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Biomass production of *Azolla microphylla* as biofilter in a recirculating aquaculture system

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Abstract. *Sumoharjo, Ma'ruf M, Budiarto I. 2018.Biomass production of* Azolla microphylla *as biofilter in a recirculating aquaculture system. Asian J Agric 2: 14-19.* This study utilized macrophyte (*Azolla microphylla* Kaulf.) as a biofilter and investigated that biomass produced in an aquaculture system could potentially be an alternative feed. This experiment was aimed to determine the *Azolla* microphylla growth rate and its efficiency in removing ammonia from a simple recirculating aquaculture system. The experimental units were set up in three different water flows, i.e. 3 lpm, 5 lpm, and 7 lpm onto the three different geometrically baseboards of Tilapia (*Oreochromis niloticus*) growing tanks (prism, rectangular and limas). The results showed that water flow did not give significant effect($P < 0.10$) on the growth rate of *Azolla*. The lower water flow (3 lpm) resulted in the highest ammonia biofiltration efficiency, which can remove ammonia up to 32.2±3.0% of the total NH_3-N and NH_4^+-N (TAN).

Keywords: *Azolla microphylla*, ammonia, biofiltration, recirculating, water flow

INTRODUCTION

The main problem in the intensification of aquaculture systems is water quality decreasing rapidly because of a high density of fish being reared with a high feed input in less water exchange. Hence, accumulation of fish metabolites, especially ammonia, tends to occur in waterbody and build-up to toxic level and affecting fish performance. Wastewater is accumulated, while feed is continuously added in a fish culture system (Rafee and Saad 2005).

In an intensive land-based fish farming system, the toxicity of excreted nitrogenous compounds is often a limiting factor (Bradfield 1985; Brune et al. 2003, Nerici et al. 2012). The toxicity of the total NH_3-N and NH_4^+-N (TAN) increases with the pH of the water because TAN enters the organism as NH_3 and the proportion of NH_3 increases with higher pH (Randall and Tsui 2002; Nerici et al. 2012). When environmental TAN level increases, the excretion of ammonium by aquatic animals decrease and the ammonium levels in the blood and tissues rise (Nerici et al. 2012). Long-term exposure to ammonia increases glycemia, lipoxygenase and unsaturated Erythrocyte Fatty Acids (Liu and Sun Pan 2008). Chronic exposure to high TAN concentrations tends to damage the fish gills, which can contribute to decreased growth because of gas exchange efficiency (Handy and Poxton 1993; Nerici et al. 2012).

In Recirculating Aquaculture Systems (RAS), biofilter is the main component and known as low-cost water treatment to keep water quality suitable for fish growth and welfare. Biofilter technology was studied intensely, however, most of them are struggling on bacterial-based biofilters, such as nitrification by nitrifiers and nutrient assimilation by heterotrophs. Smith (2003) categorized biofilters into four main types, i.e., activated sludge, aquatic plant filters, fluidized bed filters, and fixed film.

Azolla microphylla is an aquatic fern. Many reports on *Azolla* have been published, but almost all of them are related to its function as a natural feed resource. *Azolla* was recommended by FAO (2009) as feed-in small-scale aquaculture and had been used as a main component in food for tilapia (Fiogbé et al. 2014). According to Lumpkin and Placknett (1980) and Van Hove (1989), *Azolla* under good conditions presents high productivity and protein content (generally 20-30%, on a dw basis).

Growing *Azolla* seems easy (Datta 2011) because of its endosymbiotic blue algae, *Anabaena azollae*, that fixes nitrogen directly from the atmosphere (Van Howe 1989). So, *Azolla* is probably able to grow well in a relatively low nutrient environment. However, reports on *Azolla* as biofilter to remove nitrogen from the fish culture water are rare. Even though, as a macrophyte, *Azolla* should be served as a phototrophic converter at the trophic level. So, it has great potential as a biofilter for maintaining water quality in RAS as well as providing an alternative feed for growing-fishes.

This study focused on utilizing *Azolla* as biofilter in RAS. The experimental units were designed in integration of fish tank and *Azolla* growing bed to meet a series of recirculating systems. The experiment was divided into two parts; first was to analyze the effect of different water flow on *Azolla* growth rates, and the second one was to determine the optimum biomass of *Azolla* for converting nitrogen from fish waste.

