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

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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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(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:

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

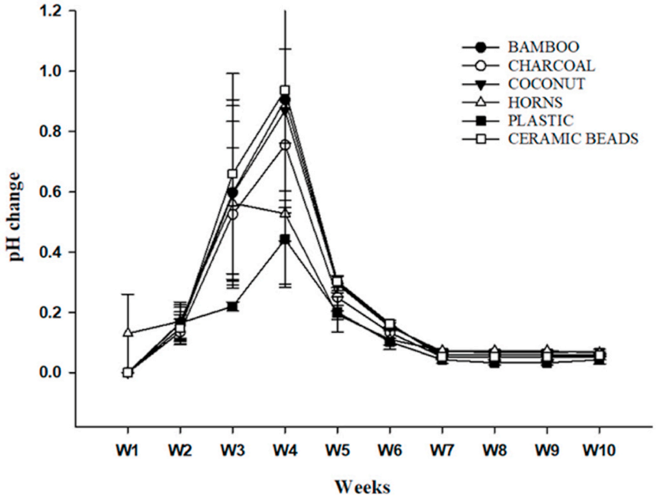

Fig. 1. Change in pH during single passage over different biofilters. The changes were measured as influent pH – effluent pH.

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

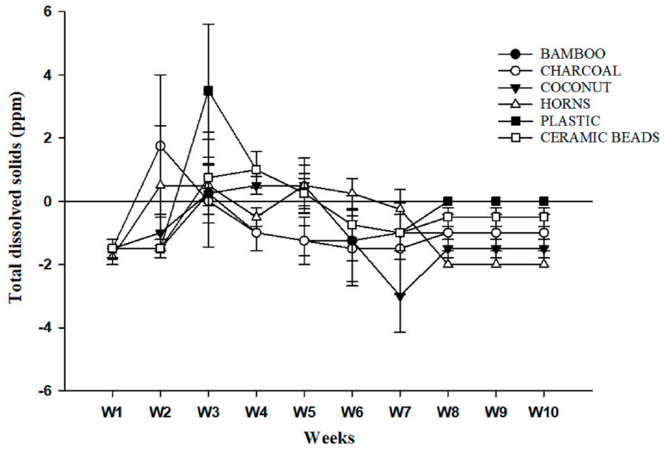

Fig. 2. Mean difference in influent and effluent total dissolved solids (TDS) measured from different biomedia per week (values below 0 reflect reduction of TDS).

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,

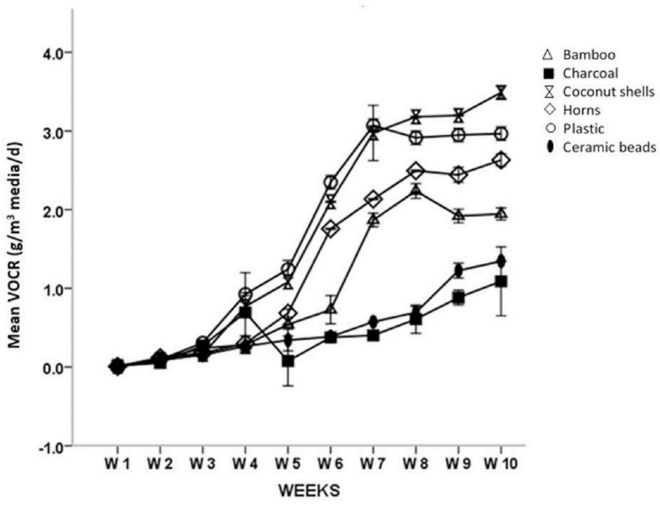

Fig. 3. Mean volumetric oxygen conversion rate in g/m^3/d observed in different biomedia at time of the experiment (calculated as dissolved oxygen in – dissolved oxygen out).

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).

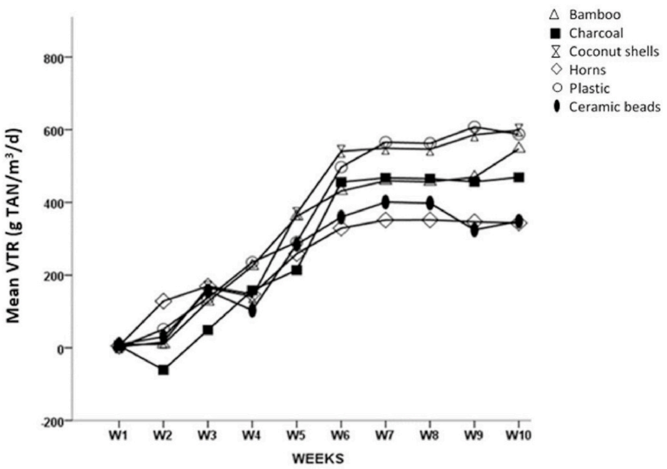

Fig. 4. Mean volumetric TAN conversion rate (g TAN/m^3/d) demonstrated by different biomedia in different experimental period.

