# The Utilization of Wastewater from Catfish Pond to Culture Azolla microphylla

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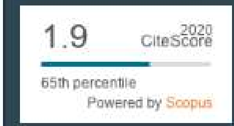


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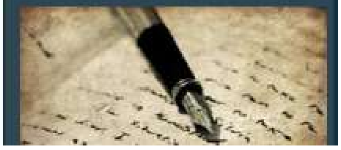

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# The Utilization of Wastewater from Catfish Pond to Culture *Azolla microphylla*

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***Abstract*— To maintain pond water quality, a large amount of wastewater is discharged. The wastewater degrades the environment and annoys nearby residents. This wastewater is the potential for growing *Azolla microphylla*, a valuable floating fern for different purposes. This research aims to observe the effect of water replacing period and mechanical aeration on the growth of *A. microphylla* and the wastewater quality. A 20-gram of *A. microphylla biomass* was inoculated in a plastic-layered wooden box (50×30×20cm) filled with catfish pond wastewater, and then designed treatments were applied for a 12-day experiment. A completely randomized design with two factorial arrangements was implemented. The first factor was the period of water replacement consisted of four levels: no replacement (E0), once in 2 days (E1), once in 4 days (E2), and once in 6 days (E3). The second factor was mechanical aeration consisted of three levels: no mechanical aeration (A0), 12-hour aeration (A1), and 24-hour aeration (A2). Parameters to be observed were Azolla biomass and water quality (temperature, pH, turbidity, and ammonium). The data set was analyzed using ANOVA followed by LSD multiple comparisons. Results revealed that interaction of the water replacing periods and the mechanical aeration significantly affected water quality (temperature, pH, turbidity, ammonium) but was not significant for the yield of *A. microphylla* biomass. The factor of water replacing period alone significantly affected the growth of *A. microphylla*. The E2 treatment was the most promising option, with a biomass yield of 804 g/m2 within 12-day cultivation.**

***Keywords—Azolla mycrophylla*; growth rate; wastewater; doubling time; ammonium; biomass yield.**

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## I. INTRODUCTION

Catfish is an important product of freshwater aquaculture in some areas of Indonesia. The fish provides people a source of protein at a fairly low price. Catfish can be easily cultured even in a small pond with high-density fish [1], [2] that many catfish ponds are frequently developed in some locations close to residential areas. Unfortunately, its odorous pond water often annoys nearby residents. Catfish eat a lot, but only 25.5% of organic matter, 26.8% of nitrogen, and 30.1% of phosphorus are converted into biomass, while most of the feed is excreted as waste in pond water [3]. When pond water needs to be flushed and replaced with freshwater, the pond wastewater discharges certainly pollute the environment [4]. Indonesian aquaculture's environmental and socio-economic problems are foreseen more complicated in the future if no proper technology is applied [5]. Some aquatic plant systems such as water hyacinth, duckweed, and Azolla are reported to treat wastewaters effectively [6], [7], [8].

The utilization of *Azolla* for reclaiming catfish pond wastewater may offer a promising solution to the problem of catfish pond wastewater management. *Azolla* contains crude protein of 27.0% and ash of 17.37% based on dry weight [9]. Azolla has been cultured for some different purposes such as biofertilizer [10]–[12], fish meals [9], [13]–[15], feed for poultry [16]–[19], cattle feed [20], [21], feedstocks for bioenergy production [22]–[26], wastewater remediation [27]–[30], $CO_2$ sequestration [31], and $CH_4$ emission reduction in paddy field [32].