MATERIALS AND METHODS

Experimental unit configuration

The experimental units were a pilot scale. Three types of tanks, with 1800-liter effective volume, were used as an experimental group. The design of the bottom of each tank varied geometrically, i.e., prism, pyramid, and rectangular. On the top of every tank were three similar trenches that had a 2-meter long biofilter bed. Every trench had three different water flow rates, i.e., 3 lpm, 5 lpm and 7 lpm. A 32 watt submersible pump was used to supply water from each fish tank to the three trenches, connected parallelly with ¾ inch PVC pipe. The water flow rate, as the treatment was adjusted, was done by the outflow head. Synchronization was carried out daily.

The fish species cultivated were tilapia (*Oreochromis niloticus*), sized 8.3±1.2 g and had 100 fishes per tank. In every trench, 50 g *Azolla microphylla* was added. The fish were fed *ad satiation* with floating pellets (CP. Prima 781-3, 31-33% raw protein).

Nutrient budgeting

In the recirculating system, the complete water from the fish tank passed over the biofilter bed (the trench) once every 2 hours, then the water was mixed continuously in the fish tank, so that there were no differences between in and outflow. Samples were taken in fish tank only. Total Ammonia Nitrogen (TAN) as nutrient input and removal rates were calculated through mass balance. To estimate TAN input per day from within the fish tanks the following can be calculated based upon the feeding rate (Timmons et al. 2002):

$$P_{TAN} = F * PC * 0{,}092$$

Where:
P_{TAN} : Production rate of total ammonia nitrogen, (kg/day)
F : Feed rate (kg/day)
PC : Protein concentration in feed (decimal value)

The constant in the ammonia generation equation assumes that protein is 16% nitrogen, 80% nitrogen is assimilated by the organism, 80% assimilated nitrogen is excreted, and 90% of nitrogen is excreted as TAN+10% as urea. In addition, the nitrogen in feces is not removed from the system but collected in the filter bed until the end of the experiment.

Water quality

Water quality parameters were monitored every three days such as temperature, pH, dissolved oxygen (DO), Total Ammonia Nitrogen (TAN), and Un-ionized Ammonia Nitrogen (NH_3-N). Lutron portable DO meter model 5510 was used to measure DO. The concentration of TAN was determined using TAONSUN spectrophotometer (Suzhou Taonsun Scientific Instruments, China).

Biomass calculation

Biomass growth of *Azolla* cultivated in biofilter units (gutter/trench) is expressed as doubling time (day^{-1}) which is calculated according to daily growth rate (DGR, %/g/day) (Zonnenveld, et al., 1991) as follows:

$$\text{DGR } (\%/g/d) = \frac{\text{Ln}(W_t)\text{-Ln}(W_0)}{t} \times 100$$

$$\text{DT } (\text{day}^{-1}) = \frac{\text{Ln } 2}{\text{DGR}}$$

Where:
DGR : daily growth rate (%/g/day)
Ln : logarithmic natural
W_t : final biomass of *Azolla* (g)
W_0 : initial biomass of *Azolla* (g)
DT : Doubling Time

Total Ammonia Nitrogen measured on the final day of the experiment will represent the nutrient output. Thus, in case of this simple RAS, whereas all the water is recirculated and there is no discharge, nutrient removal rate can be calculated with mass balance equation (Al Hafedh et al., 2003) as follows:

Waste Loading Rate (g/m^3 per day) = Ci × Q

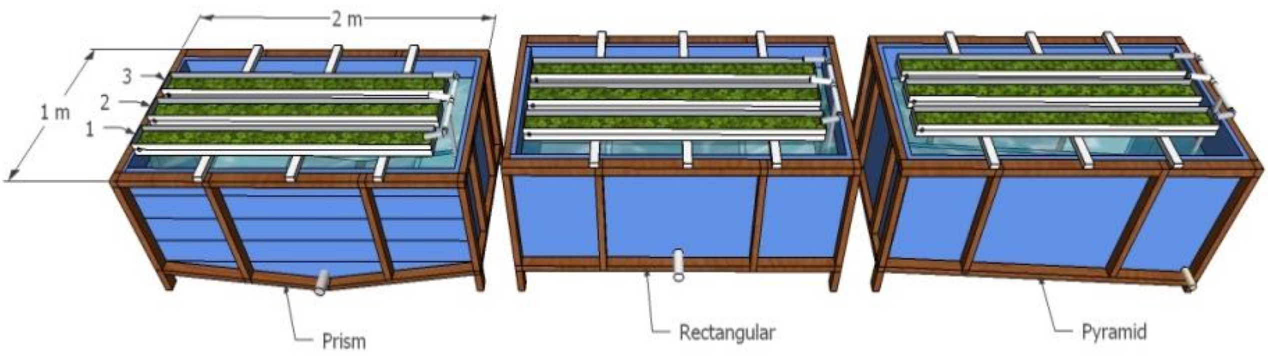

Figure 1. Sketch of experimental units configuration

Waste Removal Rate (g/m^3 per day) = $(Ci - Ce) \times Q$

$$\text{Removal efficiency } (E) = \frac{\text{waste removal rate}}{\text{Waste loading rate}} \times 100$$

