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Development of wastewater reuse system to eliminate the risk of microbial infection and toxicity to fish

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Abstract
The Recirculating Aquaculture Systems (RAS) with a biological filter is the most noticeable representative which treats internally the water polluted with dissolved organics and ammonia. It also decreases the amount of water use. This study showed lower microbial content and lesser toxic element in treated water than non-treated water. Among the treatment 1, 2 and 3, treatment 3 showed lower microbial content; *Aeromonus, Pseudomonus* (20×10^{-4} CFUml^{-1}, 30×10^{-3} CFUml^{-1}) with low toxic elements; total ammonia nitrogen concentration 0.69 ± 0.3 gm^{-3}, Nitrite-N concentration 2.1 ± 1.5 gm^{-3}, Nitrate 0.69 mg/L, CO_2 5-10 mg/L, pH 7.16 ± 0.14 than other treatments 1 (35×10^{-4} CFU ml^{-1} , 45×10^{-3} CFUml^{-1}) and treatment 2 (50×10^{-4} CFUml^{-1} , 30×10^{-3} CFUml^{-1}). Usually maximum microbial content; *Aeromonus, Pseudomonus* (250×10^{-4} CFUml^{-1}, 190×10^{-3} CFUml^{-1}) and maximum toxic effect (total ammonia nitrogen concentration 1.20 gm^{-3} , Nitrite-N concentration 6.66 gm^{-3}, Nitrate 5.0 mg/L, CO_2 15 mg/L, p^H 7.48) was observed in the control condition (Non-treated water). This study indicates that the wastewater treatment using biological filter in case of microbial infection and toxic elements. Contents difference has the potentiality to fish production in aquaculture industry.

Keywords: Recirculating aquaculture systems (RAS), Biological filters, Toxicity, Microbial infection, Total Ammonia Nitrogen (TAN) and Dissolve organic substance.

1. Introduction
If we want the betterment of aquaculture production we have to need motivating the industry toward more intensive practices. A recirculating aquaculture facility reduces water demands and discharges by reconditioning of water (Goldburg *et al.,* 2001) [1]. Better food conversions are achievable with a recirculating aquaculture system (RAS) which means less waste is generated by the feed (Lorsordo *et al.,* 1998) [2]. In recent years, there has been a growing concern over the impacts of aquaculture operations (Buschmann *et al.,* 1996; Harache, 2002; Naylor *et al.* 2000; Cranford *et al.,* 2003; Johnson *et al.,* 2004) [3, 4, 5, 6, 7]. Biofilter is the most noticeable representative of these production systems reflect a commitment to internally treat water which is contaminated by dissolved organics and ammonia. Ammonia is the principal nitrogenous waste produced by most fishes. Short-term exposure of fishes to a high concentration of ammonia causes increased gill ventilation, hyperexcitability, and loss of equilibrium, convulsions, and then death (Smart, 1978; Thurston *et al.,* 1981) [8]. Chronic exposure of fishes to a lesser concentration of ammonia causes tissue damage, decrease in reproductive capacity, decrease in growth, increase in susceptibility to disease (Thurston *et al.,* 1984, 1986) [10], and ultimately resulting in death (Randall and Wright, 1987) [11]. A major cause of nitrite toxicity is the oxidation of blood hemoglobin iron to its ferric state, forming methemoglobin (Bodansky, 1951) [12]. However, when nitrate concentrations become excessive and other essential nutrient factors are present, eutrophication and associated algae blooms can become a serious environmental problem (Russo and Thurston, 1991) [13]. Biological treatment has been considered the most feasible approach for enabling water reuse in these treatment systems (Metcalf *et al.,* 1991) [14]. To date, the majority of biological filter performance evaluations have been performed at the small, laboratory scale under conditions not adequately representative of actual production conditions (Losordo *et al.,* 2000; Eding *et al.,* 2006) [15, 16]. Biofilter evaluations at the larger pilot or commercial scales using actual waste nutrients will yield results more pertinent to actual aquaculture production conditions (Ester *et al.,* 1994; Losordo *et al.,* 2000; Brazil, 2006; Chen *et al.,* 2006) [17, 15, 18, 19]. In practice, the bacteria createa stratified