Pond wastewater contains nutrients, especially nitrogen and phosphorous derived from leftover feed, feces, and fish urine. At least two advantages can be gained if catfish pond wastewater is used for growing *Azolla*. First, *Azolla* functions as a phytoremediation that can improve pond water quality to better levels, hence preventing environmental pollution, reduced flushing water, and improving fish health if the reclaimed wastewater has to be recycled to the fishponds. Second, the biomass of *Azolla* can be utilized for animal or fish feeds, thus reducing the cost of feeds [33]. Integrated fish-*Azolla* or rice-*Azolla* farming systems are generally practiced in a single area. Azolla mat covers the surfaces where the fish or rice is grown. Research showed that the Azolla cover improves water quality parameters, lowering pH and ammonia volatilization in the rice field, increasing nitrogen recovery [12], [34]. Other research also reveals that Azolla decrease ammonia-N and phosphorus level [35]. Unfortunately, dissolved oxygen also dropped to an anoxic level (less than 2 mg/L) which could hamper the fish health [36]. The drop of dissolved oxygen (DO) is caused by photosynthetic micro-phytoplankton that cannot compete for light with Azolla, covering the pond surfaces and dying. *Tilapia*'s weight gain decreased and could be associated with the low DO effect because of the adverse effect of the Azolla cover. It was found that optimum surface cover (based on the fish yield) by *Azolla* was 25% of the total pond surface [37]. Based on the Azolla biomass, however, this meant that 70% of Azolla biomass potential was lost.

If a fish-*Azolla* aquaculture system was constructed in separated ponds, both fish and Azolla biomass gains could probably be maximized. Azolla cultivation using catfish pond wastewater in separated pond systems has not been reported. In order to develop the potential of this system, parameters such as hydraulic loading rate have to be researched. Azolla may be stressed or even die if the loading rate is too high because catfish pond wastewater contains high concentrations, particularly ammonia. In contrast, *Azolla* will grow suboptimal if the nutrient is insufficient because of too low a loading rate. In order to determine the proper hydraulic loading rate, this research was conducted. This research investigates the effects of catfish pond wastewater replacing periods (mimicking continuous hydraulic loading rates) and mechanical aerations in a batch system on the growth of *A. microphylls* and catfish pond wastewater quality improvement. The effect of mechanical aeration is also evaluated in that dissolved oxygen underneath of Azolla mat has been known very low [38].

## II. MATERIAL AND METHOD

### A. Preparation

Thirty-six plastic-lined wooden boxes, each sizing 50×30cm$^{2,}$ and 20cm depth, were prepared and placed in a plastic house, as presented in Figure 1. Catfish pond wastewater was taken from a nearby catfish growing pond whose fingerling size of fish (about 2-month-old) was used as it was. Every box was filled with 15 L (depth of around 10cm) of catfish pond wastewater. Twenty-gram (equivalent to the density of 133.33 g/m$^2$) *A. microphylla* from an available source was cultured in each box with corresponding treatment. Azolla biomass was maintained until the harvesting time at day 12.

### B. Treatment and Experimental Design

The experiment was conducted using a Completely Randomized Design with two factorial arrangements (CRD two-factor). Each box mentioned above was used as the experimental unit. All treatments combined two factors: water replacing periods (E) and mechanical aeration duration (A). The first factor consisted of 4 levels, namely: no water replacement in 12 days (E0), which is equivalent to one replacement in 12 days, once replacement in 2 days (E1), once in 4 days (E2), and once in 6 days (E3). Water replacing was carried out by draining the water in the boxes and replaced with fresh wastewater at the complementary treatment of the period. The second factor consisted of 3 levels, namely no aeration (A0), 12-hour aeration (A1), and