Where:
Ci : Total Ammonia Nitrogen measured in the fish tank (mg/L x Water Volume x1000 = g)
Ce : P_{TAN} (Production rate of total ammonia nitrogen (g)
Q : Water flow (liter per minute, lpm)

Retained nitrogen of *Azolla* is expressed as gram and can be calculated by using the following formulae:

Retained Nitrogen (RN, gram)= $(TKN_t \times W_t) - (TKN_0 \times W_0)$

$$\text{Retained Nitrogen Efficiency (RNE, \%)} = \frac{RN}{P_{TAN}}$$

Where:
TKN_t : Total Kjedahl Nitrogen at the end of experiment (g)
TKN_o : Initial Total Kjedahl Nitrogen (g)
W_t : Final biomass of *Azolla* (g)
W_0 : Initial biomass of *Azolla* (g)

Data analysis

The means on the *Azolla* growth rate, doubling time, and nitrogen retention parameters were analyzed using two-way analysis of variance (ANOVA, α = 0.1). The analysis was done using STATISTICA 8.0.

RESULTS AND DISCUSSION

Nutrient input and biomass production of *Azolla*

Total feed consumed by the fish in tanks I, II, and III were 1519 g, 1504 g, and 1313 g, respectively. The TAN production of every tank is listed in Table 1.

Based on the calculations, TAN production of all the fish tanks were evenly 3% of the total feed input. For example, tank I released 1.49 g TAN per day in 1800 liter of water. It means that 0.83 mg.L^{-1} of TAN was added and diluted in the water of the fish tank.

The TAN production was similar to the assumption of Colt (1991) that waste output of fish consuming 1000 g feed and 250 g O_2 are 30 g of TAN and 340 g CO_2 excreted via gill by ion-exchange along with 500 g fecal solid and 5.5 g PO_4-P. Then, Schneider et al. (2005) stated that the Fish-Biomass-Converter retains 20-50% feed N and 15-65% feed P. This means that 50-80% feed N and 35-85% feed P are discharged as waste.

Fish waste that was released in the water column was then recirculated and served as nutrient input for *Azolla*. The treatment with water flows showed no significant difference (P < 0.1) among 3 lpm, 5 lpm, and 7 lpm on doubling time of *Azolla*'s biomass (Figure 2).

Table 1. Feed consumption and TAN Production (P_{TAN}) in 30 days

Tank	Feed consumed (g)	P_{TAN} total (g)	P_{TAN} (g/day)
I	1519	44.7	1.49
II	1504	44.3	1.48
III	1313	38.6	1.29

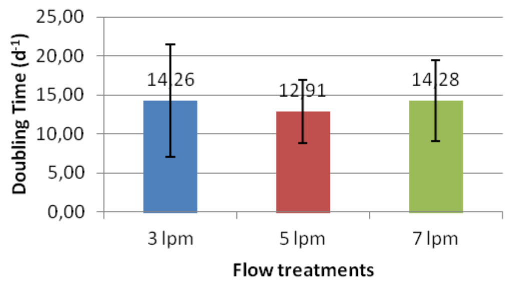

Figure 2. Doubling time of *Azolla* affected by water flow

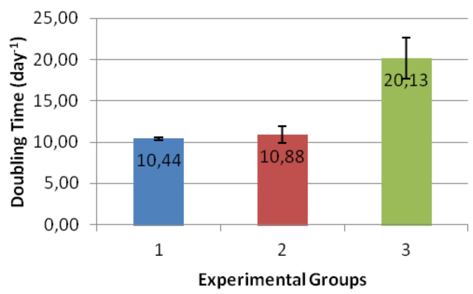

Figure 3. Doubling time of *Azolla* biomass affected by grouped-tank

Statistically, the significant difference in *Azolla*'s biomass production occurred on experimental group-tank factors. Differences in the growth rate of the *Azolla* biomass in each experimental group related to the baseboard designs. This allowed better nutrient supply in Tank I and Tank II compared to Tank III. In Tanks I and II, the average biomass growth was doubled from the initial population that occurred every 10.44 and 10.88 days while in Tank III was every 20.13 days (Figure 3).

