Nitrate accumulation trend changed from week six by demonstrating a sharp decrease to around 20 mg NO_3-N/L in week eight. Nitrate accumulation in the current study was controlled by nitrification process as well as denitrification process, which reduced nitrate concentration to 20 mg NO_3-N/L between week eight and ten. The diversity of locally available media used in this study provided adequate environment for growth of both heterotrophic and anaerobic micro zones in the RAS, for this reason and in combination with insufficient hydraulic mixing could have facilitated denitrification activity (Crab *et al.*, 2007; von Ahnen *et al*., 2015; Cr Forbis-Stokes *et al*., 2018; Hunter and Deshusses, 2020).

3.4 Conclusion and Recommendations

In conclusion, the pilot RAS was able to produce fish with limited water and relying entirely on biofiltration for water treatment. Specifically, fish growth rate was 1.785 g/d and survival rate was 98.75%, this is an indicator of a good performance. Therefore, the study has demonstrated that the developed simple pilot RAS unit can be used successfully to grow tilapia and obtain high growth rate and yield at harvest. Moreover, the study has demonstrated that the simple RAS unit can provide stable water conditions for rearing tilapia. We suggest that future studies should investigate low-cost technologies (solar driven pumps and aeration) and optimization of treatment processes (biofilter design and biofilter carrier elements) to ensure stable conditions and effective biofiltration.

By using the developed pilot scale RAS, future studies can include:-

(a), Assessment of nitrification and denitrification performance of different biomedia.

(b), Investigating bacterial colonization and microbial community in different biomedia during start-up and prolonged operation.

(c), Optimizing the water velocity to increase nitrification performance in different biofilter media.

(d), Evaluating the fish growth performance and establishing optimum carrying capacity of the low-cost RAS with different volumes of biomedia.

Acknowledgement

This study was financially supported by DANIDA through Building Stronger Universities phase III (BSU III) programme at Sokoine University of Agriculture. The authors are grateful for the financial support. We highly acknowledge the technical support and caretaking of the fish and the systems provided by Ms. Stella Genge and Mr. Mathias Shija as well as Ms. Fatuma Kindoro for her help during laboratory analysis.

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CHAPTER FOUR

PAPER THREE

Journal of Cleaner Production 366 (2022) 132929

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Evaluation of biofilter performance with alternative local biomedia in pilot scale recirculating aquaculture systems

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https://doi.org/10.1016/j.jclepro.2022.132929

Received 22 December 2021; Received in revised form 1 June 2022; Accepted 26 June 2022. Available online 29 June 2022

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ARTICLE INFO

Handling Editor: M.T. Moreira

Keywords:
Biofiltration
Biomedia
TAN removal
Recirculating aquaculture system
Water quality parameters

ABSTRACT

Plastic is commonly used as biofilter media in recirculating aquaculture systems. Because plastic is relatively expensive and may erode and emit microplastics to the environment, efforts are being made to test and develop more sustainable materials. Five alternative locally available biofilter media were compared with commercial plastic media and evaluated in duplicate in 1 m^3 two pilot scale Recirculation aquaculture system. Ammonium chloride and sodium nitrite were added to the systems for 4 weeks followed by stocking 20 kg of Nile tilapia in each system. Volumetric total ammonia nitrogen (TAN), nitrite and oxygen conversion rates were assessed for ten weeks. All biofilters with local media matured and reached full capacity after six weeks, while commercial plastic biomedia matured after seven weeks. This study found that the performance of commercial plastic biomedia was similar to performance of coconut shells in terms of volumetric TAN conversion rate (VTR), volumetric nitrite conversion rate (VNR) and volumetric oxygen conversion rate (VOCR). The highest VTR recorded in this study was 599 ± 15.8 g TAN/m^3/d from coconut shells while the lowest was 343 ± 8.9 g TAN/m^3/d from cattle horns. Biofilters with commercial plastic media had the highest VNR (704 ± 50.3 g NO_2–N/m^3/d) while media made of cattle horns was the lowest (457 ± 46.1 g NO_2–N/m^3/d). Biofilters containing coconut shells demonstrated the highest oxygen consumption around 3.0 g/m^3/d and biofilters containing charcoal consumed less than 1.0 g/m^3/d of oxygen. This study suggests that coconut shells can be used in place of plastic materials in simple recirculation aquaculture system biofiltration. This study also recommends further studies on comparing coconut shells with other biomedia and assessing its effects on water quality parameters and durability.

1. Background information

Recirculation aquaculture system (RAS) is a method of rearing fish in (indoor) tanks at high densities and controlled conditions. In RAS water is continuously cleaned and reused several times before being discharged. Water is cleaned via mechanical and biological filtration. Mechanical filtration removes particulate wastes while biological filtration removes dissolved wastes via biochemical reactions that occur during bacterial metabolism. RAS has a number of advantages over open pond culture systems such as ponds and raceways. These include the ability to completely control all the parameters in the production unit, produce higher yields on a small area of land and produce fish year-around. Moreover, RAS has advantages of reducing the quantity of water used in production units, reusing more water within the culture system, flexibility to locate production facilities near large markets and quick and effective disease control. Finally, RAS allows better control of the discharge of dissolved and particulate matter.

In recent years RAS has become more popular because of increasing scarcity of water resources as well as concerns over environmental pollution management (Ahmed and Turchini, 2021). However, application of RAS is faced by several limitations, including high generation of nitrogen compounds in the systems (Subasinghe et al., 2009). Nitrogenous compounds can be removed from fish production systems by processes that may be mechanical, physicochemical or biological (Kaleta et al., 2007; Zhu et al., 2008; Zubrowska-Sudol and Walczak, 2015). Among these, biological processes are more reliable, sustainable, economical and efficient methods of nitrogenous compounds removal, following natural decomposition routes under controlled conditions

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https://doi.org/10.1016/j.jclepro.2022.132929
Received 22 December 2021; Received in revised form 1 June 2022; Accepted 26 June 2022
Available online 29 June 2022

(Ahn, 2006; Halling-Sørensen and Jorgensen, 1993; Zhu et al., 2008).

Ammonia, nitrite and nitrate levels in recirculating aquaculture systems is mainly controlled by nitrification and denitrification processes (van Rijn, 2013; Hagopian and Riley, 1998) Nitrifying bacteria include the genera *Nitrosococcus* (Xie and Yokota, 2006), *Nitrobacter* (Xie and Yokota, 2006), *Nitrospira* (Alexander and Clark, 1965), *Nitrococcus* (Langone et al., 2014), *Nitrospina, Nitrosomonas* (Alexander and Clark, 1965), *Nitrosospira* (Schmidt and Belser, 1983) which oxidize ammonia to nitrate, through nitrite, under aerobic conditions. Recent studies have shown that *Nitrospira* is able to perform both nitrifying processes, oxidizing both ammonium and nitrite (Van Kessel at al. 2015; Wu et al., 2019; Xia et al., 2018). The end product nitrate can be reduced to free nitrogen (N2) under anaerobic conditions (Rajta et al., 2020; Schmidt and Belser, 1983; Wang and He, 2020). Heterotrophic bacteria such as *Pseudomonas*, *Rhizobium* and *Paracoccus* perform denitrification and in this process, an energy source like dissolved organic carbon (DOC) is needed (Zheng, 2018). Biological filtration is an important process in recirculating aquaculture water treatment processes (Chen et al., 2006; Colt et al., 2006; Kuhn et al., 2010), and several studies have investigated nitrification and biofilter performance in RAS (Bracino et al., 2020; Pedersen et al., 2015; Sharma et al., 2018). In RAS, bioreactors are specific sites for nitrification, though research shows that traces of nitrifying bacteria are found all over the system and therefore nitrification process takes place in other parts of the system (Schreier et al., 2010; Young et al., 2017). The performance of biofilters depends on a wide range of factors which include type and surface area of media used for bacterial enhancement, dissolved oxygen concentrations in the system, amount of organic matter, temperature, pH, alkalinity, salinity (Chen et al., 2018).

Nitrifying bacteria are known to be highly sensitive and susceptible to their environment, therefore, biological filters should consist of non-corroding material such as fiberglass, plastic, rock or ceramic that have large surface areas where nitrifying bacteria can attach (DeLong and Losordo, 2012). A biofilter with higher surface area per unit volume will be more efficient and economic compared to biofilter with low surface area. Biofilter installation in modern recirculating aquaculture systems is estimated to take 10–30% of the total cost (O'Rourke, 1996). The high cost of industrial media makes it difficult for developing countries to adopt RAS technology (Betanzo-Torres et al., 2020).

Plastic products, such as Polyvinyl chloride (PVC) and Polyethylene (PE) are commonly used carrier materials for biofilters in RAS (Hammer, 2020; Lopardo and Urakawa, 2019). Plastic filtration media in moving bed chambers are exposed to high shear forces and friction, therefore becoming a source of microplastics in system. A study on aquaculture facilities in Norway estimates that 325-ton microplastics are being released into the sea from plastic pipes used in different commercial aquaculture activities yearly. This is probably one of many uses of plastics that release microplastics into the environment and eventually into human through bioaccumulation in sea foods (Cox et al., 2019; Morgana et al., 2018).

As a way of reducing the use of plastic in filtration-systems, as well as covering the growing demand for biofilters for intensive aquaculture especially in developing countries, replacement of plastic filtration media with natural filtration media could be one possible solution. A range of natural filtration media have been tested for their efficiency in biological chambers. Earlier studies have shown that media made from locally available materials such as wood, shells, charcoal, coconut shells, husks and gravels can be used for biofiltration in bio-flock and gas filtration systems (Cruz et al., 2020; Saliling et al., 2007; Sharma et al., 2018). However, the nitrification performance of these natural materials have not been evaluated in RAS under controlled conditions.

In support to the recommendation made by Samuel-Fitwi et al. (2012), more efforts should be put on identifying locally, durable and readily available materials that can be used as cheap biological filters with superior performance characteristics. Therefore, the purpose of this study was to investigate nitrification performance of biological filters with different natural biomedia selected based on cost, availability and expected durability.

Table 1
Weight, void space and void ratio of different used biofilters. All biofilters used had 10 L total volume.

Biofilter containing Biomedia	Media Volume (L)	Weight (kg)	Void space (L)	Void ratio
Plastic	7	1.23	7.93	0.79
Horns	7	2.21	6.83	0.68
Ceramic	7	6.54	6.35	0.64
Charcoal	7	2.57	6.03	0.6
Bamboo	7	2.11	5.55	0.56
Coconut shells	7	2.69	7.47	0.75

2. Materials and methods

The experiment was conducted from February to end of April 2021 at the aquaculture unit of the Sokoine University of Agriculture in Morogoro, Tanzania. The unit is located at latitude 60 48′S and longitude 370 42′E with climatic conditions of 767 mm rainfall per annum, relative humidity and temperature ranges from 30 to 96% and 26–35.5 °C respectively, (T.M.A, 2019).

2.1. Experimental set up and operation

Two pilot scale recirculating aquaculture systems were used, each system built of a 1000 L plastic pellet tank with six parallel biofilters attached. The unit includes a sediment collector at the bottom of the pellet tank, six water pumps inside the fish tank, six water flow meters attached to each biofilter and one air pump with six air stones (Plate 1). This experiment was run for 10 weeks. At the start of the experiment, 13.3 g of ammonium chloride (NH_4Cl) and 2.3 g of $NaNO_2$ were added to each tank (900 L water in circulation) to make concentrations of approximately 4 mg/L and 2 mg/L of ammonia (TAN) and nitrite-N, respectively (Pulkkinen et al., 2018). A total of 50 g of pelleted commercial fish feed (Koudijs. Tilapia grower feed, 3.0 mm) with approximately 30% crude protein, 5.5% crude fat, 5.0% crude fiber, 14.0% ash and 11.0% moisture contents was added into each rearing tank on day one of the experiment to raise the organic content within the system (Jiang et al., 2019). Sodium bicarbonate (NaHCO3) was added as a buffer to increase pH into the system and maintain alkalinity level above 120 mg/L CaCO3 throughout the experimental period (Pedersen et al., 2012). Spiking was continuously done for four weeks, followed by stocking of Nile Tilapia (*Oreochromis niloticus*) at a stocking density of 20 kg/m^3 (Wanja et al., 2020) in order to ensure a steady and continuous supply of ammonia to the biofilters to enhance their full maturity. The same commercial feed used during spiking of organic matter (Koudijs. Tilapia grower feed, 3.0 mm) was hand fed to the fish two times a day at a feeding level of 10% of body weight at 9:00 a.m. and 4:00 p.m. Each system was operated with 10% water replacement daily.

2.2. Biomedia

Five different types of biomedia were tested in this study. As a control, a commercial biomedia (Kaldnes plastic rings in the form of pipes with a diameter of 9.1 mm and a length of 7.2 mm a cross inside and fins on the outside; inset link to product/INFO) was included. The five local products included dried cattle horns, ceramic beads made of clay, dried activated charcoal, dried bamboo sticks and dried coconut shell (Plate 2). All the biomedia were used dry to minimize the organic matter in the system. An electronic hand drill (INGCO Impact Drill, Shanghai, China) with 1 inch round saw (INGCO hole saw kit, Shanghai, China) was used to shape the locally available biomedia into similar 2.54 cm circular discs as they appear in Plate 2. The biomedia were then packed into the biofilter containers and randomly placed on the sides of the rearing

tanks in duplicate.