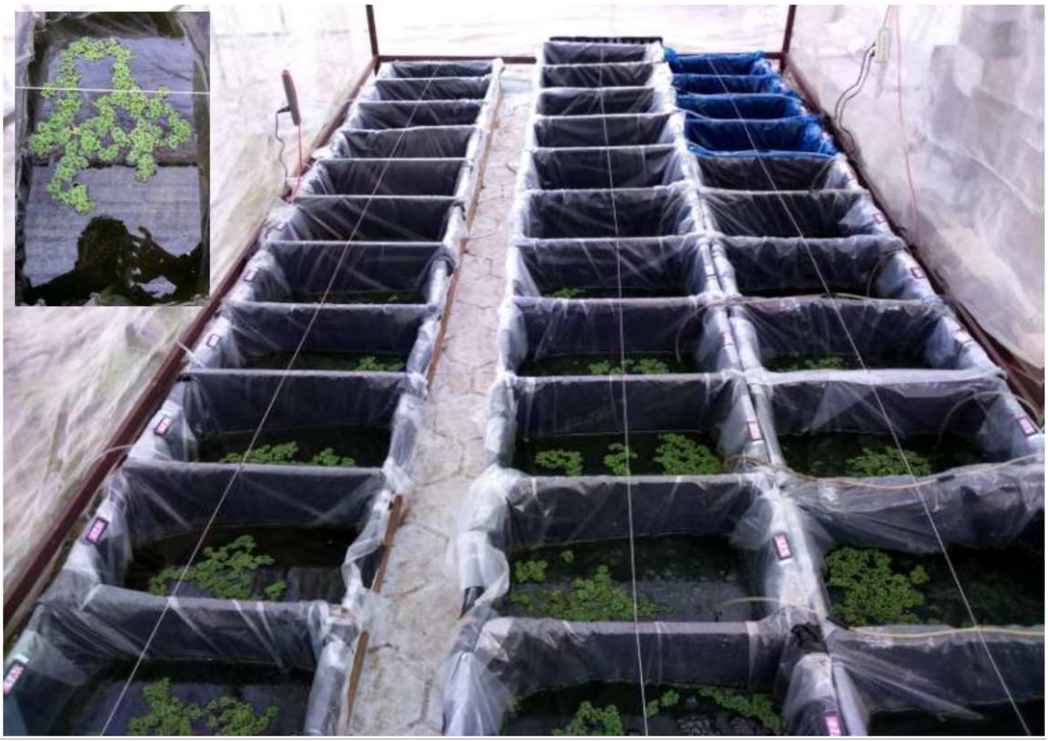

Fig. 1 Experiment lay out of Azolla cultivation with 20 grams seed for each box

24-hour aeration (A2). Mechanical aeration was carried out using small air diffusers. Each treatment combination was conducted in triplicates, totaling thirty-six experimental units. Figure 2 shows a flow chart for the whole experiment.

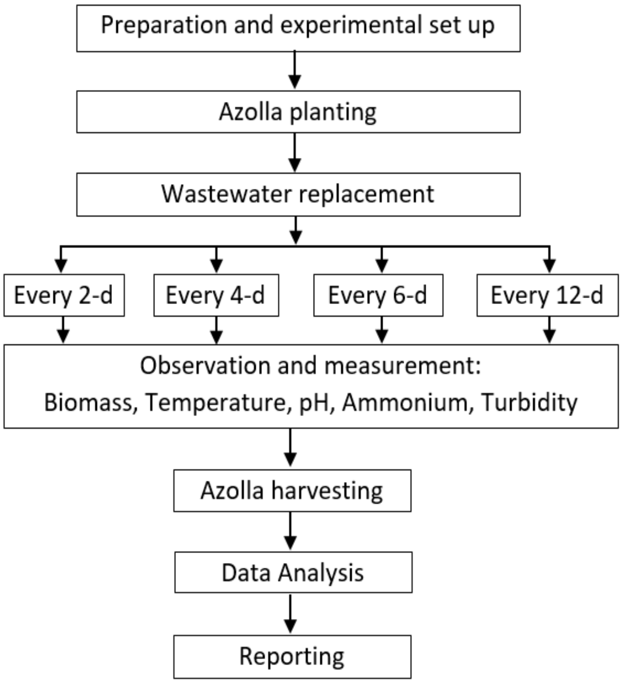


Fig. 2 Flow chart for the whole experiment

### C. Measurement and Data Analysis

Measurement was conducted in water quality and Azolla growth. Parameters corresponding to water quality including water temperature, pH, turbidity, and ammonium. The first three parameters were measured daily at 7.00 a.m. using a thermometer, pH meter, and turbidity meter. Ammonium content in the water was analyzed using Nessler reagent and followed by spectroscopy. The analysis was performed on fresh wastewater at initial filling and every replacement time on used wastewater just before water replacement.

Parameter correlating to *A. microphylla* involved the biomass yield, biomass growth rate, and biomass doubling time (gravimetric method). Observation was started from the beginning when *A. microphylla* was inoculated and terminated after 12-day of cultivation. Azolla biomass was observed every three days by taking the biomass from the box, draining, and weighing it. The development index of Azolla was measured by biomass weight, *RGR* (relative growth rate), and *DT* (doubling time) calculated as the following [39].

$$RGR = \ln (W_t/W_0)/t \quad (1)$$

$$DT = \ln(2)/RGR \quad (2)$$

where $W_0$ and $W_t$ are, respectively, the fresh weight of Azolla at zero time (weight of inoculum) and at elapsed time $t$ (in days). The unit of *RGR* is expressed in $g.g^{-1}.d^{-1}$. The collected data set was analyzed using Analysis of Variance (ANOVA), followed by using Least Significant Difference (LSD) multiple comparisons.

