Techno-economic analysis of integrated multitrophic *Clarias gariepinus* and *Arthrospira platensis* with different agitation period

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Abstract. *Arthrospira platensis* is a cyanobacteria that contains high levels of protein, fatty acids, minerals, vitamins, amino acids, and phycocyanins. The cultivation of *Arthrospira platensis* is constrained by fertilizers that have relatively high prices, so alternative fertilizers are used, one of which is catfish waste which has sufficient nutrient content for the growth of *A. platensis* which can be integrated as IMTA (Integrated Multi-trophic Aquaculture) *Clarias-Arthrospira*. Cultures of *A. platensis* on a mass scale often use open culture ponds in the form of raceways because they are easy to handle. Raceway pond agitation uses pedals that rotate for 24 hours leading to high electricity usage costs which account for 23% of total production costs. This study aims to determine the efficient stirring time on the growth of microalgae *A. platensis.* This study used 3 treatments with different stirring frequencies, namely treatment 1 stirring for 24 hours (T24), treatment 2 stirring for 12 hours (T12), and control using aeration for 24 hours (TC). After harvesting the biomass, the water from filtration has flowed into the catfish rearing container. Parameters observed were daily density *of A. platensis,* specific growth rate of *A. platensis*, revenue-cost (R/C) ratio, return of investment (ROI) and feed conversion ratio of catfish rearing that was used for *A. platensis* production. The results of the specific growth rate of *A. platensis* in this study were TC 23.625%, T24 21.162%, T12 21.105% per day. The maximum density at TC and T12 was 0.8 g.L^{-1} on day 10 while at T24 0.8 g.L^{-1} on day 9. Revenue Cost (R/C) ratio can be reached at TC 0.94, T24 1.09, T12 1.12. The result of catfish rearing used TC, T24, and T12 waste water treatments with *A. platensis* showed that the feed conversion ratio of catfish rearing was 1.38, 1.30, and 1.04 respectively. The stirring using a raceway container for 12 hours produced the best results compared to other treatments.

Key Words: catfish, raceway, R/C ratio, *Spirulina* sp.

Introduction. Aquaculture techno-economics is a complete study to scale up aquaculture business from pilot plant scale to mass scale or industrial scale, to ensure environmental-system stability and economic analysis of the feasibility of aquaculture. The development of fish farming is being intensified as an effort to meet the food needs of the community, especially protein. The Ministry of Maritime Affairs and Fisheries (KKP) launched the 2021 KKP's tagline "Mobilizing aquaculture, it is believed that it can support food security and regional economic development amid the Covid-19 Pandemic" (KKP 2021), which is to drive aquaculture by paying attention to environmental sustainability and encouraging community economic turnover (Antara 2021). The increasingly limited land and water for fish cultivation make the need for zero waste aquaculture technology that is approached with a recirculation system (Ramli et al 2020). The productivity of fish farming pond recirculation systems can be increased by using the IMTA (Integrated Multi-trophic Aquaculture) system. Integrated multi-trophic aquaculture (IMTA) is a production system of aquatic biota in which various species, of different trophic levels, are raised near each other and the by-products (organic and inorganic waste) of one cultured species are recycled to be used as nutrients for other biotas. IMTA can reduce the ecological impact around aquaculture operations, increase social perceptions of aquaculture, and provide financial benefits to aquaculture actors through product diversification, faster production cycles, and better income on IMTA products (Knowler et al 2020). The concept of fish production and remediation aquaculture commodities such as microalgae and cyanobacteria with high economic value can be an alternative for zero waste swamp aquaculture systems.