2.3. Characteristics of biomedia used

The weight of biomedia, space not occupied by biomedia (void space) in biofilters and void ratio varied from one biofilter to the other as shown on Table 1. The void space was determined by measuring the amount of water held by the biofilters including biomedia. Void ratio was calculated as the ratio of the void space to the total volume of the empty biofilter container.

2.4. Sample collection

Spiking was done after determination of background concentrations by using rapid calorimetric tests (HC879811 MColortest^{tm}. Germany for ammonia and 1.08024.0001 MQuant^{tm}. Germany for nitrite). Fifteen minutes after spiking, 15 mL of water samples were taken from the Sampling tap of each biofilter (inflow) and from each outlet of the biofilters (outflow) for analysis of ammonia, nitrite and nitrate removal. Water samples were sterile filtered (0.22 μm Sartorius filter) and kept refrigerated until analysis.

2.5. Chemical analysis

Total ammonium nitrogen (TAN) and Nitrite nitrogen (NO_2–N) were analyzed spectrophotometrically (JENWAY 7310. Bibby Scientific. Stone, Staffs, UK) at 680 nm and 545 nm, respectively (ISO, 1984; ISO, 1997). Nitrate nitrogen (NO_3–N) was analyzed using water quality parameters test stipes for nitrate (Aquacheck. HACH. Germany). Alkalinity was measured by an end point titration to pH 4.5 manually and converted to mg $CaCO_3$/L. Multimeter tool (HANNA HI 98194 PH/EC/DO. Düsseldorf, Germany) with an HI-7698194 probe which contains HI-7698194-1 pH & platinum ORP Sensor, HI-7698194-3 Four ring, stainless steel conductivity sensor and HI-7698194-2 Galvanic dissolved oxygen sensor (HANNA instruments, Germany) was used to measure water pH, dissolved oxygen, temperature, total dissolved solids and salinity. These parameters were measured in the rearing tank for system values and all biofilter influents, while the same parameters for biofilter effluent were measured from the outlet of each biofilter.

2.6. Calculations and statistics

Substrate load rate was determined by the formula;

$$LRS = 1{\cdot}44(Q_f)\frac{S_1}{V_m} \text{In} \quad g/m^3/d \tag{1}$$

where, LRS = substrate loading rate (g/m^3 (media)/d), S_1 = influent substrate concentration (g/m^3), Q_f = flow into filter (L/min), and Vm = volume of filter media (m^3). This equation effectively normalizes the substrate available to the bacteria contained within the filters.

Nitrification kinetics was determined by calculating the volumetric TAN conversion rate (VTR) and volumetric nitrite conversion rate (VNR) as described in the following formulas.

$$VTR = 1{\cdot}44(Q_f)\frac{TAN_I - TAN_E}{V_m} \text{In} \quad g \quad TAN/m^3/d \tag{2}$$

where, VTR reflects corresponding volumetric ammonium N concentrations (g N/m^3) from in- and outlet of the biofilters; Qf is the water flow into the media (m3/d) and Vm is the available volume of the carrier elements (m^3) (Malone and Beecher, 2000; Guerdat et al., 2010).

The apparent volumetric nitrite conversion rate (VNRa) was calculated as:

$$VNR_a = 1{\cdot}44(Q_f)\frac{(NO_2 - N)_I - (NO_2 - N)_E}{V_m} \text{In} \quad g \quad TAN/m^3/d \tag{3}$$

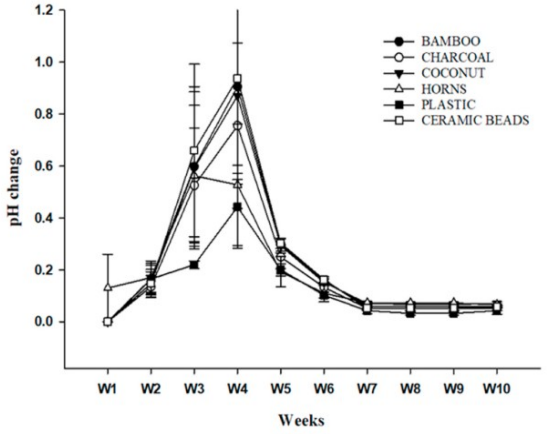

Fig. 1. Change in pH during single passage over different biofilters. The changes were measured as influent pH – effluent pH.

where, [NO2–-N] reflects corresponding volumetric nitrite concentrations (g N/m3) from in- and outlet of the biofilters; Qf is the water flow into the media (m3/d) and Vm is the available volume of the carrier elements (Malone and Beecher, 2000).

The actual volumetric nitrite conversion rate (VNR) taking the de facto oxidized TAN contribution into account can be calculated as:

$$VNR = VTR + VNRa \tag{4}$$

Oxygen consumption was evaluated as;

$$VOCR_{TOT} = \frac{\Delta O_2}{V_m} \text{In} \quad g/m^3/d \tag{5}$$

where, $VOCR_{TOT}$ is the total oxygen consumed by all bacteria in the biofilters. $\triangle O_2$ is the change in oxygen in and out of the biofilter. V_m is the volume of the biofilter used.

Void space is the volume which is not occupied by the biomedia in the biofilter. Void space divided to the total volume of the containing biofilter gives the void ratio. Clogging is minimal in biofilters with high void ratio due to the large space that allows solid wastes to penetrate. Media size, specific surface area, and void ratio are interrelated, the smaller the size of the media, the larger the specific surface area and the smaller the void ratio.

2.7. Statistical analysis

All data were analyzed by using R statistical program (version 3.9.1). The analysis of variance (one-way ANOVA) was used to analyze the data both water quality parameters (dissolved oxygen, pH, temperature, alkalinity, salinity and total dissolved solids) and parameters for nitrogen removal (VTR, VNRa and VNR). Biomedia and time (i.e; weeks) were used as fixed effects and tested using F test (command var. test ()). Differences were considered significant at $p \leq 0.05$.

3. Results and discussion

Data on biomedia performance are processed and presented as a mean of duplicate biofilters. Weekly data are also presented as a mean of two sampling days every week.

3.1. Water quality parameters

Aquatic environments are complex eco-systems with multiple water quality variables. Among these several play a fundamental role in

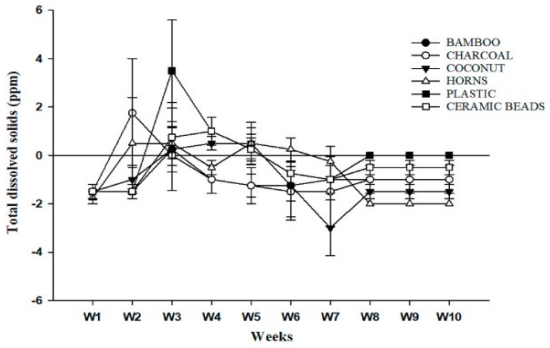

Fig. 2. Mean difference in influent and effluent total dissolved solids (TDS) measured from different biomedia per week (values below 0 reflect reduction of TDS).

Table 2
Mean ± SD weekly water quality parameters in the RAS unit as affected by all treatments.

Weeks	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Total dissolved Solids (mg/L)	Salinity (ppt)
1	5.3 ± 0.4	7.6 ± 0.2	24.3 ± 0.3	59.8 ± 8.8	0.07 ± 0.01
2	5.4 ± 0.2	7.8 ± 0.7	24.7 ± 0.8	75.7 ± 8.9	0.08 ± 0.01
3	5.2 ± 0.3	8.1 ± 0.5	25.7 ± 0	138.7 ± 21.2	0.14 ± 0.02
4	6.3 ± 0.2	7.4 ± 0.2	23.1 ± 0.5	197.0 ± 11.7	0.28 ± 0.03
5	7.0 ± 0.5	7.4 ± 0.2	23.4 ± 0.2	220.0 ± 4.4	0.32 ± 0.02
6	7.5 ± 0.3	7.3 ± 0.1	25.7 ± 0	272.7 ± 11.4	0.31 ± 0.01
7	6.7 ± 0.2	7.6 ± 0.2	25.1 ± 0	283.3 ± 5.1	0.25 ± 0.02
8	6.7 ± 0.1	8.0 ± 0.5	23.5 ± 0	283.0 ± 6.1	0.24 ± 0
9	6.3 ± 0.1	7.3 ± 0.4	25.2 ± 0.6	280.3 ± 1.9	0.24 ± 0.01
10	6.1 ± 0.1	7.3 ± 0	26.6 ± 0	277.3 ± 1.7	0.23 ± 0

aquaculture. The most important parameters affecting fish growth performance include dissolved oxygen (DO), temperature, pH, suspended solids, ammonia, nitrite and carbon dioxide (CO_2) while alkalinity is also important for the nitrifying processes (Ebeling and Timmons, 2012). Results for water quality parameters in this experiment are shown in Figs. 1 and 2 and Table 2.

Dissolved oxygen (DO) is an important parameter in water quality assessment, and it is needed by fish and other aquatic organisms for survival. In the current study, an increase of DO from 5.27 ± 0.4 mg/L in week one to 6.70 ± 0.1 in week eight was observed. Researchers have noticed a substantial effect of dissolved oxygen (DO) concentration on ammonia oxidizing bacteria (AOB) whereby, *Nitrosomonas europaea* dominates the microbial community when DO is below 0.24 mg/L (Zhang et al., 2020). *Nitrosomonas oligotropha* were found to be optimally predominant at 8.5 mg/L DO (Langone et al., 2014). Nitrification in fixed bed biofilters has been reported to stop at dissolved oxygen below 40% saturation (Pedersen et al., 2012). Therefore, this study observed values within the requirements for maximum proliferation of nitrifying bacteria. Earlier research on dissolved oxygen requirements of Nile tilapia revealed a range of 2–10 mg/L (ALY, 2007; Elnady et al., 2017) the current study observed values within the recommended ranges.

Water temperature plays an important role in the metabolic activities of aquatic organism and its changes affect the metabolism and physiology of fishes and, hence, fish productivity (Kinyage and Pedersen, 2016). Temperature in the current study was not variable. The mean value of temperature was 24.7 ± 1.1 °C. Adequate temperature is needed for both nitrifying bacterial growth and biochemical reactions in the biofilter systems (DeLong and Losordo, 2012; Ruiz et al., 2020). Temperature values revealed in the current study are ideal for the optimal growth of Nile tilapia (Ibrahim and Naggar, 2010) as well as nitrifying bacteria (Boller et al., 1994) Nitrifying bacteria can adopt a wide range of environmental temperature if acclimatized slowly (Wang et al., 2015).

Nitrification is very sensitive to pH, and this process declines significantly at pH values below 6.8 (Tomaszewski et al., 2017). Alkalinity is therefore used to balance and maintain the pH in recirculating aquaculture. In this study, system pH was stable around and ranged from 7.3 ± 0 to 8.1 ± 0.5. The system pH values reported in this study are ideal for nitrification and fish development (Guerrero and Fernandez, 2018). During startup of the biofilters, a difference in pH utilization was observed within different biofilters (Fig. 1). Biofilters containing ceramic beads biomedia were seen to have a higher pH decrease between week two and four compared to other biofilters. From week six, after biofilter maturation, all the biofilters had equal pH change. Less has been documented on the utilization of pH in biofilters.

In the current study, salinity increased gradually with time from 0.07 ± 0.01 ppt in week one to 0.32 ± 0.02 ppt in week five and then decreased slightly to 0.23 ppt. The final salinity value was significantly different from the initial salinity value due to the accumulation of ammonium chloride and sodium bicarbonate (Zhang et al., 2019) which were added in form of sodium nitrite, ammonium chloride and bicarbonate soda during the experiment. Individual biofilters did not reveal different salinity inputs to the system. In practical application, however, the temperature and salinity at which the biofilter operates is normally determined by the requirements of the species being cultured rather than specific salinity needs of nitrifying bacteria (Wang et al., 2015). The current study was, therefore, operated at acceptable water quality ranges.

Total dissolved solids increased with time from 59.8 ± 8.8 in week one to 277.3 ± 1.7 mg/L at the end of the experiment in week 10. This shows that some of the media expelled particular matter (biosolids or biofilm detachment), which, in turn, raised water conductivity. Total dissolved solids were significantly different from each biofilter at different time (Fig. 2) the contribution of dissolved solids from individual biofilters for instance charcoal in week one, ceramic beads and plastic in week two are just an additional TDS inputs into the system. In the current study, after biofilter maturation, the biofilters are seen to be responsible for eliminating TDS from the system, biofilter containing horns biomedia getting rid of a higher amount (2 ppm) while biofilters containing plastic removing the least amount of TDS (0.2 ppm) (Fig. 2 and Table 2). All of the tried biomedia were fixed in the filters except commercial plastic and bamboo which had less movement due to their low density nature. In their study on the usage of aquatic floating macrophytes (Lemna and Wolffia) as biofilter in recirculation aquaculture system (RAS) (Malone and Pfeiffer, 2006; Velichkova and Sirakov, 2013) found out that some biofilter materials applied as fixed bed filters can eliminate TDS from system water. Fixed bed biofilters are known to supplement mechanical filtration by collect organic, particles and decay materials from RAS (Pulkkinen et al., 2019).