biofilm with the faster growing heterotrophic bacteria layering over the slower growing autotrophic nitrifying bacteria (Nogueira *et al.,* 2002) [22]. Stratification reduces mass flux of substrate through the biofilm, creating an oxygen diffusion gradient thus creating favorable conditions for anoxic processes (Schramm *et al.,* 1996; Zhu and Chen, 2002) [23, 24]. The environment below a thick biofilm layer may be totally anaerobic and there is no nitrification will occur (Schramm *et al.,* 1996) [23]. Usually in a Recirculating Aquaculture Systems (RAS), autotrophic nitrifying bacteria (AB) remove ammoniaat and maintain a sufficient rate of water quality at a level adequate and prevent ammonia toxicity to the fish (Zhu and Chen, 1999) [25]. There are three ways to deal with this problem. By removing most of the carbonaceous BOD (biochemical oxygen demand) before the water enters, providing sufficient extra capacity and having a very long plug flow path through the biofilter. So the aim of this study is to reduce the risk of toxicity affect and microbial infection of fish.

2. Materials and Methods

2.1. Technical feasibility

The experimental tests were conducted on the laboratory of Fisheries & Marine Bioscience department to March to May, 2015. The technical feasibility of the water reuse system was evaluated in terms of eliminate the risk of microbial infection and toxicity to fish by using biological filter (Fig.1). Four identical biological filters filled with a pre-colonized packing media (gravels, marbel rock, pebbels, coarse sand, charcoal, cotton, net) were used. The filters functioned in parallel and received the same influent water quality, constituted from heated (20±1 °C), sand-filtered. The p^H remained between 7.5 and 8.

Fig 1: Preparation of biological filter

There are three major filtration systems usually use as independently or combined in aquaculture industry to re-use of water, treatment of water, activated sludge (bio solids) and water disinfections.

2.2. Preliminary cost estimate

A preliminary cost estimate was conducted after the technical feasibility of the water reuse system was confirmed by field experiments. The cost analysis was based on the system performance presented in Table 1

Table 1: Cost estimate for a water reuse system

Fixed costs	Cost (BDT)
Pump	2000
Media: gravels, marbel rock, pebbles, coarse sand, charcoal, cotton etc	300
Miscellaneous items: Pipes, fittings and valves, electrical components, storage tanks, paint, silicon gum, rod stand etc.	1800
Construction	1000
Subtotal Variable costs	5100
Electricity	25
Maintenance	100
Subtotal	125
Total	5225

2.3. Experimental setup

The culture system was operated for 1 month to establish a bacterial population in the filters. During that period an evaluation was conducted on the same biofilters to measure and document performance characteristics under normal operating conditions (NOC) (Guerdat *et al.,* 2010) [26]. Recirculation systems occupy a very small area and allow the grower to stock fish at high densities and produce high yields per unit area.

A recirculation system is essentially a closed system and involves fish tanks and filtration and water treatment systems. The fish are housed within tanks and the water is exchanged continuously to guarantee optimum growing conditions. Water is pumped into the tanks, through biological and mechanical filtration systems and then returned into the tanks. Not all water is 100% exchanged however as it is difficult to ensure that all waste products are converted or removed by the treatment process. Most culture systems recommend at least 5% to 10% water exchange rate per day depending on stocking and feeding rates. Regular aquarium maintenance such as filter pad replacement, substrate vacuuming or even water changes can have a negative impact on the biological filtration to some degree. If water is not properly filtered through a RO/DI unit, the chemicals and metals present in the water and can kill off the bacteria when added to the aquarium. No water changes greater than 25% of the total volume should be done at one time.

2.4. Particulate organic matter (POM)

The POM used in the experiment was collected from particle separators at the outlet of a tilapia rearing tank in a RAS. The POM was constituted mainly by fecal pellets and by non-ingested feed. After collection, the POM was concentrated by centrifugation (10,000 × g), sterilized by autoclaving (121°C, 1 atm for 20 min) and freeze-dried. Finally it was ground and sieved on a 50 mm screen to obtain a uniform powder that was easy to inject into the filters (Table 2).

Table 2: Characteristics of the biological filters

Diameter (cm)	11
Cross-sectional area (cm^2)	95
Height (cm)	85
Volume (cm^3)	8075
Flow rate (m^3h^{-1})	0.121
Water retention time (min)	4.03
Water velocity (m^{h-1})	12.74

The water retention time and water velocity are calculated ignoring the volume displaced by the packing media.