Cyanobacteria have been shown to be a rich source of biologically active secondary metabolites including antiviral, anti-inflammatory, immunostimulant agents, and their medicinal and nutraceutical properties (Deyab et al 2019; Tabarzad et al 2020). Tiwari and Tiwari (2020) have summarized and updated therapeutic applications. Cyanobacteria in drug development, with the introduction of the new term "cyanotherapeutics", after many studies have shown that these cyanobacteria have immunomodulatory, antitumor-anticancer, antiviral-antiHIV, antibacterial, antimalarial, antidiabetic activities which are specific characters for drug candidates (Naidoo et al 2020). *Arthrospira platensis* which is the only cyanobacteria that is safe for consumption free of toxins can be used to treat wastewater, including waste from fish farming because biomass can metabolize nutrients and remove pollutants from aquaculture waste efficiently (Zhang et al 2019). Treatment of industrial waste and by-products can be used for alternative culture media for *Spirulina* sp. (*Arthrospira platensis*) production as well as fish farming wastewater (Wijayanti et al 2019; Widyantoro et al 2018; Ragaza et al 2020). *Arthrospira platensis* (*Spirulina* sp.) cultivation in fish pond wastewater can have certain characteristics. Their adaptation growing in organic wastewater makes changes in the bioactive and production of important compounds. Phycocyanin is blue pigment in microalgae or cyanobacteria that have functions as antioxidant, source of food coloring, cosmetics, nutraceuticals, pharmaceuticals, and anticancer (Liu et al 2013; Tang et al 2020; Tiwari & Tiwari 2020). *Arthrospira platensis* cultured in catfish culture waste media 100% laboratory-scale bulk system can produce a maximum density of 0.867 grams dry weight per liter, the growth rate of 22.026% day^{-1} and 11.347 mg g^{-1} phycocyanin yield and able to reduce nitrogen waste by more than 80% and phosphate by more than 70% (Wijayanti et al 2020).

The design of the cyanobacterial bioreactor influences the important phyto-biochemical content contained in the post-harvest biomass. Determination of the design and culture system that is better and low-cost, will increase the practical and economic feasibility of integrating cyanobacteria in aquaculture recirculation systems, thereby increasing the potential for mass algae cultivation when scaling up this aquaculture recirculating system (Ramli et al 2020). Increasing the scale of production from a closed system to an open system is an important step for the stability of product quality and quantity, the technological and economic feasibility of the IMTA system. The pilot-scale is used for commercial-scale preparation. Open pond bioreactors are often used because they are easy and inexpensive to maintain (Mutiah & Khoirunisa 2013). There are two types of open pond bioreactors, raceway, and circular/central pivot ponds. Circular ponds where mixing is done by agitators or raceway ponds are equipped with a paddle wheel agitation system (Fazal et al 2018). De Jesus et al (2018) showed that 24-hour stirring resulted in a biomass concentration of 1.60 ± 0.04 g L^{-1}, biomass productivity of 0.054 ± 0.003 g L^{-1} h^{-1}, and a specific growth rate of 0.05 ± 0.002 h^{-1} on the coast of Bahia in the city of Salvador, Brazil, and in the city of Camaquã, Brazil it's yield density was of 0.90 ± 0.02 gL^{-1}, biomass productivity of 0.009 ± 0.001 gL^{-1} h^{-1} and specific growth of 0.02 ± 0.001 h^{-1}. However, stirring for 24 hours resulted in the use of high electrical energy.

Jonker and Faaij (2013) concluded the electric energy consumption of stirring is 23% of the total energy demand. The difference in the stirring time of *A. platensis* culture with catfish culture wastewater has never been studied before. Therefore, it is important to study differences in the length of time for stirring *Arthrospira platensis* culture media to make electricity use efficient and maximize the growth of *Spirulina* sp. in catfish culture wastewater media by knowing the highest maximum population density of *Arthrospira platensis*.

Material and Method. This study was conducted from April to November 2021 at the Laboratory of Aquaculture, Sriwijaya University, Indralaya, Ogan Ilir, South Sumatra, Indonesia (Figure 1). The experimental design used in this study was a Randomized Block Design using 3 treatments of agitation methods: control using 24-hour aeration day^{-1} (TC), stirring for 24 hours day^{-1} (T24) and stirring for 12 hours day^{-1} (T12). The replications of culture time cycling were first culture cycle, second culture cycle, and third culture cycle of integrated *Clarias gariepinus* - *Arthrospira platensis*.