In the current study, alkalinity increased gradually with time from 73.3 ± 16 in week one to a maximum value of 280 mg $CaCO_3$/L in week seven, ideal values for fish rearing and nitrification activities in RAS (Savin et al., 2012; Zheng, 2019). There were no differences in alkalinity decrease or increase observed in individual biofilters in this study,

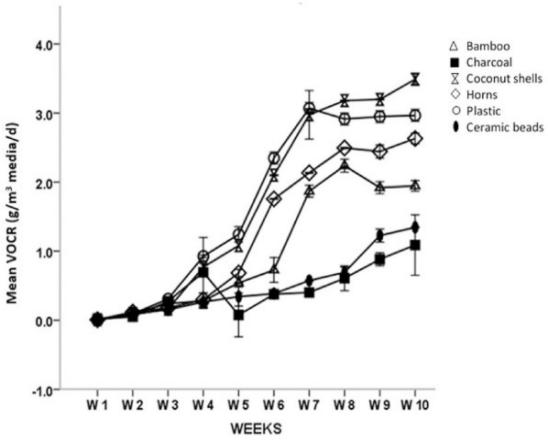

Fig. 3. Mean volumetric oxygen conversion rate in g/m^3/d observed in different biomedia at time of the experiment (calculated as dissolved oxygen in – dissolved oxygen out).

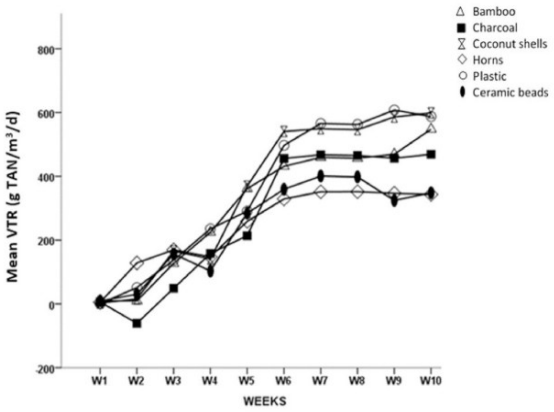

Fig. 4. Mean volumetric TAN conversion rate (g TAN/m^3/d) demonstrated by different biomedia in different experimental period.

Table 3
Description of biomedia maturity and levels of stability.

Measure for maturity					
Biomedia	VTR stability Week	VTR level at stability (g TAN/m^3/d)	VNR stability Week	VNR level at stability (g $NO2-^{N}/_{m3/d}$)	VOCR at VTR stability (g/m^3/d)
Bamboo	Week 6	431 ± 85.6	Week 7	548 ± 90.1	1.7 ± 0.25
Charcoal	Week 6	456 ± 08.1	Week 7	547 ± 51.6	1.4
Coconut shells	Week 6	541 ± 11.6	Week 6	683 ± 73.9	2.1 ± 0.01
Horns	Week 6	329 ± 43	Week 7	433 ± 71.1	1.8 ± 0.01
Plastic	Week 7	566 ± 38.8	Week 7	686 ± 33.4	3.1 ± 0.12
Ceramic beads	Week 7	401 ± 12.1	Week 7	514 ± 31.3	1.6 ± 0.01

despite the fact that denitrification leads to alkalinity increase (Ebeling et al., 2006). According to Malone and Beecher (2000), alkalinity in the recirculating system should not at any time be less than 50 mg $CaCO_3$/L and the optimal is 180 mg $CaCO_3$/L. Similar to the current study, most research on nitrification and fish rearing in recirculating aquaculture reports alkalinity values above 100 mg $CaCO_3$/L (Aich et al., 2020; Boyd et al., 2016; Xiao et al., 2019).

3.2. Oxygen utilization

In the nitrogen cycle, nitrifying bacteria utilize oxygen and alkalinity in the process of converting ammonia and nitrite into nitrate which is less toxic to fish (Francis-Floyd et al., 2020). Malone and Beecher (2000) recommended 2.5–3.0 g O2/$m_3$media/day volumetric oxygen conversion rate in recirculating aquaculture biofilters installed in grow out systems. Between weeks eight and ten of the current study, biofilters containing coconut shells, plastic and horns, demonstrated high and recommended oxygen conversation rate of 3.6 ± 0.1, 3.0 ± 0.1 and 2.8 ± 0.2 g/m^3 media/d, respectively (Fig. 3). This implies that, the particular biofilters could provide a denser and more active biofilm compared to the other tested biomedia. From the first to the fifth week, the levels of oxygen conversion in all six tested biofilters were still below the recommended amount and this shows that the nitrifying bacteria community was not fully developed (DeLong and Losordo, 2012). From the sixth to the tenth week, two of the biofilters (biofilter with charcoal and biofilter with ceramic beads) maintained a low volumetric conversion rate. This implies that charcoal and ceramic beads biomedia could not provide sufficient or conducive environment for development of sufficient quantity of bacteria compared to the other media tested (Fig. 3). A study by (Emparanza, 2009) showed factors affecting nitrification in commercial RAS with fixed-bed biofilters, and revealed that high oxygen consumption in the biofilters lead to oxygen depletion in the system hence affecting nitrification and consequently production in the RAS. As the flow to all biofilters was similar, all biomedia experienced the same reduced oxygen concentration. Hence the performance of biofilters found can by further increased if oxygen was present at higher concentrations (Szwerinski et al., 1986).

3.3. Biofilter maturation

This study evaluated six different biofilters and they had significantly different maturation trends as shown in Table 3. Biofilter maturation is achieved when the biofilter is capable of attaining its maximum capacity for converting ammonia to nitrate (Bracino et al., 2020). Different biofilters have been tested for their maturation period and the results show that most of the biofilters used in recirculating aquaculture system attain maturity between 30 and 50 days (Cruz et al., 2020; DeLong and Losordo, 2012; Zhu et al., 2016). In the current study, biofilters made from bamboo, charcoal, coconut shells and horns demonstrated the highest nitrification capacity in week six of the experiment. The rest of the biofilter showed their maturity at week seven. Regardless of the week of maturity, biofilters containing ceramic beads and horns demonstrated the lowest VTR values that were significantly different from other biofilters. Biofilters containing plastic and coconut shell media demonstrated the highest VTR values that were also significantly different from the other biofilters. In regard to VNR, all the biofilters demonstrated maturity in week seven, except biofilters with coconut shells which showed its highest VNR in week six. Volumetric oxygen conversion rates at maturity show that, each biofilter had a significantly different oxygen consumption rate from the other biofilters. Biofilter containing plastic media consumed the largest amount of oxygen, followed by coconut shells and the last was charcoal. The volumetric oxygen conversion rate reflects the amount of bacteria harbored in the specific biofilters (Boller et al., 1994). A study conducted to assess the performance of different biofilter media during biological bed maturation revealed that plastic media take 45 days to mature (Sikora et al., 2020).

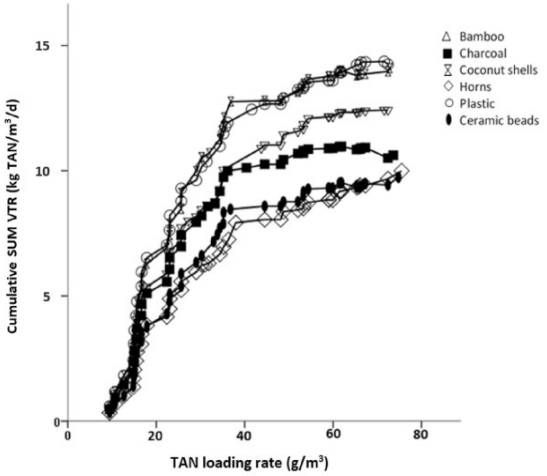

Fig. 5. The response given by different biofilters in terms of cumulative TAN in g TAN/m^3 under different TAN loading rate.

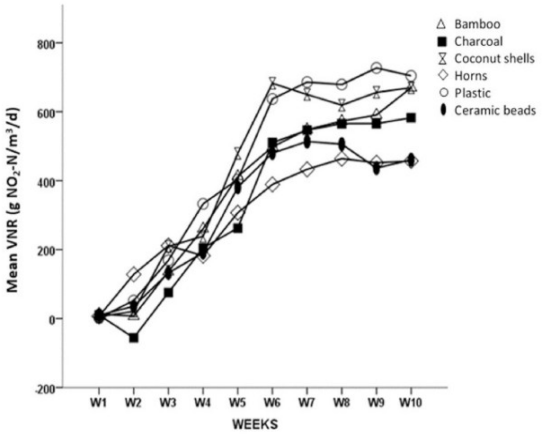

Fig. 6. Mean volumetric nitrite conversion rate (g NO2–N/m3/d) demonstrated by different biomedia in different weeks of the experiment.

3.4. Ammonia removal

Ammonia removal was assessed for the different biofilters according to equation (2). In this study, volumetric TAN conversion rate increased with time in all the tested biofilters (Fig. 4). All the biofilters revealed a sigmoid shaped trend whereby, VTR was very low at the beginning and it stabilized after week six. After stabilization (maturity), biofilters containing plastic and coconut shell biomedia showed and maintained significantly higher VTR than other biofilters. Biofilters containing ceramic beads biomedia showed the lowest VTR at the end of the study, but it did not differ significantly from the values observed for the biofilters containing horns biomedia. In comparison to other studies, the current study found higher VTR values ranging from 329 ± 43.0 to 598.65 ± 15.80 g TAN/m^3/d for the different biofilters. The highest VTR that has been observed for commercially available biological filters in recirculating aquaculture systems is 667 ± 344 (Guerdat et al., 2010). In the study by Guerdat et al. (2010), three biological filters were tested and one element revealed much higher VTR compared to the current study. A study carried out by (Savin et al., 2012) on filter system performance in a tilapia recirculating system reported that VTR could be as high as 2000 g TAN/m^3/d. Another study by (Malone et al., 2006) reported VTR of more than 2000 g TAN/m^3/d which is much higher compared to the VTR values obtained in the current study.

3.5. Cumulative VTR for specific biofilter in response to TAN loading rate

TAN loading rate was calculated as shown in formula no. 1. The effect of TAN loading rate on VTR is presented. Relationship between VTR and substrate loading rate has been shown to be positively correlated in previous studies (von Ahnen et al., 2015; Guerdat et al., 2010). The relationship is only true if the tested biomedia are already colonized. In the current study, uncolonized biomedia were used in the study, therefore, revealing a negative correlation at startup under high TAN loading and subsequently a positive correlation after maturation. Following this observation, cumulative TAN was used to explain the continuous effect of TAN loading rate on VTR for the whole experimental period in the current study (Fig. 5). All tested biomedia experienced exponential VTR increase under TAN loading rate between 0 and 40 g/m^3/d. VTR for biofilters containing charcoal biomedia did not sequentially increase at TAN loading rate beyond 40 g/m^3/d. Other biofilters demonstrated gradual cumulative increase of VTR. In the general view, biofilters containing plastic biomedia ended up with the highest cumulative VTR, which was not significantly different from the biofilters containing coconut shells.

3.6. Nitrite removal

Changes in nitrite concentration during single passage over the biofilters were used to evaluate nitrite removal by the different biofilters tested in this study as shown in Fig. 6. Fig. 6 shows that the VNR values were similar in the biofilter media from week one to week six. Similar to VTR, VNR exponentially increased from week one to week 6, after which the different biofilters demonstrated their differences in VNR. At the sixth week of this trial, biofilters containing horns as biomedia demonstrated the lowest VNR which was significantly different from the VNR for other media. Biofilters containing coconut and plastic biomedia revealed the highest VNR values which were not significantly different from each other, but statistically different from the VNR of all other biofilters. Many researchers do not evaluate biofilter performance in terms of VNR, therefore, information on VNR is limited. A previous study on filter system performance in a tilapia recirculating system reported VNR values ranging from 500 to 4000 g NO_2/m^3/d (Guerdat et al., 2010). This observation corresponds well to the findings in the current study.

3.7. Differences in biofilter performance

The performance of tested biomedia was based on nitrification. Nitrification involves bacterial oxidation of ammonium to nitrite and further to nitrate as shown on equations (6) and (7) (Ebeling and Timmons, 2012).

