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AGUS HARYANTO <agus.haryanto@fp.unila.ac.id>

[IJASEIT] Submission Acknowledgement

1 message

IJASEIT <ijaseit@gmail.com> Mon, Jul 27, 2020 at 11:35 AM
To: Mr Agus Haryanto <agus.haryanto@fp.unila.ac.id>

Mr Agus Haryanto:

Thank you for submitting the manuscript, "Utilization of Wastewater from Catfish Pond to Culture Azolla microphylla" to International Journal on Advanced Science, Engineering and Information Technology. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Manuscript URL:
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Username: agusharyid65

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

IJASEIT
International Journal on Advanced Science, Engineering and Information Technology

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[IJASEIT] Revision Required

2 messages

Rahmat Hidayat <rahmat@insightsociety.org> Thu, Feb 25, 2021 at 8:49 AM
To: Mr Agus Haryanto <agus.haryanto@fp.unila.ac.id>

Mr Agus Haryanto:

We have reached a decision regarding your submission to International Journal on Advanced Science, Engineering and Information Technology, "Utilization of Wastewater from Catfish Pond to Culture Azolla microphylla".

Our decision is to: Revision Required

Please update your abstract into 220-250 words and your reference 70% in (2017-2020) from journal indexed by Scopus. Citation and Reference in Paper must using Mendeley with IEEE Style.

Please submit your revision in 10 days. More than 10 days of paper will be rejected from the system. Re-upload your revision into journal system NOT via email.

Editor

Reviewer A:
This paper already has novelty and good contributions according to research objectives. Abstract is good and already describes the objectives, methods and research results well. In the introductory section, updated research has been seen related to the description of relevant research. However, the Introduction is still not effective, it is enough only 4-5 paragraphs containing background, gap analysis and research updates and research objectives. The method is good, but still needs to be improved by adding a flow chart so that the stages of the research are seen properly and systematically. Making tables is not good, correct them according to the 2021 template. The conclusions are good and in accordance with the objectives of the research you are doing.

International Journal on Advanced Science, Engineering and Information

Technology
http://insightsociety.org/ijaseit/index.php/ijaseit

AGUS HARYANTO <agus.haryanto@fp.unila.ac.id> Thu, Feb 25, 2021 at 5:24 PM
Reply-To: agus.haryanto@fp.unila.ac.id
To: SUGENG TRIYONO <sugeng.triyono@fp.unila.ac.id>, striyono2001 <striyono2001@yahoo.com>

Kabar baik pak. Revisinya lumayan.
Besok Jumat ya kita ketemu.
😋😋
AH
[Quoted text hidden]

Gmail AGUS HARYANTO <agus.haryanto@fp.unila.ac.id>

Revision has been uploaded

2 messages

AGUS HARYANTO <agus.haryanto@fp.unila.ac.id> Mon, May 31, 2021 at 7:05 AM
Reply-To: agus.haryanto@fp.unila.ac.id
To: Rahmat Hidayat <rahmat@insightsociety.org>

Dear Mr. Rahmat Hidayat,

First of all, thank you very so much for your valuable comments.
Just to let you know that I have revised the paper according to your comments. The abstract has been improved to 245 words. And the references have been updated to include 75% recent scopus indexed sources published within 2017-2021. The revised version has just been re-uploaded via the OJS system on 31 May 2021.
I hope this revision fulfills the requirements of IJASEIT.
Again, thank you very much.

Best regards,
Agus Haryanto,
Agr. Eng. Dept.
University of Lampung

Mail Delivery System <Mailer-Daemon@legion.in-hell.com> Mon, May 31, 2021 at 7:06 AM
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To: Rahmat Hidayat <rahmat@insightsociety.org>

Cc:
Bcc:
Date: Mon, 31 May 2021 07:05:48 +0700
Subject: Revision has been uploaded
Dear Mr. Rahmat Hidayat,

