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Phytoremediation of Eel (*Anguilla bicolor bicolor*) rearing wastewater using amazon sword (*Echinodorus amazonicus*) and water jasmine (*Echinodorus palaefolius*)

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ABSTRACT

Management of aquaculture wastewater is still the major problems in fisheries, especially in intensive systems. Intensively aquaculture activities often increase organic matter and nutrients (N and P) in the water. The study was conducted to evaluate the phytoremediator performance of *Echinodorus amazonicus* and *Echinodorus palaefolius* in removing inorganic nitrogen and orthophosphate from aquaculture wastewater with the recirculation system. This study used a completely randomized design with three treatments and 3 replications. The treatments of this study were P1: *E. amazonicus*, P2: *E. palaefolius* and P3: control (without aquatic plants). The tested fish were elvers which average weight 6.98 ± 0.19 g, and fish density was 4 gL^{-1}. Elvers reared in an aquarium that containing 48 L in a recirculation system. The paste feed which protein level of 45.25% was given at satiation 3 times/day. Elvers reared for 60 days. The results showed that *E. palaefolius* significantly reduced concentrations of total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3) and orthophosphate (PO_4) with an efficiency of 27.10 ± 2.42%; 45.03 ± 9.77%; 20.94 ± 1.29% and 14.19 ± 3.05%, respectively higher than *E. amazonicus* and control. The best result of elver's performance (SGR and FCR) was in treatment P2 (*E. palaefolius*), i.e. 1.19 ± 0.18% and 1.57 ± 0.30. Based on the results of this study, it can be concluded that the use of *E. palaefolius* as phytoremediator in eel culture with recirculation system can removed inorganic nitrogen and orthophosphate more optimal compared to other treatment.

Keywords: *aquaculture wastewater, nutrient removal, phytoremediation, Echinodorus amazonicus, Echinodorus palaefolius*

1. Introduction

Increasing freshwater fish production could be achieved through intensificationor increase in fish density per unit area of culture tank. High stocking density in intensive fish culture leads to the high quantity of feed given to fish thus results in accumulation of organic matter in fish tank. Accumulation of organic matter causes the formation of toxic compounds for fish, nutrient mineralization from organic matter and high absorption of oxygen, which further decreases the water quality and creates the negative impact on fish health and production. Waste accumulation may lead to declining water quality that affects the physiological process, behavior, growth, and mortality of fish (Bureau and Hua 2011; Davidson *et al*. 2013; Davidson *et al*. 2016). Similarly,eel culture production continues to be further increased through intensification to meet the demand of the consumer.

Conventional water quality management can be done through periodic water exchange, yet it is less effective, costly, and requires quite huge amount of water. One of themanagement efforts to improve water quality and optimize the use of fish culture water can be done by performing phytoremediation technology through recirculation. Wastewater from fish

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culture that contains organic matter will decompose into inorganic materials that can be used by plants as nutrient for growth (Effendi *et al*. 2015). The principle of the recirculating system is the reuse of water that has been removed from aquaculture activity. Recirculating system provides benefits since it is able to minimize water use, function as pH buffer, and reduce organic matters like ammonia and nitrite (Suzuki *et al*. 2003).

The result of study performed by Brune *et al*. (2003) showed that about 25% of total nitrogen in feed given to fish is used for growth, while 60% of it is released in the form of $NH_3$and 15% of it is released in the form of feces. Therefore, the potential of ammonia waste enters fish culture water amounted to 75% of feed nitrogen. Approximately 70-80% of nitrogen in nitrogen in transformed into ammonia, both through direct excretion and mineralization by bacteria. Around 33% of nitrogen contains in fish diet will be excreted by fish and can be recycled (Crab *et al*. 2007).