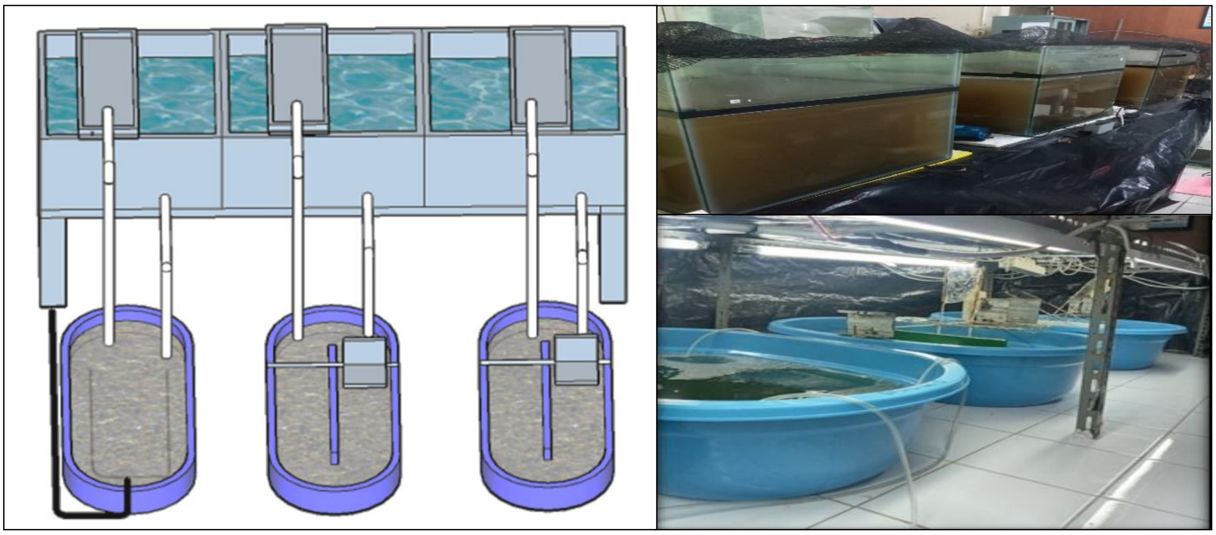

Figure 1. Design of integrated *Clarias gariepinus* - *Arthrospira platensis* culture in this study.

Preparation of Arthrospira platensis growing media. Wastewater comes from catfish rearing ponds which are kept for 14 days using a 40 x 40 x 40 cm aquarium. The density of catfish reared is 500 fish/m^3 with a size of 12±1 cm with a salinity of 4 ppt (Sitio et al 2017) and a pH of 7.5-8 (Ndubuisi et al 2015). During the maintenance catfish were fed floating pellets with a protein content of 31-33%. Feed is given 2 times a day (morning and evening) at satiation. No water changes are made during fish rearing. After rearing the catfish, the wastewater from catfish farming is taken and filtered using a 30-40 micron cloth to clean the wastewater from dirt and insoluble feed residue.

Culturing and harvesting Arthrospira platensis. *A. platensis* used was first cultured as initial stock inoculum (Aquaculture Laboratory culture collection at Sriwijaya University) with a minimum density of ± 1.0 g L^{-1}. Then the *A. platensis* seeds were put into the catfish culture wastewater media, with an initial density of ± 0.1 g L^{-1}. The lighting time with the help of fluorescent lamps is 12 hours (Oktafiani & Hermana 2013; Budiardi et al 2010). Agitation was carried out using a mill with a speed of 5-6 rpm. The control had aeration using an aerator for 24 hours, treatment 1 was stirred for 24 hours, while in treatment 2 the stirring was for 12 hours (06:00 am – 6:00 pm).

When *A. platensis* has passed the peak phase of maximum density or exponential phase, harvesting of *A. platensis* is carried out using a cloth filter measuring 30-40 microns (Wijayanti et al 2020). *A. platensis* was filtered with a cloth then weighed for the wet weight and then put into a porcelain crucible, then dried in an oven at 40°C for 18 hours (Afriani et al 2018 modification), then mashed in a mortar.