## III. Results and Discussion

### A. Water Temperature

The average daily water temperature ranged from 26.0 to 30.0°C with an average of 28.18°C. The water temperature was a little low because the data set was collected in the morning. Before and just after noon, the water temperature increased to about 33°C, which is normal for tropical locations. Though the interaction between the water replacing period and mechanical aeration significantly influenced daily average temperature, the differences were too small. Within this range of temperature, the growth of *A. microphylla* should not be adversely affected by the existing water temperature because it is tolerant to high temperatures (40 °C) [40].

Effect of interaction between water replacing period and mechanical aeration duration on the water temperature at day 12$^{th}$ was significant at 5% level (Figure 3). Figure 3 suggests that, in general, more aeration duration has resulted in the decreasing water temperature. With 24-hour aeration, the water temperature could be maintained stable even for boxes without water replacement treatment. This phenomenon is unsurprising because the air bubble diffused into the water took the heat out of the water. Figure 3 suggested that for more frequent water replacement or shorter water replacement periods (2 and 4 days), aeration may not be needed because water temperatures were not significantly different. However, for less frequent water replacement (12 days), 24-hour aeration was needed because water temperature increased.

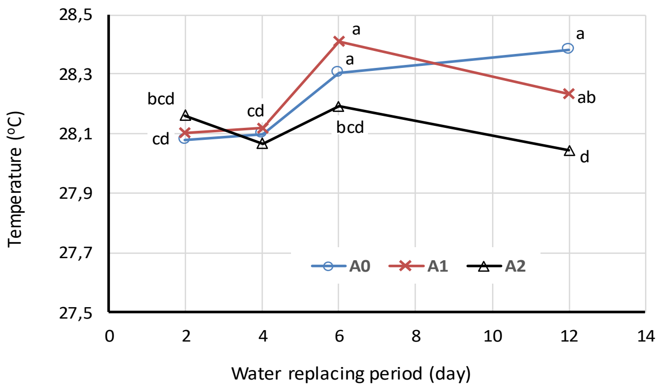


Fig. 3 Effect of water replacing period and aeration duration on water temperature at day 12th. (Values followed by common letters indicate no difference at 5%).

### B. Water pH

Water acidity or pH was monitored daily at the same time as the water temperature measurement. Initially, wastewater from the catfish pond has an almost neutral pH, namely 7.5. All the experimental units showed the water pH increased so that it becomes slightly basic (ranging from 7.71 to 8.60), but it should not be harmful to Azolla yet, because Azolla can survive within a wide pH range of 3.5-10 [35]. The daily pH values were fairly stable, though little fluctuations were still visible.

The interaction between the water replacing period and mechanical aeration duration on the average pH value was significant at 5% level. Figure 4 indicated that extending the water replacing period increased water pH, and the same situation happened for aeration duration. However, when water was replaced more frequently (once in 2-day), the effect of aeration duration on the increase of pH was not as much as on longer water replacing period (12-day or no replacement during 12-day cultivation).

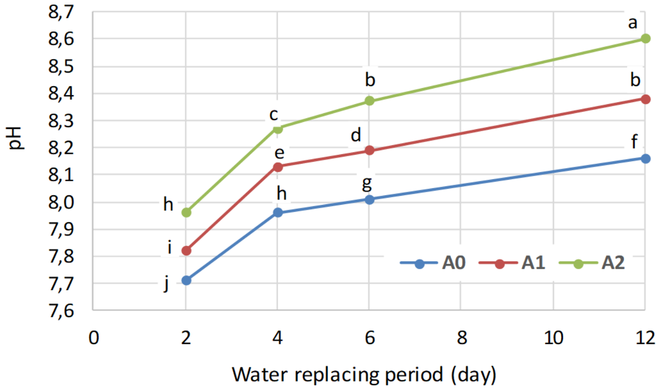


Fig. 4 Effect of water replacing period and aeration duration on the average pH. (Values followed by same letters are not statistically different at 5%).