Phytoremediation is the use of plants to decrease, extract, or remove organic and inorganic compounds from waste. The advantage of phytoremediation compared to other waste treatment technology includes the natural process, lower cost, organic matter reduction, the synergy between plants, cultured fish, and environment, and does not require high technology (Paz-Alberto and Sigua, 2013). Several previous studies had performed phytoremediation on waste from freshwater fish culture using aquatic plants such as water hyacinth (Syahputra 2005), *Pistia stratiotes* (Herniwati *et al.* 2013, Madhurina *et al.* 2014), *Hydrilla verticilata* (Rahman *et al.* 2011) and *Lemna perpusilla* (Mkandawire *et al*. 2007, Crishmada & Mardiyati 2011). Application of system which combines recirculation and phytoremediator (plant with the ability to assimilate N and P nutrient produced from biofilter) results inless water use and water quality that remains good. Yet, phytoremediator commonly used is still vegetable plant like water spinach, spinach, and tomato (Ebeling *et al*, 2006). This phytoremediator is technically useful but economically provides relatively less benefit due to the low price of vegetable product. Hence, it is necessary to find other phytoremediators, which is able to improve the water quality of culture media besides having quite high economic value, such as freshwater aquarium plant.

2. Materilas and Methods

Tank preparation

The tanks used in this study were 18 rectangular aquaria at a size of 60x40x40 cm. the recirculating system was designed with the water height of 25 cm. Considering the aquatic plants, double bottom technic was applied at the bottom of aquarium with bottom layer consisted of a series of: PVC pipe as cantilever, fine gauze, dacron as filter, and sand as planting media for aquatic plant. In this research, no water exchange was applied, yet the addition of water was done to maintain the height of water level in aquarium which continued to decrease due to evaporation.

Experimental fish and plant

Fishused in this study were eels (*Anguilla bicolor bicolor*) with an average weight of 6.98±0.19 $gfish^{-1}$. Eels were obtained from eel farmer in Tulungagung. Prior to treatment, eels were adapted in fiber tank at a size of 100x100x100 cm for 30 days. The stocking density of elver was 4 gL^{-1} (Harianto et al, 2017). Eels were maintained for 60 days. During culture, eels were fed paste feed with dietary protein (45.15%) (Handajani *et al*, 2018) to at satiation. Feeding frequency was three (3) timesa day, namely in the morning (07.00), at noon (13.00) and in the afternoon (19.00) (Affandi*et al* 2013). Aquatic plant density in each aquarium was 80 gram (Akinbile & Yusoff, 2012). Freshwater aquarium plantsused as phytoremediators were *Echinodorus amazonicus* and*Echinodorus palaefolius*. These plants were collected from freshwater aquarium plants in the region of Batu-Malang. Before treatment, the plants were adapted in fiber tank of 150x50x50 cm in a flow-through system for 14 days.

Experimental design and data analysis

The research was done by experimental method in the laboratory. The experimental design used completely randomized design (CRD). This research consisted of three (3) treatments, namely P1: *Echinodorus amazonicus,* P2: *Echinodorus palaefolius,* and P3: without aquatic plants. Three replications were applied in each treatment. To determine the effect of treatment on each parameter tested, ANOVA and F-test were performed using the program of SPSS ver.21 at confidence level of 95%, and further followed by Tukey Multiple Range test.

Data collection

Before treatment, the measurement of water quality included the parameter of:temperature, pH, DO, alkalinity, TSS, TAN nitrate (NO_3), nitrite (NO_2), and orthophosphat (PO_4).Water quality measurement was done for 60 days (2 months) after aquatic plants were put in the system. The parameter of water quality that were measured daily consisted of NO_3, NO_2, PO_4, and TSS were measured twice a week (14 days) (Muangkeow *et al*, 2007). Measurement of TAN, nitrite, nitrate, and orthophosphate was conducted using spectrophotometer which referred to the method of APHA (2006). Water quality was measured in the laboratory of water quality and proximate analysis was performed in the laboratory of fish nutrition University of Muhammadiyah Malang.

To investigate the growth performance of eel, calculation of survival rate, SGR, feed conversion, protein retention and lipid retention was performed.

The calculation of nutrient removal was done every two weeks, include TAN, NO2, NO3, PO4 (Endut et al 2011). Measurement of aquatic plant parameter was wet biomass weight, at the beginning and the end of the research.