Daily density measurement. In this study, the initial density of *A. platensis* was measured using 1 milliliter of *A. platensis* culture samples in each treatment with 3 replications. The 1 milliliter water sample was put into a pre-weighed aluminum foil bowl. The wet samples were then dried in an oven at 40°C for 18 hours. The dried water sample after the oven treatment was reweighed to obtain dry weight biomass. The dry weight of *A. platensis* biomass was then converted to g L^{-1}. Measurement of the density of *A. platensis* was carried out in each treatment every day at the same hour.

Arthrospira platensis daily growth rate. The daily growth rate (μ) of *A. platensis* can be calculated using the Vonshak formula μ = ((ln Nt- ln No)/t) x 100%, where Nt is the biomass concentration at t days culture and No is the initial biomass at 0 day culture (Vonshak 2002).

Extraction and measurement of phycocyanin yield. The dry biomass of *A. platensis* was taken at 0.04 g after passing through the exponential phase (harvest) for preparing phycocyanin extraction. The method of phycocyanin extraction followed previous studies (Wijayanti et al 2020). The absorbance of the extracted supernatant was measured using a spectrophotometer at a wavelength of 615 nm and 652 nm for determining phycocyanin yield with Bennett and Bogorard formulas : C-phycocyanin (mg mL-1) = ((OD 615) - 0.474 (OD 652))/5.34; and Phycocyanin yield (mg g-1) = (C-phycocyanin x Solvent volume)/Dried Biomass (Bennett & Bogorad 1973).

Reduction of total nitrogen content. Reduction of total nitrogen content in the media was carried out at the beginning and end of the study (1 day after the peak phase in each treatment).

Business analysis. Profit calculation is done by R/C Ratio and ROI, which is calculating the selling price of catfish and *A. platensis* dry biomass divided by production costs including feed costs, electricity usage for biomass production, and drying time for one production cycle in each treatment.

$$RCR = \frac{\text{Total Revenue}}{\text{Total Production Costs}} \qquad ROI = \frac{\text{Total Profit x 100\%}}{\text{Total Production Costs}}$$

Statistical analysis. The values of maximum density, growth rate, phycocyanin yield, benefit-cost ratio, reduction in total nitrogen content of each treatment medium were analyzed for variance (ANOVA). If it was significantly different, then the analysis was continued using the Honest Significant Difference test. The density of *A. platensis* is presented in graphical form. Specific growth rate data, phycocyanin yield, reduction of total nitrogen, R/C ratio, ROI, and FCR are presented in tabular form.

Results and Discussion

Density of A. platensis. The daily density of *A. platensis* for each culture treatment have been shown in Figure 2. The time of achieving the highest density of *A. platensis* in each treatment was eight days after cultivation start.

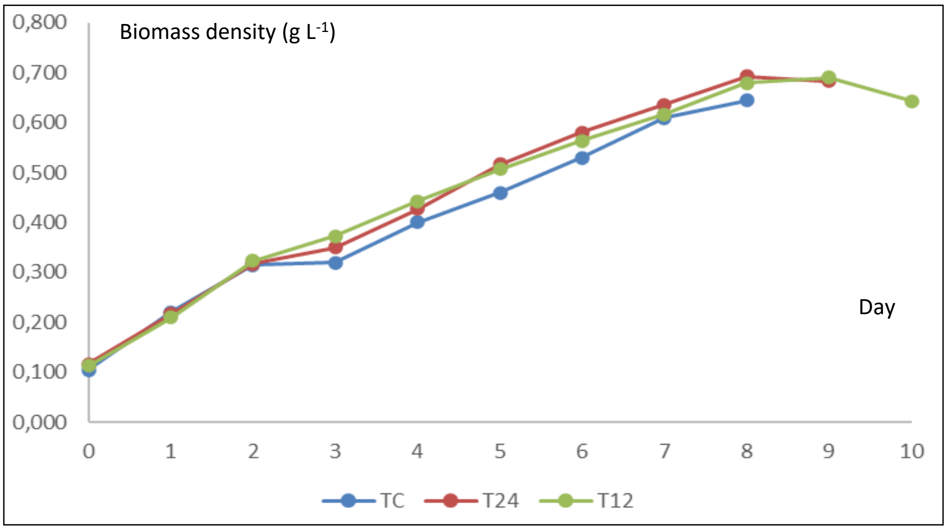

Figure 2. Biomass density of *Arthrospira platensis* for 10 days.