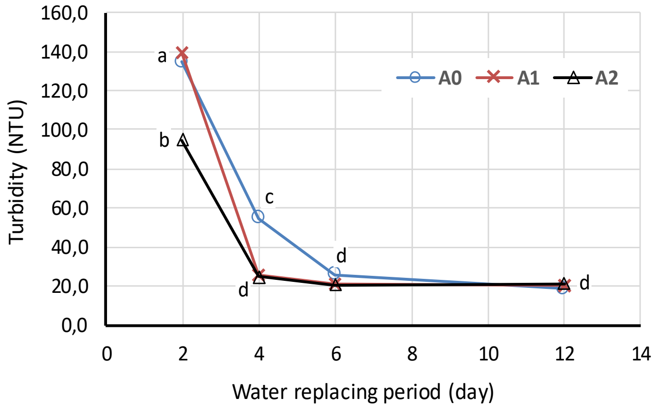


Fig. 5 Effect of water replacing period and aeration duration on pond wastewater turbidity. (Values followed by common letters mean no difference at 5% level)

## C. Water Turbidity

Soluble organic solids cause turbidity in pond wastewater. The turbidity of fresh pond wastewater was 178 NTU on average. The value decreased to 95-140 NTU when the water was replaced once in 2 days (E1), and to around 25-55 NTU for replacing 4 days (E2), and further decreased to around 20 NTU with longer replacing periods. At every end of the designed water replacement period, the water was replaced with fresh pond wastewater, and turbidity turned back to the initial level. Final turbidity at every time before wastewater replacement was presented in Figure 5. The interaction between the water replacing period and mechanical aeration duration on wastewater turbidity was significant at 1% level. Figure 5 also suggested extending the water replacement period from 2 to 4 days dropped water turbidity and level off (21 NTU) at a longer period (6 and 12-day water replacement period). Even with no aeration, turbidity dropped from 140 NTU (2-day replacing period) to 55 NTU (4-day replacing period). For the 2-day water replacement period, 12-hour aeration was not needed because turbidity was not significantly different from that with no aeration. Likewise, 24-hour aeration was not needed for a 4-day water replacement period because turbidity was not significantly different from that with 12-hour aeration. Overall, six and 12-day water replacement periods may be unnecessary because turbidity already leveled off at about 21 NTU either with or without additional mechanical aeration. However, for aquaculture purposes, acceptable turbidity was less than 25 NTU, meaning that the treatments of 4-day water replacing the period with aeration or less frequent water replacement will be better methods if the Azolla culture system was incorporated with aquaculture [41].

## D. Ammonium Content

Fresh pond wastewater initially had high ammonium content (about 178-180 mg/L on the average) and then dropped to certain levels within 2, 4, 6, 12-day periods of time. Based on statistical analyses, the interaction between mechanical aeration duration and water replacing period on pond wastewater ammonium content was significant at 5% level. Water replacement period alone significantly affected ammonium content at 1% level, while aeration duration did not.

Extending wastewater replacing periods from 2 to 4 days was not effective. However, extending the water-replacing period from 4 to 6 or 12 days was very effective, in that ammonium dropped from 16.25 mg/L to 4.94 mg/L on average (Figure 6). The insignificant effect of aeration on ammonium concentration could be attributed to the shallow water depth used in this experiment (only about 10 cm). The shallow water depth was favorable for ammonia volatilization. The important thing is determining optimal ammonium concentration, which is enough for *A. microphylla* growth, yet not odorous.

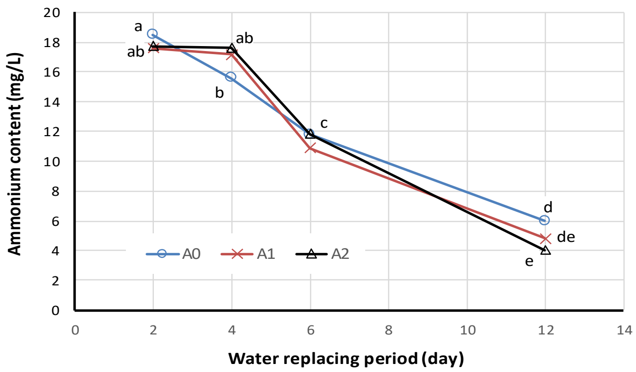


Fig. 6 Effect of water replacing period and aeration duration on ammonium level at day 12th. (Values followed by common letters mean no difference at 5% level)