Ammonia oxidizing bacteria:

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O + 84\ \text{kcal/mol ammonia} \quad (6)$$

Nitrite oxidizing bacteria:

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- + 17.8\ \text{kcal/mol nitrite} \quad (7)$$

The nitrification performance of different biofilters depends on the ability of the media to form biofilm and ensure sufficient transfer from the water into the biofilm. In this study, coconut shells and plastic carriers performed better compared to other four tested media. Bacteria attachment is supported by available surface area in the provided biomedia (Colt et al., 2006). This study had highest void ratio in biofilters containing plastic media, followed by coconut shells and horns. The same biofilters are found to have higher oxygen utilization. These two

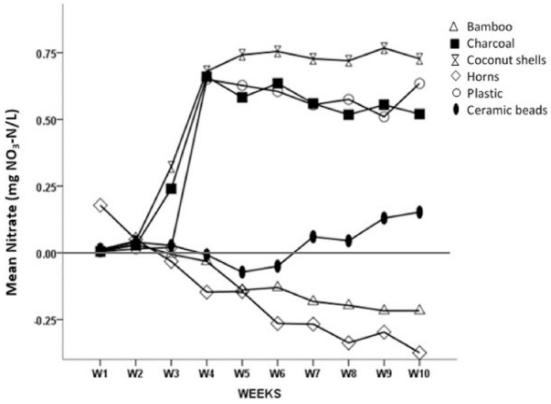

Fig. 7. Amount of nitrate (mg NO_3 -N/L) released into the RAS system by deferent biofilters at different period of the experiment (Biomedia with values below 0 mg NO_3–N/L showing negative nitrate contribution to the system).

indicators show that biofilters containing plastic and coconut shell biomedia contained higher number of nitrifying bacteria and hence the higher VTR and VNR. Additional microbial processes were observed in biofilters containing horns, bamboo and ceramic suggesting heterotrophic N-assimilation and/or denitrifying bacteria as shown in section 3.8.

3.8. Nitrate accumulation in the RAS system

As expected, and in line with findings by Pedersen et al. (2012), the mean nitrate concentration level in the pilot RAS increased from 0.0 mg NO_3–N/L during the first week to 55 mg NO_3–N/L in the fifth week. However, hereafter it substantially dropped to 25 mg NO_3–N/L between week eight and ten. This concentration was contributed by each biofilter as shown in Fig. 7. Biofilters containing coconut shells produced significantly more nitrate, than other tested biofilters. Biofilters with plastic and charcoal biomedia also produced a considerable amount of nitrate into the system. System nitrate concentration donated by biofilter containing ceramic beads was very low while biofilters containing bamboo and horns biomedia took a negative impact on nitrate production. Effluent water from biofilters containing bamboo and cattle horns biomedia were found to reach lower nitrate concentration compared to influent water, suggesting partial denitrification process which occurred in the biofilters. Studies have reported similar decrease in nitrate concentration in the RAS (Kuhn et al., 2010; Sikora et al., 2018). Kuhn et al. (2010) did not observe an increase in the concentration of nitrates in the RAS system inoculated with nitrification bacteria, this was associated with the incomplete nitrification process. The nitrate concentration accumulation curve presented by (Seo et al., 2001) is similar and comparable to the trend observed in biofilters containing plastic, coconut shells and charcoal biofilters in the present study. Towards the end of the experiment, the total accumulated nitrate concentration decreased in RAS systems. This decrease may be associated with development of heterotrophic bacteria in some of the biomedia used in this experiment.

4. Conclusion and recommendation

A comparison of different biomedia to be used in biofilters for TAN and nitrite removal in recirculating aquaculture system showed distinct differences. Out of the five locally available media evaluated in comparison to the commercial media (plastic), only coconut shells could compete with the commercial plastic biofilter by demonstrating VTR and VNR which were not significantly different. The other locally available biomedia which performed better in terms of TAN and nitrite

KEY

1. Rearing tanks
2. Flow regulators
3. Water flow meters
4. Sampling tap (biofilter influent)
5. Air pumps
6. Biofilters
7. Overflow pipes
8. Metal support and ladder
9. Total drainage pipes (waste collector)

Plate 1. Side view of the experimental RAS unit used in the experiment. Showing all the functional parts of the system and water circulation was from 1, 2, 3, 6, 7 and back to 1.

Plate 2. Natural materials used for biofiltration.

removal is charcoal with similar performance as the plastic media. The remaining biomedia used in this study, horns and bamboo, reduced nitrate from the system, but their performance is significantly lower than the plastic media, coconut shells and charcoal. This study, performed under oxygen limiting conditions therefore, concludes that coconut shells have potential to be used as biological filters in recirculating aquaculture systems. Further studies focusing on comparison of coconut shells with other commercial biomedia and assessment the durability and performance of coconut shells in fresh, brackish and salt water are recommended. Here studies could look into parameters such as clogging, anaerobic zones, need of backwash and maintenance and relate this to size of coconut beads and hydraulic.

CRediT authorship contribution statement

Mang'era Samwel Mnyoro: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Renalda N. Munubi:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Lars-Flemming Pedersen:** Conceptualization, Supervision, Resources, Writing – review & editing. **Sebastian W. Chenyambuga:** Supervision, Funding acquisition, Project administration, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Funding for this study was provided by DANIDA through Building Stronger Universities phase III (BSU III) programme at Sokoine University of Agriculture. The technical support and caretaking of the fish and the systems provided by Ms. Stella Genge and Mr. Mathias Shija as well as Ms. Fatuma Kindoro for her help during laboratory analysis, is highly acknowledged.

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CHAPTER FIVE

PAPER FOUR

Capacity of Different Biomedia for Removing Ammonia and Nitrite in Recirculating Aquaculture Systems

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Abstract

Recirculating Aquaculture System (RAS) is a prominent method for intensive fish farming. One of the most important functional parts of RAS is biofilters. Biomedia type and surface area are among the factors determining the effectiveness of a biofilter. This experiment was done to evaluate the ability of coconut shells to support nitrification in RAS in comparison to other cheap but commercially available biomedia (Foam, Leca and plastic). The trial was done in a pilot scale RAS for eight weeks. Water quality parameters were monitored and the performance of different biofilters were assessed in terms of volumetric TAN conversion rate (VTR), volumetric nitrite conversion rate (VNR) and bacterial activity by using hydrogen peroxide (H_2O_2) degradation method. The results indicate that significantly higher VTR (310 ± 21 g TAN/m^3/d) was obtained from biofilter

containing foam biomedia, and significantly lower VTR was obtained from biofilters containing plastic beads biomedia (178.8 ± 63 g TAN/m^3/d) and coconut shells (142 ± 80 g TAN/m^3/d) . Biofilters containing foam biomedia also indicated significantly higher VNR (257 ± 22.2 g NO_2-N/m^3/d) while biofilters containing plastics revealed the lowest (90 ± 5.2 g NO_2-N/m^3/d). H_2O_2 degradation rate constant (*k*) was found to be 0.76 ± 0.1 h^{-1}, 1.0 ± 0.01 h^{-1}, 1.4 ± 0.1 h^{-1} and 2.3 ± 0.3 h^{-1} for biofilters containing plastics, leca, coconut shells and foam biomedia, respectively. It is concluded that foam is a better biomedia for RAS, followed by coconut shells and leca. In order to support the global efforts for avoiding the use of plastic materials in food production, this study recommends the use of coconut shells and leca for biofiltration in RAS.

Key words: Biofilter, Ceramic beads, Coconut shells, Foam, Nitrification kinetics, Plastic beads

5.0 INTRODUCTION

Strict environmental regulations which aim at making the world a better place for current and future generations, have forced inland aquaculture to shift from extensive pond based production system to confined intensive aquaculture systems, with the aim of reducing or eradicating the release of effluent water into the environment (Hai *et al*., 2018; Angel *et al*., 2019; Leal *et al*., 2019; Lulijwa *et al*., 2020). Moreover, the need to meet the growing demand of fish for human consumption has made the intensification of fish production from aquaculture a necessity. On these grounds, recirculating aquaculture is being promoted as an efficient intensive aquaculture production technology that is well suited for environmental conservation purposes (Hai *et al*., 2018). Recirculating aquaculture systems (RAS) allow intensive production of high quality fish with limited amount of water and space and in areas close to markets, thus, giving assurance of high economic

returns (Timmons and Ebeling, 2010). However, the wide use of RAS is facing challenges, including delays in biomedia maturation and availability of sufficient amount of biomedia in some developing countries (Xiao *et al*., 2019).

Recirculating aquaculture system is based on the use of mechanical and biological filters for removal of organic waste products and ammonia, respectively. Nitrifying bacteria attached in the biofilters convert toxic ammonia into nitrite and finally to nitrate which is harmless. Many commercial biomedia and bioreactors have been developed for removal of ammonia, especially in large scale commercial RASs. Some of the commonly used biomedia include; polystyrene microbeads, kaldnes beads, plastic bio blocks and sand (Erkmen, 2000; van Rijn, 2013; Hayder *et al*., 2017; Pulkkinen *et al*., 2018; Betanzo-Torres *et al*., 2020; Mnyoro *et al*., 2021). These biomedia are modeled into different sizes and shapes to give a desired surface area for specific operations, allowing bacteria biomass to grow primarily on the protected surface (Boller *et al*., 1994). Commonly used reactors set-ups are moving bed, fixed bed and trickling bioreactors (Bracino *et al*., 2020). The use of plastic as biomedia in recirculating aquaculture systems has dominated the sector because of its availability, durability and easiness to alter the forms and density. Studies have reported that plastic biomedia is capable of hosting sufficient amount of nitrifying bacteria and, hence, adequate nitrification can take place (Pfeiffer and Wills, 2011; Bracino *et al*., 2020).

In recent years, debates have arisen on the risk associated with plastic erosion and release of microplastics into the environment (do Sul and Costa, 2014; Ma *et al*., 2020; Wu *et al*., 2020; Xiong *et al*., 2021; Zhou *et al*., 2021). These discussions pose potential challenges to the use of plastic as biomedia and for other uses associated with food production. Together with this challenge, the relatively high cost of plastic media is the main

limitation for their application in RAS, especially in developing countries. Thus, developing countries have not been able to import considerable amounts of these biomedia from developed countries where they are produced. Therefore, there is a need to identify alternative materials that are cheaper and readily available in developing countries as biomedia in recirculating aquaculture systems. This study was conducted to evaluate the performance of four different biomedia for removing ammonia and nitrite. The tested biomedia were Coconut shells and Leca ceramic beads (non-plastic generated), and Foam and Plastic beads (plastic generated). These materials were selected based on previous research (Watari *et al*., 2021; Mnyoro *et al*., 2022), availability, cost, surface area and durability (Bagaswari and Moersidik, 2018; Oladimeji *et al*., 2020).

5.1 Materials and Methods

This trial was carried out using a RAS facility (plate 1) of the Aquaculture section, Institute of aquatic resources, Technical University of Denmark for eight consecutive weeks.

5.2 Description of Experimental RAS unit

The RAS unit (Plate 1) comprised of two rearing tanks (each with capacity of 5 m^3), twelve 90 L biofilter tanks, twelve 44 Watt adjustable biofilter pump, one HYDROTECH® drumfilter, one 7.5 m^3 sump, three 1.5 hp RAS water pump (water transports), an oxygen cone and four EXPO-NET® Bio-blocks trickling filters placed in the sump.

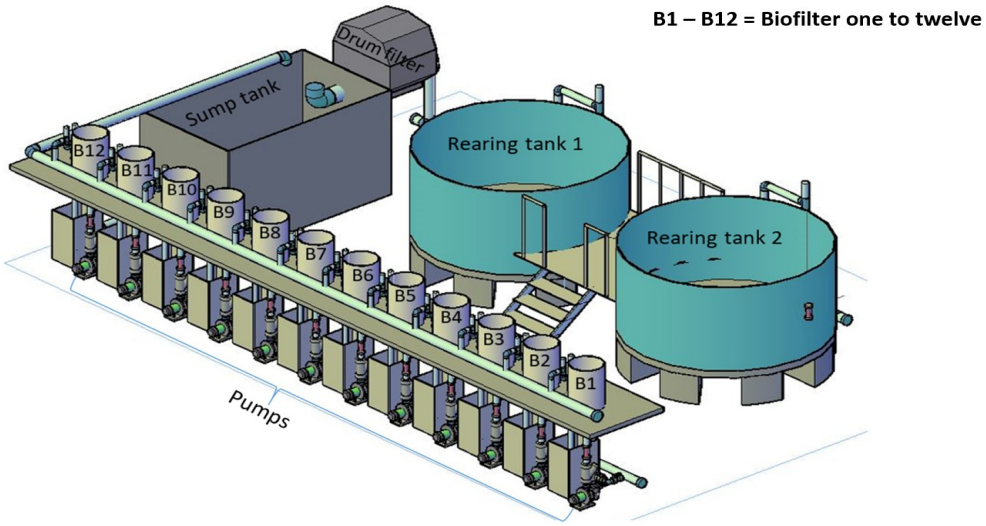

Plate 1: Structure of the system used in this trial

5.2.1 Biofilter specification

Each biofilter had an inlet connected to the common RAS inlet pipe (all at the same level) and an outlet feeding the main outlet to the sump. The inlet (fitted with valve) was connected to a pump that transport water through a series of valve-flowmeter-valve to ensure that specific water volume goes through the biofilter at a given elevation speed. Two colanders were installed in each biofilter, one bellow and one above the biomedia. A perforated air pipe was embedded on the lower colander fitted in a diameter of 29 cm of biofilter-cone for air supply. This holds biomedia and allows water up-flow movement. The upper colander was placed 15 cm below the outlet pipe to ensure provision of similar condition (fixed bed) for all biomedia. The biofilters were operated as shown in Table 1.