The maximum density in treatment TC was 0.645 g L^{-1} on day 8, in treatment T24 it reached 0.693 g L^{-1} on day 8, in treatment T12 it reached 0.690 g L^{-1} on day 9. The maximum density of *A. platensis* on treatments have been shown in Figure 3. It can be seen that the highest density of *A. platensis* was in the T24 treatment with a biomass of 0.693 g L^{-1} and T12 with an average biomass of 0.690 g L^{-1}, while the lowest density was in the TC treatment, 0.645 g L^{-1}.

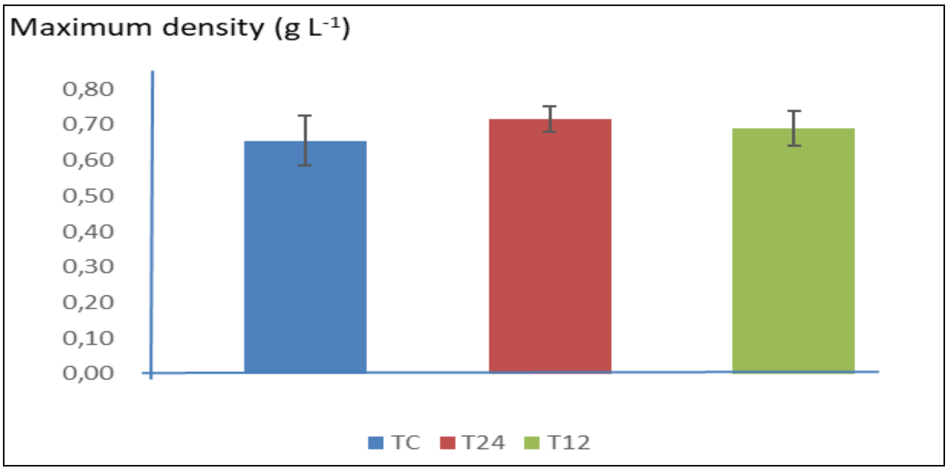

Figure 3. Maximum density of *A. platensis* biomass.

Table 1

Maximum density (g L^{-1}) of *A. platensis* in treatment stirring on *A. platensis* culture media from *Clarias gariepinus* waste water

Treatment of stirring media	*Group cycle*			*The effect of treatment (T)*
	1	*2*	*3*	
TC	0.80	0.65	0.52	0.66 ± 0.14
T24	0.80	0.68	0.67	0.72 ± 0.07
T12	0.80	0.66	0.61	0.69 ± 0.10
Mean	0.80 ± 0.00	0.66 ± 0.02	0.60 ± 0.08	

Based on Table 1, the highest density of *A. platensis* was seen in replicate 1 (first culture cycle) with a biomass of 0.80 g L^{-1} and after that, it decreased gradually to 0.66 g L^{-1}, 0.60 g L^{-1}. The high and low density of A. platensis was determined by the nutrients that were still present in the cultivation medium. The N ratio affects the growth of microalgae and the level of light intensity that enters the media. The maintenance of *A. platensis* describes the growth phase from the first day to the 10th day slowly, because of cells adapting to the new environmental conditions. All treatments had an average increase on day 8. This explains that the growth of *A. platensis* cells undergo division. The existence of cell division causes the growth of *A. platensis* to run quickly because the growth medium of *A. platensis* is rich in nutrients that are needed for its growth. On day 8 the maximum density of *A. platensis* culture was reached. After the 8th day, all treatments experienced a decline (death) phase on average. This is caused the number of nutrients in the media had decreased. However, *A. platensis* cells were still able to divide but the number was not as large as in the growth phase.