First of all, thank you very so much for your valuable comments.
Just to let you know that I have revised the paper according to your comments. The abstract has been improved to 245 words. And the references have been updated to include 75% recent scopus indexed sources published within 2017-2021. The revised version has just been re-uploaded via the OJS system on 31 May 2021.
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Best regards,
Agus Haryanto,
Agr. Eng. Dept.
University of Lampung

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AGUS HARYANTO <agus.haryanto@fp.unila.ac.id>

JOURNAL PROCESSING FEE - 12695

4 messages

Rahmat Hidayat <mr.rahmat@gmail.com>
Reply-To: mr.rahmat@gmail.com
To: Mr Agus Haryanto <agus.haryanto@fp.unila.ac.id>

Fri, Apr 30, 2021 at 11:46 AM

Dear Authors,

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We are happy to inform you that since Volume 5 (2015) *International Journal on Advanced Science, Engineering, Information and Technology* (IJASEIT) has been indexed in **Scopus**. The Scientific committees of IJASEIT agree that your manuscript is **accepted** to be published in IJASEIT.

Tittle	Utilization of Wastewater from Catfish Pond to Culture Azolla microphylla
Authors	Sugeng Triyono, Aprian Mandala Putra, Muhammad Amin, Agus Haryanto

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Best Regards

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Rahmat Hidayat

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AGUS HARYANTO <agus.haryanto@fp.unila.ac.id> Sat, May 1, 2021 at 3:17 PM
Reply-To: agus.haryanto@fp.unila.ac.id
To: mr.rahmat@gmail.com

Dear Mr. Rahmat,

Thank you for this information. We are really happy.
Just to let you know that we have completed the payment on April 7, 2021.
Please find attached receipt of payment.

Thank you.

Regards
Agus Haryanto

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Rahmat Hidayat <mr.rahmat@gmail.com> Sat, May 1, 2021 at 4:56 PM
Reply-To: mr.rahmat@gmail.com
To: Mr Agus Haryanto <agus.haryanto@fp.unila.ac.id>

Well received with thanks.
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AGUS HARYANTO <agus.haryanto@fp.unila.ac.id> Fri, Apr 8, 2022 at 2:40 PM
Reply-To: agus.haryanto@fp.unila.ac.id
To: striyono2001 <striyono2001@yahoo.com>

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REAPONSES TO THE REVIEWER COMMENTS:

EDITOR Comments:

Comments	Action
Please update your abstract into 220-250 words	Abstract has been updated with 248 words
Reference 70% in (2017-2020) from journal indexed by Scopus.	References have been updated with most are Scopus indexed and from 2017-2020
Citation and Reference in Paper must using Mendeley with IEEE Style.	Citation has been updated following IEEE style.

REVIEWER A Comments:

Comments	Action
This paper already has novelty and good contributions according to research objectives. Abstract is good and already describes the objectives, methods and research results well.	Thank you for the very motivating comments
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The method is good, but still needs to be improved by adding a flow chart so that the stages of the research are seen properly and systematically.	Flow chart is added
Making tables is not good, correct them according to the 2021 template.	Table has been recreated according to the 2021 template
The conclusions are good and in accordance with the objectives of the research you are doing.	Thank you for the very motivating comments

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

Sugeng Triyono[1], Aprian Mandala Putra[1], Mohammad Amin[1], Agus Haryanto[1,#]

[1]*Agricultural Engineering Department, Faculty of Agriculture, The University of Lampung. Soemantri Brojonegoro Rd #1, Bandar Lampung, 35145 Indonesia.*
E-mail: striyono2001@yahoo.com
E-mail: striyono2001@yahoo.com

**Corresponding author: Agus Haryanto*
E-mail: agus.haryanto@fp.unila.ac.id

***Abstract*— In order to maintain pond water quality, large amount of wastewater is flashed which pollutes environment and its odor annoys nearby residents. This wastewater is potential for growing Azolla microphylla, a valuable floating fern for feed. The objective of this research is to observe the effect of water replacing period and mechanical aeration on the growth of A. microphylla and quality of the wastewater. 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 was applied for a 12 day experiment. The experimental used a completely randomized design with two factorial arrangement. First factor was 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). 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 production of Azolla biomass and water quality (temperature, pH, turbidity, and ammonium). 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 found to be the most promising option, with biomass yield of 804 g/m^2 within a 12-day of cultivation.**

