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Evaluation on Biofilter in Recirculating Integrated Multi-Trophic Aquaculture

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Abstract *- Integrated multi-trophic aquaculture pays more attention as a bio-integrated food production system that serves as a model of sustainable aquaculture, minimizes waste discharge, increases diversity and yields multiple products. The objectives of this research were to analyze the efficiency of total ammonia nitrogen biofiltration and its effect on carrying capacity of fish rearing units. Pilot-scale bioreactor was designed with eight run-raceways (two meters of each) that assembled in series. Race 1-3 were used to stock silky worm (*Tubifex *sp) as detrivorous converter, then race 4-8 were used to plant three species of leaf-vegetable as photoautotrophic converters, i.e; spinach (*Ipomoea reptana*), green mustard (*Brassica juncea*) and basil (*Ocimum basilicum*). The three plants were placed in randomized block design based on water flow direction. Mass balance of nutrient analysis, was applied to figure out the efficiency of bio-filtration and its effect on carrying capacity of rearing units. The result of the experiment showed that 86.5 % of total ammonia nitrogen removal was achieved in 32 days of culturing period. This efficiency able to support the carrying capacity of the fish tank up to 25.95 kg/lpm with maximum density was 62.69 kg/m^3 of fish biomass production.*

Keywords *— aquaculture; multi-tropihc; integrated; productio; sustainable*

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I. Introduction

FAO (2010) claims that aquaculture accounted for 46 percent of total food fish supply, a slightly lower proportion than reported in The State of World Fisheries and Aquaculture 2008. On the other hand, aquaculture is required to grow in response to demand for increased cheaper protein resources. However, in practices, aquaculture faces major problems in feed nutrient retention, where only 25-30% of feed nutrients converted for energy and growth (Avnimelech, 1999; Rakocy, *et al.*, 2006; Losordo, *et al*,. 2007), the rest is excreted in water column that would otherwise build up to toxic levels and finally decreasing carrying capacity in the fish rearing units. Actually, Fish can be grown at very high density in aerated–mixed ponds. However, with the increased biomass, water quality becomes the limiting factor, due to the accumulation of toxic metabolites, the most notorious of which are ammonia and nitrite (Avnimelech, 2006). It is estimated that 85% of phosphorus, 80-88% of carbon, 52-95 % of nitrogen (Wu, 1995) and 60% of mass feed input in aquaculture will end up as particulate matter, dissolved chemicals, or gases (Masser, *et al*., 1999). That why in conventional aquaculture often replace 5-10 % of water every day. Moreover, in recent years, environmental regulation and land limitation become the most consideration in aquaculture development.

Integrated multi-trophic aquaculture (IMTA) is a new concept of aquaculture that different to polyculture terminology. With the multi-trophic approach, aquaculture of fed organisms (fin-fish or shrimp) is combined with the culture of organisms that extract either dissolved inorganic nutrients (seaweeds) or particulate organic matter (shellfish) and, hence, the biological and chemical processes at work are balancing each other (Chopin, 2006). This concept seems to become a future of aquaculture systems and operations. FAO (2012) states that one-third of the world's farmed food fish harvested in 2010 was achieved without the use of feed, through the production of bivalves and filter-feeding carps.

IMTA usually operated in open water-based aquaculture, such as mariculture or cages in lakes or reservoirs. While land-based aquaculture, water and land use are rapidly becoming a strong factor driving the adoption of recirculating technologies. A fish farm can take full advantage of IMTA once the nutrient discharge by the fed (fish) component is fully balanced by the harvest of the xtractive components (seaweeds and suspension- and deposit-feeders) (Troell et al., 2009). Therefore, the biological filter components play an important role in such systems. Its efficiency in removing nutrient waste from fish tanks is the main goal to design the biofilter systems.

Because of relatively high cost, built recirculating aquaculture systems should be designed such that it is efficient, cost-effective and simple to operate. This research was an effort to develop biofiltration subsystems and to analyze its efficiency in removing nutrient waste and increasing carrying capacity to a pilot scale of integrated multi-trophic reciculating aquaculture system.

II. Material and Methods

2.1. IMTA System Description

A pilot scale of IMTA was set up for raising two species of fish in different trophic level, i.e.; climbing perch (*Anabas testudineus* Blk) and nile tilapia (*Oreochromis niloticus*). Fish tank construction made from wood coated with fiberglass. The biofilter system was placed in series with the fish tanks. The biofilter systems consisted of eight run-raceways (2 meters in length and 13 cm in width of each) with effective volume was 140 liters.