According to Budiardi et al (2010), the death phase is due to limited nutrition and light supply, old cell age, environmental factors that are no longer supportive, and contamination by other microorganisms. According to Haryati (2008), the decrease in growth of *A. platensis* occurs due to reduced nutrients caused by the increase in *A. platensis* biomass, as a result, biomass needs to compete for nutrients in the culture medium. In addition, the denser the biomass results in the reduced intensity of light entering the culture media so that it can interfere with the biomass to carry out photosynthesis. The pinwheel and aeration aid in the agitation of the medium containing nutrients and algae so that all cells are thoroughly exposed to the nutrients and light supplied. In addition, the agitation caused by the velocity of the bubbles produced was not good because not all parts of the cells were exposed to the nutrients from the medium. High bubles aeration can also give less than optimal results on biomass density in the *Arthrospira platensis* culture (Jung et al 2019)

Specific growth rate. The specific growth rate describes the rate of growth of algal cells per unit time which can be used as a benchmark to determine the carrying capacity of the media on algae growth (Santosa 2010). The value of the specific growth rate of *A. platensis* for each treatment is shown in Table 2 and Figure 4.

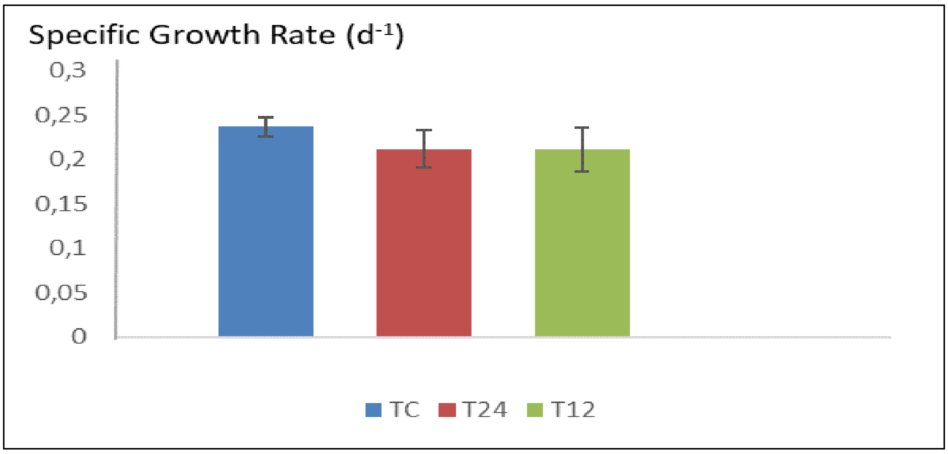

Figure 4. Specific growth rate of *A. platensis* in growth phase.

Table 2

Specific growth rate (% per day) of *A. platensis* in treatment stirring and on *A. platensis* culture media from *Clarias gariepinus* waste water

Treatment of stirring media	*Group cycle*			*The effect of treatment (T)*
	1	*2*	*3*	
TC	25.99	22.69	22.19	23.63 ± 2.07
T24	25.99	19.27	18.22	21.16 ± 4.22
T12	25.99	20.97	16.35	21.11 ± 4.82
Mean	25.99 ± 0.00	20.98 ± 1.71	18.92 ± 2.98	

Based on the results in Table 2, the control treatment gave the best specific growth rate value of 23.63% per day. The 12 hours stirring gave the lowest growth rate value of 21.11% per day. Although, the specific growth rate of *A. platensis* biomass in all of the results of the treatment is not different significantly. The high growth rate is caused by sufficient carbon dioxide which is diffused with aeration bubbles. According to de Godos et al (2014), the growth rate is influenced by the limitations of carbon dioxide in the media. Under phototrophic growth conditions in raceway pond cultures, the carbon dioxide becomes very limited. It can be at least 5% of the carbon required by algal cultures is released directly into the atmosphere from culture media.