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

Introduction

Catfish is an important product of fresh water aquaculture in some areas of Indonesia. The fish provides people a source of protein with fairly low price. Catfish can be easily cultured even in a small pond with a high density fish [1] that a lot of catfish ponds are frequently developed in some locations closed to residential areas. Unfortunately, its odorous pond water often annoys nearby residents. It is because catfish eats a lot, but only 25.5% of organic matter, 26.8% of nitrogen and 30.1% of phosphorus are converted into biomass while most portion of the feed is excreted as waste in pond water [2]. When pond water needs to be flushed and replaced with fresh water, discharging of the pond wastewater certainly pollutes environment [3]. Environmental and socioeconomic problems of Indonesian aquaculture are foreseen more complicated in the future if no proper technology is applied [7]. Some aquatic plant systems such as water hyacinth [8], duckweed, and Azolla [9], [10] are reported to treat wastewaters effectively.

Utilization of Azolla for reclaiming catfish pond wastewater may offer a promising solution to the problem of the catfish pond wastewater management. Azolla is a free floating fern which can be grouped into two sub-genera, Euazolla and Rhizosperma [11]. Euazolla is characterized by the presence of three megaspore floats and consists of *A. caroliniana*, *A. filiculoides*, *A. mexicana*, *A. microphylla* and *A. rubra*. Rhizosperma consists of *A. pinnata* and *A. nilotica* possessing floats in the megaspore apparatus [12]. Azolla contains crude protein of 25.78% and ash of 5.76% [13]. Azolla has been cultured for some different purposes such as biofertilizer [12], [14]–[17], fish meals [18]–[20], feed for chicken [13], [21], [22], cattle feed [23], goat feed [24], [25], pig feed [26], biogas substrate [27], [28], biofuel feedstock [10], [29]–[32], wastewater remediation [33]–[38], CO_2 sequestration [39], and CH_4 emission reduction in paddy field [40]. Recently, Azolla is also used as biofilter for recirculating aquaculture system [41], [42].

Pond wastewater contains nutrients especially nitrogen and phosphorous derived from leftover feed, feces, and urine of fish. At least two advantages can be gained if catfish pond wastewater is used for growing *Azolla*. First, Azolla functions as phytoremediation that can improve pond water quality to better levels, hence preventing environmental pollution, reduced flashing water, and improving fish health if the reclaimed wastewater has to be recycled to the fish ponds. Second, biomass of Azolla can be utilized for animal or fish feeds, thus reducing cost of feeds [43]. 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 improved water quality parameters, lowering pH and ammonia volatilization in rice field, hence increasing nitrogen recovery [44]. Other research on tilapia-Azolla aquaculture system also showed that nitrate, ammonium, phosphate decreased [45]. Unfortunately, dissolved oxygen also dropped to an anoxic level (less than 2 mg/L) which could hamper the fish health [46]. The drop of dissolved oxygen (DO) is caused by photosynthetic micro-phytoplankton could not compete for light with Azolla which covers the pond surfaces, and died. Further, in that research, the weight gain of tilapia decreased and it could be associated with the low DO effect, because of adverse effect of the Azolla cover. It was found that optimum surface cover (based on the fish yield) by Azolla was 30% of the total pond surface [45]. 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 be probably 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 stress or even die, if loading rate was too high because catfish pond wastewater contains high concentrations particularly for ammonia. In contrast, Azolla will grow under suboptimal if the nutrient is not enough because of too low loading rate. In order to determine proper hydraulic loading rate, this research was conducted. This research aims to investigate 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. microphylla* and catfish pond wastewater quality improvement. Effect of mechanical aeration is also evaluated in that dissolved oxygen underneath of Azolla mat has been known very low [45].