Table 1: Specifications and size of Biofilter used to assess ammonia and nitrite removal

Item Description	Size	Unit
Total biofilter volume	90	L
Water volume (strainer-outlet)	66,5	L
Media filling (ratio: 30 %)	20	L
Velocity (in a biofilter)	12	m/h
Lower colander diameter	29	cm
Upper colander diameter	35	cm
Water flowmeter, Wasser® 250-2500 L/h	1500	L/h
Air flowmeter, BROOKS INSTRUMENT® 0-25 L/min	5.0	L/min
Water pump, AQUA MEDICS®	44	Watts
Biofilter Inlet/Outlet pipe diameters	04	cm

2.1.2 Biomedia specification and arrangement

Four different bio-elements were randomly tested in triplicate. These were; coconut shells crushed to approximately 10 - 30 mm (obtained from Tanzania), granulated polyurethane foam (SKU: 100L - G, Skumhuset, Denmark), Leca ceramic beads (LECA® Large 10 - 20 mm, Leca, Denmark) and RK plastic beads (RK BioElements®, RK Plast, Denmark) with density of 1.20 g/cm^3. These media are shown in Plate 2 and described in Table 2.

Table 2: Biomedia types, their density and weight corresponding to the volume used

BIOMEDIA TYPE	DENSITY	WEIGHT/20 L
RK plastic	>1	5.40 kg
Leca	<1	6.90 kg
Foam	<1	3.28 kg
Coconut shells	>1	11.46 kg

All media used in this trial were washed and soaked in clean water for two days to get rid of soil and other loose particles on their surfaces before being installed into the bioreactors. Plastic screens were used to hold all the bio-elements under the water level in

order to ensure maximum utilization of the surface area of each bio-element. The system was provided with a separate up flow biofilter (0.40 m^3 biofilter) filled with 0.2 m^3 1.2 RK PLAST (750 m^2/m^3), KSK Aqua ApS – Saddle-Chips, and sponge. The mentioned biomedia were already colonized to support nitrification at the beginning of the trial. The supportive biofilter was shut down after three weeks of the trial, after observing evidence of nitrification activities in the biofilters. A two-millimetre screen was placed at the collective outlet of all the biofilters to trap any escape of the small biomedia. During this trial, 0.833% and 0.42% of the total volume of Foam and Leca biomedia, respectively, escaped from biofilters and were trapped and taken out of the system.

Plate 2: Type of biomedia used in the study

5.2 Experimental Design

2.2.1 Experiment one

The first experiment was conducted for six weeks. The experiment was designed to monitor the performance of different biomedia from the start of the experiment to maturity. The rearing tanks were stocked with Rainbow trout (*Onchorhynchus mykiss*) at a density of 0.067 kg/m^3. Three millimetre pelleted commercial feed containing 35% crude protein was fed to the fish. Feeding was done for 12 hours daily by the use of belt feeders. A total of 0.8 kg of feed was provided in week one and two, 1.2 kg in week three, 1.8 kg in week four, 2.4 kg in week five and 3.0 kg in week six. Water quality parameters were maintained at the levels that meet the specific requirements for Rainbow trout optimal growth (MacIntyre *et al*., 2008). System makeup water replacement was maintained at 100 L/h, equivalent to 2.62 m^3/day.

Five litres of water were taken from the rearing tanks at weekly intervals for determination of system total chemical oxygen demand (COD_{TOT}), dissolved chemical oxygen demand (COD_{DISS}), five days' total biological oxygen demand ($BOD_{5\text{-}TOT}$), dissolved biological oxygen demand ($BOD_{5\text{-}DISS}$), alkalinity and nitrate. Filtration (0.45 µn) was done for dissolved chemical oxygen demand (COD_{DISS}) and dissolved biological oxygen demand ($BOD_{5\text{-}DISS}$) samples. Sulphuric acid (4 M) and Allyithiourea nitrification suppression variant (ATU 0.1%) were used to preserve COD and BOD samples, respectively. Fifteen ml of water were sampled from biofilter influent and effluent and filtered (mesh size 0.2 µn) for ammonia and nitrite analysis two times a week. All the samples were preserved at 4 °C, and analysis was done within the next 24 hours.

5.2.2 Experiment two

The second trial was designed and conducted for two weeks, immediately after the first experiment, to monitor the performance of different biomedia after maturity by eliminating organic matter and heterotrophic organisms from the system as well as deactivating the heterotrophic bacteria attached to the biomedia by starvation. This action provided a greater chance for autotropic bacteria in the biofilters to show their optimal performance. Fish were removed from the system, the biofilters were locked into their separate loop (Plate 3) to prevent biofilter washback and minimize bacterial disturbance. A large amount of system water (90 %) was exchanged and the whole system was opened back to normal circulation. A large amount of organic materials and heterotrophic bacteria were eliminated from the system. Synthetic ammonia was added into the system in form of ammonium chloride (NH_4Cl) at a rate of 20 g/m^3 NH_4CL. Ammonium chloride was added into each rearing tank by the use of a 12-hour belt feeder. The amount of synthetic ammonia provided corresponded to the ideal amount released into the system by feeding the fish at week six before destocking the system (Ip and Chew, 2010; Huang, 2019).

Water pH and alkalinity were optimized by the use of sodium bicarbonate. Water sampling was done in week two of this trial for three consecutive days (Table 3).

The biofilters were decoupled from the flow-through systems, turned into internally circulated loops, forming smaller individual systems, each containing one biofilter (66.5 L), one sump (90 L), two aerators (one in the biofilter and one in the sump) and one pump as shown in Plate 3.

Table 3: Spiking of hydrogen peroxide, ammonia and nitrite

Day	Parameter	Chemical spiked	Amount added (g)	Target concentration
One	Total Ammonia Nitrogen (TAN)	Ammonium chloride (NH_4Cl)	2.99	5 mg TAN/L
Two	Nitrite	Sodium nitrite ($NaNO_2$)	1.17	5 mg NO_2-N/L
Three	Bacterial activity	Hydrogen peroxide (H_2O_2)	53.06	10 mg/L

For Ammonia-N and Nitrite-N samples, one turnover (6 minutes) was allowed before sampling. Fifteen milliliters of water were sampled from the sump after every third turnover (20 minutes) for 30 consecutive turnovers (200 minutes). The same procedure was repeated by adding sodium nitrite to an initial nitrite concentration of 5 mg/L. For hydrogen peroxide degradation, initial samples were taken before spiking. After spiking, sampling was done after 5, 10, 15, 20, 25, 30, 40, 50 and 60 minutes in each biofilter (Pedersen *et al*., 2019a).

5.3 Laboratory Analysis

Total and dissolved chemical oxygen demand (COD_{TOT} and COD_{DISS}) were determined spectrophotometrically using Merck test kit (1414). Total and dissolved five days' biological oxygen demand ($BOD_{5\text{-}TOT}$ and $BOD_{5\text{-}DISS}$), were analyzed by following the

procedure described in the Danish standards for water analysis (1991). Total ammonium nitrogen (TAN), Nitrite nitrogen (NO_2 —N) and nitrate nitrogen (NO_3 —N) were measured spectrophotometrically at 680, 545 and 340 nm, respectively, following Danish standards for water analysis (1991) and ISO 7890 (1986). Alkalinity was measured by an end point titration to pH 4.5 using Mettler Toledo T50 auto-titrator (Glostrup, Denmark). Water pH, oxygen and temperature were measured using Hach Lange HQ40 multimeter (Düsseldorf; Germany) with an IntelliCALTM PHC1010 pH electrode and an Intellical LDO101 Laboratory luminescent optical dissolved oxygen sensor.

Bacteria activity

The hydrogen peroxide degradation assay described by Arvin and Pedersen (2015) was used to assess bacterial activities. The method quantifies enzymatic reactions based on the existence and activeness of particle-bound bacteria (Hosetti and Frost, 1994). The higher the bacterial activity in the media, the higher the degradation gradient of hydrogen peroxide concentration. Hydrogen Peroxide concentration was measured spectrophotometrically using the method described by Pedersen *et al*. (2019). The degradation kinetics can be described as a first order reaction by the exponential decay equation: $C_t = C_0 \cdot e^{-kt}$, with k being the descriptive reaction rate constant (per h), C_0 the initial concentration of H_2O_2 (mg/L), C_t the concentration at time "t" in hours (h) (Arvin and Pedersen, 2015).

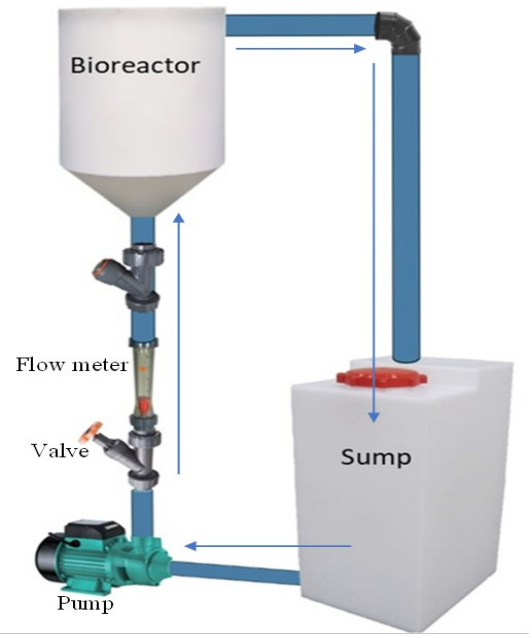

Plate 3: Closed loop system of individual biofilters

5.4 Computation of Ammonia and Nitrite Removal

Nitrification kinetics was determined by calculating the volumetric TAN conversion rate (VTR) and volumetric nitrite conversion rate (VNR) as described in the following formulas.

$$VTR = 1 \cdot 44 \left(Q_f\right) \frac{TA\,N_I - TA\,N_E}{V_m} \text{ In g TAN/m}^3\text{/d} \quad \text{.....} \quad \text{(i)}$$

$$VTR = \frac{\left(\Delta TAN\right) * Q}{V_{media}} \quad \text{In g TAN/m}^3\text{/d} \quad \text{.....} \quad \text{(ii)}$$

Where, VTR reflects corresponding volumetric ammonium N concentrations (g N/m^3) from inlet and outlet of the biofilters; Q_f is the water flow into the media (m^3/d) and V_m is the available volume of the carrier elements (m^3) (Malone and Beecher, 2000; Guerdat *et al*., 2010).

The apparent volumetric nitrite conversion rate (VNRa) was calculated as:

$$VNR_a = 1 \cdot 44 \left(Q_f\right) \text{¿¿¿ In g } NO_2^- \text{N/m}^3\text{/d} \quad \text{.....} \quad \text{(ii)}$$

Where, [NO_2^-N] reflects corresponding volumetric nitrite concentrations (g N/m^3) from inlet and outlet of the biofilters; Q_f is the water flow into the media (m^3/d) and V_m is the available volume of the carrier elements (Malone and Beecher, 2000).

The actual volumetric nitrite conversion rate (VNR) taking the de facto oxidized TAN contribution into account was calculated as:

VNR = VTR + VNRa ……..… …….. (iv)

$$VNR = \frac{(\Delta NITRITE) * Q}{V_{media}}$$ In g $NO_2^-N/m^3/d$ ……..……. (v)

5.5 Statistical Analysis

Data was normalized and normality was assessed by Shapiro–Wilk test. Analysis of variance (Two-way ANOVA) was done for all parameters for nitrogen removal (VTR, VNRa and VNR). The biomedia and time were used as fixed factors in the model. The statistical analyses were done by using R statistical programme version 3.9.9. Tukey post-hoc test was carried out for multiple comparisons, as appropriate. Mean differences were considered significant at $p \leq 0.05$.

5.6 Results

5.6.1 Water quality parameters measured in the RAS unit

Water quality parameters were measured for six weeks. Water temperature, dissolved oxygen, pH and alkalinity values in the RAS are shown in Table 4. The mean temperature during the trial was 4 ± 0.5 °C, dissolved oxygen was 9.9 ± 0.8 mg/L, pH was 7.2 ± 0.2 and alkalinity was 125.19 ± 8.6 mg $CaCO_3$/L. Total and dissolved chemical oxygen demand, total and dissolved biological oxygen demand increased lineally from week one to six. Total chemical oxygen demand increased from 17.6 ± 0.6 mg/L in week one to 39.1 ± 0.4 mg/L in week six. Dissolved chemical oxygen demand increased from 15 ± 0.8

mg/L to 30.2 ± 0.3 mg/L. The initial value for total biological oxygen demand was 2.5 ± 0.4 mg/L in week one and it increased to 6.6 ± 0.5 mg/L in week six, while dissolved biological oxygen demand increased from 1 ± 0.2 mg/L in week one to 4.9 ± 0.3 mg/L in week six.