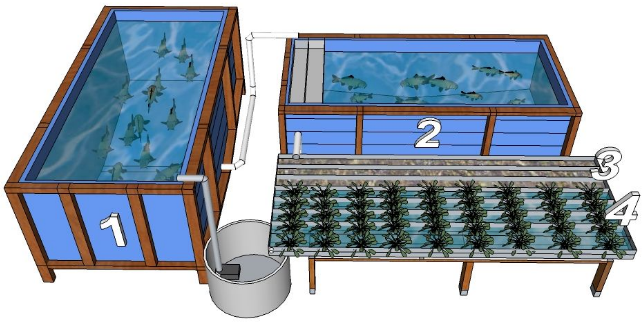

Figure 1. Sketch of pilot scale integrated multi-trophic aquaculture configuration.

Where; 1= climbing perch's tank as carnivorous, 2 = nile tilapia's tank as herbivorous; 3. Silky worm's raceways; and 4 = plant's raceways as photoautotrophic.

Table 1. Experimental biofilter characteristics

Unit	Description
Volume	140 liters
Height of water level	6,5 cm
Hydraulic retention time	28 minutes
Media type	PVC Bio-net 1 mm diameter Polystyrene sheet
Filter coefficient	7.95
Turn over duration	16.46 hours

2.2. Experimental conditions

The experiment was conducted for 5 weeks between June and July 2012 at Laboratory of Fish Genetic and Reproduction of Fisheries and Marine Science Faculty, Mulawarman University Samarinda.

The rearing tanks consisted of a 1,09 m^3 for growing a 8,0 kg/m^3 of climbing perch (*Anabas testudineus*) weighing 40,2±3,36 grams, and a 0,98 m^3 rearing tank was being stocked 6,58 kg/m^3 of nile tilapia (*Oreochromis niloticus*) weighing 29,3±12,46 grams. Floating pellets containing 32 % protein were used to feed the fish at satiation rate. Fish was weighed at the end of experiment (at 32 days) The number and weight of fish taken out from each of the culture tanks was recorded for calculating fish growth parameter. Fish dead during experiment was replaced with the same size to keep the constant number of fish in the tanks. Death time and fish size were recorded to figure out the survival rate parameter.

Water flow maintained at 5 liters per minutes throughout the experiment units including nutrient waste (effluent) discharged from fish tank to bioreactor. Silky worm (*Tubifex* sp) that stocked at the bioreactor spread out 3 individual/cm^2 in three raceways (raceway 1-4). While spinach (*Ipomoea reptana*), green mustard (*Brassica juncea*) and basil (*Ocimum basiliucum*) were hydroponically planted 40 plants of each at raceway 4-8. Planting lay out were conducted in completely randomized block design regarding to flow direction and used rafting technique where the plants floated by polystyrene sheets.

2.3. Water Quality

Water was sampled twice a week at five points based on organism areas, i.e.; (1) inlet of bioreactor or the 1st raceway (outlet of nile tilapia's tank), (2) inlet of phototrophic or the 4th raceway, (3) outlet of bioreactor, (4) outlet of climbing perch (*A. testudineus*) tank, and (5) in the nile tilapia (*O. niloticus*) tanks. Samples were analyzed for TAN (total ammonia nitrogen), NO2-N (nitrite-nitrogen), NO3-N (nitrate-nitrogen), and PO4-P (ortho-phosphate) by using Genesis Spectrophotometer (). Water temperature, pH, DO (Dissolved oxygen), alkalinity and CO_2 (carbon dioxide) were also measured, following standard methods (APHA, 1992)

2.4. Calculations

Calculation steps to determine biofilter efficiency.

Total Ammonia Nitrogen (TAN) production calculated based on nitrogen mass balances using value for TAN produced per kg of feed (Timmons, *et al.*,2002) :

$$P_{TAN} = F \times PC \times 0,092$$

Where: P_{TAN} = total ammonia production rate (kg/day); F is feed rate (kg/day); *PC* is the protein content of feed (decimal value). 0,09 constant in ammonia generation equations assumes that protein is 16% nitrogen, 80% nitrogen is assimilated by the organism, 80% assimilated nitrogen is excreted, and 90% of nitrogen excreted as TAN+10% as urea.

Then, TAN loading rate calculation based on Wheaton (1977), ammonia accumulation factor (C) due to recirculation determined by following equation.

$$C = \frac{C_{limit.TAN}\left(\frac{g}{m^3}\right)}{C_{TAN}\left(\frac{g}{m^3}\right)}$$

Where: $C_{limit.TAN}$ is allowable ammonia concentration, C_{TAN} is single pass ammonia concentration that determined with, C_{TAN} = P_{TAN} (gm/d)/water flow rate, Q (m^3/hari), and TAN loading rate determined with equation.