I. Material and Method

A. Preparation

Thirty six plastic-lined wooden boxes each sizing $50 \times 30 cm^2$ and 20cm depth were prepared and placed in a plastic house, as presented in Figure 1. Catfish pond wastewater taken from a nearby catfish growing pond whose fingerling size of fish (about 2-month old) was use as it was. Every box was filled with 15 L (depth of around 10cm) of catfish pond wastewater. Twenty gram (equivalent to 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.

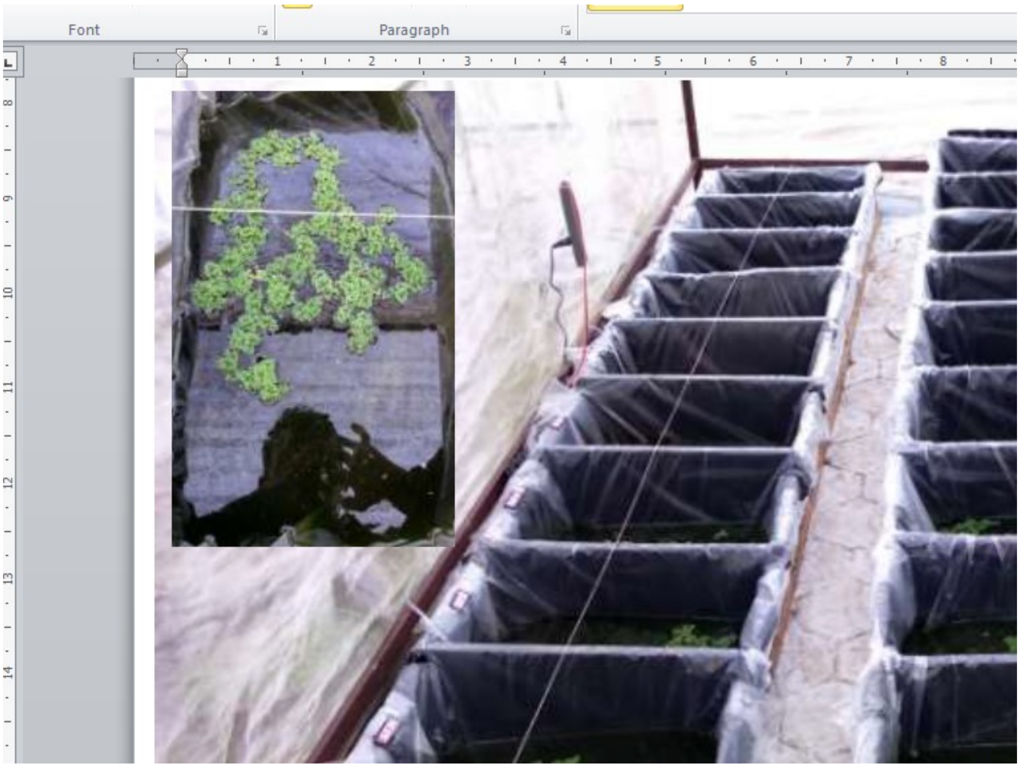

Figure 1. Experiment lay out of Azolla cultivation with 20 grams seed for each box

B. Treatment and Experimental Design

The experiment was conducted using Completely Randomized Design with two factorial arrangement (CRD two-factor). Each box mentioned above was used as the experimental unit. All treatments combined 2 factors, namelly 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 equivalent to once 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 corresponding treatment of time period. The second factor consisted of 3 levels, namely no aeration (A0), 12-hour aeration (A1), and 24-hour aeration (A2). Mechanical aeration was carried out by using small air diffusers. Each treatment combination was conducted in triplicates, totaling of 36 experimental units.

C. *Measurement and Data Analysis*

Measurement was conducted on water quality and Azolla growth. Parameters corresponding to water quality involved water temperature, pH, turbidity, and ammonium. The first three parameters were measured daily at 7.00 a.m using thermometer, pH meter and turbidity meter, respectively. 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 as well 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 [47], [48].

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

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

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 by using Analysis of Variance (ANOVA), and then followed by using Least Significant Difference (LSD) multiple comparisons.