Nutrient removal

During the experiment, nutrient removal within the water occurred. The amount or quantity of nutrient removal can be calculated using the formula as follows (Zhou *et al*. 2006):

$$\text{Removal efficiency (\%)} = \frac{(Na - Nb)}{Ca} \times 100$$

Description:

Na = nutrient concentration in influent(mgL^{-1})

Nb = nutrient concentration in effluent (mgL^{-1})

3. Results and Discussion

Water quality and Nutrient removal

Results of water quality measurement during culture period (60 days) which included temperature, pH, dissolved oxygen, alkalinity, TSS, TAN, NO_2, NO_3 and PO_4 are presented in **Table1**.

Table 1. Water quality during culture period (60 days)

Parameter	P1 *(E. amazonicus)*	P2 *(E. palaefolius)*	P3 (without plant)
Temperature (^0C)	28.5 – 30.0	29.0 – 30.5	28.5 – 29.5
pH	7.1 – 7.5	7.2 – 7.5	7.0 – 7.8
DO ($mg\ L^{-1}$)	4.5 – 5.9	4.5 – 6.1	4.3 – 5.8
Alkalinity ($mg\ L^{-1}$)	46 – 105	58 – 104	60 – 100
TSS ($mg\ L^{-1}$)	1.44 – 2.54	1.2 – 1.98	2.18 – 3.98
TAN ($mg\ L^{-1}$)	0.12 – 0.23	0.12 – 0.22	0.13 – 0.23
NO_2 ($mg\ L^{-1}$)	0.011 – 0.073	0.013 – 0.067	0.013 – 0.089
NO_3 ($mg\ L^{-1}$)	15.27 – 49.15	13.31 – 48.29	26.71 – 53.14
PO_4 ($mg\ L^{-1}$)	1.13 – 7.72	1.07 – 5.68	1.39 – 8.79

Culture of eel using phytoremediation technology is strongly influenced by several environmental factors such as temperature, pH, dissolved oxygen, alkalinity and TSS. The temperature value was in a constant state during culture period.

The average temperature in this study was still within the good condition for eel growth (28.5–30.0^{0}C). According to KKP (2011), the optimal temperature range for eel is 27-31^{0}C, which was confirmed by the study result of Luo *et al* (2013) that eel was able to grow properlyat temperature of 28-33^{0}C. The value of pH during observation period was between the range of 7.1 – 7.8, this value was still within the optimum range for eel growth, namely 6.0 – 8.0 (Tseng & Wu, 2004), 7.0 – 8.0 (KKP, 2011).

Dissolved oxygen during the research ranged of 4.3-6.1 mgL^{-1}, this value was within the tolerance range for eel growth, that is > 4 mgL^{-1} (KKP, 2011), while according to Herianti (2005), dissolved oxygen for eel growth amounted to > 3 mgL^{-1}. Dissolved oxygen also has the function to break down organic matter into inorganic nutrient to be further used by plants. Alkalinity during eel culture had a range of 46 – 105 mgL^{-1}, which was also still within the tolerance limit in eel culture. Study result of Chaudhary & Pillai (2008)mentioned that optimal alkalinity for eel growth ranged of 58 – 123 mgL^{-1}. The value of TSS during observation had a range from1.2 – 3.94 mgL^{-1}.

In the control treatment, TSS value was higher than the treatments with aquatic plants, because there was not filtration system of waste load from in the culture medium. The range of TSS value in this study was still appropriate value for eel, because in fish culture the value of TSS should be <100 $mg\ L^{-1}$(Palero, 2010). The value of TSS is closely related to water turbidity. The higher value of TSS leads to high turbidity of waters. Turbidity is affected by the existence of organic matter, inorganic matter, and other suspended or dissolved material (Khalil *et al*, 2016).