2.6. Pumps
Two pumps were used to supply effluent to each group of filters. All three filter systems were individually supplied by internal power filter pump, SP-2500L (Fig. 2), power of the each pump was 18W which was needed for laboratory use.

Fig 2: Internal power filter pump (SP-2500L)

Fig 3: Biological filter

2.7. Plumbing and flow rates
The filters were supplied by way of one manifold system for each group of filters. The inlet pipes from the manifold to the filters were lengthened to a minimum of 1.5 m to accommodate the requirement of a fully developed flow profile within the pipe and provide for more accurate flow measurements in this study. Inlet pipes for all filters were of the same diameter for flow measurement purposes. Flow to each filter was individually controlled using a valve directly in front of the filter inlet. Flow rates within each group were equalized with the total variation in flow rates between all filters being no greater than 10%. Flow rates were recorded at the time of sampling and adjusted after sampling was complete, if required.

2.8. Operation
Step1: Two aquariums were used for the experiment. One aquarium was used for culture fish and for the polluted water. A pump was used for circulate the polluted water through a plastic pipe.

Step 2: By using a pump, polluted water moves to the biological filter through a pipe (Fig. 3). Then the polluted water treated into the filter and the treated water stocked into another aquarium.

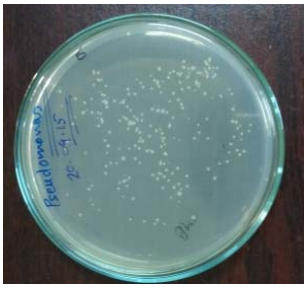
Step 3: At the last step the treated water was stocked in the second aquarium and then the treated stocked water supplied to the first polluted aquarium by using another pump through a pipe.

2.9. Sampling and analysis
During the conditioning period prior to data collection, occasional samples were taken to monitor water quality. Twelve days prior to the start of data collection, regular samples were taken to identify stable water quality conditions within the system. Sampling and water quality analysis were conducted as described below.

2.9.1. Water quality
Grab samples were taken at the pump outflow sample port and each filter's respective sample port located directly after the filter exit. Inlet pipes for the three pumps were located within 1m of one another, and approximately 4m below the water surface to insure identical water quality was delivered to each of the filter systems. Inlet dissolved oxygen (DO) concentrations taken at the pump outflows of the pumps for the filter systems were compared in preliminary tests. The DO concentrations were nearly identical and it was decided based on these comparisons to use samples taken from the outflow of pump as the inlet data for each of the filter systems. To eliminate possible introduction of any settled material in the sample port into the water sample, water was wasted at the sampling port for 10s prior to any sampling. Sample bottles were then rinsed three times with water from the sample ports before being filled. All water samples were analyzed for total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2^-N), nitrate-nitrogen (NO_3^-N), alkalinity (as mg $CaCO_3$ L^{-1}), pH, DO, temperature, chemical oxygen demand (COD), and total organic carbon (TOC).

3. Results
3.1. Microbial content analysis
When the water quality of the tank declines, fish are likely to become stressed, which increases their susceptibility to disease. Microbial content analysis was performed according to current protocols in the Central Laboratory of Jessore University of Science and Technology (Table. 3).

Table 3: Microbial content analysis

Pre – Treatment	Post – Treatment
	
Fig 6: High microbial content	**Fig 7:** Reduced microbial content

Microbial content was high in the non-treated or polluted water of the fish tank and it was visibly reduced by using biological filter (Table. 4).

Table 4: Microbial content in water

Treatment		Non - treated water (No. of colony CFU^{ml-1})	Treated water (No. of colony CFU $^{ml-1}$)
Number of treatment	**Selective media**		
Treatment1	Aeromonus	220×10^{-4}	35×10^{-4}
	Pseudomonus	173×10^{-3}	45×10^{-3}
Treatment2	Aeromonus	250×10^{-4}	50×10^{-4}
	Pseudomonus	190×10^{-3}	30×10^{-3}
Treatment3	Aermonus	150×10^{-4}	20×10^{-4}
	Pseudomonus	100×10^{-3}	30×10^{-3}

Counts of cultivable heterotrophic free living and attached bacteria were performed by the spread plate method on Marine Agar (Difco 2216) sterilized by autoclaving (121 °C, 1 atm for 20 min). Dilutions were performed in 34 g l^{-1} sterile sodium chloride solution. Plates were set up in duplicate for each dilution. Only plates having between 20 and 300 colonies were considered. Incubation time was 10 days at 25 °C. Bacterial concentrations were expressed as CFU per ml for free living bacteria.