II. Results and Discussion

A. *Water Temperature*

The average daily water temperature ranged from 26.0 to 30.0ºC with average of 28.18ºC. The water temperature was little low because the data set was collected in the morning. Before and just after noon, the water temperature was increasing to about 33ºC which is normal for tropical locations. Though the interaction between water replacing period and mechanical aeration significantly influenced daily average temperature, the differnces were too small. Within this range of temperature, growth of *A. microphylla* should not be adversely affected by the existing water temperature, because it is tolerant with tropical temperature [49].

Effect of interaction between water replacing period and mechanical aeration duration on the water temperature at day 12th was significant at 5% level (Figure 2). Figure 2 suggests that in general more aeration duration has resulted in the decreasing water temperature. With 24 hour aeration, 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 water. Figure 2 suggested that for more frequent water replacement or shorter water replacement period (2 and 4 days), aeration may not be needed because water temperatures were not significantly different. But for less frequent or longer water replacement (12 day), 24 hour aeration was needed because water temperature tended to increase.

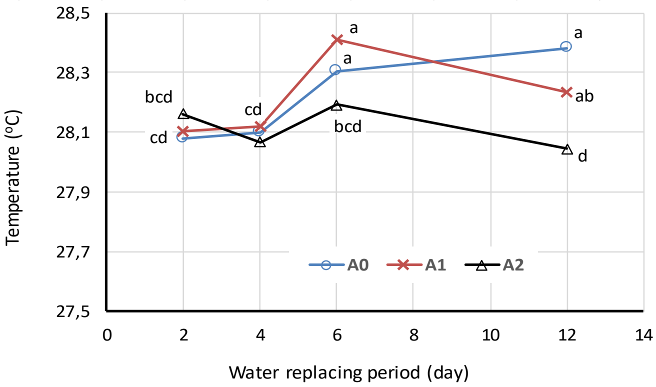

Fig. 2 Effect of water replacing period and aeration duration on water temperature at day 12th. (Values followed by common letters indicate no difference at 5%).

B. *Water pH*

Water acidity or pH was monitored daily at the same time as the water temperature measurement. Initially, wastewater from catfish pond has almost neutral pH, namely 7.5. All the experimental units showed the water pH increased so that become 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 (Hasan and Chakrabarti, 2009). The daily pH values were fairly stable, though little fluctuations were still visible.

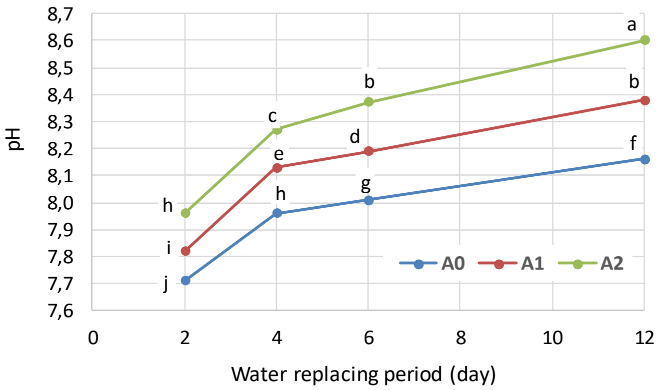

Fig. 3 Effect of water replacing period and aeration duration on the average pH. (Values followed by common letters are not statistically different at 5%).

Effect of interaction between water replacing period and mechanical aeration duration on the average pH value was significant at 5% level. Figure 3 indicated that extending water replacing period increased water pH, and the same situation happened for aeration duration. But 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).

C. Water Turbidity

Turbidity is caused by soluble organic solids in pond wastewater. Turbidity of fresh pond wastewater was 178 NTU on the 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 period of 4 days (E2), and further deceased to around 20 NTU with longer replacing periods. At every end of 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 on Figure 4. Effect of interaction between water replacing period and mechanical aeration duration on wastewater turbidity was significant at 1% level. Figure 4 also suggested that extending water replacement period from 2 to 4 days dropped water turbidity, and level off (21 NTU) at 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 2 day water replacement period, 12 hour aeration was not needed because turbidity was not significantly different from that with no aeration. Likewise for 4 day water replacement period, 24 hour aeration was not needed because turbidity was not significantly different from that with 12 hour aeration. Overall, 6 and 12 day water replacement periods may be not needed 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 period with aeration, or less frequent water replacement will be better methods if Azolla culture system was incorporated with aquaculture [50].