Total ammonia load into bioreactor, $L_{TAN\ in}$ (gram TAN/day) = $P_{TAN} \times C$

The final ammonia concentration that measured at the outlet of bioreactor. Thus, TAN loading out of bioreactor (gm/day) is

$$L_{TAN\ out} = C_{TAN \cdot out}\ (gm/m^3) \times Q\ (m^3/day).$$

$C_{TAN \cdot out}$ is total ammonia nitrogen concentration out of bioreactor, Q is water flow rate. Thus, Ammonia biofiltration efficiency (%) can able determined by following equation.

$$\%TAN_{removal}\ (\%) = \frac{L_{TAN.out}\left(\frac{g}{m^3}\right)}{L_{TAN.in}\left(\frac{g}{m^3}\right)} \times 100$$

Carrying capacity (loading density) and fish biomass density.

According to TAN biofiltration efficiency, hydraulic recirculation rates (*R*), feeding rates, and tanks volume. The maximum carrying capacity of the fish tanks without water exchanges determined by Westers (1997) equation.

$$LD = \frac{\%TANrem \times V_{tank} \times ANO_3}{FR \times P_{TAN} \times 4{,}2 \times R \times 1000}$$

Where LD is fish loading density (kg/lpm), Eff_{TAN} is TAN biofiltration efficiency, V_{tank} is fish tanks volume (liter), ANO_3 is allowable nitrate nitrogen, FR is feeding rate (%/BW/d), P_{TAN} is TAN production (g/d); 4,2 constant is come from 1 molecule of TAN generate 4,2 molecules of NO_3; R is recirculation rates ($^{-}$hour)

Therefore, maximum fish density can be expressed with this equation.

$$D = \frac{LD \times R}{0{,}06}$$

Where D is fish density (kg/m^3); LD is loading density (kg/lpm), R is recirculation rates, and 0,06 represents m^3 from 1,0 lpm × 60 minutes = 0,06 m^3.

III. RESULTS AND DISCUSSION

3.1. Fish performance and TAN Production

During the 32 days of grow out period, climbing perch feed consumption is very small compared to tilapia, which 0,5 kg of feed while tilapia can spend 1.96 kg of feed.

For the total growth during the 32 days of grow out period, the average climbing perch and tilapia has reached the size of 46.0±8,47 gm and 42,4±27,73 gm, respectively. Based on unpaired t test assuming not the same variance, the growth of these two species were significantly different ($P < 0.05$). According to total feeding rate, TAN production rate was 72,5 gm TAN/kg feed. It means that 2,94 % of TAN produced per kg feed, this value was not significant different with the standard of the estimation TAN production that published by Malone, *et al* (1990), which is in the same feeding rate could generate 2,74 % of TAN/kg feed.

Nitrogen dynamics represented by TAN, nitrite and nitrate concentration fluctuated during the experiment. TAN tends to decrease during experiment, nitrite started rising at day-16 while nitrate also increased during experiment. In 32 days experiment, nitrification process seemed to follow the first order reaction, when at sufficiently low substrate concentration, the relationship become linear (Chen et al., 2006). However, at the experiment showed that nitrite oxidation rate to nitrate appears did not have linear correlation, nitrite accumulation occurred in day-20 and made nitrate production become slower. The accumulation of nitrite suggested that ammonium and nitrite oxidations did not proceed at the same rates in the batch experiments (Sesuk et al., 2009).

Oxidation of ammonia is usually the rate limiting step in the conversion of ammonia to nitrate (Chen et al., 2006). Thus value of ammonia oxidation are the rate limiting parameters in describing nitrification (Wheaton et al., 1994).