Table 4: Mean ± standard deviation of water quality parameters as measured in the RAS during the experiment

Time (Weeks)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Alkalinity (mg $CaCO_3$/L)	COD_{TOT} (mg/L)	COD_{DISS} (mg/L)	$BOD_{5\text{-}TOT}$ (mg/L)	$BOD_{5\text{-}DISS}$ (mg/L)
1	16.8 ± 0	10.4 ± .4	7.4 ± 0	126.81 ± 7.3	17.6 ± .6	15 ± .8	2.5 ± .4	1 ± .2
2	16.55 ± .1	10 ± .1	7.3 ± .1	129.25 ± 4	21.2 ± .2	18 ± .6	3.9 ± .1	1.5 ± .1
3	16.6 ± 0	10 ± .7	7.2 ± .1	122 ± 3.3	25.35 ± .2	23.2 ± .2	4.6 ± .1	1.75 ± .2
4	16.6 ± .4	10.1 ± 1	7.1 ± 0	115 ± 11.5	31.9 ± .4	25.75 ± .4	5.1 ± 0	3.1 ± 1
5	15.7 ± .6	9.3 ± .1	7.0 ± 0	129.65 ± 7.4	36.05 ± .5	28.45 ± .6	6.2 ±1.3	4.5 ± .2
6	15.7 ± .4	9.8 ± .3	7.1 ± .1	128.45 ± 5.2	39.1 ± .4	30.2 ± .3	6.6 ± .5	4.9 ± .3
Mean	** Expression is faulty ** ± .5	** Expression is faulty ** ± .8	** Expression is faulty **±.2	** Expression is faulty ** ±8.6	** Expression is faulty **±7.3	** Expression is faulty ** ±5.4	** Expression is faulty ** ±1.5	** Expression is faulty ** ±1.4

Total ammonia nitrogen, nitrite and nitrate accumulated in the RAS as shown in Fig. 1. TAN accumulated from 0.1 ± 0.09 mg/L in week one to 0.5 ± 0.1 mg/L, followed by a drop to 0.4 ± 0.1 mg/L in week six. Nitrite accumulated from 0.15 ± 0.1 mg/L to 0.35 ± 0.18 mg/L in week one and five, respectively and slightly dropped from 0.35 ± 0.1 mg/L to 0.3 ± 0.05 mg/L in week six. Nitrate gradually increased from 10.0 ± 5.1 mg/L to 37.5 ± 2.0 mg/L between week one and five, followed by an exponential increase to 78.1 ± 4.2 mg/L in week six.

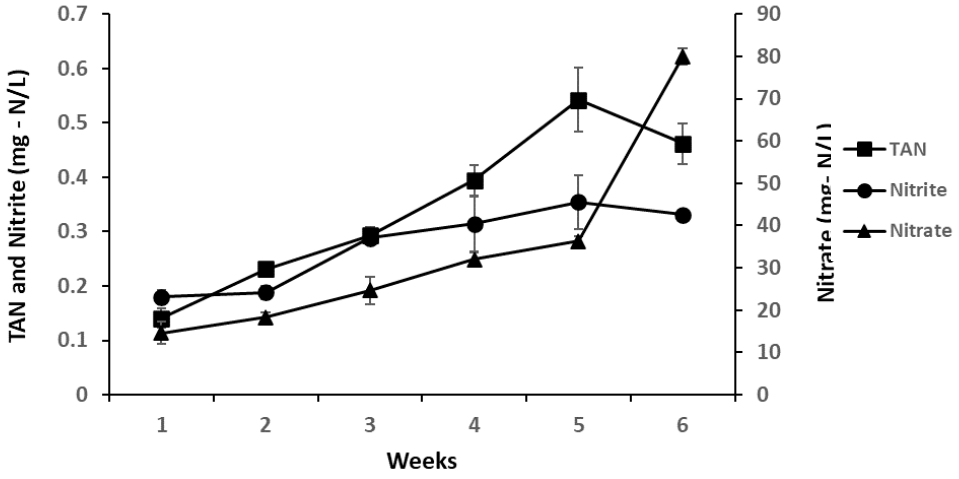

Figure 1: Mean accumulated TAN, Nitrite and Nitrate concentrations in the system during the six weeks of the experimental period

5.6.2 Mean daily variation of TAN and nitrite during experiment one and two

During this trial, TAN and nitrite concentration trends were monitored for 24 hours within a day and it was revealed that between midnight (00:00 hours) and 10:00 hours in the morning, the amount of nitrite concentration in the system was higher compared to the amount of ammonia concentration during trial one (Fig. 2A). For the rest of the day, ammonia concentration was higher compared to nitrite concentration. Diurnal variation of TAN and nitrite concentrations in trial two revealed that TAN was always higher in the system compared to nitrite, with a significantly higher diference between 12:00 and 00:00 midnight (Fig. 2B).

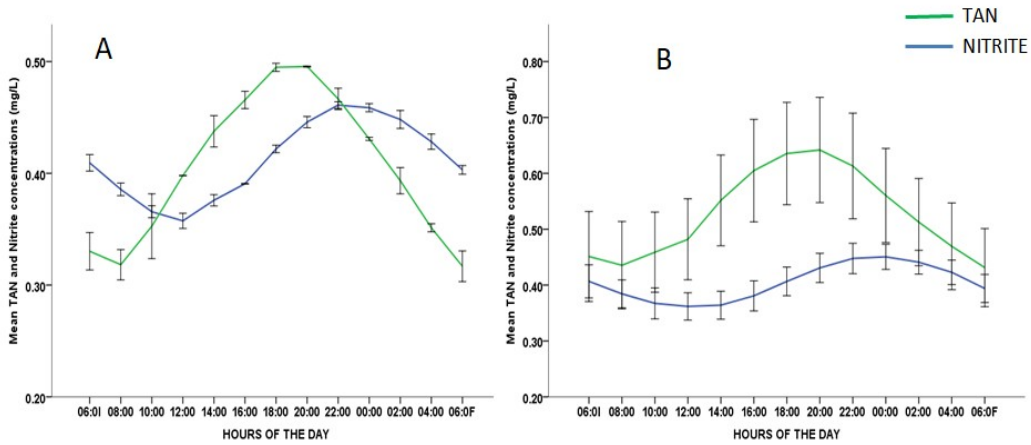

Figure 2: The mean diurnal change of TAN and nitrite concentrations in the system during experiment one (A) and experiment two (B)

5.2 Volumetric TAN and Nitrite Conversion Rates from Start-up to Stable State

Volumetric TAN conversion rate (VTR) and volumetric nitrite conversion rate (VNR) in different biofilters were measured weekly and the results are as shown in Fig. 3A and 3B. The type of biomedia used had significant effect on the VTR and VNR recorded. Volumetric TAN conversion rate from the biofilters containing foam biomedia incresed from 35 ± 7 g TAN/m^3/d to 313 ± 78 g TAN/m^3/d from week one to five (Fig. 3 A). Biofilters containing plastic beads media started with VTR of 12.7 ± 3 g TAN/m^3/d and increased steadily and obtained a steady state in week five with VTR of 178.8 ± 63 g TAN/m^3/d. Biofilters containing coconut shells had a VTR value of 12.5 ± 7 g TAN/m^3/d in week one and the nitrification increased to 142 ± 80 g TAN/m^3/d in week five. Biofilters containing leca showed a VTR value of 9.5 ± 15 g TAN/m^3/d in week one and 144 ± 72 g TAN/m^3/d in week five.

Volumetric nitrite conversion rates measured in different biofilters demonstrated an increasing trend from the start to week five of the trial (Fig. 3 B). During the first 15 days of the trial, the VNR measured in all tested biofilters was not statistically different from

each other. From week three, to the last week (week six), VNR measured in biofilters containing foam biomedia revealed the highest and significantly different values (68 ± 23 g NO_2-N/m^3/d to 257 ± 22.2 g NO_2-N/m^3/d) compared to other biofilters. Volumetric nitrite conversion rate in biofilters containing coconut shells, leca and plastic beads increased from around 40 g NO_2-N/m^3/d in week three to a around of 120 g NO_2-N/m^3/d in week five, followed by a decrease of VNR measured in biofilters containing Plastic beads in week six (90 ± 5.2 g NO_2-N/m^3/d).

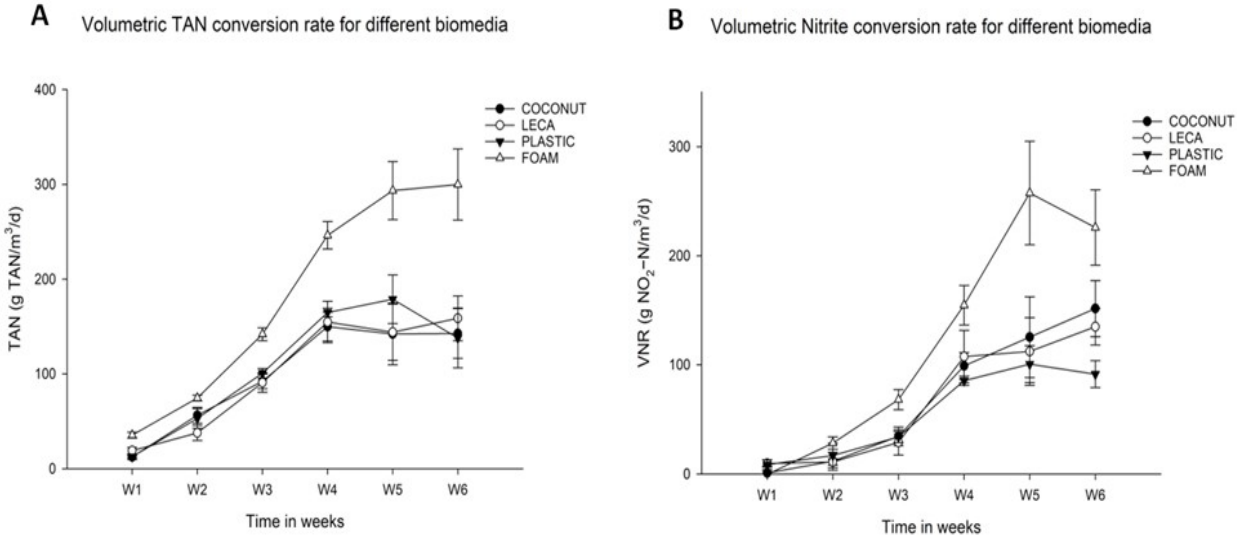

Figure 3: Volumetric TAN and nitrite conversion rate for different biomedia over time (A = VTR per time and B = VNR per time)

Nitrification kinetics at stable state

The starting concentrations after spiking for all the biomedia used in this trial were 4 mg/L and 5 mg/L for TAN and NO_2-N, respectively (Fig 4 A and B). After 200 minutes of closed circulation, the TAN and nitrite concentrations in the systems connected to biofilters containing foam, coconut shells and leca biomedia decreased to undetectable levels. Concentrations of TAN and nitrite in the systems containing Plastic beads biomedia were reduced to 0.5 ± 0.1 mg TAN/L and 2.7 ± 0.1 mg NO_2-N/L, respectively. Systems with biofilters containing foam biomedia showed a quicker and significant TAN and nitrite concentration reduction rate compared to the other systems as shown in Fig. 4.

With regard to equations 2 and 4 mentioned in section 2.4, the zero (0') order nitrification kinetics for volumetric TAN conversion rates were found to be 4.6 ± 0.3, 3.8 ± 0.3, 3.3 ± 0.3 and 1.7 ± 0.2 g/m^3/d for biofilters containing foam, coconut shells, leca and plastic beads, respectively. The calculated first order rate constant k_{1a} (1' order) for volumetric TAN conversion rates were 0.05, 0.04, 0.04 and 0.01 m/d for biofilters containing foam, coconut shells, leca and plastic, respectively. On the other hand, the 0' order nitrification kinetics for VNR were 5.1 ± 0.8, 4.5 ± 0.6, 3.7 ± 0.2 and 1.4 ± 0.2 g/m^3/d for biofilters containing foam, coconut shells, leca and Plastic beads, respectively. The first order rate constant k_{1a} was 0.04, 0.03 and 0.02 for biofilters containing foam, coconut shells and leca, respectively. The concentration of nitrite in biofilters containing plastic beads biomedia did not reach substrate dependant VNR levels (Fig. 4B), therefore, the 1' order VNR was not calculated for these biofilters.