Aquaculture activity produces organic matter and nutrient in substantial quantity. In general, most of the nitrogen waste (60-90%)is in the form of soluble form (particularly ammonia), while it is 25-85% for phosphorus (Van Rijn 2012). In this study, ammonia was measured in the form of total ammonia nitrogen (TAN). The concentration of TAN, NO_2 and NO_3in control was higher than that in treatments (with aquatic plants) since the waste from eel culture in control was not remediated. The range of TAN, NO_2 and NO_3 in this research was 0.12-0.23 mgL^{-1}; 0.011-0.089 mgL^{-1}; and 13.31-53.14 mgL^{-1}, respectively (Table 1), which was lower compared to the study conducted by Akinbille *et al* (2013)who used *Eichhornia crassipes* and *Pistia stratiotes*as remediation agent with TAN, NH_3, and NH_4value obtained of 1.34 mgL^{-1}; 1.79 mgL^{-1}; 0.14 mgL^{-1}; 0.2 mgL^{-1}; 0.13 mgL^{-1}; and 0.22 mgL^{-1}, respectively. Plants in aquaponics system utilize ammonium as the main source of inorganic nutrient through plant roots (Endut *et al*. 2011).

Phosphorus is one of the major nutrients required by plants (Salisbury and Ross 1995). Orthophosphate (PO_4) is inorganic compound dissolved in water and can be directly absorbed by plants (Vaillant *et al*, 2004). Orthophosphate is not toxic to fish. However, high phosphorus content in waters may trigger the growth of algae, resulting in algae bloomingwhich leads to oxygen depletion and mass death of fish. The concentration of orthophosphate in this study ranged from 1.07-8.79 mgL^{-1}. Tolerance for orthophosphate concentration in eel culture ranges of 150 mg/L (Suzuki et al, 2003).

Statistical analysis result for nutrient removal efficiency during culture period indicated significantly different result between treatments. Treatment P2 (*E.palaefolius*) resulted in the highest value of nutrient removal efficiency (TAN, NO_2, NO_3, and PO_4). More complete data are presented in Table 2.

Table 2. The average of nutrient removal efficiency (%)

Parameter	P1 (*E. amazonicus*)	P2 (*E. palaefolius*)	P3 (Without plant)
TAN (%)	$22.19±1.94^{b}$	$27.10±2.42^{c}$	$2.18±0.19^{a}$
NO_2 (%)	$29.40±3.95^{a}$	$45.03±9.77^{b}$	$18.15±4.83^{a}$
NO_3 (%)	$11.95±1.24^{b}$	$20.94±1.29^{c}$	$1.85±1.9^{a}$
PO_4 (%)	$4.75±3.16^{a}$	$14.19±3.05^{b}$	$3.96±3.48^{a}$

Different superscriptlettersshow significantly different effect between treatments (P<0.05)

There is a trend of nutrient removal efficiency every two weeks in each treatment (Figure 1). The efficiency of NO_2 and PO_4 removal in all treatments tended to decrease every 2 weeks. The efficiency of NO_3 removal tended to decline for all treatments, except for a treatment without aquatic plant in week 6th. There was an increasing value of nutrient removal efficiency in control treatment.