In contrast, the first cycle gave different specific growth rates with the third cycle of culture. In the first cycles of culture, the biomass had better conditions than in the third cycle. The biomass of *A. platensis* needs the micronutrient for growth. The wastewater medium is less micronutrient for enhancing growth. It can be suggested for adding the micronutrient such as vitamins and trace minerals in the culture media for increasing the performance of *A. platensis* cells, especially for enhancing the growth. According to Borowitzka and Vonshak (2017), potential problems with media recovery are selective enrichment of cultured contaminating organisms or unwanted cell morphologies; build-up of specific ions that negatively impact algal growth; establishment of auto-inhibition; cell disruption during harvest resulting in increased dissolved organic matter; flocculants residues lead to flocculation of cells in the culture. For the medium to be recovered may require some pretreatment or conditioning before being returned to the culture tank. Most large commercial algae producers have successfully implemented moderate recovery.

Reduction of total nitrogen content. Ammonia in the form of ammonium is used by *A. platensis* as a nitrogen source to stimulate its growth. Nitrogen needed for microalgae growth is generally absorbed in the form of nitrate (NO_3^-). Nitrate is further reduced by nitrite reductase to nitrite and then reduced to ammonium (NH_4^+) so that it can be synthesized through various amino compounds in *Spirulina* sp. (Widyantoro et al 2018). The percentage of nitrogen reduction have beeen shown in Table 3.

Table 3

Reduction of total nitrogen content (%) in *A. platensis* culture media

Treatment of stirring media	*Group cycle*			*The effect of treatment (T)*
	1	*2*	*3*	
TC	97.54	98.82	98.76	98.38 ± 0.73
T24	99.28	99.19	99.01	99.16 ± 0.14
T12	92.75	95.71	98.07	95.51 ± 2.66

Based on the results in Table 3, the percentage reduction of nitrogen content in treatment T24 (99.161%) is higher than in treatment TC (98.373%), and T12 (95.507%). However, based on analysis of variance between treatments and groups, the percentage reduction of nitrogen content had no significant effect. *A. platensis* in T24 treatment was able to utilize more nitrogen content. In this study *A. platensis* in wastewater of *Clarias gariepinus* catfish can reduce total nitrogen media by more than 95%. The study of *A. platensis* in wastewater of Nile tilapia (*Oreochromis niloticus*) reared in biofloc system media can remove the nitrogen, such as nitrate nitrogen uptake of 99%, 80% of total ammonia nitrogen (TAN) and 90% of nitrite nitrogen for 10 days (de Moraes et al 2021). Ammonia can remove 87.77 ± 1.01% by *Spirulina platensis* cultured in wastewater of *Pangasius* catfish reared for 10 days (Wijayanti et al 2019). Thus, given the results obtained, besides being able to grow in wastewater, *A. platensis* can also be used in bioremediation processes of aquaculture wastewater.

Phycocyanin yield. The results of the phycocyanin analysis included C-phycocyanin and phycocyanin extract yields. Based on the research, the highest concentration of phycocyanin was obtained from TC which was an average of 0.464 mg mL^{-1}. While the lowest levels of C-phycocyanin were in treatment 1 with an average of 0.39 mg mL^{-1}. The yield value of phycocyanin *A. platensis* for each treatment is presented in Table 4.

Table 4

Phycocyanin (%) of *A. platensis* dry biomass at 10 days after cultivation

Treatment of stirring media	*Group cycle*			*The effect of treatment (T)*
	1	*2*	*3*	
TC	5.098	1.192	5.315	3.87 ± 2.32
T24	5.043	2.391	2.318	3.25 ± 1.55
T12	3.300	2.697	4.350	3.45 ± 0.84

The analysis of variances at the treatment effects of phycocyanin yield (Table 4) showed that the stirring time and the different groups were not significantly different from the phycocyanin yield. The highest phycocyanin yield was obtained in the treatment using an aerator for 24 hours day^{-1} (3.87%) and the lowest was in the stirring treatment using a propeller for 24 hours day^{-1} (3.25%). According to Zhou et al (2017), pH value and salinity of culture media conditions can affect protein levels in *A. platensis* cells, differences in salinity affect the external osmotic pressure of *A. platensis* cells causing changes in cell composition, especially phycocyanin. Sharma & Tiwari (2011) stated that the environment includes nutrient availability, pH, salinity, light, and temperature can influence the growth and bio pigments accumulation of cyanobacteria biomass.