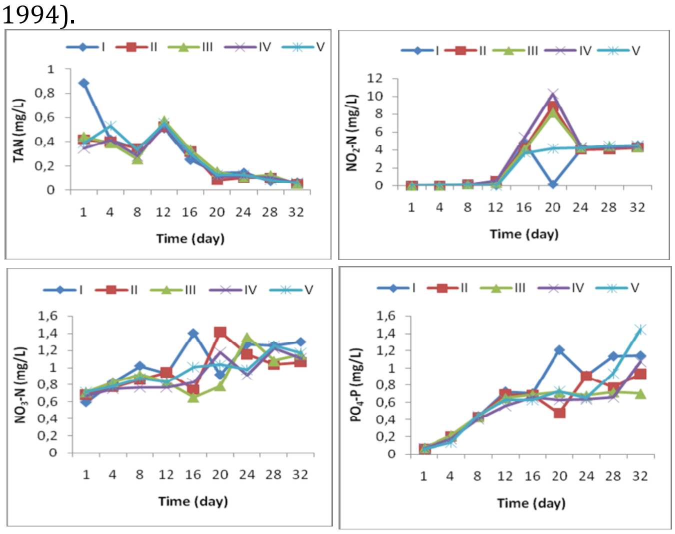

Figure 2. Nitrogen dynamic of TAN, NO_2-N, NO_3-N and PO_4-P

A. *Effects of Biofiltration efficiency to carrying capacity*

The production capacity of fish that can be produced by the IMTA system is analyzed through a combination of two major limiting factors i.e., dissolved oxygen and total ammonia nitrogen. Model calculations then consider other controlling factors such as feeding rate of water flow, the amount of water circulation, and the efficiency of the biofilters. Based on the concentration of dissolved oxygen, systems can accommodate a maximum density of 25.8 kg/m^3 of climbing perch, while the tilapia is still capable of supporting up to a maximum density of 47.7 kg/m^3. The results of these calculations based on the value of the oxygen fish need oxygen concentration available and the remaining oxygen is not used for respiration. The more fish the greater oxygen needed to supply the needs of fish (Colt, 1991; Wester, 1997).

The difference in capacity between trophic I (climbing perch) and trophic II (nile tilapia) is strongly influenced by the IMTA system configuration, in which the layout like tilapia are in a position after filtration and before tilapia tank, it makes the climbing perch get a supply of water containing higher oxygen, whereas theirs oxygen demand themselves lower. The types of labyrinth fish (with additional respiratory system) such as climbing perch are not sensitive to the concentration of oxygen in water (Zonnenfeld, 1991).

In IMTA system, water was recirculated continuously, where water with higher oxygen concentration from climbing perch's tank flows into the tilapia's tank thus providing a greater influence on the capacity of tilapia production. However, in the three-week maintenance period, the fish still need oxygen to be supplied from the flow of water out of the tank with flow rates 5 liters /min, but then the concentration of oxygen is already close to zero, and tilapia loss of appetite. To overcome this added bubble jet aeration system, but it also only lasted for two weeks. Thus, changes made to the aeration system keeps the water fountain with a height of 1 meter, the system is able to maintain the DO concentration in the tank of tilapia with an average of 1.3 mg/l.

Modeling fish densities can be done if the oxygen demand is not a problem in the system. Brune, et al. (2003) states that if the concentration of oxygen is sufficient for the needs of the fish and the stripping of CO_2 through aeration, the NH_3 will be a limiting factor within 24 hours. Therefore, the density of the fish will be strongly influenced by the nitrogen removal efficiency in the system. Based on the average values of temperature and pH, the fraction of unionized ammonia (NH_3) is only 1.91% on average in the tank of climbing perch and 1.76% at the tilapia tank. Biofiltration efficiency of TAN was 86.5% overall. Model density of fish made on the basis of the efficiency of biofiltration of ammonia and dissolved oxygen demand estimated of 62.69 kg/m³ fish biomass. However, the water quality parameters will begin to limit the carrying capacity allowed for waste degradation, accumulation of ammonia, carbon dioxide, and suspended solids (Timmons and Ebeling, 2007).

Carrying capacity calculation procedure was based on the calculations made by Losordo and Hobbs (2007) as shown in the following worksheet.

Flow calculations represent the factor analysis procedure with ammonia production as a limiting factor to the efficiency of biofilters as independent variables (Wheaton, 1977; Wester, 1997; Drenan II, 2006; Ebeling, 2006; Timmons and Ebeling, 2007). Production of ammonia was generated by the calculation of Drenan II (2006) at 3.06 g/day was not much different when using the equation of Ebeling (2006), which was equal to 3.28 g/day.

Through the process of biofiltration with trophic level detrivorous (Tubifex sp) and phototrophic (spinach, mustard greens, and basil) on a scale integrated aquaculture systems multi-trophic pilot was able to absorb the ammonia waste by 86.5% of TAN. This value is higher than ever published by Graber and Junge (2009) that 69 % of nitrogen removal by the overall system could thus be converted into edible fruit in hydroponic system design only. Therefore, based on the calculation of production capacity due to TAN removal efficiency, a culture system like this can result in fish biomass of 62.69 kg/m3. However, according to Timmons and Ebeling (2007) stated that do not get stuck on the calculation of the mathematical models because you can kill fish, so to be safe, it is recommend stocking half of the results of these calculations (only 31.34 kg/m³ of fish biomass is recommended).