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).

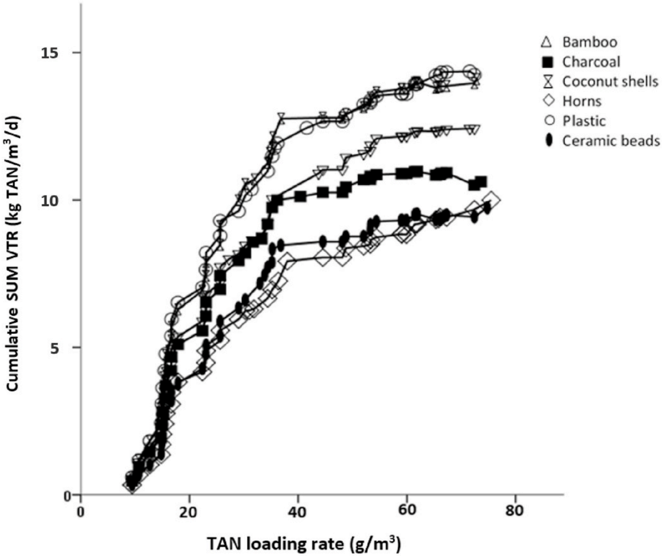

Fig. 5. The response given by different biofilters in terms of cumulative TAN in g TAN/m^3 under different TAN loading rate.

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.

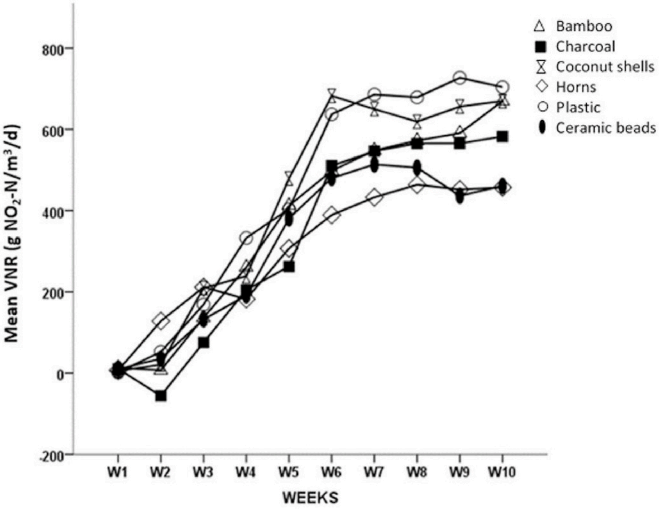

Fig. 6. Mean volumetric nitrite conversion rate (g NO2–N/m3/d) demonstrated by different biomedia in different weeks of the experiment.

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:

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

Nitrite oxidizing bacteria:

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

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

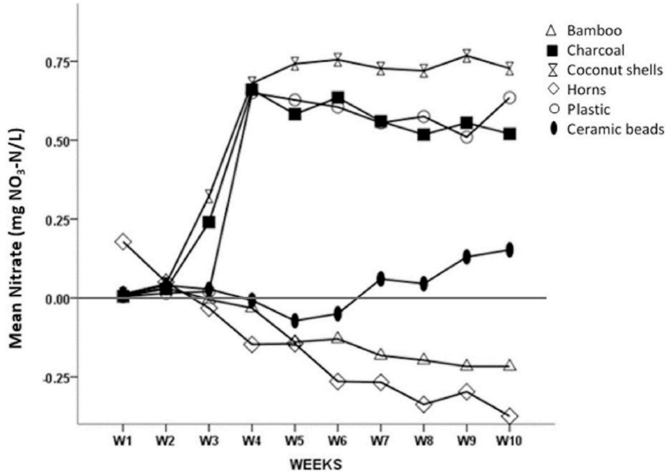

Fig. 7. Amount of nitrate (mg NO_3 -N/L) released into the RAS system by deferent biofilters at different period of the experiment (Biomedia with values below 0 mg NO_3–N/L showing negative nitrate contribution to the system).

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.

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