Business analysis. The results of the feasibility analysis for semi-mass production are obtained from production costs with the assumption that the purchase price of catfish is IDR 19,000 kg^{-1}, the price of feed is IDR 11,000 kg^{-1}, the price of electricity is IDR 1,445 kWh^{-1}, the price of salt is IDR 5,000 kg^{-1} and the selling price of catfish IDR 22,000 kg^{-1}, selling price of *A. platensis* IDR 500 g^{-1}, with investment in tools such as a dynamo (4 watts) IDR 18,000 with a service life of 4 years, a mill of IDR 30,000 with a service life of 4 years, two aerators (10 watts) IDR 37,000 with a service life of 3 years, containers IDR 80,000 with a service life of 6 years, Tube lamp (32 watts) IDR 47,000 with a service life of 4 years and Asahi cloth IDR 18,000 with a service life of 4 years, analysis of R/C Ratio and ROI in each treatment and group. Table 5 shows the business analysis.

R/C Ratio (Return Cost Ratio) is a comparison between total revenue and production costs. There are three criteria in the R/C ratio, namely: R/C ratio > 1, then the business is profitable, R/C ratio = 1, then the farm is breaking even, and R/C ratio < 1, then it is not efficient. ROI (Return On Investment) is a comparison between the total profit and production costs in percent. There are three criteria in ROI: ROI is positive, the business is profitable, ROI = 0, then the farm breaks even, and ROI is negative, so it is not efficient.

Honestly Significant Difference test showed that the R/C ratio and ROI in treatment TC (aeration for 24 hours day^{-1}) were significantly lower than treatments T24 and T12. Treatments T24 and T12 were not different significantly, although the highest value was in the T12 treatment. In the T12 treatment, the R/C Ratio and ROI were the highest, while in the control treatment the R/C Ratio and ROI were the lowest compared to other treatments, the low profit in the aeration treatment was due to the use of electricity up to 46% of the total production cost, so it was less economical if done in cultivation.

Table 5

R/C and ROI in *A. platensis* culture integrated with *Clarias gariepinus* rearing systems, and the feed conversion ratio of the catfish rearing

Treatment of stirring media	*Mean of R/C value ($HSD_{\alpha 0.05= 0.069}$)*	*Mean of ROI value ($HSD_{\alpha 0.05= 6.882}$)*	*Feed Conversion Ratio (FCR) in catfish rearing*
TC	0.942 ± 0.050 ^{a}	-5.756 ± 5.025^{a}	1.38 ± 0.33
T24	1.098 ± 0.059 ^{b}	9.821 ± 5.907^{b}	1.30 ± 0.30
T12	1.120 ± 0.068 ^{b}	12.013 ± 6.842^{b}	1.04 ± 0.40

In T12's first production cycle, the average cost of feed was IDR 3,458, catfish was IDR 12,780 and catfish earn was IDR 21,294, product *A. platensis* flour was IDR 9,850 with a total income of IDR 31,144 with a profit of IDR 1,585 from production costs of IDR 29,559 (*A platensis* in 30 liters of media and 20 fish). *A. platensis* in 12,000 liters of media and 8,000 fish will get an income of IDR 12,457,696 and a profit of IDR 5,527,788 with a production cost of IDR 6,929,908 in 1 cycle, if in 1 year of production there are 26 cycles then the profit obtained will reac IDR 143,722,495.

Conclusions. The utilization of catfish culture wastewater for the culture of *A. platensis* with different treatments and stirring time gave a significant effect on business analysis, but no effect on the maximum density and the growth rate of *A. platensis*, the reduction of total nitrogen content in the media, and the yield of phycocyanin. The best growth rate and total nitrogen reduction were obtained in treatment T24 (stirring using a mill for 24 hours per day). The result of the business analysis was better using a raceway system with wheel rotation than using an aeration system. The treatment stirring wheels 12 hours per day is the best result for scaling up *Arthrospira platensis* production at an outdoor integrated pond system.

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Conflict of interest. The authors declare that there is no conflict of interest.

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