The result is inversely proportional to the TAN reduction pattern, which means that during the 1st day until the 20th day there was active ammonia assimilation by *Azolla*, and at the peak multiplication of the biomass the assimilation rate of N decreases, resulting in the TAN concentration in the water to rise again.

Azolla has been known to have the ability to fix nitrogen from the air, so that it can survive and keep growing under low nutrient conditions in the water. However, from the results of this study, there is a correlation between minimal TAN concentration and the rate of assimilation of N by *Azolla*. The TAN concentration in water should remain at a value of> 0.1 mg.L^{-1} to maintain the rate of assimilation of N. If the TAN concentration <0.1 mg.L^{-1}the assimilation of N tends to be slower or even stopped so that in this phase will result in cessation or decrease in growth rates of *Azolla*.

Based on the results of *Azolla* Total Kjeldahl Nitrogen (TKN) analysis on the 30th day showed that the average *Azolla* protein content in each treatment was different but not significant (P> 0.10). The level of protein content present in *Azolla* in this study was relatively good (28.8 %) compared to the results of the tests with the duckweed (*Lemna minor*) in the same experimental design which reached 25.7% (Sumoharjo 2015). Therefore, it could be an alternative feed for herbivorous fish such as tilapia. The high levels of this protein content are influenced by *Azolla*'s ability to convert nutrients from the water into *Azolla* biomass. The nitrogen retention by *Azolla* showed considerable value in each trial and showed significant differences between treatments as well as groups (Table 2).

The highest nitrogen retention was achieved by the 3 lpm treatment of 11.27 ± 6.95 gN, followed by the 5 lpm treatment of 9.23 ± 7.28 gN, and the lowest was the 7 lpm treatment which only retained N of 7.97 ± 6.22 g.

TAN removal efficiency

The TAN conversion rates were determined as overall retained nitrogen of *Azolla* from P_{TAN} of every tank as part of the experimental group (Figure 4). The results of this experiment showed that the efficiency of the TAN removal by *Azolla* was still lower than the treatment using *Lemna minor* which reached 48% but was much higher than *Spyrogyra* sp which retained 2.91% N of TAN produced by tilapia (Sumoharjo 2015). Determining how much TAN removal will greatly determine the potential level of the use of a phototrophic organism as a biofilter for the use of water quality management in RAS.

Water quality and nitrogen dynamics

Water quality characteristics, such temperature ranged between 27.3 to 30.7ºC, while pH and TAN tended to decrease during experiment (Figure 5). The proportion of NH_3 increased with higher pH. It could be because TAN enters the organism as NH_3 (Randall and Tsui 2002). Therefore, the toxicity of TAN (the total NH_3-N and NH_4^+-N) increased in line with the increase of pH of the water. Fortunately, pH during the experiment tended to decline from 7.9 ± 0.2 at the beginning to 6.6 ± 0.2 at the end of experiment. So that the proportion of un-ionized ammonia (NH_3) was low and in a tolerable concentration for Tilapia. The toxic level of NH_3 for short-term exposure usually are reported in between 0.6 to 2 mg.L^{-1}, while the maximum tolerable concentration is to be 0.1 mg.L^{-1} (Pillay 1992). Moreover, the specific growth rate (SGR) of tilapia exposed to un-ionized ammonia nitrogen over 0.068 mg NH_3 was significantly reduced. The specific growth rate and the increase of the unionized ammonia concentration increased the feed conversion ratio (El-Syafai 2004).

Table 2. Variance analysis of retained nitrogen by *Azolla*

Source	Degr. of	RN SS	RN MS	RN F	RN p
Treatments	2	16.4019	8.2009	8.7203	0.034805*
Groups	2	276.2516	138.1258	146.8737	0.000180*
Error	4	3.7618	0.9404		
Total	8	296.4152			