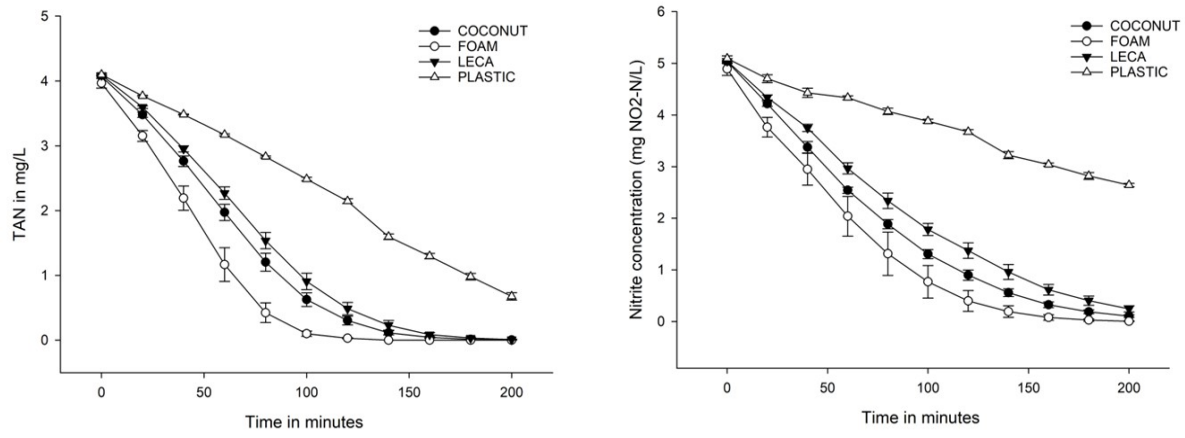

Figure 4: Change in TAN and Nitrite concentration in different biofilters over time after spiking of ammonium chloride and sodium nitrite (A = TAN concentration in the system and B = Nitrite concentration in the system)

5.3 Mean Nitrate and Alkalinity Changes during Ammonia and Nitrite Spiking

Nitrate and alkalinity changes were assessed during spiking of ammonia and nitrite for different systems containing different biomedia as shown in Table 5. After ammonia

spiking, 200 minutes of looped circulation resulted into change of nitrate concentration. Biofilter containing coconut shells biomedia revealed the highest nitrate concentration increase, followed by the systems containing leca, foam and lastly Plastic beads. Alkalinity decrease was also observed within 200 minutes of closed circulation after ammonia spiking. Alkalinity utilization in the biofilters was not very variable, but biofilters containing leca biomedia revealed the highest alkalinity utilization while the biofilters containing Plastic beads showed the lowest value. The same trend of nitrate increase was also seen after nitrite spiking. Systems with biofilters containing coconut shells and leca had the highest nitrate concentration increase compared to the systems containing foam and Plastic beads biomedia. Alkalinity change after nitrite spiking was very low in all systems compared to the change seen during ammonia spiking.

Table 5: Nitrate increase and alkalinity decrease in 200 minutes closed loop during ammonia and nitrite spiking for different biofilters

Biofilter	**Nitrate increase during ammonia spiking (mg NO_3/L)**	**Alkalinity decrease during ammonia spiking (g $CaCO_3$/L)**	**Nitrate increase during nitrite spiking (mg NO_3/L)**	**Alkalinity decrease during nitrite spiking (g $CaCO_3$/L)**
Coconut	4.1 ± 1^{a}	33.3 ± 2.2^{a}	3.1 ± 0.2^{a}	0.7 ± 0.9^{a}
Foam	3.1 ± 0.7^{a}	32.0 ± 0.2^{a}	2.7 ± 0.5^{b}	4.4 ± 4.9^{a}
Leca	4.0 ± 0.7^{a}	33.6 ± 2.4^{a}	3.2 ± 0.1^{a}	2.6 ± 2.4^{a}
Plastic	2.7 ± 0.8^{b}	27.5 ± 2.5^{b}	1.9 ± 0.2^{c}	1.6 ± 2.5^{a}

5.4 Hydrogen Peroxide Degradation

Hydrogen peroxide degradation decreased over time as shown in Fig. 5 A. The lowest Hydrogen peroxide (H_2O_2) removal rate was observed in biofilters containing Plastic beads media (3.0 ± 0.3 mg/L reduction of H_2O_2 concentration after 30 min) with a mean degradation rate constant (k) of $0.76 \pm 0.1\ h^{-1}$. Biofilters containing leca biomedia had a

3.7 ± 0.36 mg/L H_2O_2 reduction after 30 min with a mean degradation rate constant (k) of 1.0 ± 0.01 h^{-1}. Biofilters containing coconut shells showed a reduction of 4.5 ± 0.11 mg/L H_2O_2 after 30 minutes and a mean degradation rate constant of 1.4 ± 0.1 h^{-1}. The biofilters containing foam biomedia revealed the highest reduction of H_2O_2 (5.8 ± 0.16 mg/L) after 30 minutes with a mean degradation rate constant of 2.3 ± 0.3 h^{-1}. Hydrogen peroxide degradation in the system water without the biofilters was also assessed to serve as a control and the result is shown in Fig. 5 B. The result indicated that there was no H_2O_2 reduction.

The recorded hydrogen peroxide degradation was as shown in Fig. 5. The degradation was different for all the tested biomedia. The initial concentration recorded in all the tested media was around 10 mg/L and after one hour, the concentrations were low and between more than 6 mg/L and 1 mg/L for different biofilters. The concentration of H_2O_2 in the system water remained constant around 10 mg/L for one hour.

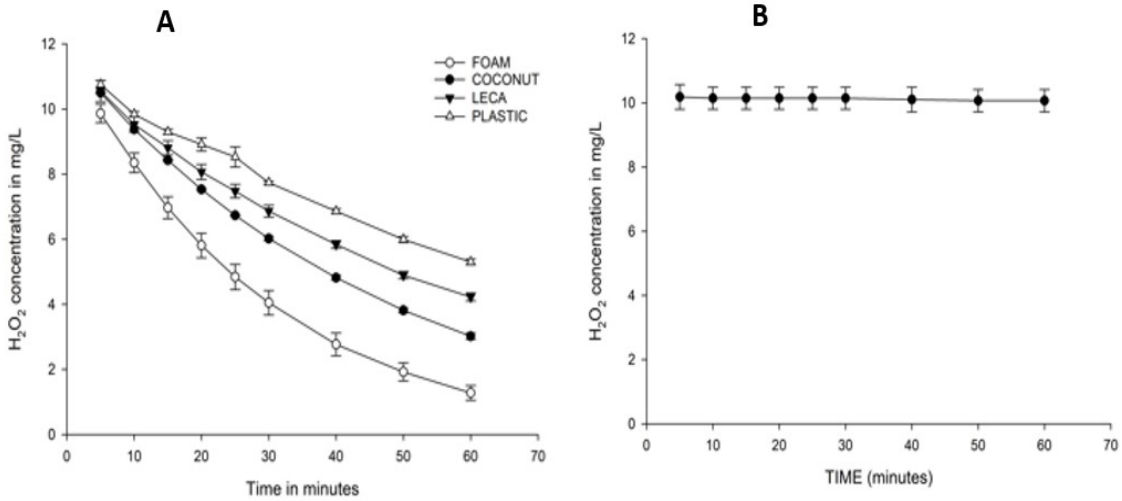

Figure 5: Degradation of H_2O_2 (A –in the system with biofilters and B - in the system water without biofilters) over time

5.5 Discussion

5.5.1 Water quality parameters

Water quality is important for the welfare of aquatic organisms. Poor water quality affects physiology, growth development and productivity, leading to pathological vicissitudes and organ impairment, or causes death in severe cases (Guerrero and Fernandez, 2018). The fatal effects of poor water quality are associated with an increase in the incidence of disease (MacIntyre *et al*., 2008). In this study, water quality parameters were maintained at recommended levels for normal growth of rainbow trout. In their collective review, MacIntyre *et al*. (2008), recommended dissolved oxygen range of between 5 and 10 mg/L at 15 - 20 °C for rainbow trout.

Biological oxygen demand ($BOD_{5\text{-}TOT}$ and $BOD_{5\text{-}DISS}$) and Chemical oxygen demand (COD_{TOT} and COD_{DISS}) increased exponentially from week one to week six. The initial values of BOD and COD were ideal and within the expected values in recirculating aquaculture system (Rojas-Tirado *et al*., 2017). The increase in these parameters in the system indicated an increase in microbial activity caused by increased presence of bacteria community in the system (heterotrophic and autotrophic bacteria) (Rojas-Tirado *et al*., 2017; Safwat, 2018; Ruiz *et al*., 2020).

In any biofilter experiment, TAN, nitrite and nitrate concentrations are expected to increase during the initial phase of the experiment before biofilter maturation. This period is followed by a drop in TAN and nitrite concentrations in the system with a concomitant sharp increase of nitrate concentration (Villaverde *et al*., 2000; DeLong and Losordo, 2012; Jiang *et al*., 2019). This phenomenon was also demonstrated in the current study, whereby TAN, nitrite and nitrate concentrations followed exactly the same trends. The decrease of nitrite and increase of nitrate was due to nitrification process. Nitrification

involves complete oxidation of ammonia to nitrate through nitrite. This is a two-step reaction which involves ammonia nitrifying bacteria (AOB) converting ammonia to nitrite and nitrite oxidizing bacteria (NOB) responsible for nitrite to nitrate conversion (Hagopian and Riley, 1998). In a biofilter start-up, a film of AOB develops faster compared to that of NOB, resulting in the amount of nitrite to increase in the system while ammonia concentration is reduced. After development of both the AOB and NOB films, complete nitrification reaction occurs and, therefore, the nitrate concentration keeps raising in the system.

Diurnal concentration of TAN and nitrite observed during biofilter start-up interchangeably diverge from each other until the nitrification process reaches its optimality (Rojas-Tirado *et al*., 2017). In the current study, the same interchangeable pattern of increase of TAN and decrease of nitrite was observed. Previous studies have shown clearly that water quality, specifically the concentration regime of the limiting nutrient (TAN and/or nitrite N), is extremely important in defining the start of nitrification and consequently the amount of these nutrients retained in the system (von Ahnen *et al*., 2015). At a stable nitrification state, the diurnal TAN and nitrite concentrations in RAS have a normal distribution nature because the concertation is controlled by both substrate loading rate and nitrification.

5.5.2 Volumetric TAN and Nitrite conversion rates

The steady state volumetric TAN and nitrite conversion rate for all tested biofilters were obtained in week five. Throughout the study period, biofilters containing foam biomedia revealed significantly higher VTR values compared to the other biomedia. The VTR values observed in all other biomedia from start-up to stable state, were not significantly different from each other. Volumetric nitrification rates of all tested biomedia types in

biofilters increased with the influent TAN concentration which is synchronized with the feeding schedule (Chen *et al*., 2006). The differences of VTRs among the four different media types in the biofilters were due to the difference in material properties of the biomedia (Smet *et al*., 1996).

The volumetric nitrite removal observed from start-up to stable state in this trial indicated higher and significantly different performance of biofilters containing foam biomedia from week three to six. The VNR performance of the other tested biomedia varied slightly in week four and five, though the differences were not significant. In the last week of the first trial (week six), biofilters containing plastic beads biomedia recorded a significantly lower VNR compared to all other tested biomedia. In other biofiltration trials documented, foam has shown an extra ordinary nitrification performance, just as it has been demonstrated by the current study. Beneš *et al*. (2020) reported 218 ± 33 g TAN/m^3/d as the highest biofiltration capacity of foam. Chu and Wang (2011) compared polyurethane foam and biodegradable polymer as carriers in moving bed biofilm reactor for treating wastewater and observed higher performance of polyurethane foam in terms of total nitrogen removal (65 to 95 %). However, the use of foam in aquaculture food production may not be fully supported because of its biodegradability and ecotoxicity nature (Skleničková *et al*., 2020).

Nitrification performance of three different forms of plastic media were tested by Pfeiffer and Wills (2011) and they reported a mean VTR of 166.4 ± 26.5 g TAN/m^3/d at higher substrate concentration and stable state. This findings concur with to the nitrification ability of plastic beads biomedia observed in the current study. Suhr and Pedersen (2010) compared nitrification performance of plastic beads biomedia in submerged fixed bed biofilters (FBB) (the same set-up as the current study) and moving bed biofilters (MBB).

In their study they obtained a volumetric TAN removal of 92 g TAN/m^3/d for the FBBs which is less compared to the values observed in the current study.

The use of coconut shells for aquaculture biofiltration has not been exploited. Trials have been done on the use of coconut shells in air filtration and aquaponics and all the trials reported positive results (Ramírez-López *et al*., 2003; Jordan *et al*., 2018; Mulay and Reddy, 2021). This study found a similar nitrification performance between biofilters containing coconut shells and leca. The two biomedia were found to be better in VTR and VNR compared to Plastic beads biomedia in the current study. The study conducted by Li *et al*. (2020) showed that ceramic biofilters performed better (VTR; 93 ± 3) compared to plastic (VTR; 45 ± 5 g/m3/d). The reported values were lower compared to the findings in the current study. However, the findings reported by Li *et al*. (2020) are in agreement with the results of the current study in showing that ceramic biofilters perform better than plastic biofilters.