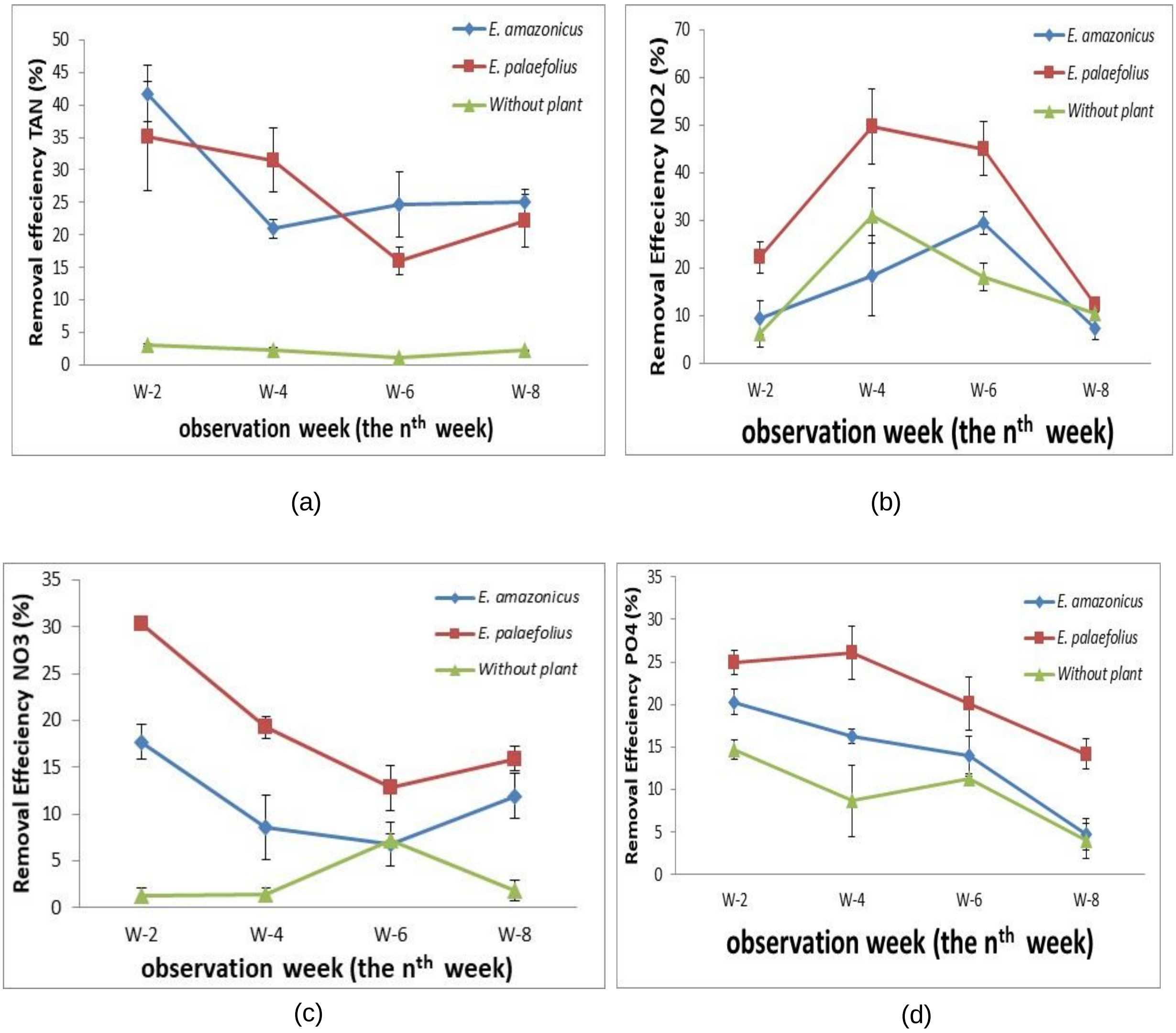

Figure 1. Removal efficiency (%) TAN (a), NO_2 (b), NO_3 (c) and PO_4 (d).

Change in nutrient removal efficiency in the three treatments showed that each plant has different ability to utilize nutrient. The ability of *E. palaefolius*in removing nutrient from eel culture wastewater was higher than that of *E. amazonicus*. It was found that *E. palaefolius*was able to removethenutrient of TAN of 35.13% in week- 2, $NO_2$of 49.65% in week-4, NO_3 of 30.31% in week-2, and $PO_4$of 26.13% in week-4. Compared to the study conducted by Chrismada and Mardiati (2011), it was found that L. *perpusilla* hasabsorbtive capacity of3.9 mg NO_3 $m^{-2}day^{-1}$and 6.7 mg PO_4 $m^{-2}day^{-1}$in water media originated fromSaguling Dam in day-3 of study with removal rate of N-$NO_3$of 74.05% and P-$PO_4$of 73.36%. Result of this study showed lower value, yet the concentration of TAN, NO_2, NO_3,and PO_4obtained was still within the optimum range for eel to live.