## E. Azolla Biomass

Figure 7 shows the condition of Azolla biomass on the 12th day just before harvesting. From the figure, it is clear that boxes with water replacement once in 2- or 4-day produce Azolla biomass with a higher density as compared to those with longer water replacement periods (6- and 12-day). At the harvest time, treatment of the water replacing period significantly affected the *A. microphylla* biomass yield at a level of 5%, while mechanical aeration did not.

Table 1 presents fresh biomass weight (*FW*) along with relative growth rate (RGR) and doubling time (DT) of *A. microphylla* for 12 days culture observed every 3-day.

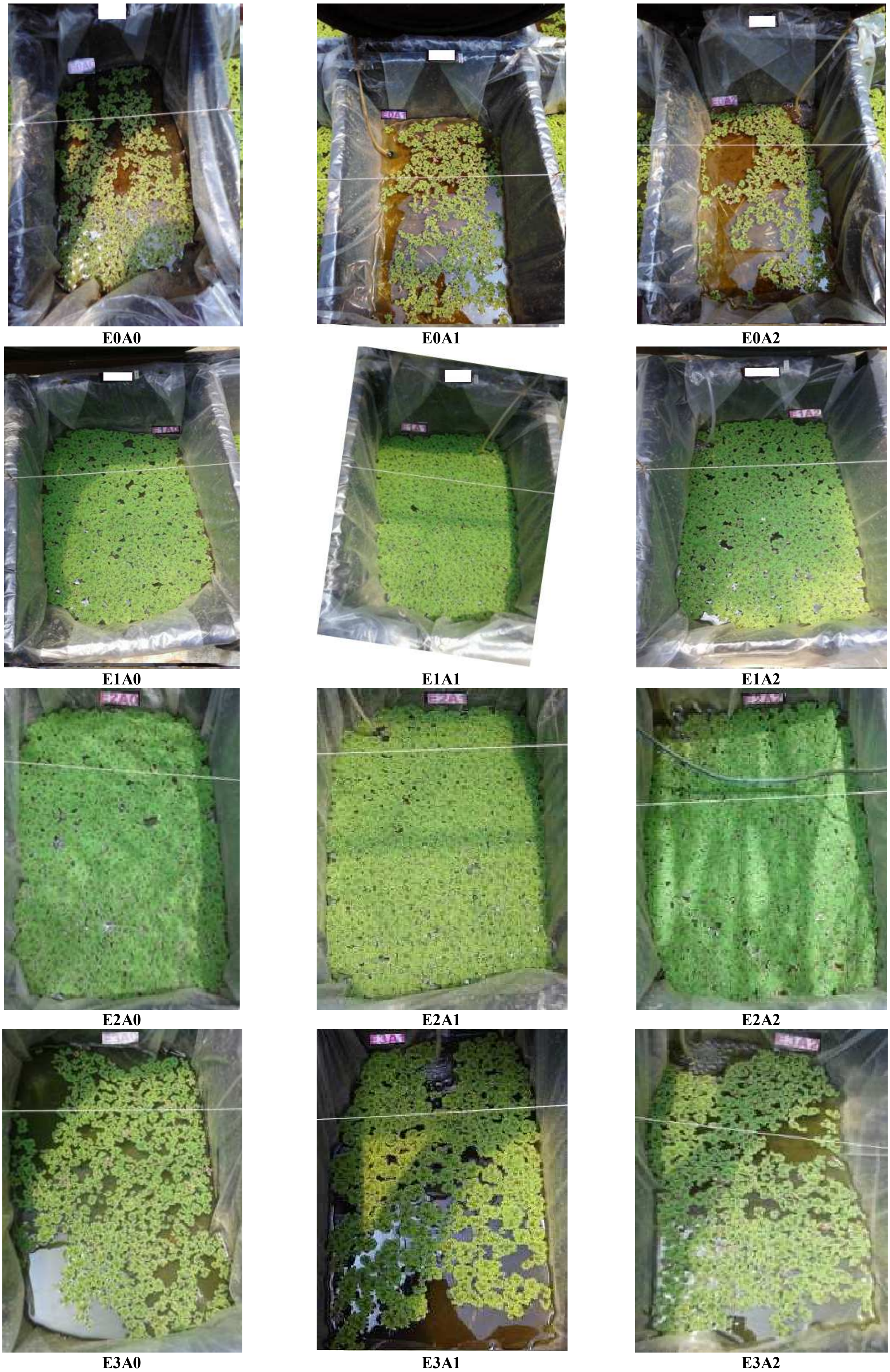


Fig. 7 *Azolla* population from different treatments at day 12.

TABLE I

EFFECT OF WATER REPLACING PERIOD ON BIOMASS FRESH WEIGHT (FW), RELATIVE GROWTH RATE (RGR), AND DOUBLING TIME (DT) OF AZOLLA

| Parameters | Treatments of Water Replacing Periods | Day of Measurements 3 | 6 | 9 | 12 |
|---|---|---|---|---|---|
| *FW (g)* | E1 | 171.2 (a) | 341.4 (a) | 484.4 (b) | 782.7 (b) |
| | E2 | 178.5 (a) | 319.3 (b) | 496.3 (a) | 804.5 (a) |
| | E3 | 175.6 (a) | 319.3 (b) | 229.4 (c) | 292.6 (c) |
| | E12=E0 | 174.1 (a) | 308.9 (b) | 232.6 (c) | 188.9 (d) |
| *RGR* ($g.g^{-1}.d^{-1}$) | E1 | 0.083 (a) | 0.157 (a) | 0.143 (b) | 0.148 (b) |
| | E2 | 0.097 (a) | 0.149 (b) | 0.146 (a) | 0.150 (a) |
| | E3 | 0.092 (a) | 0.148 (b) | 0.060 (c) | 0.065 (c) |
| | E12=E0 | 0.089 (a) | 0.143 (b) | 0.062 (c) | 0.029 (d) |
| *DT (d)* | E1 | 8.48 (a) | 4.43 (b) | 4.84 (b) | 4.70 (c) |
| | E2 | 7.22 (a) | 4.66 (a) | 4.75 (c) | 4.63 (d) |
| | E3 | 7.59 (a) | 4.70 (a) | 11.56 (a) | 10.60 (b) |
| | E12=E0 | 7.95 (a) | 4.84 (a) | 11.34 (a) | 24.82 (a) |