5.5.3 Nitrification kinetics at stable state

Based on the standards established for evaluating a specific set of operating conditions in RAS trials (Chen *et al*., 2006; Fangyong and Benben, 2010), conversion rates for TAN and nitrite were measured at low and high substrate loading to assess 0′-order and 1′-order kinetics in the different biofilters tested. Biofilters containing foam revealed the highest substrate degradation (kinetic values), followed by coconut shells and leca, while biofilters containing plastic beads biomedia, showed the lowest removal kinetics. High kinetic values are suggestive of greater substrate consumption (TAN removal) and more active microbial mass per unit volume of media which is anticipated as a result of rising supply of substrate concentrations (Pfeiffer and Wills, 2011).

Experimental results on performance of different biological filters including ceramic and plastic beads done by Fangyong and Benben (2010) reported that, under substrate

unlimiting conditions, the maximum TAN removal rate of ceramic beads biofilters was 7.45 g/m^3/d while plastic beads was 3.08 g/m^3/d. These values are higher compared to the findings in the current study, but both studies agree that ceramic beads seem to be better filters in TAN and nitrite removal compared to plastic beads. Studies have shown that nitrification kinetic values fluctuate depending on reactor type and application (Hall, 1999; Pfeiffer and Wills, 2011).

5.5.4 Mean nitrate and alkalinity changes during ammonia and nitrite spiking

Complete nitrification results into increase in nitrate concentration (Hagopian and Riley, 1998). During ammonia spiking in this study, nitrate concentration changes in the system was highest for the biofilter containing coconut shells biomedia, but did not differ significantly from the changes shown by the systems containing the other types of biomedia. The system containing Plastic beads biomedia recorded the lowest nitrate concentration change. The changes in nitrate concentration at nitrite spiking were lower for all systems compared to the changes during ammonia spiking. Nitrate concentration changes in the systems containing foam, leca and coconut shells were higher compared to the concentration change in the system containing Plastic beads biomedia. The accumulation of nitrate in each individual system reflects the activeness of nitrification process. From that perspective, nitrification levels were similar in all systems, but was quite lower in the system containing plastic beads biofilters.

The alkalinity changes in the systems containing foam, leca and coconut shells biofilters were higher, but they did not differ significantly after ammonia spiking. This implies similar nitrification in the systems containing foam, coconut shells, and leca. The alkalinity depletion in the system containing plastic beads biomedia was significantly lower, indicating low nitrification (Boyd *et al.*, 2016). The mean alkalinity changes in all the systems during nitrite spiking were not statistically different from each other. The

stoichiometric equation for complete oxidation of ammonium to nitrate, including cellular synthesis, shows that per mole of ammonia removed, a significant amount of oxygen is required and a substantial amount of alkalinity is destroyed by the production of hydrogen ions (Biesterfeld *et al*., 2003). Accepted stoichiometric ratios for alkalinity destruction are between 7.07 to 7.4 grams of alkalinity consumed per gram of ammonia (NH_3-N) oxidized to nitrate (NO_3-N) in a closed system (Figueroa and Silverstein, 1992; Biesterfeld *et al*., 2003; Boyd *et al*., 2016).

5.5.5 Microbial activity measured by Hydrogen peroxide degradation

Nitrification in different biofilters can differ due to the difference in the amount of nitrifying bacteria hosted in the biomedia (Schmidt and Belser, 1983). Hydrogen peroxide decomposition assay, a new fast tool to describe microbial activity, was used for evaluating microbial activity in each biofilter (Pedersen *et al*., 2019; Rojas-Tirado *et al*., 2018). This study revealed significant different bacterial activities among the biofilter. The biofilter containing foam biomedia showed the highest bacterial activity, followed by those containing coconut shells, leca and lastly plastic beads. As a control, system water without biofilters was tested and no bacterial activities were found. Abucayon *et al*. (2014) investigated bacteria catalase activity through H_2O_2 decomposition and they concluded that, a single colony of a free-living biofilm is capable of decomposing 10.6 ± 0.7 µg/L H_2O_2 in one minute. This implies that, in one hour, at least 0.042 mg/L H_2O_2 should be decomposed in presence of bacteria. This observation concurs with the findings of the current study that the more the bacteria colonies, the more the H_2O_2 decomposition.

5.6 Conclusions

The study evaluated volumetric nitrification performance of four different biomedia (foam, coconut shells, leca and plastic beads), all operated in fixed bed biofilters with

identical conditions. It is concluded that biofilters containing foam biomedia are more robust, in both start-up and ammonia and nitrite removal, followed by biofilters containing coconut shells and leca ceramic beads. Biofilters containing plastic beads have generally low performance in terms of ammonia and nitrite removal. Furthermore, foam biomedia have higher bacterial activity, followed by coconut shells, leca and lastly plastic beads. Therefore, the foam media provide suitable environment for nitrifying bacteria attachment compared to the other tested biomedia. Due to the environmental challenges facing the application of plastic products, especially in food producing industries it is recommended that coconut shells and leca ceramic beads should be use as biomedia in recirculating aquaculture systems in place of plastic material. However, further studies are required on coconut shells and leca ceramic beads to optimize their utilization.

Author contributions

All authors conceived the idea for the research. M.S.M. and L.F.P. designed the trial. S.W.C. ensured funding for the research and edited the manuscript. M.S.M. conducted the experiments, collected the samples and performed the laboratory analyses. M.S.M., L.F.P. and R.N.M. processed and analyzed the data and wrote the draft. All authors reviewed the final version of the manuscript.

Declaration of Competing Interest

All authors assert that they have no known conflicting interests or personal relationships that could have affected the work reported in this article.

Acknowledgements

This study was funded by DANIDA through “Building Stronger Universities Phase III (BSU III) programme at Sokoine University of Agriculture. The authors are very grateful for the financial support. The technical and analytical support provided by Rasmus

Frydenlund Jensen, Ole Madvig Larsen, Brian Møller, Ulla Sproegel, all from DTU Aqua Section for Aquaculture, Hirtshals, Denmark is highly appreciated. We thank Mr. Nicolai Skoven, the product specialist at LECA who provided the leca biomedia used in this study.

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CHAPTER SIX

6.0 GENERAL DISCUSSION

6.1 Effect of Water Velocity on the Effective Removal of Ammonia and Nitrite in RAS

In RAS the removal of ammonia and nitrite in biofilters is enhanced by the activeness of nitrifying bacteria hosted in the biofilters. The welfare of nitrifying bacteria greatly depends on the supply of substrate (ammonia, oxygen, etc) and transport limitation due to a water boundary layer between attached bacteria and the TAN in the bulk liquid (Prehn *et al*., 2012). Biomedia in fixed bed biofilters are immobile and, therefore, static biofilm. This means that the biofilm is not directly in contact with substrate (Chen *et al*., 2006). The diffusive boundary layer (DBL) is a reedy layer of liquid at contact with a solid surface generated by the hydrodynamic flow around (Sulpis *et al*., 2019). The nature and thickness of DBL is determined by the structure of the biofilm, surface structure of the carrier material and the flow velocity over the filter surface (Stoodley *et al*., 1997; Rasmussen and Lewandowski, 1998; Abbasi *et al*., 2015). Changing the flow velocity is, therefore, one of the ways for altering the transfer of substrate into active biofilter. This, in turn, affects the effective removal of ammonia and nitrite through nitrification (Prehn *et al*., 2012; Mnyoro *et al*., 2021). Slow substrate transport can be significantly reduced by increasing water flow to the biofilter (Reino and Carrera, 2017; Shai *et al*., 2017).

This study investigated and proved a positive correlation between water flow velocity and nitrification ability of biofilters. Flow velocities above 10 m/h were found to be better compared to lower velocities and this finding can be applied to commercial as well as low-cost RAS operating with lower hydraulic flow velocities. Commercial RAS operating

with significantly more complex filter geometry can easily improve biofilter performance by increasing flow velocity.

6.2 Evaluation of A Simple and Low-Cost RAS Technology Ideal for Use in Developing Countries

The main aim of sustainable aquaculture is to produce large quantity of fish while maintaining minimal disturbance to natural resources. This can only be achieved by employing intensive production systems with a minimum ecological impact (Martins *et al*., 2010). Recirculating aquaculture system (RAS) is the most common intensive aquaculture production system used mostly in developed countries. Essentially RAS is an indoor system that allows farmers to control environmental conditions throughout the production circle. The RAS unit removes the pollutants such as ammonia in the fish tank by using mechanical and biological filters and allows to reuse water and stock fish at higher densities. The RAS technology has minimal effluents and, therefore, reduces environmental impacts.

In an effort to adapt this technology in developing countries, some farmers have tried to import and operate RAS facilities (Agenuma, 2010; Ozigbo *et al*., 2014). In their review on aquaculture in Africa, Adeleke *et al*., (2021), mentioned that unreliable power supply and high cost of operating power generators make the operation of RAS a non-viable option in developing countries. Moreover, the costs associated with construction of a RAS unit is exorbitantly high and not affordable to most farmers in developing countries. Currently, there only a few farms in Africa that utilize RAS for fingerlings and table fish production (Ozigbo *et al*., 2014; Menezes and Murekezi, 2021). AS an effort to promote the adoption of RAS technology and the transformation of aquaculture sector from extensive production to intensive fish farming, this study developed a simple and cheap

RAS applicable to small-scale farmers and training institutions (Paper II). The developed simple model RAS can be scaled to be applicable for middle scale producers. The pilot RAS was able to produce fish with limited water exchange and relied entirely on biofiltration for water treatment. The fish reared in the simple RAS showed higher fish growth rate (1.785 g/d) with 98.75 % survival rate which reflects good performance. The study has demonstrated that the developed simple pilot RAS unit is relatively cheap compared to available commercial RAS units and can be used successfully for tilapia production.

6.3 Suitability of Coconut Shell as Biomedia in RAS

Coconut shell is a hard-agricultural waste and is available in plentiful quantities throughout tropical countries worldwide. This material has been tested and proved to be better in performance as a biomedia for removal of ammonia and nitrite in RAS (Mnyoro *et al*., 2022). It has been reported that biofilters made from coconut charcoal can be effectively used as biomedia (Wrębiak *et al*., 2018). The higher fixed carbon content of coconut shell leads to the production to a high-quality solid residue which is used as activated carbon in wastewater treatment as it has good ability for bacteria attachment (Babel and Kurniawan, 2004).

Underhill and Prosser, (1987) investigated factors affecting attachment of nitrifying bacteria to solid surfaces. Their study revealed that, ammonium oxidizing bacteria attached preferentially to cation exchange surfaces while the nitrite oxidizing bacteria colonized anion exchange surfaces more extensively. According to Richter *et al*., (2005), uncleaned coconut shell would contain remains of coconut water which naturally contains chloride, malate, and potassium as the major ions. This implies that, coconut shells have cation exchange surfaces and, therefore, can support proliferation of ammonium oxidizing

bacteria. Cleaned coconut shells will not contain remains of ions; therefore, will support all nitrifying bacteria.

CHAPTER SEVEN

7.0 CONCLUSIONS AND RECOMMENDATIONS

7.1 General Conclusions

The main objective of this study were to develop a simple and low-cost RAS ideal for use in developing countries using suitable locally available biomedia for ammonia and nitrite removal in RAS. Therefore, the following conclusions have been made:

1. This study has verified under near-practice conditions that water velocity strongly affects the TAN removal rate. An increase in biofilter water velocity increases nitrification rate. At low velocities, TAN concentration has low effect on the nitrification rate. Nitrite concentrations in RASs increase as water velocity is reduced.
2. The developed simple model RAS was able to produce fish with limited water exchange and relied entirely on biofiltration for water treatment. The developed model RAS was found to be relatively cheap compared to imported RAS facilities. The experiment has demonstrated thet;
    (a), The simple model RAS can provide stable water conditions,
    (b), High fish performance can be achieved in the simple model RAS,
3. Out of the five locally available media evaluated in comparison to the commercial media (plastic), only coconut shells could equally compete with the commercial plastic biofilter in terms of ammonia and nitrite removal. This study concludes that coconut shells can be used in place of plastic media as biological filters in recirculating aquaculture systems.
4. Foam biomedia has been found to be more robust, followed by biofilters containing coconut shells and leca ceramic beads in start-up, ammonia and nitrite

removal, and bacterial activity. Biofilters containing plastic has shown a generally low performance.

7.2 Recommendations

This study recommends the following studies to be undertaken by the help of the developed low-cost model RASs.

(a), More trials on different sized coconut shells and come up with the appropriate size and shape for commercial applications

(b), Investigating bacterial colonization and microbial community in coconut shell biomedia during start-up and prolonged operation

(c), Optimizing water velocity and aeration to increase nitrification performance in coconut shells biomedia

(d), Evaluating fish production performance and establishing optimum carrying capacity of RAS unit with different volumes of coconut shells biomedia

(e), Development of coconut shredder for easy shredding and sizing of coconut shells. This will enhance further studies on the application of coconut shell biomedia including durability, applicability in brackish and salty water, and so forth. This study also calls for further studies on leca ceramic beads to optimize its utilization.

This study recommends adaptation of the low-cost RAS and the use of coconut shells as biomedia in RAS.

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