Growth performance of eel

Based on Table 3, specific growth rate (SGR) ranged of 0.94% – 1.19%. The range of feed conversion rate (FCR) was 1.57-3.91. Protein retention had a range of 18.88% - 41.12% and lipid retention ranged of 29.27% – 47.31%. Survival rate of all treatments was 100%. Result of statistical analysis showed that significantly different effect was found between treatments in all parameters. The use of *E. palaefolius*plant resulted in highest value of SGR, protein retention, and lipid retention of 1.19±0.18%; 41.12±3.51%; and 47.31±6.65%, respectively, compared with *E.amazonicus*plant and control.

Table 3. Growth performance of eel

Parameter	P1 (*E. amazonicus*)	P2 (*E. palaefolius*)	P3 (Without plant)
SR (%)	100	100	100
SGR (%)	0.94±0.21^{ab}	1.19±0.18^{b}	0.65±0.30^{a}
FCR	2.09±0.67^{ab}	1.57±0.30^{a}	3.91±1.09^{c}
Protein Retention (%)	37.18±4.01^{b}	41.12±3.51^{b}	18.88±2.69^{a}
Lipid Retention (%)	42.47±7.10^{b}	47.31±6.65^{b}	29.27±2.44^{a}

Different superscript letters show significantly different effect between treatments (P<0.05)

Based on the data of eel growth performance (Table 3), the use of aquatic plant as phytoremediator was able to decrease FCR as well as increase SGR, protein retention, and lipid retention. This finding showed that the use of aquatic plant (*E. amazonicus* dan *E. palaefolius*) in this study did not create inhibitory effect on survival rate and the growth of eel. Increasing growth performance of eel indicated that the role of aquatic plant in phytoremediation is necessary since the environment in which the fish live is improving as seen from the optimum value of water quality parameter during culture period (Table 1). Study result of El-Shafai *et al* (2007) proved that the application combination of recirculating system and *Lemna gibba* aquatic plant was able to increase the growth and decrease FCR in Tilapia culture. Similarly, result of study performed by Ghaly *et al* (2004); Endut *et al* (2011); Effendi *et al* (2015), by using aquatic plant as phytoremediator in recirculating system was found to increase the growth performance of the commodity cultured.

Growth performance of plants

During culture period of 60 days, freshwater aquarium plants grew well. The growth performance of aquatic plants was seen from the weight gain, daily growth rate, nitrogen retention, and phosphorus retention in plant tissue. Data of aquatic plant growth are presented in Table 4.

Table 4. Growth performance of plants during 60 days of culture

Parameter	P1 (*E. amazonicus*)	P2 (*E. palaefolius*)
Weight gain (gram)	9.33±2.52	32.67±8.96
Daily growth rate (g/day)	0.16±0.04	0.54±0.15
N retention (%)	2.59±1.22	21.47±2.97
P retention (%)	3.97±0.84	6.36±1.08

The success of phytoremediation system cannot be separated from the growth of plant as phytoremediation agent. During the observation period, the growth of aquatic plants in phytoremediation system showed positive responseas indicated by the increasing biomass growth, nitrogen retention, and phosphorus retention in plants. Aquatic plants used in this study were found to have the ability to utilize eel culture wastewater as nutrient source for growth. This statement was confirmed by the occurrence of nutrient removal by plants every observation period (2 weeks) (**Figure 1**). The growth of *E. palaefolius*was faster than that of *E. amazonicus*, as well as its nitrogen and phosphorus retentions which were also higher.

4. Conclusion

Treatment using aquatic plant *E. amazonicus*and and *E.palaefolius*as phytoremediator highly affected the declining concentration of TAN, NO_2, NO_3 and PO_4 in eel culture wastewater. The ability of *E. palaefolius*in utilizing the nutrient of fish culture waste was found to be better than that of *E. amazonicus*. The growth performance of eel maintained with phytoremediator *E. amazonicus*and *E. palaefolius*was better than the growth of eel treated without aquatic plants.

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