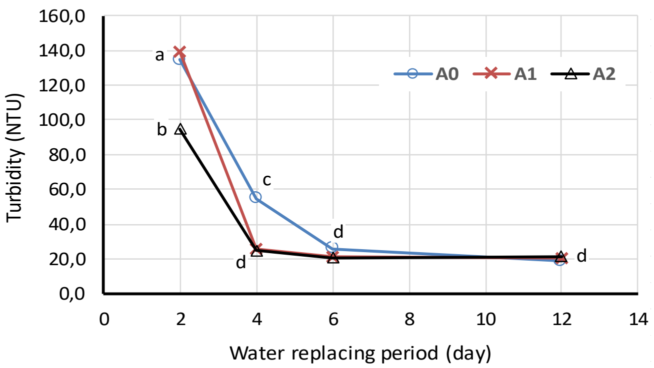

Fig. 4 Effect of water replacing period and aeration duration on pond wastewater turbidity. (Values followed by common letters mean no difference at 5% level)

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 analyzes, effects of 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 so effective. But extending 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 the average (Figure 5). 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 to determine optimal ammonium concentration which is enough for *A. microphylla* growth, yet not odorous.

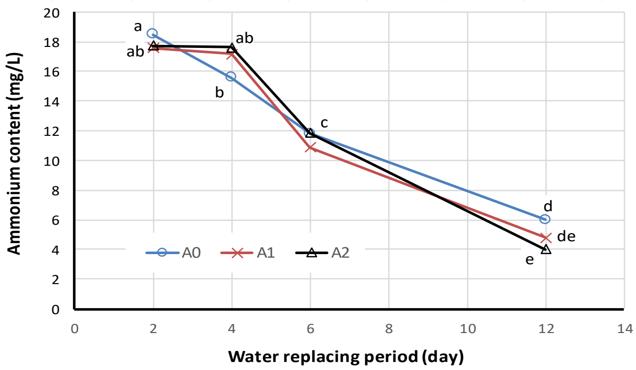

Fig. 5 Effect of water replacing period and aeration duration on ammonium level at day 12th. (Values followed by common letters mean no difference at 5% level)

E. Azolla Biomass

Figure 6 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 higher density as compared to those with longer water replacement periods (6- and 12-day). At the harvest time, treatment of water replacing period significantly affected the A. microphylla biomass yield at level of 5%, while mechanical aeration did not (Table 1).

Table 1 presents biomass fresh weight (*FW*) along with relative growth rate (RGR) and dobling time (DT) of *A. microphylla* during 12 days culture observed every 3-day. 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 12^{th} 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 the production of *A. pinnata* and *A. carolinina* biomass, about 9.7 t/ha, which is planted as dual crop alongwith rice paddy and with fertilizer application of 20