IV. CONCLUSIONS

A pilot-scale of integrated multi-trophic aquaculture production systems set up in this study generally works well for a single production cycle (32 days). Although there is no water exchanges, but the subsystem designed biofilter still able to maintain optimum water conditions for the survival and growth of fish. Maximum production capacity of fish that can be produced from an integrated multiple trophic with a total volume of 2.2 m³ of water was 25.30 kg of climbing perch and 39.58 kg of tilapia; 772.1 grams of spinach, 333.6 grams of basil, and 217, 6 grams of mustard for 28 days, and 789,533 individual of silky worm (*Tubifex* sp) for 32 days.

Table 2. Worksheet calculation of system's carrying capacity

No.	Description	Value	Units	Formula
1.	Fish tanks volume (Vt)	2.07	m^3	P x L x T
2.	Filter volume (Vf)	0.14	m^3	P x L x T
3.	Total volume system (Vs)	2.21	m^3	$V_t + V_f$
4.	Flow rate (Q)	7.20	m^3/day	(5 lpm*1440)/1000
5.	Recirculation rates (R)	0.14	$Hour^{-1}$	0.06*Q (Lpm)/Vs(m^3)
6.	Tanks retention time (RTt)	3.57	Hour	1/R
7.	Filter retention time (RT)	28	Minute	V_f (L)/Q (Lpm)
8.	Total feed (TF)	2.46	kg	Experimental result
9.	Feeding rate/day (F)	0.08	kg	Experimental result
10.	Protein content (PC)	32	%	Experimental result
11.	TAN inlet concentration (Cin)	0.54	g/m^3	Experimental result
12.	TAN outlet concentration (Cout)	0.47	g/m^3	Experimental result
13.	Allowable TAN (TANlimit)	2.0	g/m^3	Experimental result
14.	Feeding Rate (FR)	3.40	%/bw/day	Experimental result
15.	Allowable NO_3 (ANO_3)	200	g/m^3	Wester (1997)
16.	Circulation Percentage (R)	0.96	%	Wheaton (1977)
	PROSES			
17.	TAN Production (P_{TAN})	2.27	g/d	F*PC*0,095*1000
18.	TAN single pass concentration (Ci)	0.31	g/m^3/d	P_{TAN}/Q
19.	TAN accumulation factor (C)	6.35		TAN_{limit}/Ci
20.	TAN loading rate ($LTAN_{in}$)	14.40	g/d	P_{TAN}*C
21.	Hydraulic Retention Time (HRT)	0,47	hour	V_f (m^3)/Q (m^3/day)*24 hour
22.	Exchange Rate (R)	0.14	kali/hr	0.06* Q (lpm)/V_{tank} (liter)
	OUTPUT			
23.	TAN concentration in the filter outlet (Cout)	0.27	g/ m^3	Experimental result
24.	TAN loading out (LTANout)	1.94	g/day	C_{out} (g/m^3/day)*Q (m3)
25.	*TAN Removal Rate* (%TAN_{rem})	86.5	%	(L_{TANin}-L_{TANout})/L_{TANin})*100
26.	*Carrying capacity (LD)*	25.95	kg/lpm	(%TAN_{rem}*100*Vs*1000*A_{NO3})/(%FR*TAN_F*4.2*R*1000)
27.	Fish density (D)	**62.69**	kg/ m^3	(LD*R)/0.06
28.	Total biomass (TB)	129.77	kg	D (kg/m^3)*V_{tank} (m^3)
29.	% biomass of perch (%BIB)	0.39		Experimental result
30.	% biomass of tilapia (%BIN)	0.61		Experimental result
31.	Max. biomass production of perch (PM)	50.61	kg	TB*%BIB
32.	Max. biomass production of tilapia (PM)	79.16	kg	TB*%BIN
33.	Max. biomass production of perch (PM)	**25.30**	kg	1/2*PM (Timmons & Losordo, 2007)
34.	Max. biomass production of tilapia (PM)	**39.58**	kg	1/2*PM (Timmons & Losordo, 2007)

Given that the investment cost for the installation of recirculation system is considerably high, the cultured species has to be selected for those who are fast growth and high economic value, as well as a uniform seed size. Although aiming for sustainability of local fish, but given the low growth rate, then the selection of climbing perch (*Anabas testudineus*) in aquaculture systems may be less favorable. For the types of plants that are used to absorb nitrogen waste, spinach (*Ipomoea reptana*) and basil are recommended to be used, although the price is relatively low, but the rate of growth (harvest every 14 days) and the high absorption rate of nutrients added value in terms of economic benefits and health.

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