Note: *: Significant difference on 90% of reliability

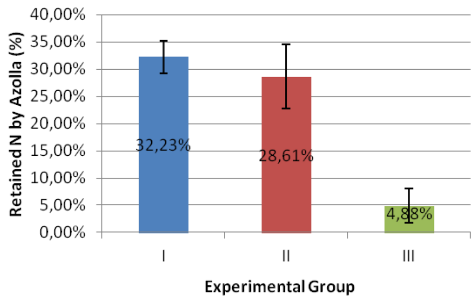

Figure 4. TAN Removal efficiency

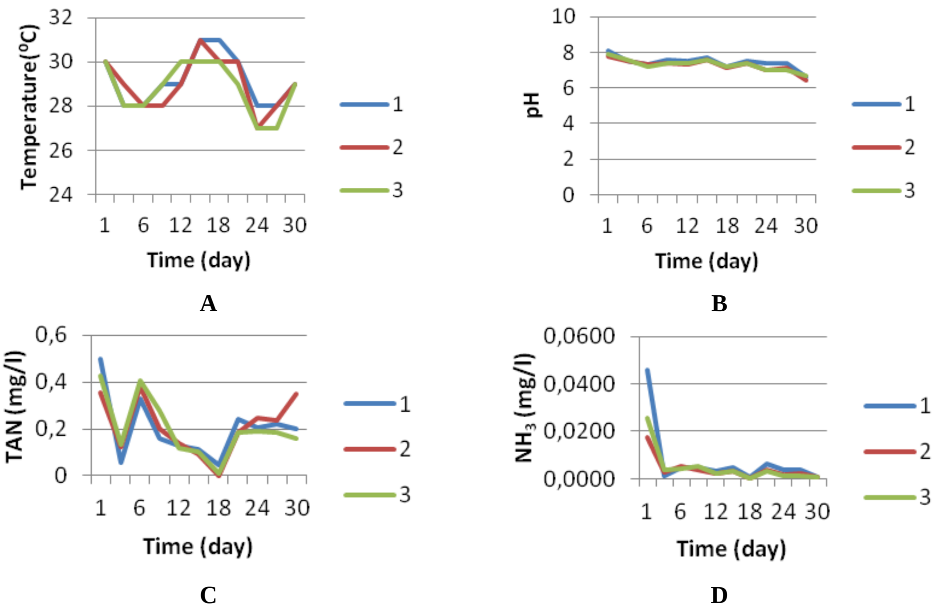

Figure 5. Water quality characteristics: A. Temperature, B. pH, C. TAN, D. The proportion of NH_3

Table 3. Means and standard deviations of water quality characteristics

Water quality parameters	Unit	Tank		
		I	II	III
Temperature	ºC	29.2±1.2	29.0±29.0	28.8±1.2
pH		7.4±0.4	7.3±0.4	7.3±0.3
DO	mg.L	4.1±1.4	3.6±1.2	4.0±1.7
CO2	mg.L	21.0±11.4	23.2±13.3	22.0±11.7
TAN	mg.L	0.20±0.1	0.21±0.1	0.20±0.1
NH3	mg.L	0.007±0.013	0.004±0.005	0.005±0.007

Carbon dioxide (CO_2) is another factor that may affect feed behavior (Trand-Duy et al. 2008). In an intensive culture system, CO_2 may not have an adverse effect on fish unless its concentration reaches 100 mg.L^{-1} (Balarin and Heller 1982). Nile tilapia can tolerate CO_2 concentration above 20 mg.L^{-1} (Wedemeyer 1996). In this study, CO_2 was 30.2±3 mg.L^{-1} on the first day then decrease to 15.2±1.1 on the 30th day of the experiment (Table 3). CO_2 tended to decrease during the experiment because the turbulences that occurred in inflow and outflow of the *Azolla* reactor may strip CO_2 to atmosphere. Moreover, algae and *Azolla* thrived in the reactors play a role in removing CO_2 out of the system.

Concentration of dissolved oxygen (DO) during the experiment ranged between 2,2 to 6,2 mg.L^{-1}(Table 3). The lowest DO concentration occurred on the last day of the experiment. Accumulation of sludge in the *Azolla* reactor played a role in decreasing DO gradually. This may happen because there is no sludge disposal from the system. DO should be maintained above 3.0 ppm and 5.0 ppm for warm and cold-water fish, respectively (Buttner et al. 1993). However, most species of fish are distressed when DO falls to 2-4 mg.L^{-1} (Floyd 2003).

The lower extreme value of DO (less than 0.8 mg.L^{-1}) was obtained from an experiment in which there were no significant differences between the yields of Nile tilapia raised in ponds with two aeration regimes (Teichert-Coddington and Green 1993). Thus, practical threshold of DO for Nile tilapia was not higher than 10% of saturation (0.8 mg.L^{-1} at 26°C) (Trand-Duy et al. 2008).

In conclusion, *Azolla microphylla* can be grown well in RAS. The assimilation rate of TAN by *Azolla* decreased after its peak biomass production (when doubling time was achieved). Therefore, harvesting must be done 15 to 18 days after cultivation. As a biofilter, it provides a mini-ecosystem that serves as nutrient controller for aquaculture practices. The lower water flow rate the higher nitrogen retention, although, there was no significant effect of water flow rates on the *Azolla* growth response. It has enough protein content, hence has potential as feed source for herbivorous fishes.

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