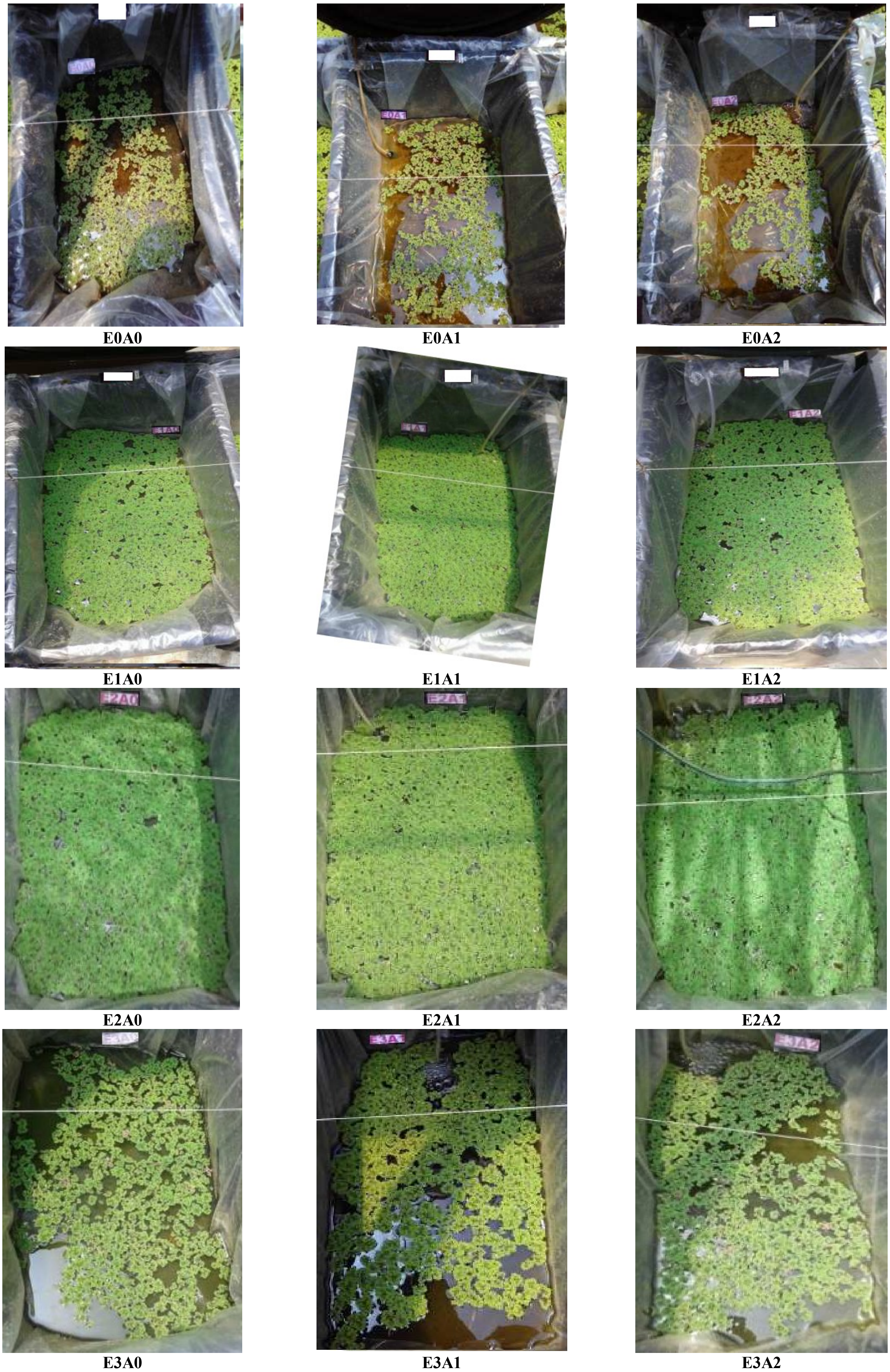

Fig. 4 Azolla population from different treatments at day 12.

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

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

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

TABLE III
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 day.	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	[47]
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).	[51]
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).	[52]
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.	[17]
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.	[53]
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.	[54]

kg/ha [55]. Azolla nourished nutrients primarily nitrogen from 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 replacemen of 2- and 4-day. When the water was replaced at any longer periods of time, 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 replacemen (6- and 12-day), Azolla's life was started to be suppressed after the 6th day, and did not develop anymore (Figure 6).

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 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 at 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. (2012) also reported a similar patern of Azolla growth with high rate in the first week (9.37 ± 1.95%), and then decrease. According to reference [48] Azolla can grow rapidly with RGR up to 0.350 $g.g^{-1}.d^{-1}$. Whereas, reference [51] reported a 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 published works.

III. CONCLUSIONS

Based on the above discussions, one important conclusion is that *A. microphylla* can be cultured by 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 fish pond 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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