3.2. Toxicity analysis of water

Water quality parameters monitored for the length of the study are summarized in Table. 5.

Table 5: Toxic parameters analysis of Treated and Non-treated water

Variables	Treated water	Non-treated water
TAN concentration (gm^{-3})	0.69 ± 0.3	1.20
Nitrite-N concentration (g gm^{-3})	2.1 ± 1.5	6.66
Nitrate (mg/L)	0.69	5.0
DO concentration (g gm^{-3})	5.8 ± 0.8	8.1
Temperature (°C)	28.9 ± 2.3	31.6
ph	7.16 ± 0.14	7.48
Alkalinity (as g CaCO3 gm^{-3})	261 ± 70	422
TOC (g gm^{-3})	25.1 ± 16	58.5
COD (g gm^{-3})	80 ± 12	98
Carbon dioxide (mg/L)	5-10	15
Oxygen (mg/L)	4	2
BOD (g gm^{-3})	15.6	29
$_2$S (mg/L)	0.001	0.005

Variations in total ammonia nitrogen (TAN) and NO_2^-N concentrations were expected per experimental design.

4. Discussion

The result of the present study showed the advantage of using biological filter for the development of water re-use system to eliminate the risk of microbial infection and toxicity to fish. Others are designed to allow denitrification on internal carbon sources which are produced in the RAS (Van Rijn *et al.,* 2006) [29]. In the latter case, bacterial fermentation processes play an important role in supplying carbon compounds for denitrification whereby most of the organic carbon is eventually oxidized to CO_2. Therefore, not only nitrogen but also organic carbon is removed by means of this treatment combination (Eding *et al.,* 2003; van Rijn *et al.,* 1995) [32]. Moreover a novel form of anaerobic ammonia oxidation (anammox) bacteria (Strouss *et al.,* 1997, 1999) [29, 30] can contribute to ammonia removal, but their presence inside biofilters has not been examined to date (Tal *et al.,* 2003). Increased alkalinity was used as a pH buffer in addition to providing a source of inorganic carbon for the autotrophic nitrifying bacteria in the form of sodium bicarbonate ($NaHCO_3$). Variations in pH and alkalinity were due to balancing the effects of feed rates, CO_2 production, and $NaHCO_3$ additions. Various linear and non-linear statistical models were examined. Using the Akaike's Information Criterion (AIC) as a basis for model selection, the AIC number may be derived using a mixed analysis in SAS and penalizes for adding predictor variables into models (Akaike, 1974) [34]. Smaller AIC numbers represent models more appropriate for response prediction. In solution, ammonia maintains equilibrium between an ionized (NH_4^+) and unionized (NH_3) form. Unionized ammonia-nitrogen (NH_3) is toxic to most aquacultured aquatic organisms and must be controlled within the production system (Meade, 1985) [35]. Other water quality parameters – including pH (a measure of the amount of acid in water), temperature and dissolved oxygen, biochemical oxygen demand (BOD), alkalinity, hardness – determine the degree of toxicity. Biochemical oxygen demand (BOD) is the amount of dissolved oxygen needed by aerobic biological organisms in a body of water to break down organic material present in a given water sample at certain temperature over a specific time period. This is not a precise quantitative test, although it is widely used as an indication of the organic quality of water (Clair N. Sawyer, *et al*., 2003) [36]. If the microbial population deoxygenates the water, however, that lack of oxygen imposes a limit on population growth of aerobic aquatic microbial organisms resulting in a longer term food surplus and oxygen deficit. (Reid and George K, 1961) [37]

3. Conclusion

Water treatment technology has seen a dynamic development in recent years with new treatment methods rapidly emerging. This study shows that the water re-use system or recirculating water system (RAS) for the removal of microbial content and toxicity from water, is technically feasible and environmentally friendly which is the most unique and important feature of this system.

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