Note: values followed by common letters at the same column indicates no difference at 1% level (P<0.01)

TABLE II

COMPARISON OF RELATIVE GROWTH RATE (RGR) AND DOUBLING TIME (DT) OF AZOLLA

| ***RGR*** **($g.g^{-1}.d^{-1}$)** | ***DT*** **(d)** | **Culture condition** | **References** |
|---|---|---|---|
| 0.148 | 4.70 | Catfish wastewater pond, water was replaced once in two days. Cultivation in plastic house for 12 days. | This work |
| 0.150 | 4.63 | Catfish wastewater pond, water was replaced once in four days. The max RGR is observed at day 6th. The DT is calculated during 12-day cultivation in plastic house. | This work |
| 0.130 | 5.4 | Cultured in polyhouse for 14 days at 30±2°C | [39] |
| 0.162-0.214 | 3.24-4.28 | Nitrogen fertilizer application of ammonium sulphate and Urea each corresponds to 40 kg N/ha. RGR is highest without fertilizer and lowest with Urea. DT was calculated by Eq. (2). | [42] |
| 0.231-0.252 | 2.75-3.00 | Four isolates of *A. microphylla* were dual cultured with rice at 10 days after rice transplanting with a rate of 500 kg/ha. The RGR was estimated from chart at day 20, and DT was calculated by Eq. (2). | [43] |
| 0.129-0.153 | 4.52-6.58 | Inoculation rate 50 to 200 g/m$^2$. RGR is highest at 50 g/m$^2$ inoculation rate with DT 4.52. DT is not statistically different at that inoculation rate range. | [44] |
| 0.042 | 16.43 | *Azolla pinnata* cultivated with different types of water in zippered PE plastic bag for four weeks. The values are calculated from data observed at week two for Azolla cultivated in distilled water. | [45] |
| 0.173 | 4.00 | Ten grams *A. pinnata* were cultivated in a greenhouse using plastic pots filled with 1 kg soil and 3 liters tap water for 25 days. The values are calculated from data observed at day 15. | [46] |

Treatments of 2- and 4-day water replacing periods (E1 and E2) showed an excellent effect on the growth of *A. microphylla* with a consistent growing till the harvest time at the 12th day. From 20 g (133.33 g/m$^2$) biomass initially put on the culture, *A. microphylla* was growing up to 117.45 g (782.96 g/m$^2$ or 7.83 t/ha) when the water was replaced once in 2 days, and to 120.67 g (804.45 g/m$^2$ or 8.04 t/ha) when the water was replaced once in 4 days. This yield is comparable to *A. pinnata* and *A. carolinina biomass*, about 9.7 t/ha, which is planted as dual crop along with rice paddy and fertilizer application of 20 kg/ha [47]. Azolla nourished nutrients, primarily nitrogen from the water, in the forms of ammonium and nitrate. When nitrogen concentration was sufficient, *A. microphylla* could grow normally. Ammonium concentrations were around 16.51 mg/L and 16.25 mg/L for the water replacement of 2- and 4-day. When the water was replaced at any longer periods, such as 6-day, the nitrogen concentration depleted, and the growth of *A. microphylla* was hampered, or even some parts of biomass decreased because of death. With longer water replacement (6- and 12-day), *Azolla*'s life was started to be suppressed after the 6th day and did not develop anymore (Figure 7).

Table 1 shows the relative growth rate (*RGR*) of Azolla biomass at 3-daily observation intervals. In the first 3-day interval, it was seen that the difference in growth rate due to the influence of the water replacement period was not significant at a value of around 0.083-0.097 $g.g^{-1}.d^{-1}$. The condition of the water medium is still the same except for the box with a water replacement period of once in 2 days. In addition, Azolla seeds may still be in the adjustment period so that the E1 box where water has been replaced the day before still has not had a significant effect. On the observation of day

6th, the growth increased to around 0.191-0.199 $g.g^{-1}.d^{-1}$ and did not differ for the 4, 6 and 12-day water replacement periods. The difference occurred at the water replacement of 4-day with *RGR* 0.230 $g.g^{-1}.d^{-1}$. At the observation of the 9th day, the effect of the water replacement period began to look very prominent where the box with longer water replacement periods (6- and 12-day) experienced negative growth, while the box with the water replacement period 2- and 4-day showed a good growth rate until the day to 12 (the last day of Azolla cultivation). Nordiah *et al.* [45] also report a similar Azolla growth pattern with a high rate in the first week (9.37 ± 1.95%), and then decrease. Our result on the Azolla growth rate is comparable to that reported in the references. For example, Kösesakal and Yıldız [48] reveal that *A. pinnata* has a growth rate of 0.148 $g.g^{-1}.d^{-1}$ and 0.120 $g.g^{-1}.d^{-1}$ for *A. caroliniana*. In addition, [42] reports a higher growth rate of 0.162 to 0.214 $g.g^{-1}.d^{-1}$ with the highest value for Azolla cultivated without fertilizer addition. Table 2 compared our result on the RGR and DT of Azolla with the values found in published works.

## IV. Conclusion

Based on the above discussions, one important conclusion is that *A. microphylla* can be cultured using catfish pond wastewater. Interaction of the water replacing periods and the mechanical aeration significantly affects water quality (temperature, pH, turbidity, ammonium), but is not significant for the yield of *A. microphylla* biomass. The factor of water replacing period alone significantly affected the growth of A. *microphylla*. The optimum growth of *A. microphylla* was found when the wastewater was replaced once in 4 days (E2 treatment) with relative growth rate of 0.150 $g.g^{-1}.d^{-1}$, biomass yield of 804 $g/m^2$, and doubling time 4.63 day within a 12-day of cultivation. This option would maintain fishpond water with fairly good quality (pH 8.12, turbidity 35 NTU, and ammonium 16.25 mg/L). Mechanical aeration significantly affected some water quality parameters (temperature, turbidity and pH), but did not significantly affect the *A. microphylla* growth.

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